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Virus removal within a soil infiltration zone as affected by effluent composition, application rate, and soil type

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ABSTRACT

The column studies presented in this paper simulated the infiltrative surface of onsite wastewater systems where effluent is applied and where a biomat may form. Two bacteriophages, MS-2 and PRD-1, were used as surrogates for human pathogenic enteric viruses during two tracer tests. A vacuum manifold was used to simulate the drainage effects of an underlying unsaturated soil profile, allowing for the collection of percolate samples at 4 cm immediately below the infiltrative surface. The impact of effluent applied (septic tank effluent (STE) or a simulated ground water), soil type (medium sand or sandy loam), hydraulic loading rate (5 or 25 cm/day) and method of application (four equivalent daily doses or 24 equivalent micro-doses per day) on the removal of viruses were investigated.

These unsaturated mini column experiments demonstrated that the removal of viruses within an infiltrative surface zone (of ~4 cm) generally improved over time under the conditions studied. An exception occurred in sand-filled columns dosed with STE where the removal of PRD-1 decreased after a period of effluent application. Statistical analysis conducted on the calculated percent removal demonstrated that the quality of the effluent applied to the infiltrative surface is important for removal of MS-2 and PRD-1. Hydraulic loading rate also proved important in the removal of viruses. At the time of tracer test 2, columns dosed at the higher HLR (25 cm/day) had higher percent removals for both MS-2 and PRD-1. Soil type altered the removal of PRD-1 at the time of the second tracer test, at which time sandy loam had higher removal rates for PRD-1. No significant differences were observed between columns dosed four times daily and those dosed 24 times daily for either bacteriophage at either of the tracer test time points.

These data suggest that over a relatively short period of operation the infiltrative surface of soil based wastewater treatment systems can achieve much higher removal then initially measured shortly after startup.

1. Introduction

Wastewater systems for onsite and small-scale applications are commonly designed for application of primary treated wastewater into natural soil where it infiltrates and percolates through the vadose zone before it recharges the underlying ground water. These systems are widely used due to their high purification performance with respect to organics, solids and nutrients, with relatively low cost and limited operation and maintenance requirements (Siegrist et al., 2001). However, with such systems increasingly being used as permanent solutions for wastewater treatment and at

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increasing numbers and densities, there is a growing awareness and concern over system performance with respect to removal of bacteria and viruses. Since human pathogens are known to exist in sewage effluents, their removal during soil treatment of wastewater is essential in preventing contamination of ground water, which may be used for drinking water purposes.

Fig. 1 presents a generalized schematic of a soil-based treatment system wherein soil functions as an in situ porous media biofilter (PMB). In these systems, soil is commonly used to treat STE which contains high levels of organic matter, nutrients and microbes. Alternatively, soil may be used to treat a higher quality effluent (e.g., sand filter effluent) that contains substantially lower levels of organic matter, nutrients, and microbes. Important zones for treatment in these systems include (1) the biozone or biomat, which may be formed at the infiltrative surface, (2) the vadose zone, the depth of which may vary from one foot or less to many hundreds of feet, and (3) the saturated ground water zone. The biogeochemical characteristics of the receiving soil and ground water system may or may not be altered by the applied effluent. The ground water transport distance to drinking water wells from a soil treatment system can vary from less than a hundred meters to many kilometers.

The column studies presented in this paper were designed and conducted to simulate the unsaturated infiltrative surface zone of a soil treatment system where effluent is applied and where a biozone or biomat may form. In order to address the issue of virus fate and transport, information on the attachment and inactivation/die-off behavior has been gathered in the laboratory using material and temperatures representative of field conditions following established methods (Harvey, 1997; Van Cuyk et al., 2001; Navigato, 1999; Loveland et al., 1996).

The removal of virus during soil treatment of wastewater depends on attachment and inactivation processes (Yates, 1995; Navigato, 1999). However, the factors that control attachment and inactivation are not readily available or well understood, particularly for soil systems used to treat domestic wastewater effluent onsite. This paper describes a series of laboratory experiments that were carried out to enhance the understanding virus removal in soils receiving domestic wastewater effluents.

Two viruses with bacterial hosts (bacteriophages), MS-2 and PRD-1, were used in this research as surrogates for human viruses. Both of these bacteriophages have been employed as surrogates (Bales et al., 1993; Nicosia et al., 2001; Powelson and Gerba, 1994; Straub et al., 1992; Yahya et al., 1993a,b and others). The coliphage MS-2 is similar to that of human enteroviruses, including poliovirus while PRD-1 is similar to adenovirus (Ryan et al., 1999; Bales et al., 1991). Bacteriophages were first evaluated as water tracers in a river by Wimpenny et al. (1972). These authors note the advantages of using bacteriophages as surrogates, including: (1) the phage is non-pathogenic to man, (2) it is specific to its host bacteria, (3) the assay is simple and rapid, and (4) they have good survival characteristics.

