Vadose Zone Processes and Chemical Transport

Evaluating Microbial Purification during Soil Treatment of Wastewater with Multicomponent Tracer and Surrogate Tests

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ABSTRACT

Soil treatment of wastewater has the potential to achieve high purification efficiency, yet the understanding and predictability of purification with respect to removal of viruses and other pathogens is limited. Research has been completed to quantify the removal of virus and bacteria through the use of microbial surrogates and conservative tracers during controlled experiments with three-dimensional pilot-scale soil treatment systems in the laboratory and during the testing of full-scale systems under field conditions. The surrogates and tracers employed included two viruses (MS-2 and PRD-1 bacteriophages), one bacterium (ice-nucleating active *Pseudomonas*), and one conservative tracer (bromide ion). Efforts have also been made to determine the relationship between viruses and fecal coliform bacteria in soil samples below the wastewater infiltrative surface, and the correlation between *Escherichia coli* concentrations measured in percolating soil solution as compared with those estimated from analyses of soil solids. The results suggest episodic breakthrough of virus and bacteria during soil treatment of wastewater and a 2 to 3 log (99–99.9%) removal of virus and near complete removal of fecal coliform bacteria during unsaturated flow through 60 to 90 cm of sandy medium. Results also suggest that the fate of fecal coliform bacteria may be indicative of that of viruses in soil media near the infiltrative surface receiving wastewater effluent. Concentrations of fecal coliform in percolating soil solution may be conservatively estimated from analysis of extracted soil solids.

More than 25% of the U.S. population and 37% of all new development is served by on-site and small-scale wastewater systems. Wastewater treatment for these on-site and small-community applications commonly relies on infiltration and percolation of septic tank effluent through soil to achieve purification before recharge to ground water (Crites and Tchobanoglous, 1998; USEPA, 1997; Jenssen and Siegrist, 1990). These porous media–based systems (wastewater soil absorption systems) are widely used due to their high purification performance resulting from the complex interactions of hydraulic and purification processes (Siegrist et al., 2001; McCray et al., 2000; Ausland, 1998; Schwagger and Boller, 1997). However, in many settings, there is an increasing awareness and concern over performance of these systems with respect to pathogenic bacteria and virus. Since human pathogens are known to exist in sewage effluents, their removal in soil treatment systems is essential in preventing contamination of ground water sources. There have been incidences of disease outbreaks due to contaminated source water in ground water systems (Craun, 1985; USEPA, 2000). In fact, some researchers have noted that septic systems are the most frequently reported cause of ground water contamination associated with disease in the USA (Powelson and Gerba, 1994). Thus, fundamental understanding of the relationship between design parameters and environmental conditions and their effects on purification performance is required to use soil treatment systems effectively while preventing ground water contamination.

Despite the need for understanding, research into pathogen transport and fate in porous media or soil-based wastewater treatment systems has been limited. Most work has assessed microbial purification by tracking the behavior of fecal coliform bacteria presuming they are indicators of human pathogens. However, due to their size, surface properties, and survivability in the environment, the behavior of fecal coliform bacteria may not adequately mimic that of many human pathogens, particularly viruses, and may be poor predictors of waterborne disease outbreaks (Craun et al., 1997). Studies of viruses in wastewater soil absorption systems have been limited and are normally based on the use of viral surrogates, such as bacteriophages, due to their availability, ease of assay, and lack of pathogenicity. Commonly employed bacteriophages have been MS-2 and PRD-1. Both of these bacteriophages have been found previously to adsorb poorly to soils (Bales et al., 1991; Yahya et al., 1993) and have been extensively used as viral surrogates (Adams, 1959; VanDuin, 1988; Bales et al., 1991; Emerick et al., 1997; Schijven et al., 1999). Previous studies have reported significant (24–83%) reductions of enteric virus in septic tanks, probably due to sedimentation with suspended solids (Roa et al., 1981; Payment et al., 1986; Higgins et al., 1999). Additionally, field-scale studies have demonstrated high removal efficiencies for viruses (Table 1). The concentrations of viruses and duration of additions have been either intentionally high to challenge a wastewater soil absorption system or have been set at levels more closely representative of those found in septic tank effluent when a

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Abbreviations: BT_{10}, time to 10% breakthrough; BT_{50}, time to 50% breakthrough; cfu, colony forming units; INA, ice-nucleating active; pfu, plaque forming unit; STE, septic tank effluent; TOC, total organic carbon.

Table 1. Results of field studies of virus treatment in wastewater soil treatment systems.

Investigator	Location	Study characteristics	Virus and concentrations	Method of assessment	Findings
This study (five sites)	Colorado	0.5 to 2.7 cm/d hydraulic loading rate (HLR) of septic tank effluent (STE) to mature absorption systems	spiking of STE with MS-2 and PRD-1 at approximately 1×10^5 plaque forming units (pfu)/mL	soil core collection and extraction	99.9% removal after 60 cm
Nicosia et al. (2001)	Florida	3.2 or 6.3 cm/d HLR of STE to infiltration cells	spiking of STE with bromide and PRD-1 at 6.0×10^{10} or 1.6×10^{11} pfu/mL	stainless steel pan lysimeters	$\log_{10}$ removals of PRD-1 after 60 cm of unsaturated fine sand were 1.43 and 1.91 at high HLR and 2.21 at low HLR
Higgins et al. (1999)	Massachusetts	3 cm/d HLR of STE to a buried sand filter constructed of medium sand	indigenous MS-2 at 3×10^4 pfu/mL in raw wastewater and 7.8×10^3 in STE applied to sand	pressure-free pan lysimeters placed during sand placement in buried, lined cells	74.44% removal in septic tank, 99.17% removal in 30 cm of soil, 98.45% removal in 60 cm, 99.79% removal in 152 cm†
Oakley et al. (1999)	California	variable loading (0.81–6.5 cm/d) of STE to a soil absorption system in clay loam	indigenous ϕX174 at 1×10^0 to 1×10^4 pfu/mL in STE	suction-lysimeters augured and driven into intact natural soil	1-log removal in recirculating gravel filter, 100% removal in 60 cm of soil
Anderson et al. (1991)	Florida	onsite soil absorption systems and subdivisions on fine sandy soils	indigenous virus present in STE at 0.06 to 43.7 most probable number (MPN) of infectious units per L	soil cores and extraction plus ground water samples	no enterovirus were detected in soil samples below the soil infiltration area at four homes; at one home, virus was detected in shallow ground water at a 0.6- to 0.9-m depth right under the system but not 3 m downgradient from it
Gilbert et al. (1976)	Arizona	secondary effluent land applied at 100 m/yr with cyclic flooding onto fine loamy sand	indigenous enterovirus at 1×10^3 to 7×10^3 pfu/100 L in municipal effluent	ground water sampling and analysis	99.99% removal in 3 to 9 m of soil

† Percent removals are based on the STE levels applied to the soil.

resident of a dwelling would be shedding viruses and infectious bacteria.

Research was initiated at the Colorado School of Mines to study the hydraulic and purification behavior of wastewater soil treatment systems and to quantify the effects of infiltrative surface character and vadose zone soil depth (Van Cuyk et al., 2001). The focus of this paper is on the fate and transport of a multicomponent surrogate and tracer mixture in laboratory pilot-scale three-dimensional lysimeters and in full-scale mature soil treatment systems. The overall goal of the research is to quantify the removal of MS-2 and PRD-1 bacteriophages under subsurface transport conditions representative of soil-based wastewater treatment systems and to compare this transport with that of indicator bacteria.

MATERIALS AND METHODS

Surrogate and Tracer Materials and Analyses

Two bacteriophages, MS-2 and PRD-1, were used in this study. Bacteriophage MS-2 is an icosahedral single-stranded RNA coliphage with an average diameter of 25 nm and an isoelectric point (pH_{iep}) of 3.9 (Powelson et al., 1990). Bacteriophage PRD-1 is an icosahedral double-stranded DNA phage with a diameter of 62 nm, a pH_{iep} of <4.5, and a lipo-protein coat. The primary host of PRD-1 is *Salmonella typhimurium* (Ryan et al., 1999; Bales et al., 1991). Liquid samples and extracted soil core samples were analyzed for these bacteriophages following the plaque forming unit (pfu) technique as described by Adams (1959). For this assay, all samples were serially diluted in phosphate-buffered saline, plated on a layer of soft agar containing the appropriate bacterial host, and incubated overnight at 37°C. Plates were enumerated by counting plaques formed in the host lawn. Bacteriophages MS-2 and PRD-1 and their host bacteria, *E. coli* and *S. typhimurium*, were obtained from stocks at the United States Geological Survey in Boulder, CO.

Ice-nucleating active (INA) bacteria are dead *Pseudomonas syringae* containing high molecular weight proteins in the outer membrane that function as nucleation sites for the formation of ice crystals at subzero temperatures up to −2°C (Lindlow et al., 1978). The ability of this to cause freezing of 10-μL volumes of water at higher temperatures forms the basis of its use as an environmental tracer (Strong-Gunderson and Palumbo, 1997). Ice-nucleating active bacteria was purchased from York Snow (Victor, NY). The INA activity was measured following the freeze-drop assay described by Vali (1971) and the concentration of INA bacteria was calculated using a most probable number method (American Public Health Association, 1992). No ice-nucleating activity was found in background septic tank effluent (STE) samples.