Many researchers have developed models to describe the transport or removal of virus or microorganisms in the vadose or saturated zone (Anders and Chrysikopoulos, 2005; Bechdol et al., 1994; Corapcioglu and Haridas, 1984; Yates and Ouyang, 1992; Sim and Chrysikopoulos, 1999; Teutsch et al., 1991; Yates and Yates, 1988; Mathess and Pekdeger, 1985). None of these models incorporate the impact of the infiltrative

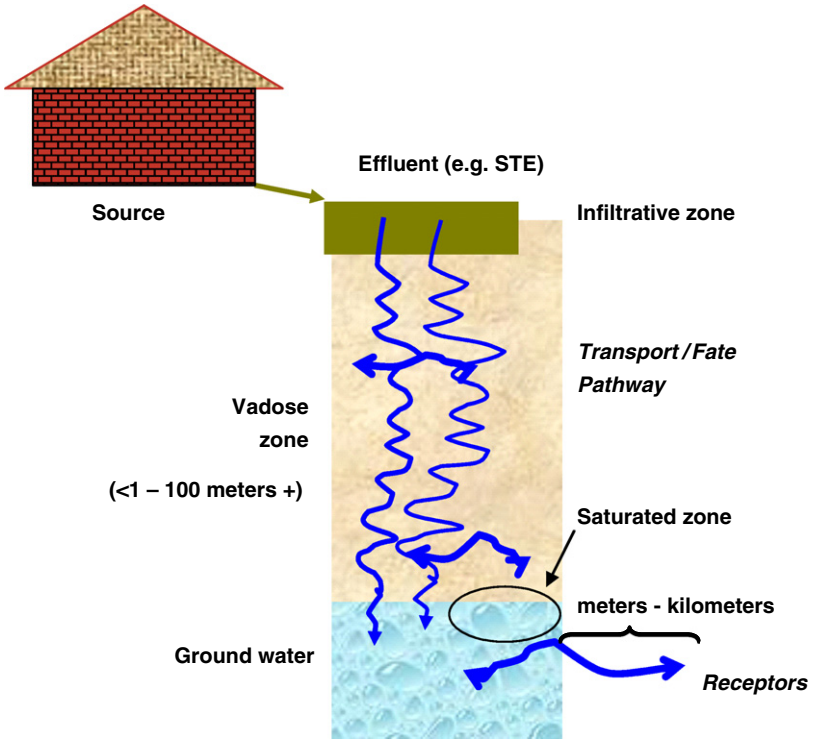

Fig. 1 – General schematic showing treatment zones within a soil-based treatment system. STE = septic tank effluent.

Table 1 – Soil physical properties

Soil properties	Units	Medium sand	Sand loam
Dry bulk density[a]	g/cm^3	1.70	1.68
Porosity (average in columns)	%	0.30	0.24
Organic carbon content[b]	%	0.00130 (0.0169)[c]	0.226 (±0.009)
pH		6.8	6.5
d10, d60	mm	0.22 mm, 0.60 mm	
Metal elemental composition	Dry weight %[d]	Al 8.182 (0.779)	Al 7.574 (0.602)
		Ca 0.360 (0.268)	Ca 2.476 (0.309)
		Fe 4.138 (0.642)	Fe 3.432 (0.625)
		Mg nd	Mg 0.952 (0.344)
		Si 33.076 (1.012)	Si 28.21 (0.806)

[a] Dry bulk density = dry soil mass/volume of soil.
[b] Mean percent organic carbon content (±1 standard error).
[c] ±0.0015 (from Sheldon 1999).
[d] Mean percent (five replicates) measured by scanning microanalysis (±1 standard error).

surface/clogging zone and its development on hydraulic and purification performance of PMBs for the removal of virus. Limited work has been performed to better understand the impact of design and operation (i.e., effluent quality, method of effluent delivery) on system purification performance. There is limited understanding how the infiltrative surface of these systems develops over time and how the engineering and design of these systems impact their overall performance. Models that are utilized to predict the transport of virus must include the loss of virus from soil solution or ground water due to attachment based on the physical and chemical properties of the soil and the ground water. This paper presents a summary of research completed that looks at soil type, effluent quality and system design parameter (hydraulic loading rate (HLR) and dosing regime) and system purification performance, with respect to virus removal. Additional details and related work can be found in Van Cuyk (2003).

2. Materials and methods

2.1. *Viruses*

Two bacteriophages, MS-2 and PRD-1, were used in this study as models for human pathogenic enteric viruses. 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). PRD-1 is an icosahedral lipid phage with a diameter of 62 nm, a pH_{iep} of < 4.5 and a *Salmonella typhimurium* host (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 (PBS), plated with the bacterial host on a layer of agar and incubated overnight at 37 °C. Plates were enumerated by counting plaques formed in the host lawn. MS-2 and PRD-1 bacteriophages and host bacteria were obtained from the United States Geological Survey in Boulder, CO. Viruses were added to applied effluent (i.e. STE or artificial ground water (AGW)) at target concentrations of ~10^4–10^7 PFU/mL.