Fecal coliform and bacteriophage analyses on soil core samples were performed aseptically in duplicate by taking a known weight (approximately 4 g) of moist soil and adding 40 mL of 1.5% beef extract solution to yield a final dilution of approximately 1:10 (sand–beef extract). Method 9221A of the American Public Health Association (APHA) suggests extraction for coliform bacteria in sediments and sludges using 10% phosphate-buffered saline (PBS). However, a comparison of extraction methods conducted at the bench scale at the Colorado School of Mines using six different extractants (including PBS) proved beef extract to be the most efficient method for removing the coliform bacteria and both bacteriophages (Masson, 1999). Powelson et al. (1990) also found beef extract to be the most efficient eluant at desorbing virus. Following the addition of beef extract, samples were shaken for 2 min at 350 rpm and then allowed to settle for 1 min at which time the liquid sample was analyzed. An aliquot of liquid (typically 1 mL) was withdrawn from mid-depth of a sterile 50-mL conical (Masson, 1999) and analyzed directly (for low levels) or

Table 2. Experimental conditions evaluated during three-dimensional pilot-scale studies.

Lysimeter	Lysimeter code	Infiltrative surface character	Depth to ground water	Effluent application rate
			cm	cm^3 per d per cm^2 of total area
L2	AF-90	aggregate-free (open via chamber)	90	8.4
L3	AL-90	aggregate-laden (2- to 4-cm-diameter gravel)	90	5.0
L4	AL-60	aggregate-laden (2- to 4-cm-diameter gravel)	60	5.0
L5	AF-60	aggregate-free (open via chamber)	60	8.4

diluted as needed (for high levels) for fecal coliform bacteria and added surrogates. Analysis for fecal coliform bacteria was performed according to the membrane filtration method (APHA Method 9222D). The pfu assay was conducted for quantification of bacteriophages. All sample dilutions were plated in duplicate. Results are expressed as colony forming units (cfu) or organisms per gram of soil, based on the dry weight of the soil. Bromide (added as KBr) was employed as a conservative solute tracer and measured using an ion chromatograph (Dionex [Sunnyvale, CA] AI-450) or an ion selective electrode (Cole-Parmer, Vernon Hills, IL).

Multicomponent Surrogate and Tracer Testing Methods

Three-Dimensional Pilot-Scale Systems

Surrogate and tracer testing was completed during controlled laboratory experiments with three-dimensional tanks packed with medium sand and loaded with STE for 48 wk (Van Cuyk et al., 2001). This lysimeter experiment examined the effects on hydraulic and purification performance associated with infiltrative surface character (aggregate-free [i.e., chambered infiltrative surface] vs. aggregate-laden [i.e., gravel-laden infiltrative surface]) and soil vadose zone depth (60 or 90 cm) (Table 2, Fig. 1). Van Cuyk et al. (2001) describe the setup and operation of the pilot system and only highlights are given here. Four lysimeters (L2–L5) were established using the same sand medium (grain size that is 10% finer by weight [d_{10}] = 0.22 mm, grain size that is 60% finer by weight [d_{60}] = 0.60 mm; pH = 6.8; total organic carbon [TOC] = 0.017 dry wt. %; and K_{sat} = 0.032 cm/s). Lysimeters L2 and L5 had an aggregate-free infiltrative surface (IS) with 1650 cm^2 of infiltration area and either a 90- or 60-cm sand depth, respectively. Lysimeters L3 and L4 had aggregate-laden IS (approximately 1 to 3 cm of gravel) with 1650 cm^2 of infiltration area and either a 90- or 60-cm soil depth, respectively. The lysimeters received either 5.0 cm/d (L3 and L4) or 8.4 cm/d (L2 and L5) of the same medium-strength STE collected from a condominium complex (total suspended solids = 69 mg/L; 5-d carbonaceous biochemical oxygen demand [cBOD$_5$] = 227

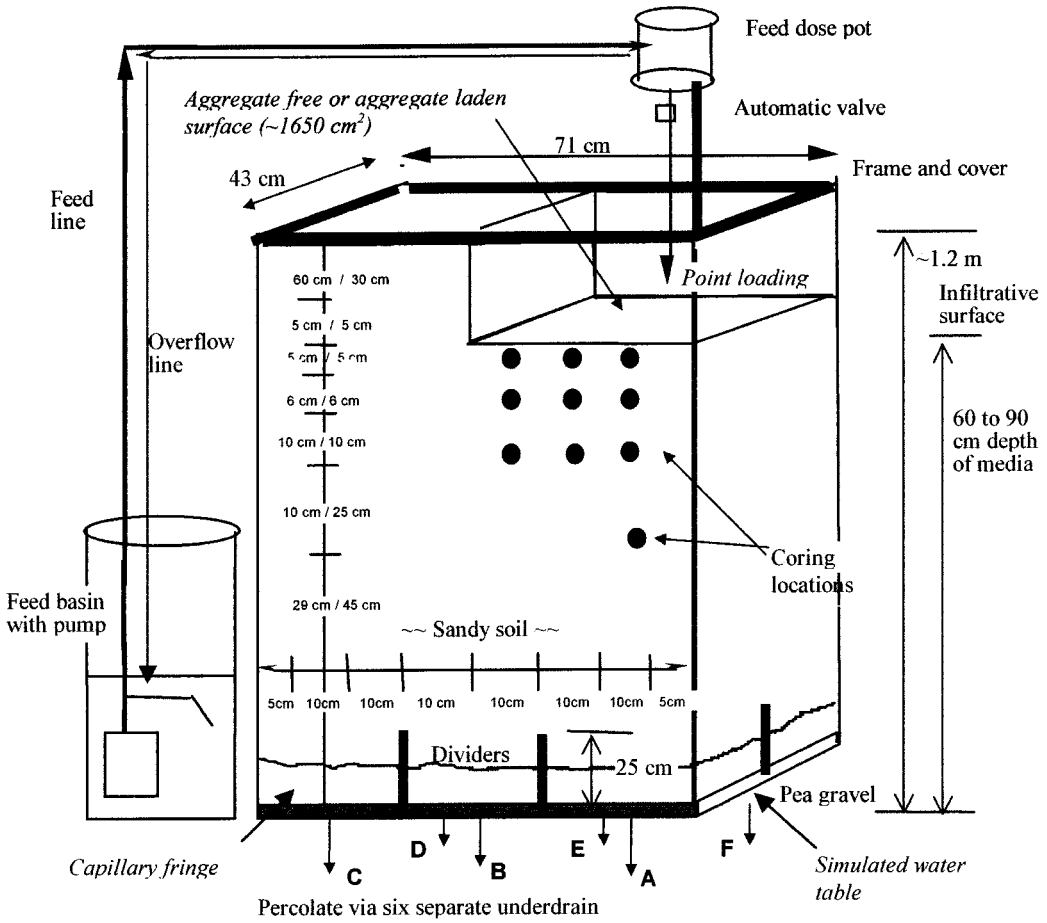

Fig. 1. Schematic of the three-dimensional pilot-scale soil treatment system.

mg/L; total nitrogen = 57 mg N/L; total phosphorus = 4.6 mg P/L; and fecal coliforms = 5.4×10^5 cfu/100 mL). The lysimeters were point-loaded with STE four times daily (8, 12, 16, 20 h) at 1.25 cm/dose (aggregate-laden) or 2.1 cm/dose (aggregate-free) from an orifice at a rate of 2 L/min. Vertical partitions (25 cm in height) divided the bottom of each lysimeter area (approximately 3050 cm^2 of total outflow area) into six equal area compartments (A to F) that allowed two-dimensional characterization of the percolate outflow volume and composition. The lysimeters were cloaked to prevent algal growth and operated at a temperature of 18 to 22°C. Percolate from each lysimeter compartment (A to F) was collected over a 24-h period once each week and characterized for water quality parameters (BOD_5, total suspended solids, total P, total N, and fecal coliform [FC]) and outflow distribution. After setup and before daily loading with STE, the lysimeters were dosed with clean water (City of Golden tapwater). A bromide tracer test was completed during this clean water loading period (Week 0) to establish hydraulic properties of each lysimeter before STE application. Multicomponent surrogate and tracer tests were conducted after 8 and 45 wk of STE loading. These two time points were selected to represent a period of startup (Week 8) and a period when treatment processes were established and performance was relatively stable (Week 45).