2.2. *Unsaturated mini-columns*

Static columns were packed with approximately 10 g of sand or sandy loam in 50 cm^3 glass syringes (Popper and Sons). See Table 1 for porous media characteristics. After packing, columns were upflow saturated with deionized water and approximately 5 pore volumes were throughput before allowing downflow of effluent or water. Column experiments were designed to evaluate the effects of porous media type, applied effluent composition, HLR, and application frequency, following a full 2^4 factorial design as shown in Table 2. Following this design, effluent (STE or AGW at pH 7) was dosed onto the sand or sandy loam columns at a rate of either 5 or 25 cm/day. AGW was prepared in the laboratory following the method of Struse et al. (2002). The final composition of the AGW was: 0.83 mg/L KCl, 1.0 mg/L $NaNO_3$, 1.28 mg/L $FeCl_3$, 68.8 mg/L $MgCl_2$, 104 mg/L $CaSO_4$ (see Table 3). STE was collected weekly from a multifamily housing unit located on the Colorado School of Mines campus and stored quiescent and cloaked at 18 °C. Properties of the STE and AGW are shown in Table 3. Columns were dosed with equal total daily volumes, for a total of either 4 or 24 doses per day. A vacuum was pulled under each column during each dosing period and for 5 min following dosing. This applied vacuum pulled a consistent suction equivalent to that of approximately 60 cm of unsaturated soil (see Fig. 2 for unsaturated mini column apparatus). All dosing and delivery of effluent was automated using a programmable Chrontrol device. All columns for conditions studied were run in triplicate.

During the column experiments, virus removal tests (called tracer tests (TT)) were conducted by adding MS-2 and PRD-1 to the STE or AGW to be applied to the column. Bacteriophages were spiked into the column dose pots (see Fig. 2) containing the STE or AGW. Effluent with viral surrogates added was dosed (in the prescribed fashion as shown in Table 2) for a 24-h period. Dose pot samples were collected and analyzed at

Table 2 – Replicated 2^4 full factorial design of unsaturated mini-column experiments

Column #	Effluent	Porous media	HLR (cm/day)	Dose (#/day)
Run 1				
1–3	AGW	Med. sand	5	4
4–6			25	4
7–9			25	24
10–12			5	24
Run 2				
1–3	STE	Med. sand	5	4
4–6			25	4
7–9			25	24
10–12			5	24
Run 3				
1–3	STE	Sandy loam	5	4
4–6			25	4
7–9			25	24
10–12			5	24
Run 4				
1–3	AGW	Sandy loam	5	4
4–6			25	4
7–9			25	24
10–12			5	24

Note: Soil depth in mini-columns ~4 cm, representing the infiltrative surface zone.
AGW = artificial ground water, STE = septic tank effluent, HLR = hydraulic loading rate. All columns run in triplicate.

Table 3 – Properties of septic tank effluent and artificial ground water used in mini column studies

Parameter	STE[a]	AGW[b]
Alkalinity (mg $CaCO_3$/L)	209	40
$cBOD_5$ (mg/L)	276	—
COD (mg/L)	504	21.7
DOC (mg/L)	4	—
TS (mg/L)	585	237.5
TSS (mg/L)	60	5
FC (CFU/mL)	2.2×10^5	ND
pH	~6.8	6.0

— indicates value not measured: ND indicates non-detectable (below 1/100 mL).
[a] Septic tank effluent, average values presented.
[b] Artificial ground water.

the start ($t = 0$) and at the end ($t = 24$ h) of delivery of the surrogates. Little soil-free inactivation was observed in this 24 h period, but in cases where measured concentrations of added virus differed in the liquid samples collected at the start and at the end of the 24 h period, average values were used for the initial concentration of virus in removal calculations. Bench scale inactivation studies conducted in soil-free mini columns containing AGW or STE were conducted prior to this study and limited inactivation of growth of MS-2 and PRD-1 was seen over a 9 day period. A 10% reduction in virus concentration was observed and this reduction was similar for both AGW and STE (data not shown). At the end of the addition of surrogates, all effluent delivery lines were flushed with virus-free AGW or STE. Percolate samples were continuously collected from each column at 6 h intervals during tracer tests, until low levels of viruses (less than 1 PFU/mL) were measured in samples, at which point collection of samples occurred at 12 or 24 h intervals. Samples were collected from Erlenmeyer flasks place in an ice bath and delivered into conical vials and stored at 4 °C until analysis. These samples were plated for MS-2 and PRD-1 infective units (PFU) within 48 h of collection. The first virus tracer test commenced at a time point that represents 1 week of loading at either 5 or 25 cm/day. The second tracer test commenced after week 6 of column operation. Surrogate tracer tests occurred at these time points in order to gain an understanding of the response of the infiltrative surface to the effluent application and operation and to understand the removal of virus at the infiltrative surface during system start-up.

Percent removal of viruses in the columns was calculated using the following equation:

$$100 \times \frac{[(V_t)(Co)] - [\sum_{j=1}^{n}(V_j)(C_j)]}{[(V_t)(Co)]} = \%\ \text{Removal}, \quad (1)$$

where V_t is the total volume of the dose containing bacteriophages (mL), Co is the concentration of bacteriophage added to the dose volume (PFU/mL), V_j is the volume of column outflow collected over a sampling time (mL), C_j is the concentration of viruses measured in the column outflow (PFU/mL) and n is the number of outflow samples collected. Since the viruses were detected using the PFU assay, which only measures infective virus particles, the removal calculation incorporates the removal of viruses due to sorption onto the soil matrix as well as any inactivation or loss of infectivity.