During bromide tracer tests at Weeks 0, 8, and 45, the 100 mg Br/L (added as KBr) was added to the applied effluent for three consecutive days (12 doses total) with bromide concentrations measured in the percolate. Percolate samples were collected four times daily (0800, 1200, 1600, 2000 h) during tracer testing. Bromide breakthrough and elution curves were developed. The time to 10% breakthrough ($BT_{10} = C_e/C_o = 0.10$) and to 50% bromide breakthrough ($BT_{50} = C_e/C_o = 0.50$) were used as indicators of the flow regime in the lysimeters. During the bromide tracer tests at Weeks 8 and 45, INA bacteria and MS-2 and PRD-1 bacteriophages were added to the STE dosed to each lysimeter for 3 d and the percolate concentrations of each were monitored for 21 d. The lysimeters were dosed with MS-2 at 1×10^7 pfu/mL (Weeks 8 and 45), PRD-1 at 1×10^3 pfu/mL (Week 8) or 1×10^7 pfu/mL (Week 45), and INA bacteria at 1×10^7 pfu/mL. Percolate collected from each of the six underdrains over 4-h intervals was sampled and analyzed for added constituents.

After 48 wk of operation, soil cores were collected at 11 locations within each of the lysimeters and analyses were made for soil chemical and microbial properties as well as the added tracer and surrogates. A 28-mm hole saw created an opening in the three-dimensional tank wall through which a 2.5-cm-diameter copper tube (precleaned and ethanol rinsed) was horizontally inserted to obtain a 20-cm-long sand core. The sand in the tube, excluding the 2.5 cm closest to the lysimeter wall, was extruded into a precleaned glass beaker and mixed before subsamples were taken and stored at 4°C pending analyses for water content and added surrogates as described below.

Full-Scale Systems

Testing of a similar multicomponent surrogate and tracer mixture, bacteriophages MS-2 and PRD-1 and bromide, was also conducted at the field scale at five sites to assess the operating soil treatment systems' bacteriophage removal performance as measured at 60 to 90 cm below the infiltrative surface. Systems with evidence of wastewater ponding at the infiltrative surface, daily flows ranging from 0.5 to 2.7 cm/d, accessible septic tank, and homeowner consent were selected for field site testing. Five home sites were selected (Fig. 2, Table 3) according to these criteria: four homes (Sites 2, 12, 14, and 16) with chambered infiltrative surfaces and one home (Site 10) with gravel-laden infiltrative surface. Home sites were located near Brighton (24 km northwest of Denver, CO, elevation of approximately 1650 m above mean sea level) or Silverthorne, CO (approximately 100 km west of Denver in Summit County, elevation approximately 2800 m above mean sea level).

Before the addition of the surrogates and tracer mixture, STE samples were collected for background levels of bromide, MS-2, and PRD-1. There was no detection of any of these surrogates or tracers in the STE. Stock solutions of bromide (added as KBr), MS-2, and PRD-1 were added to the dosing chamber to obtain final concentrations targeted at 500 mg Br/L of bromide and 1×10^6 pfu/mL of both MS-2 and PRD-1.

Following surrogate or tracer addition to the wastewater dosing tank, amended wastewater was mixed using a submersible pump that recirculated effluent within the tank for approximately 10 min. After this period of mixing, five grab samples of the STE in the dosing tank were collected to characterize the time zero conditions. Subsequently, STE samples were collected from the dosing tank every 2 to 7 d to characterize the concentrations of surrogates and tracers being dosed onto the soil (drain field) over time.

Soil sampling (described below) was conducted at the time when the surrogate and tracer mixture was anticipated to have traveled to the target depth. This estimate of the time required for effluent to infiltrate and percolate to a depth of 60 cm was made based on the daily flow, area of infiltrative surface, and an effective porosity for the soil based on the following relationship:

$$T_r = (A_{is} D N_e)/Q \quad [1]$$

where T_r is travel time required for effluent to reach the depth of interest (days), D is depth of interest (m), A_{is} is infiltrative surface area (m^2), Q is daily flow (m^3/d), and N_e is effective porosity (v/v). This relationship assumes uniform application and infiltration and is thus a first approximation of travel times. For Site 2, A_{is} was determined from the as-built drawings to be 36.8 m^2, the depth of interest to evaluate was 0.60 m, the average daily flow was 1.0 m^3/d, and the effective porosity was estimated at $N_e = 0.20$ v/v. For these conditions, the time required for applied effluent to percolate to a 60-cm depth below the infiltrative surface was approximately 5 to 20 d. To ensure that adequate time was allowed for the surrogates or tracers to be distributed in the first system evaluated (Site 2) and to infiltrate or percolate into the soil, soil core sampling was initiated 25 d after introduction of the surrogates or tracers. Results from the initial test at Site 2 led to coring at the other four sites (10, 12, 14, and 16) at 7 to 9 d following introduction of the surrogates or tracer.

At Sites 2 and 16, soil cores were taken at two spatially separate locations and soil subsamples were collected in duplicate at depths of 0 to 5, 10 to 15, 25 to 30, and 55 to 60 cm below the infiltrative surface. This entailed hand excavation from the ground surface to the top of the chamber. An access hole was cut in the top of the chamber. A thin-tube sampling probe with a pre-cleaned stainless steel or acetate liner (5 cm in diameter by 15 cm long) was then driven into the undisturbed soil and an intact core was retrieved within the sleeve. The core was capped with plastic end caps, labeled, and placed in a cooler containing blue ice. The casing was driven further into the probe hole and then the push probe (after cleaning with 90% v/v ethanol in water followed by a deionized water rinse) was inserted into the probe hole and driven another depth interval (nominally 15 cm). This process was repeated

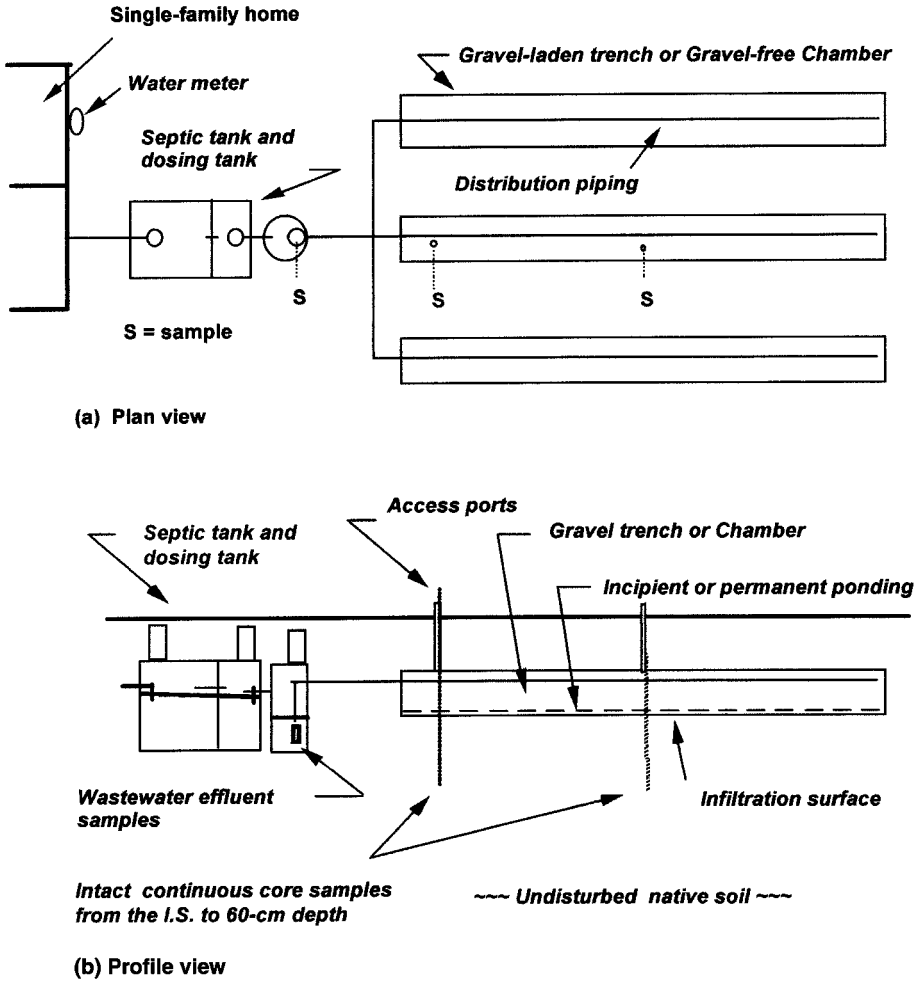

Fig. 2. Illustration of field full-scale system with monitoring locations. IS, infiltrative surface.

until a depth of 60 to 75 cm was reached or cobbles and dense soil prevented further penetration. Using double casing and thin-tube sampling methods, soil extraction and analysis were conducted for Br^-, MS-2, PRD-1, fecal coliform, and water content. At Sites 10, 12, and 14 soil core samples were collected via direct-push soil core collection with the GeoProbe 54DT and GeoProbe/Terraprobe sampling techniques (Geoprobe Systems, Salina, KS). A 1.2-m-long by 5-cm-i.d. dual-tube assembly with polyethylenterephthalate (PETG) liners was used to collect continuous undisturbed samples from the ground surface to 60 cm below the infiltrative surface. The dual-tube assembly is comprised of an outer stainless steel core barrel (8-cm i.d.), an inner stainless steel core barrel (6-cm i.d.), and PETG liners (5-cm i.d.) inserted into the inner core barrel. The inner core barrel with PETG liner and soil core is then retrieved to the surface. Upon retrieval to the surface, the PETG liner with the intact soil core was removed from the sampler, capped, and stored at 4°C before transporting to the Colorado School of Mines laboratory for analyses. A clean PETG sleeve was then replaced into the inner core barrel and reinserted into the outer core barrel retained in the subsurface. The dual-tube assembly was then again advanced approximately 1 m and the process repeated until a continuous core to the desired depth was obtained. The core was capped with foil, labeled, and placed in a cooler containing blue ice. All samples were stored at 4°C until laboratory analysis was performed at the Colorado School of Mines. In the lab, the cores were carefully opened and the outermost soil media was removed and wasted. Subsamples of the interior of the core were then taken with sterile utensils at up to four intervals that corresponded ap-

Table 3. Summary of full-scale system characteristics.