Column dismantling occurred after ten weeks of operation for all columns except columns in run 2. The run 2 columns were dismantled following 11 weeks of operation. This period of time allowed for a maturation of the infiltrative surface, while still assessing the start-up period of operation. Prior to dismantling, column dosing was halted and the columns "rested" for approximately 2.5 h. The soil was then aseptically removed from each column, mixed using sterile utensils and divided into three subsamples. The first portion was used for water content analysis. This soil was added to preweighed aluminum weigh boats, dried at 105 °C overnight and percent water content calculated on a dry weight basis (APHA, 1998). The second portion was added to a sterile conical and extracted using 1.5 wt% beef extract solution (1:10 w/w soil: beef extract), shaken on an orbital shaker for 2 min at 250 rpm, followed by 1 min of settling after which a supernatant subsample was taken and analyzed for fecal coliform bacteria (via membrane filtration, APHA, 1998), heterotrophic plate count (HPC) (APHA, 1998) and MS-2 and

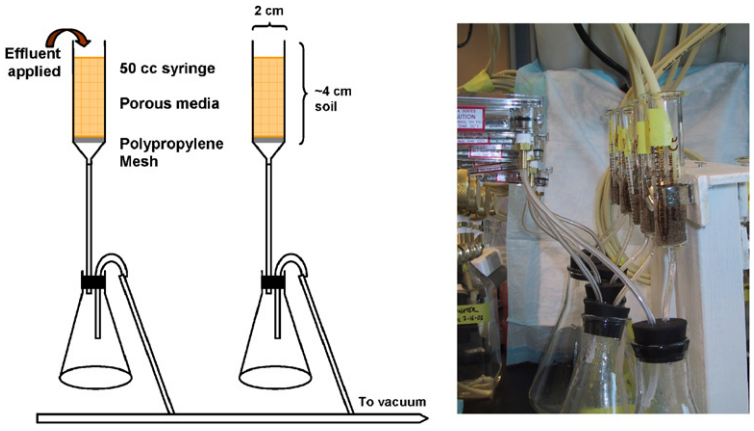

Fig. 2 – Unsaturated mini-column apparatus. Vacuum manifold designed to simulate ~60 cm of unsaturated soil.

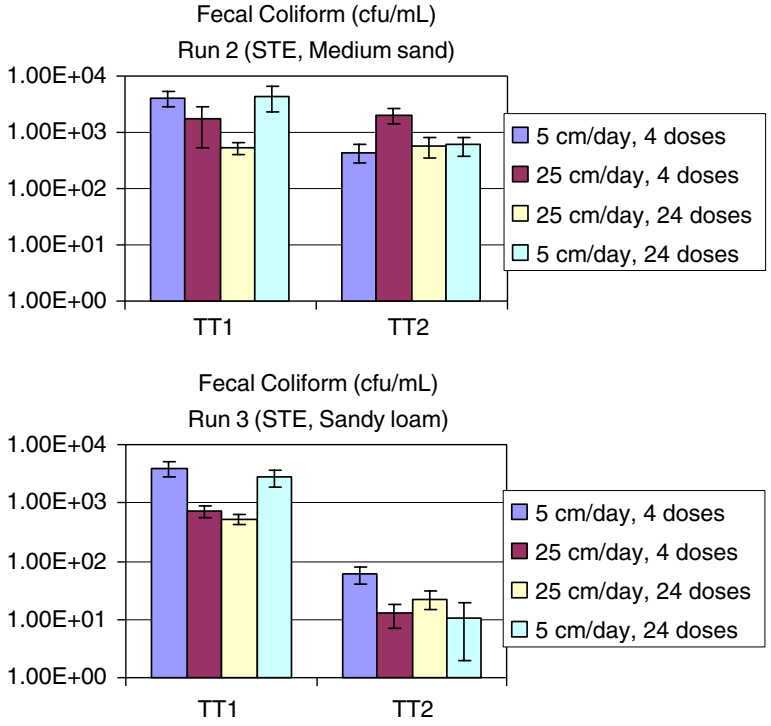

Fig. 3 – Concentration of fecal coliform in percolate from columns dosed with STE (run 2 in medium sand and run 3 in sandy loam) just before the start of tracer test 1 (TT1) and tracer test 2 (TT2). Average values for triplicate columns are shown with standard error bars. (Input concentration ~2.2×10^5 CFU/mL.) Percent removal of fecal coliform presented in text.

PRD-1 bacteriophage. Concentration of bacteria and virus found in the extracted soil supernatant were converted to number per gram dry soil via the water content values. The third portion of soil was stored at 4 °C for possible future analysis. Determination of total organic carbon (TOC) followed APHA (1998) methods.

Statistical analysis was conducted using analysis of variance (ANOVA). In all cases the confidence limit was 95%. This work was performed using Microsoft excel spreadsheets and MiniTab statistical software.

3. Results and discussion

3.1. *Unsaturated mini-column experiments*

At the start of tracer test 1 (week 1 of operation) the 5 and 25 cm/day columns processed an average of approximately 60 and 200 pore volumes, respectively. By the time of tracer test 2 (week 6) the 5 and 25 cm/day columns processed an average of approximately 350 and 1400 pore volumes, respectively.

Given the different compositions of STE vs. AGW, the mass of organic matter, nutrients, and bacteria applied to the columns varied markedly which likely impacted biofilm formation and biomat development in the porous media within the columns.