Site	Infiltrative surface type†	Age	Estimated HLR‡	Depth to infiltrative surface	Ponding depth
		yr	$(cm/d)^2$	m	
2	chamber	10	2.7	approximately 1.2	yes§
10	gravel	9	0.8	0.91–1.06	approximately 7 cm
12	chamber	4	0.5	0.76–1.00	5 cm
14	chamber	4	0.5	0.39–0.49	wet¶
16	chamber	3	0.6	0.45–0.68	30 cm

† Chamber systems are gravel-free at the infiltrative surface. Gravel systems have aggregate at the infiltrative surface.
‡ Hydraulic loading rate.
§ Ponding is defined as presence of wastewater above the infiltrative surface.
¶ Depth of ponding not measured.

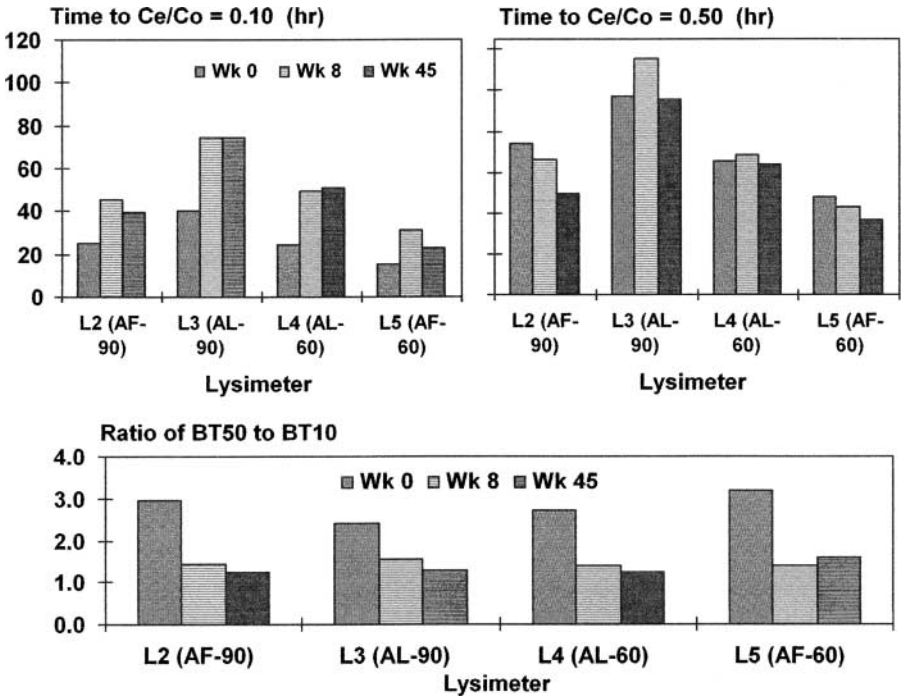

Fig. 3. Time to appearance of bromide tracer in the percolate at 10 and 50% of the applied concentration (BT_{10} and BT_{50}, respectively) and their ratio at system start-up after 8 and 45 wk of operation. AF-60, aggregate-free infiltrative surface with 60-cm vadose zone; AL-90, aggregate-laden with 90-cm vadose zone.

proximately to those used in the laboratory lysimeter study (e.g., 0–5 cm, 10–15 cm, 25–30 cm, and 55–60 cm below the infiltrative surface). All laboratory analyses for water content, fecal coliform bacteria, and surrogates were performed within 24 h of sample collection as described in the analytical methods section.

Ancillary Testing

An additional study was completed to understand the survival or inactivation of the bacteriophages (MS-2 and PRD-1) in the wastewater environment over time. In this bench-scale test, two of the samples collected from the dosing tank following addition of the multicomponent mixture were stored in the dark at either 4 or 20°C and concentrations of both MS-2 and PRD-1 were measured over a period of 26 d. An additional study was also conducted to allow a comparison of bulk solids and soil percolate sampling methods. In this work, controlled laboratory experiments were conducted in small columns made from 50-cm^3 syringes filled with the same low organic content (TOC = 0.017% dry wt.) clean medium sand (d_{10} = 22 mm, d_{60} = 0.60 mm) with *E. coli* added at 10^5 cfu/mL in a sterile phosphate-buffered saline solution. The minicolumns were dosed four times daily (every 6 h) in an automated fashion at total hydraulic loadings of approximately 5 cm/d. A second column experiment was run under the same conditions using a different sand media containing a higher carbon content (TOC = 0.225% dry wt.). Concentration of *E. coli* was measured in both the percolate and the soil extracted samples.

RESULTS

Three-Dimensional Pilot-Scale System Tests

Results from pilot-scale investigations for bromide tracer testing in all four lysimeters for Weeks 0, 8, and 45 are shown in Fig. 3. These tests revealed a temporal increase in BT_{10} (from 15 to 40 h at Week 0 to 23 to 74 h at Week 45) and a concomitant reduction in BT_{50} to BT_{10} ratio (from 2.4 to 3.2 at Week 0 to 1.3 to 1.6 at Week 45). Changes that were observed in infiltration and percolation behavior over time were consistent with the development of a wastewater-induced clogging zone at the infiltrative surface (Siegrist, 1987; Siegrist and Boyle, 1987; Schwagger and Boller, 1997).

Bacterial and viral surrogate tests revealed very little INA bacteria detected (data not shown) and >99% removal (based on total pfu added) of MS-2 and >95% removal of PRD-1 at Weeks 8 and 45, independent of lysimeter character (surface character and media depth). Figure 4 summarizes results for bacteriophage removal in each of the four lysimeters. In all lysimeters, an increase in the total percent removal of MS-2 and PRD-1 occurred between Weeks 8 and 45. Breakthrough of the bacteriophages was observed in all lysimeters at both time points except for MS-2 in L2 at Week 45 where MS-2 was not detected in the percolate. It is noted that removal includes both inactivation and virus sorption. Bench-scale laboratory studies conducted by adding both bacteriophages and INA bacteria to STE showed little degradation of infectivity in the case of virus or ice nucleation in the case of INA bacteria following a 16-h period of incubation at both 8 and 20°C.

Soil coring (Week 48) and analysis of sand extracts for added surrogates showed low levels of virus, which suggests either rapid die-off or inactivation in these lysimeters or inefficiency in recovery of these surrogates from sand media. Figure 5 shows water content, fecal coliform, and PRD-1 values measured in the extracted soil cores from two of the four lysimeters (L2 and L5). Extraction of soil cores showed low levels of virus as

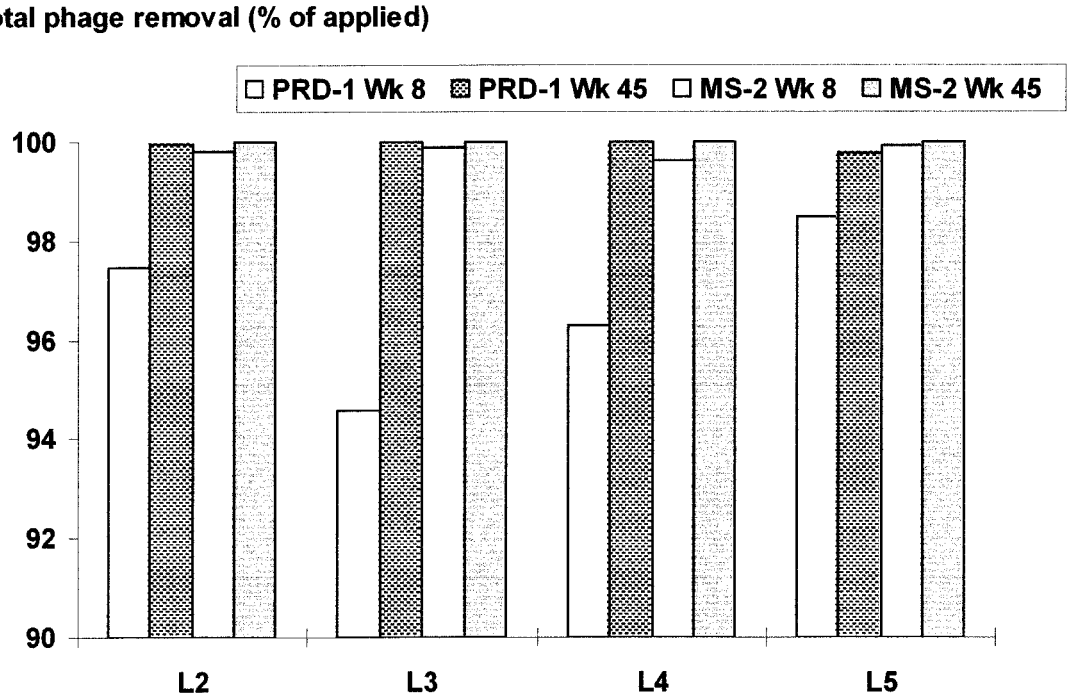

Fig. 4. Percent removal of total applied PRD-1 and MS-2 bacteriophage after 8 and 45 wk of operation. L2, open, 90-cm vadose zone; L3, gravel, 90 cm; L4, gravel, 60 cm; L5 open, 60 cm.

compared with influent (dosed) values of 1×10^7 pfu/mL. The highest densities of virus were found at 3 to 8 cm below the infiltrative surface in all the lysimeters and ranged from 0 to 700 pfu/g soil. Bacteriophage MS-2 was not detected in any of the extracted soil cores. There appears to be a relationship between levels of fecal coliform bacteria and PRD-1 levels detected in these cores. Higher levels of bacteria and viruses were observed in the 10 cm below the infiltrative surface with a decrease in virus and bacteria densities with increasing depth. In general, water content was relatively low as would be expected in an unsaturated system, with a range of 6.11 to 13.94% (dry wt.) and an overall average of 7.47% for all lysimeters. Water content generally decreased with depth in all the lysimeters. In L2 and L5 (both chambered systems), the water content values had less fluctuation between depths and a smaller variance between water content than L3 and L4 (both gravel). Lysimeters L3 and L4 also had higher average values for water content than L2 and L5. This corresponds with the hydraulic data collected from the bromide tracer tests showing longer retention times for the gravel systems.

Full-Scale System Tests

The concentrations of conservative tracer (bromide) and viral surrogates (MS-2 and PRD-1) measured in the dosing tank at one home site over time are presented in Fig. 6 (data representative of all field sites). These results show a decline in the bromide concentration in the dosing tank that is consistent with the decline in concentration expected based on dilution due to incoming STE containing no tracer. After 5 d, the bromide concentration had declined by 99%. Similarly, the levels of MS-2 and PRD-1 declined during this initial 5-d period (Fig. 6). However, between 12 and 20 d, the bromide concentrations dropped toward zero (nondetectable at <1 mg/L) while the MS-2 and PRD-1 levels remained relatively unchanged. It is noted that the variability in concentrations from time point to time point was nearly 1 log compared with 11% for analytical variability between duplicate analyses.

Soil coring showed no bromide in any of the extracted soil cores from Site 2. This is probably the result of the decline in the concentration of bromide with time due to dilution in the dosing chamber and the length of time before sample coring commenced (Fig. 6). This effectively resulted in the bromide migrating into and through the depth interval of interest before collection of the soil cores. Soil core values for MS-2 and PRD-1 are shown for Home Sites 10, 12, 14, and 16 in Fig. 7. An overall trend of lower levels of surrogate virus with increasing depth below the infiltrative surface was observed. A 3-log removal of the added MS-2 and PRD-1 was observed in all extracted soil core samples at the 55- to 60-cm-depth location. Fecal coliform bacteria also declined with depth below the infiltrative surface.

The removal of bacteriophages was conservatively estimated based on the assumption that all bacteriophage detected in the extraction of soil solids were mobile in the soil pore water. Based on the average dry weight percentage water content at each home site, the pfu/g dry soil values were converted to pfu/mL of pore water. These estimated pore water values were then compared with the dose concentration of MS-2 or PRD-1. The estimated pore water concentrations correspond to 98.2 to 99.9% removal efficiency for both MS-2 and PRD-1 within the top 15 cm below the infiltrative sur-

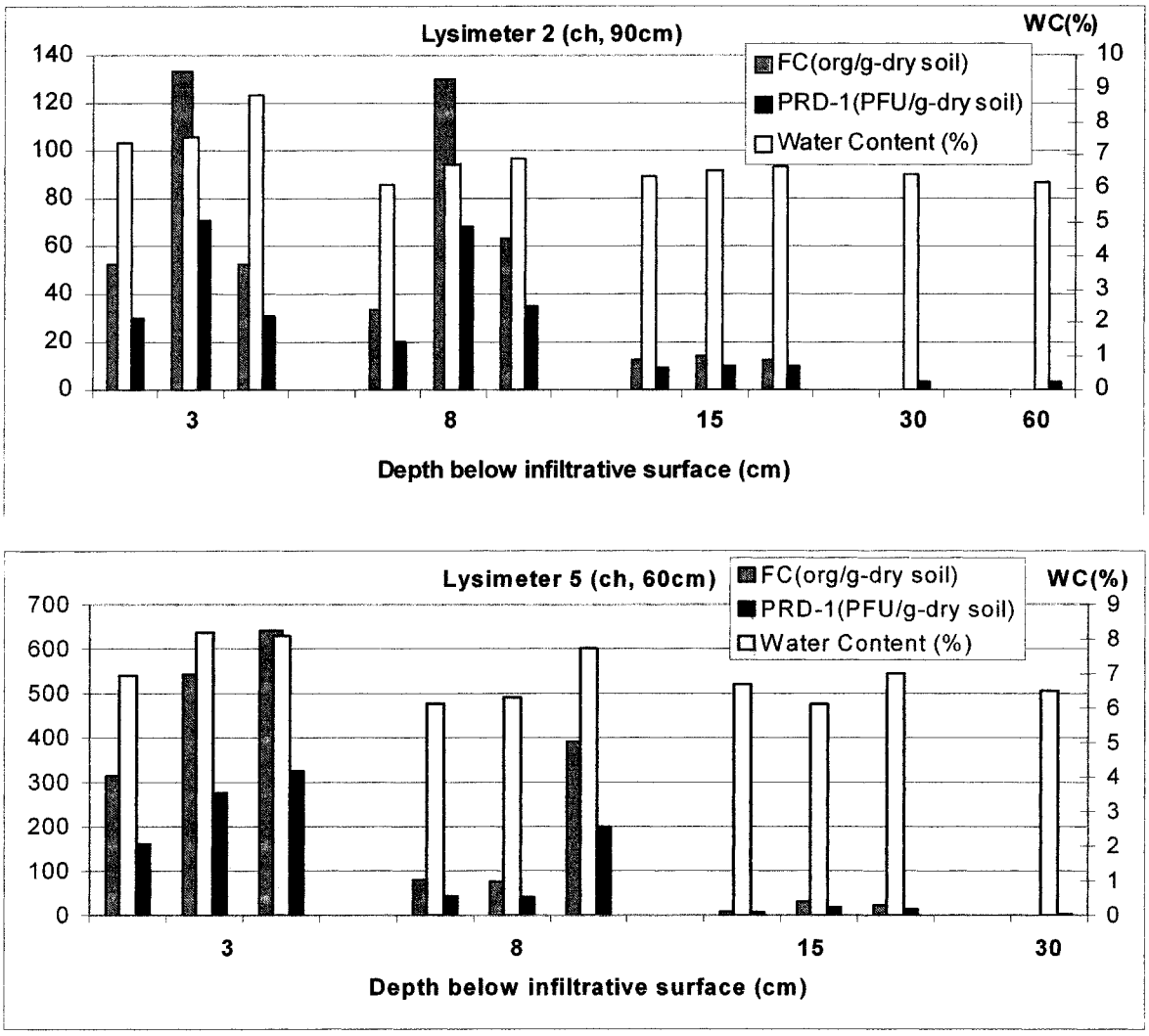

Fig. 5. Soil core extracted densities of MS-2, PRD-1, and fecal coliform (FC). No MS-2 was detected in any of the lysimeter core samples. Influent concentrations for lysimeter study (dosed at Week 45, soil cores taken at Week 48) were 1×10^6 PFU/mL. Blank columns represent nondetectable levels.

face and 99.5 to 99.8% for MS-2 and 99.1 to 99.9% for PRD-1 at 30 cm below the infiltrative surface. Because the conservative tracer was not detected at the 55- to 60-cm interval, treatment efficiencies were not estimated for this depth. Considering that the extracted bacteriophages may not have all been mobile in the pore water it is reasonable to conclude that a 3-log removal of the applied viral surrogates was achieved at a total depth of 30 cm (maximum sample depth where surrogate or tracers were detected). Achievement of 3-log removal of virus after STE infiltration at 1 to 3 cm/d and percolation through 60 to 90 cm of natural soil was also shown in previous field studies (Higgins et al., 1999; Oakley et al., 1999; Anderson et al., 1991; Gilbert et al., 1976). Work presented by Nicosia et al. (2001) showed only 1- to 2-log removal of PRD-1 in 60 cm of unsaturated fine sand.