Fig. 3 shows the number of fecal coliform bacteria quantified in the percolate of columns dosed with STE (runs 2 and 3) as measured just prior to addition of viruses to the effluent (prior to both tracer tests). A single composite sample was collected over a 12 h interval and the number of fecal

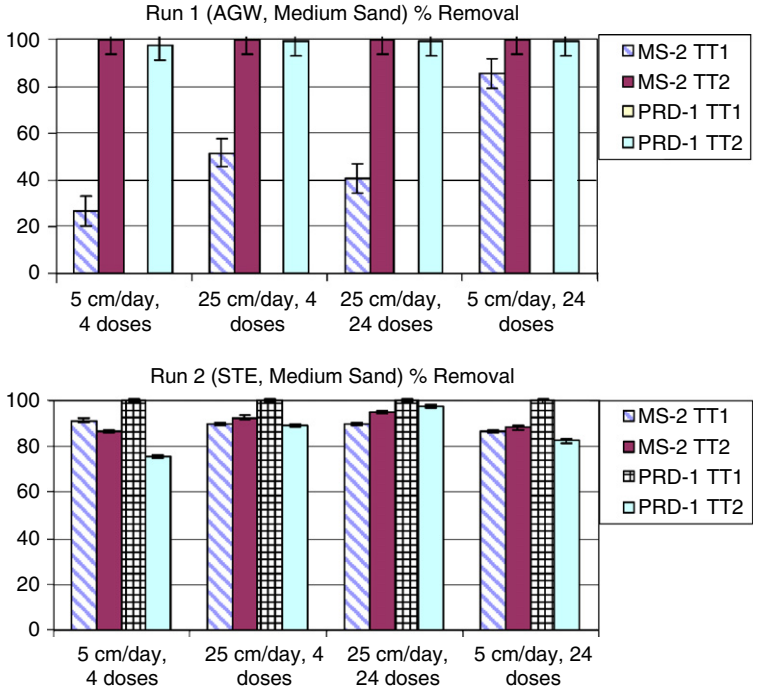

Fig. 4 – Percent removal from columns in Run 1 and 2 for MS-2 and PRD-1 at tracer test 1 TT1) and 2 (TT2). Average values for triplicate columns are shown with pooled standard error. No removal of PRD-1 was observed in run 1 during TT1.

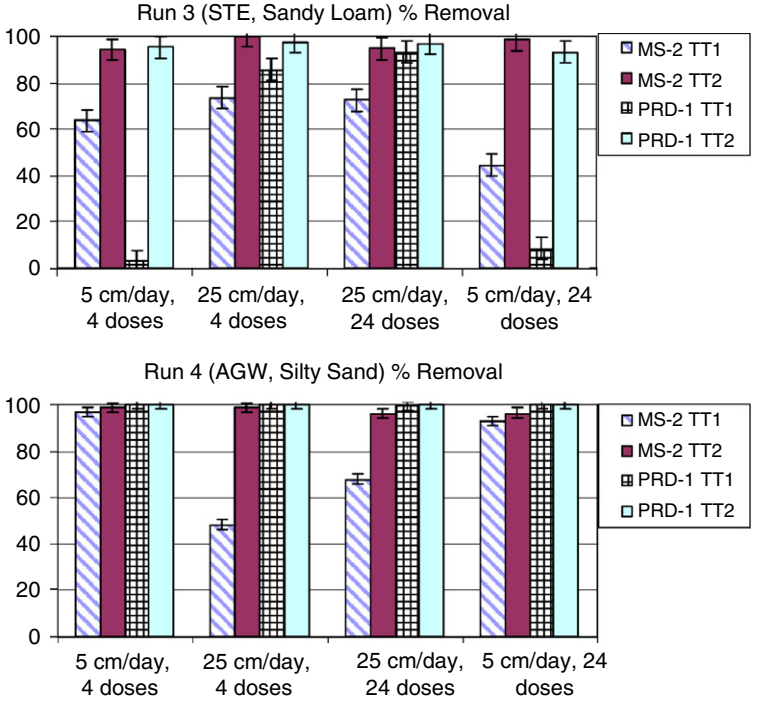

Fig. 5 – Percent removal from columns in Run 3 and 4 for MS-2 and PRD-1 at tracer test 1 (TT1) and 2 (TT2). Average values for triplicate columns are shown with pooled standard error.