Ancillary Testing

Results of the bench-scale inactivation test conducted with the STE collected from Field Site 2 are shown in Fig. 8. These data show little temperature effect on PRD-1 but a marked effect on MS-2. The results of the dosing tank measurements and the bench-scale test with STE from the study site demonstrate a slight increase in the concentrations of MS-2 and PRD-1 measured in the dosing chamber (temperature of the chamber was measured at approximately 8°C). Thus, future field and/or laboratory testing should include intensive monitoring of the temporal changes in surrogate or tracer concentrations.

The relationship of MS-2 and PRD-1 to fecal coliform concentrations is of interest, since fecal coliform bacteria are often used as indicators of microbial contamination. A comparison of fecal coliforms with MS-2 and PRD-1 values from soil cores collected at Site 2 indicated that, under the conditions examined, fecal coliforms in soil extracts may be a reasonable indicator for the presence of virus at the same location. Data from the additional four homes (Sites 10, 12, 14, 16) confirm this relationship as all soil core extracts with virus also had fecal coliform concentrations. Fecal coliform values

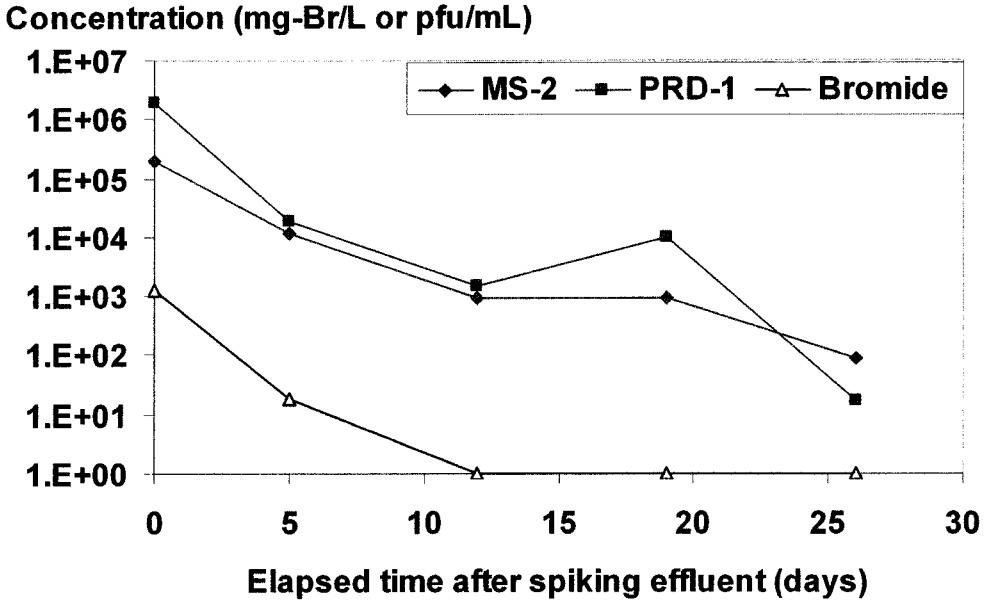

Fig. 6. Concentrations of MS-2 and PRD-1 bacteriophages and the conservative tracer (bromide) in the dosing chamber at Field Site 2 over time.

were higher than virus (in pfu/g soil) in 97% of the MS-2 samples and 94% of the PRD-1 samples (Fig. 9).

One issue to be addressed when using soil core collection is how the densities of virus or bacteria extracted from solids compared with those being transported in the soil water. To understand the relationship between bulk soil solids and soil percolate sampling, controlled laboratory experiments were conducted using two types of sand media and known concentrations of *E. coli* (1×10^5 cfu/mL). Results from this work (presented in Fig. 10) suggest that in both types of sand media at this concentration of *E. coli*, values obtained from soil core extracts would be higher (therefore a more conservative measure) compared with the concentrations actually contained in the percolate and soil water. This relationship is probably due to sorption of bacteria on soil solids. Further testing on various laboratory and field soil samples at different levels of influent bacteria and virus will aid our ability to understand with confidence the relationship between soil core and pore water concentrations at different environmental conditions.

DISCUSSION

Retardation of virus in the three-dimensional pilot system was calculated using the following expression (Powelson et al., 1993):

$$\Theta_w = \Theta_{Br}/R_{Br} \quad [2]$$

where Θ_w is the travel time for infiltration water to reach the depth of interest (60 or 90 cm), Θ_{Br} is the travel time for bromide, and R_{Br} is the bromide retardation. If we assume that R_{Br} equals 1, then:

$$\Theta_{vir} = \Theta_{Br}R_{vir} \quad [3]$$

By comparing the retention time (BT_{10}) of bromide and virus, R_{vir} can be calculated. Values for R_{vir} for PRD-1 at Week 8 were found to range from 1 to 3 depending on the lysimeter (L2 = 1.0, L3 = 2.4, L4 = 1.7, and L5 = 3.0). The term R_{vir} could not be calculated for PRD-1 at Week 45 or for MS-2 due to the limited breakthrough (high removal). Similar retardation rates were reported by Powelson et al. (1993), who found that virus transport was retarded ($R_{vir} = 1.9$) initially but increased ($R_{vir} = 0.47$) after 4 d of flooding (saturated conditions). Previously, Powelson et al. (1990) found R_{vir} of 0.75 in unsaturated sand-filled columns dosed at rates of 0.13 m/d, which the authors claim suggests charge exclusion from part of the water volume in this system. These investigators also observed greater R_{vir} with secondary effluent versus tertiary and suggested that this may possibly be due to greater amounts of organic matter deposited by secondary effluent, which could result in greater adsorption of virus.

The high removal and high retardation of viruses observed may be attributed to the development of an active biomat, even as early as 8 wk after start-up as observed in the three-dimensional lysimeters. This clogging layer may be important because of its effect on the hydraulic regime of these systems and its biogeochemical activity (treatment and purification performance). With increased biomat development of the infiltrative surface a more even infiltrative surface distribution of intermittent doses, both spatially and temporally, is observed. Soil coring of the lysimeters showed higher densities of virus extracted from the sand media near the infiltrative surface and the 15 cm directly below it, suggesting a more biogeochemically active zone. A similar trend of increased virus concentrations below the infiltrative surface was also observed at the field test sites.

Assuming that virus die-off follows first-order kinetics (e.g., Bitton and Harvey, 1992), the number of viruses remaining in the soil at the end of a given time period can be described as:

$$C_t = C_o \exp(-kt) \quad [4]$$

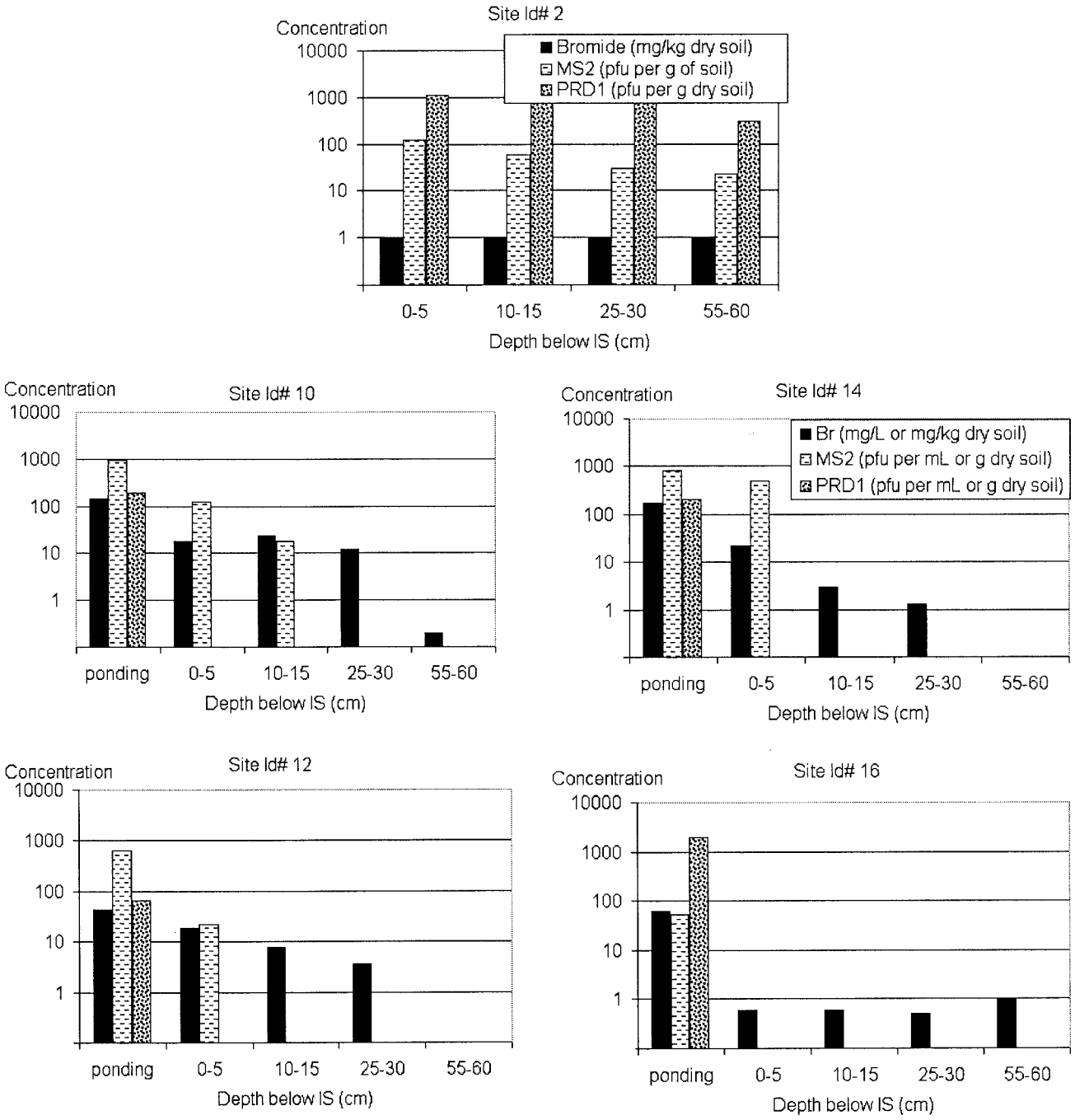

Fig. 7. Bromide, MS-2, and PRD-1 levels in soil core extracts collected at Field Sites 2 (sampled 21 d following tracer addition), 10, 12, 14, and 16 (sampled 7 to 9 d following addition of surrogates and tracer to the septic tank effluent [STE] being applied). Detection limits are: bromide at 0.1 mg/L (ponding) or mg/kg dry soil and MS-2 and PRD-1 at 1 plaque forming unit (pfu)/mL (ponding) or g of dry soil. Zero values (blank bars) represent nondetects. Site 2 was wet with no ponding; bromide was not detected in soil cores. IS, infiltrative surface.

where C_t is the concentration of viruses at time t, C_o is the initial concentration of viruses (10^7 pfu/mL dosed onto lysimeters), k is the first-order rate coefficient for net die-off rate of virus (at 20°C), and t is time in days (soil cores were taken 28 d following first dose of virus at Site 2 and 7 to 9 d at Sites 10, 12, 14, and 16).

Based on water content values in the three-dimensional pilot-scale work that ranged from 6 to 13% (dry-weight basis), soil pore water concentrations (pfu/mL) were estimated from extracted values. Rate constants (k, Eq. [4]) of 0.26 to 1/d were calculated for PRD-1 in this soil at 20°C. This is within the range of average die-off rates for viruses (0.04–3.69/d) presented by Reddy et al. (1981).

A virus removal model is being tested with data generated from this work. Figure 11 presents predicted removal in 60 cm of sand medium with virus concentration of 1×10^7 pfu/mL in STE applied at 5 cm/d. This virus removal model assumes: (i) first-order removal, with respect to concentration; (ii) clogging of the infiltrative

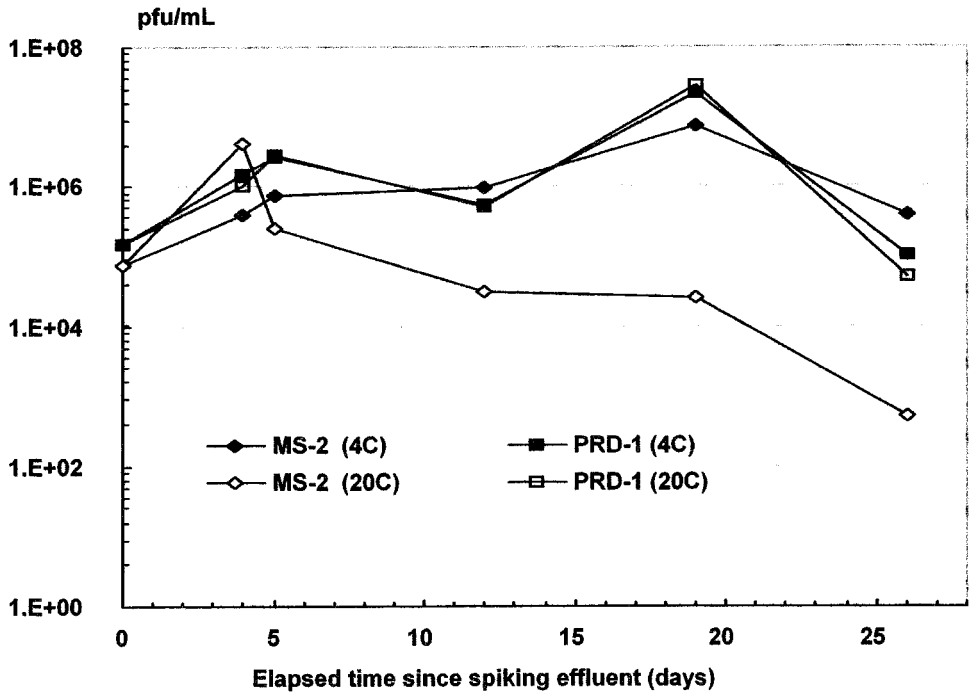

Fig. 8. Bacteriophages MS-2 and PRD-1 in Field Site 2 septic tank effluent (STE) during incubation at 20 and 4°C. Note that all samples were run in duplicate and the average percent difference was 11%.

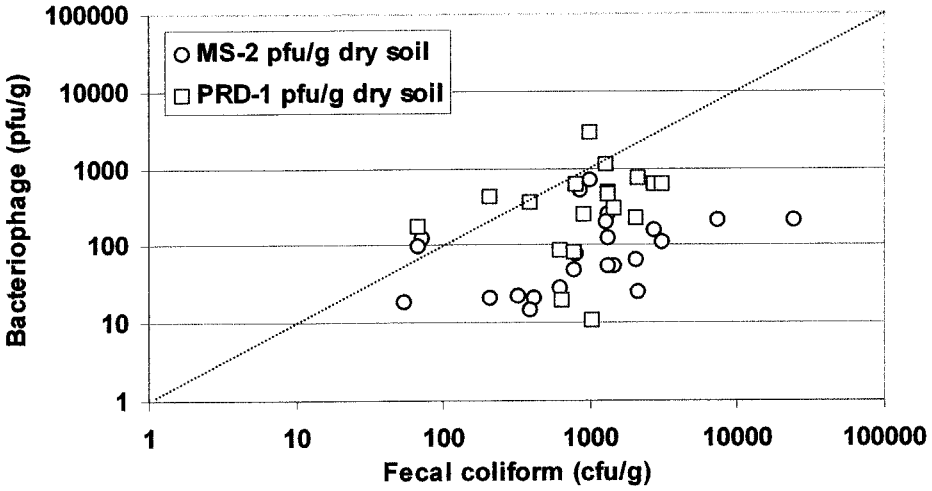

Fig. 9. Relationship between soil fecal coliform and bacteriophages levels measured in co-located field soil core samples. Dashed line represents 1:1 relationship. The MS-2 samples were less than fecal coliform in 97% of samples; PRD-1 were less than fecal coliform (FC) in 94% of samples.

surface zone with operation, with no change to the vadose zone removal rates; and (iii) initial k values in both infiltrative zone and vadose zone compartments of 0.1/h. In this simulation, the k value increased over time in the infiltrative zone but remained constant in vadose zone. The effective porosity of soil was assumed to be 0.2 v/v (with a slight increase over time in the infiltrative zone). Average retention time (t) in each soil zone was calculated using the following expression:

$$t = (LN_e\mathrm{ISU})/q \quad [5]$$

where L is length of soil including infiltrative surface and vadose zone (60 cm), N_e is effective porosity (0.2–0.3 v/v), ISU is infiltrative surface utilization (or volumetric utilization efficiency [VUE] in the vadose zone compartment), and q is application rate over design infiltration area (0.208 cm/h, which is a hydraulic loading rate [HLR] of 5 cm/d). The term ISU is the fraction of the infiltrative surface or filter/soil volume with which the wastewater comes in contact. These values (percentages) will increase with time of operation and volume of effluent applied. A significant increase in the ISU will occur as the result of clogging (biomat) development at the infiltrative surface. Figure 11 shows the increased importance of reactions occurring in the infiltrative zone with time of operation as well as improved removal efficiency of virus added with time.