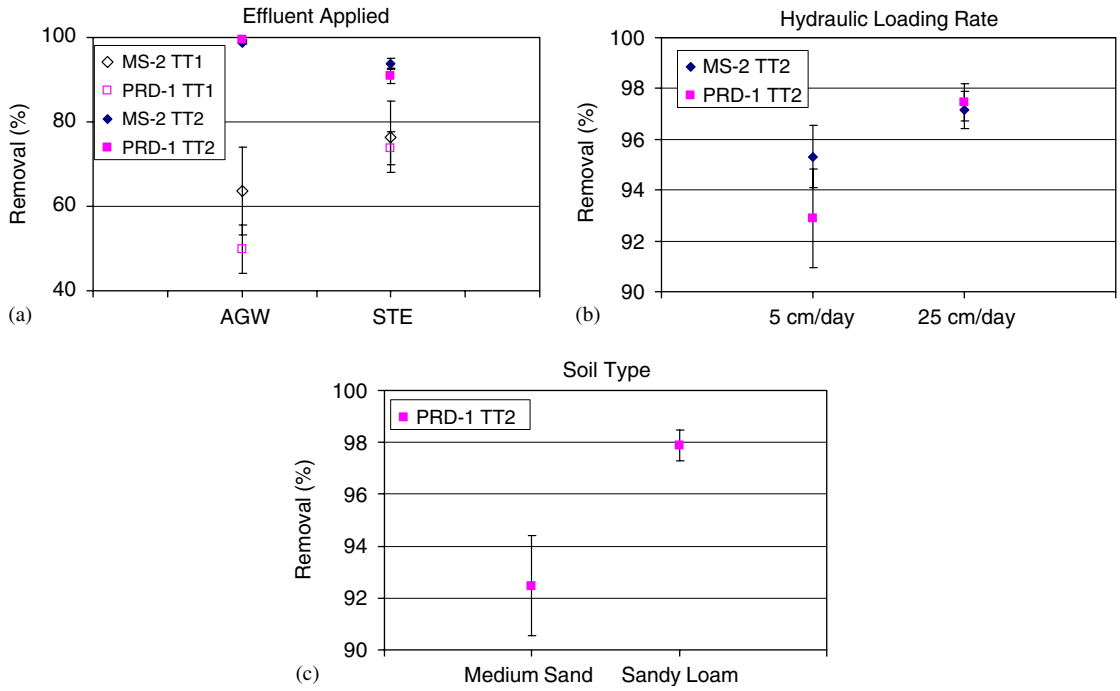

Fig. 6 – Three factors shown to have importance in the removal of viruses in unsaturated mini columns are effluent applied, HLR and soil type. Averages are shown with pool standard error ($n = 48$, CI = 95%). (Note difference in scales.) Only data with statistically significant differences are presented in this figure.

coliform bacteria was measured. These results show a decrease in fecal coliform breaking through (improved removal) in the columns filled with sandy loam with increased time of operation (from tracer test 1 at week 1 to tracer test 2 at week 6 of operation). Little change was seen from tracer test 1 to tracer test 2 in the columns dosed with STE and filled with medium sand. Percent removal of coliform bacteria increase slightly from tracer test 1 to tracer test 2 in both Run 2 (98.79% at TT1, 99.59% at TT2) and Run 3 (99.09% at TT1 and 99.98% at TT2).

The percent removal of MS-2 and PRD-1 in the unsaturated mini columns are shown in Figs. 4 and 5. Runs 1, 3 and 4 show improved removal of added viruses under all conditions with increased time of operation, that is, from tracer test 1 (week 1) to tracer test 2 (week 6). The only incidence where a decrease in removal was observed with time was in Run 2 (STE, medium sand) where a decrease in the removal of PRD-1 was seen from the first tracer test to the second. No removal of PRD-1 was observed in the columns in Run 1 (AGW, sand) at the first tracer test (TT1), but significant removal was observed in these same columns after only five additional weeks of continued operation (at TT2, week 6 of operation).

Two-way ANOVA was conducted on the percent removals calculated from these experiments in order to elucidate which factors are important in the removal of viruses through these columns. The quality of the effluent applied proved to be important for removal of MS-2 and PRD-1. Fig. 6a shows that when all columns are compared based on effluent applied, there is an increase in the percent of MS-2 and PRD-1 removed from columns loaded with AGW or loaded with STE from tracer test 1 to tracer test 2. This figure also shows that for both bacteriophages the first tracer test had higher removal when dosed with STE as compared to AGW, but by tracer test 2 those dosed with AGW had significantly higher removal of added phage. This shows an initial benefit (at the time of tracer test 1) of having columns dosed with STE, perhaps by more rapid development of attachment sites (e.g., by organic matter deposition) that result in an increased virus removal as suggested by Bales et al. (1993). By the second tracer test, the removal is improved in columns dosed with either type of effluent as compared to the first test, but the removal in AGW dosed columns is higher. The AGW may stimulate the microbial communities present (as seen in unsaturated column dismantling data) and also beneficially improve the attachment of viruses in the system. The addition of AGW to these columns may have been enough to result in changes in the total biological activity, much like that found in the sand filtration/soil clogging literature (Gupta and Swartzendruber, 1962; Frankenberger et al., 1979).

HLR also proved to be important in the removal of viruses. While Fig. 6b shows no effect of HLR on removal at the time of the first tracer test, by tracer test 2, columns dosed at the higher HLR (25 cm/day) have improved removal of both MS-2 and PRD-1. The literature suggests the opposite: that an increase in HLR leads to an increase in transport of the viruses (deeper penetration into the porous media). These mini columns represent a small portion (i.e., the infiltrative surface) of such systems, where it appears that higher HLR is more beneficial to removal of viruses, perhaps due to accelerated soil grain "conditioning." The HLR can also be discussed with respect to total loading of constituents. Total mass loading of constituents at the higher HLR is expected to

Table 4 – Overall average percent removal of MS-2 and PRD-1 in unsaturated mini-column runs 1–4

	MS-2		PRD-1	
	TT1	TT2	TT1	TT2
Run 1 (sand, AGW)	50.90 (28.50)[a]	99.99 (0.002)	0	98.71 (1.004)
Run 2 (sand, STE)	89.28 (2.85)	90.54 (4.22)	99.89 (0.039)	86.22 (9.97)
Run 3 (sandy loam, STE)	63.60 (20.11)	96.94 (5.70)	47.68 (44.99)	95.73 (2.79)
Run 4 (sandy loam, AGW)	76.51 (21.04)	97.46 (1.74)	99.87 (0.43)	99.98 (0.006)

TT1 = tracer test 1; TT2 = tracer test 2.
[a] Values in parentheses represent one standard deviation.

be five times the amount loaded at the lower HLR. More total mass of constituents will be loaded onto the columns at the time of the second tracer test (TT2).