The USEPA is proposing to require a targeted risk-based regulatory strategy for all ground water systems. This Ground Water Rule will provide opportunity to

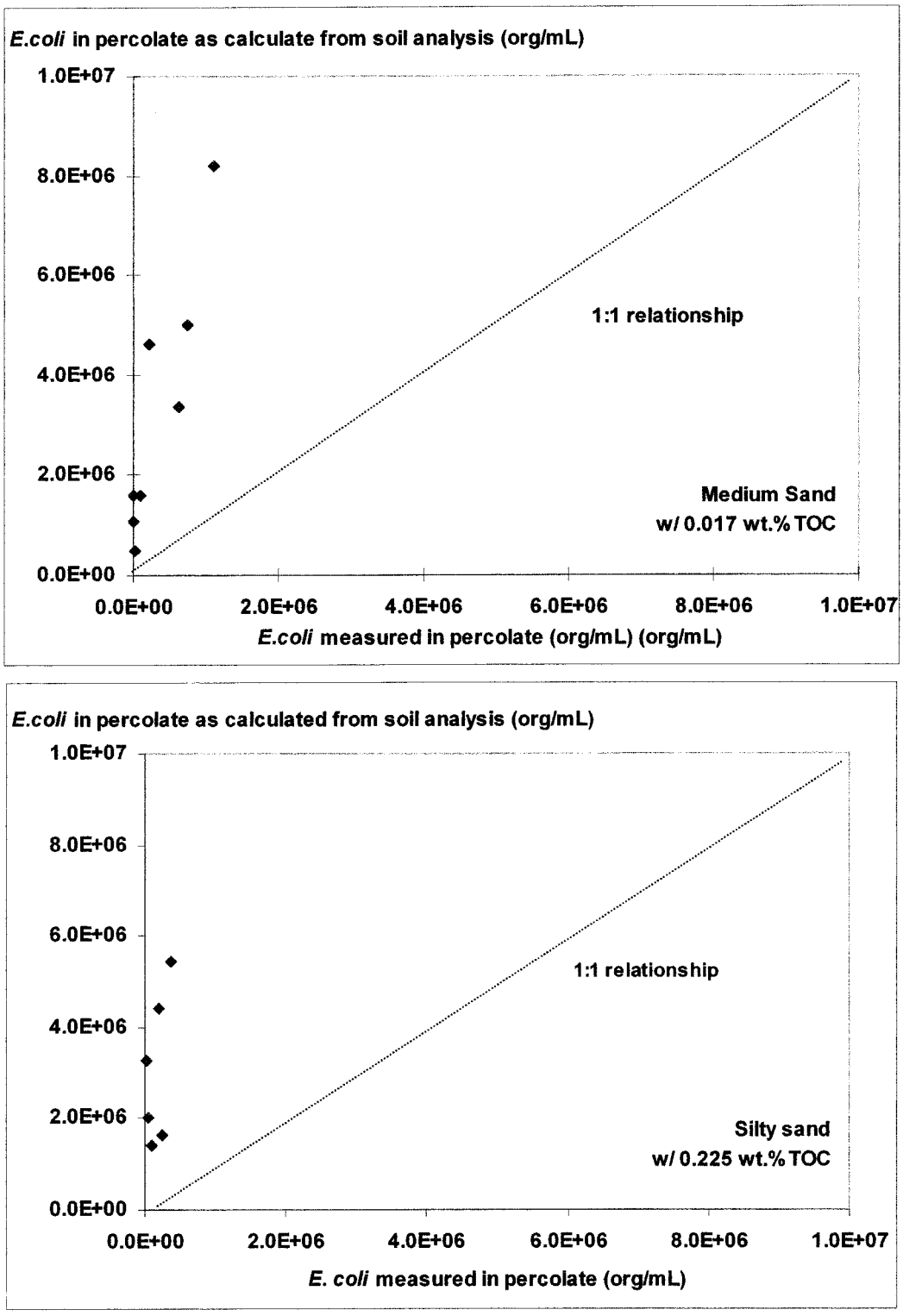

Fig. 10. Soil solid versus percolate values for *E. coli* added to columns filled with medium sand (0.017 wt. % total organic carbon [TOC]) or silty sand (0.225 wt. % TOC). Dashed line represents 1:1 relationship where percolate sample value equals soil solid extracted value.

reduce public health risk associated with the consumption of waterborne pathogens from fecal contamination (USEPA, 2000). The two main goals of this rule are to define and categorize which drinking water wells are susceptible to viral contamination and to develop regulatory means to protect drinking water wells from viral contamination. This rule will contain a requirement for correction of fecal contamination by eliminating the source of contamination, providing an alternative source water or providing treatment that achieves at least 99.99% (4-log) inactivation or removal of viruses. It has been found that virus transport distance and transport

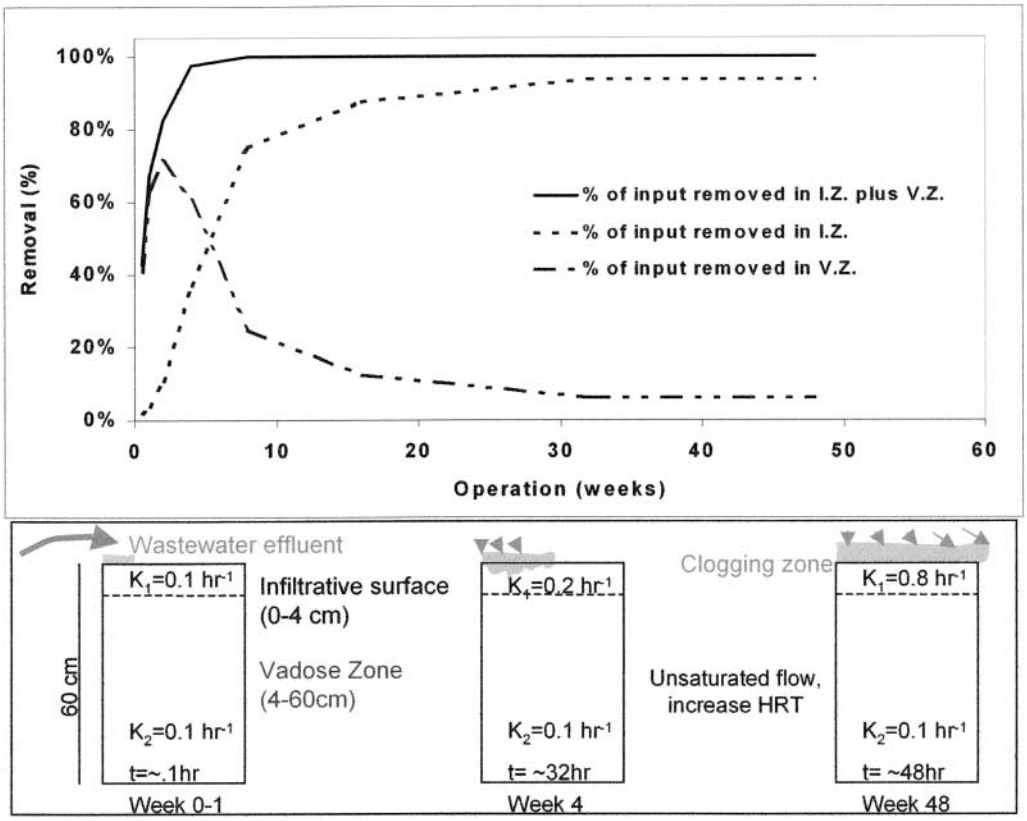

Fig. 11. Virus removal simulation (assuming first-order removal with respect to concentration) with increasing time of operation. Conditions: 1×10^7 plaque forming unit (pfu)/mL of virus added in septic tank effluent (STE) dosed at 5 cm/d. IZ, infiltrative zone (0–4 cm); VZ, vadose zone (4–60 cm).

time estimated by models are very sensitive to the choice of attachment and inactivation rate coefficients (Yates, 1995; Navigato, 1999). These parameters and the processes that control attachment and inactivation are not readily available or well understood. While the research presented here demonstrates that a 3-log reduction of MS-2 and PRD-1 can occur in the vadose zone below a soil-based wastewater system receiving STE at the above-stated conditions, further research is being conducted to understand what controls the natural disinfection of virus in the subsurface, primarily in the region with on-site wastewater soil treatment systems.

CONCLUSIONS

In this investigation an attempt was made to quantify the removal of added human viral surrogates in conditions representative of on-site wastewater soil treatment systems. Based on the results of this study the following conclusions have been drawn. First, intermediate three-dimensional laboratory studies using a multicomponent surrogate and tracer mixture showed a high removal of constituents early on and following one year of operation with wastewater. Episodic release of virus was observed during the early period of operation (Week 8) of the lysimeters. This behavior was not observed at the later time period (Week 45), which may be attributed to the development of a biomat at the infiltrative surface. Second, bench-scale analysis completed with mini-columns and two types of soil media (clean sand with low TOC and silty sand with higher TOC) showed that estimated concentrations of fecal coliforms in percolating water can be conservatively estimated based on analysis of bulk soil solids. Third, a methodology for using a multicomponent mixture of virus surrogates and a conservative tracer to assess virus purification in a wastewater soil absorption system was successfully applied under field conditions. Three-log reductions in the applied MS-2 and PRD-1 viral surrogate concentrations were achieved at 30 to 60 cm below the infiltration surface. Fourth, under the conditions examined, the performance and virus treatment efficiency measurements made for the chamber systems were comparable with those determined for gravel systems, even though the chambers were estimated to receive loadings of 1.23 cm/d (0.30 gpd/ft^2) as compared with 0.74 cm/d (0.18 gpd/ft^2) for the gravel system. Lastly, a strong relationship was observed between fecal coliform concentrations measured in soil core samples compared with MS-2 and PRD-1 virus concentrations. These data suggest that the presence of fecal coliform in soil cores may indicate the presence of viruses, but if no fecal coliform bacteria are detected there is probably no virus present.

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