There were no observed differences in removal of surrogates as a function of dosing, i.e., there were no differences seen between columns dosed four times daily and those dosed 24 times daily with either bacteriophage at either of the tracer test time points.

Soil type altered the removal of PRD-1 at the time of the second tracer test, at which time sandy loam had higher removal rates for PRD-1. Soil type did not have a significant impact on MS-2 removal at the time of either tracer test and did not have a significant impact on PRD-1 removal at the first tracer test. Fig. 6c shows that those columns filled with sandy loam had higher removal rates for PRD-1 at the time of tracer test 2.

These data suggest that over a relatively short period of time (after 10–11 weeks of operation) the infiltrative surface of soil based wastewater treatment systems can achieve much higher removal then initially measured shortly after startup. Table 4 shows tracer test 1 had removals of less than 1 log for most conditions (only Run 2 had higher removal, i.e., 2 log removal for PRD-1). By the time of the second tracer test overall removal had improved under most conditions, with some exceptions. 1 log removal most frequently observed in addition to 3 and 4 log average removals. The 3 and 4 log removals were seen for MS-2 in run 1 (sand, AGW) and for PRD-1 in run 4 (sandy loam, AGW).

3.2. *Dismantling of unsaturated columns*

Dismantling of these columns occurred following a time period of dosing representative of approximately 10 weeks of operation. Total volumes dosed in run 2 are slightly higher due to the fact that they were left in operation ~1 week longer than the other columns.

Water content values measured at the time of dismantling are shown in Fig. 7. ANOVA conducted on water content values determined that soil type had the only significant impact on the measured value. Fig. 8 shows higher percentage water content in columns packed with sandy loam versus those packed with medium sand ($n = 24$, 95% confidence level). No other factors had a significant impact on the water content of the columns at the time of dismantling.

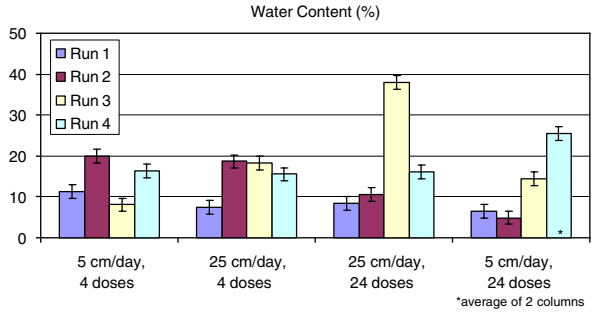

Fig. 7 – Water content (% dry wt.) measured in columns at time of dismantling. Averages of triplicate columns shown with standard error. Run 1 = AGW, medium sand; Run 2 = STE, medium sand; Run 3 = STE, sandy loam; Run 4 = AGW, sandy loam.

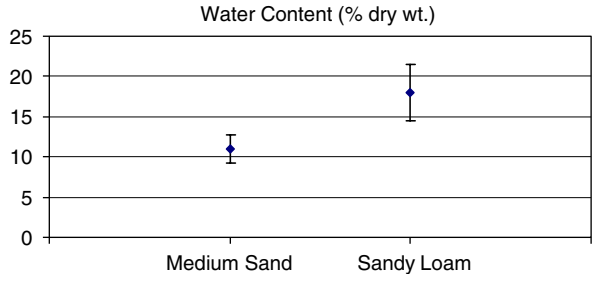

Fig. 8 – Water content (% dry wt.) versus soil type at the time of column dismantling. Average shown with standard error ($n = 24$, CI = 95%).

TOC levels in soil samples revealed a significant effect of both soil type and effluent type on the magnitude of organic matter accumulated in the infiltrative surface zone (shown in Fig. 9). As expected, higher levels were observed in columns filled with the sandy loam soil and in those columns dosed with STE. HLR and dosing regime had no statistically significant impact on the TOC values measured at the end of unsaturated mini column experiments. The HLR may not significantly impact the TOC values because the difference between the high and low HLR is five-fold (5 versus 25 cm/day), while the COD values between the STE and AGW differ by a factor of 23 (504 and 22 mg-COD/L, respectively).

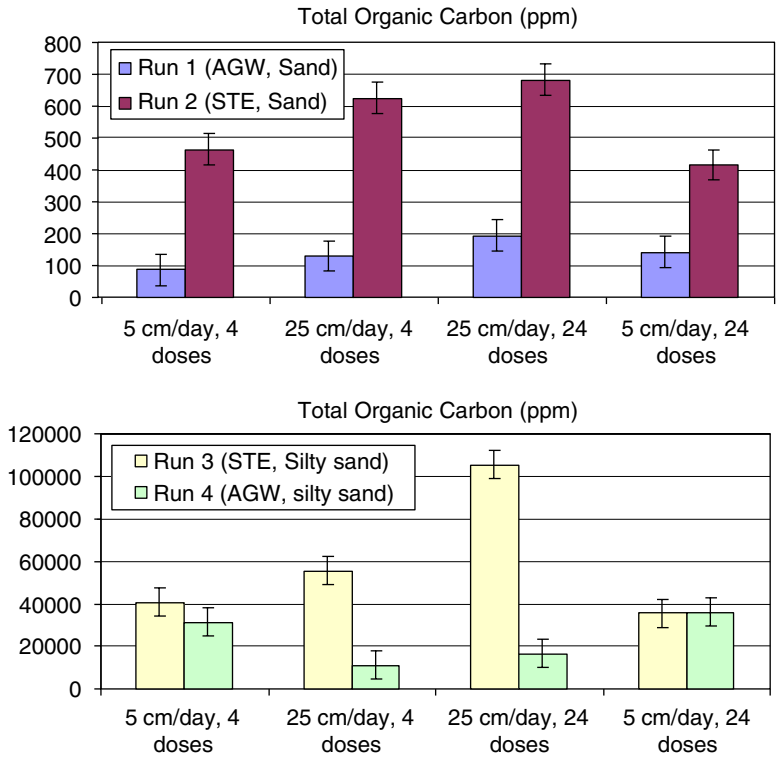

Fig. 9 – Total organic carbon (ppm dry weight) measured in soil samples from dismantled columns. Averages of triplicate columns are shown ± standard error of mean. Note difference in y-axis scales.

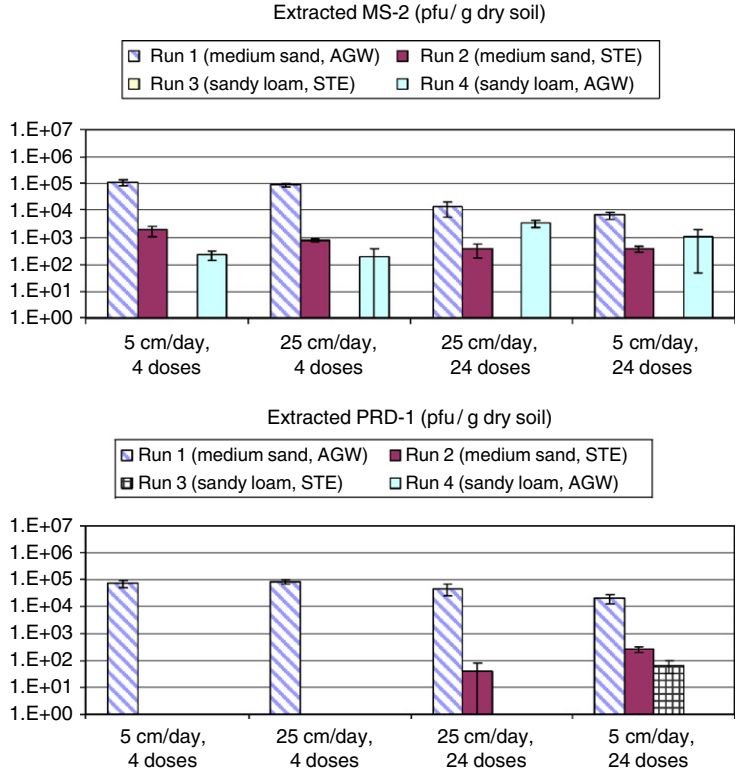

Fig. 10 – Values for MS-2 and PRD-1 measured in extracted soil columns. Averages of triplicate columns are shown ± standard error.

O_MRKO_MRK

Values of MS-2 in soil samples (Fig. 10) show that only one run (Run 3, sandy loam, STE) had no MS-2 measured in beef extracted samples. The presence of organic matter on the soil in combination with that added by STE dosing may have yielded ineffective extraction of this bacteriophage or the MS-2 may no longer be infective and is therefore not detectable. Percent removal in these columns did not vary greatly from other column conditions. Run 3 columns extracted for PRD-1 also yielded no measurable extracted virus.

4. Summary and conclusions

The fate of surrogate viruses in the infiltrative surface zone of a soil treatment system was evaluated under varying conditions of effluent applied, soil type, HLR and method of dosing. Conclusions from this research include:

- The infiltrative surface can achieve removal of virus with ranges from less than one to 3 log removal of viruses. Higher HLRs (25 cm/day) improved the removal of both MS-2 and PRD-1 in the infiltrative surface. Higher removal of PRD-1 was seen in sandy loam versus medium sand.
- Higher levels of TOC were measured in columns loaded with STE at a HLR of 5 cm/day versus AGW loaded at 25 cm/day (when comparing in either sand or sandy loam), although higher removal of both MS-2 and PRD-1 was seen at the time of the second tracer test in the columns loaded with AGW at 25 cm/day, versus those in the same soil dosed with STE at 5 cm/day.
- Microdosing, when comparing AGW dosed 24 times/day versus columns dosed with STE 4 times daily, resulted in higher removal of both MS-2 and PRD-1 at the time of the second tracer test.

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