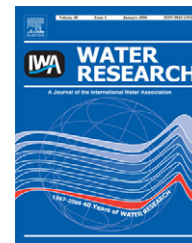


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# Fate and transport of viruses during sewage treatment in a mound system

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## ABSTRACT

Studies undertaken to assess the performance of filter materials to remove phosphorus in decentralised sewage systems have not reported on the broader performance of these systems. This study aimed to identify virus fate and transport mechanisms at the laboratory scale for comparison with field experiments on a mound system amended with blast furnace slag. Inactivation was a significant removal mechanism for MS2 bacteriophage, but not for PRD1 bacteriophage. Column studies identified rapid transport of PRD1. Laboratory studies predicted lower removal of PRD1 in a full scale system than was experienced in the field study, highlighting the importance of considering pH and flow rate in pathogen removal estimates. The results highlight the necessity for studying a range of organisms when assessing the potential for pathogen transport.

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## 1. Introduction

A broad range of treatment options are available for decentralised sewage systems, but high rates of failure of traditional septic tanks (USEPA, 2002) are discouraging innovation in favour of centralised sewerage installation. Uptake of alternative technologies has also been limited where the ability to provide centralised management is limited by geographic or economic constraints. A further concern to regulatory authorities is the increasing potential for failure as the complexity of systems and treatment processes increases, the belief that householders will not

maintain them, and the greater human and environmental risks arising from such failures.

Soil mound systems are one type of low-maintenance system, and can be enhanced with various materials to facilitate phosphorus removal. These materials facilitate ion exchange and precipitation of phosphorus through provision of increased surface area, pH and chemicals such as aluminium, iron and calcium compounds. Amendment materials investigated for their nutrient removal capabilities have included red mud (Ho et al., 1991), blast furnace slag (Johansson, 1999), fly ash (Cheung and Venkitachalam, 2006), zeolite (Sakadevan and Bavor, 1998) and lightweight

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Nomenclature		EC	electrical conductivity
$\alpha$	fraction of viruses subject to higher inactivation rate in biphasic inactivation model	$k_{att}$	attachment coefficient
ARW	artificial rain water	$k_{det}$	detachment coefficient
C	virus concentration	pfu	plaque-forming units
$C_0$	virus concentration at time = 0	t	time
DO	dissolved oxygen	SD	standard deviation
		$\mu$	inactivation rate
		$\mu_1$	higher biphasic inactivation rate
		$\mu_2$	lower biphasic inactivation rate

expanded clay aggregate (Ausland et al., 2002). However, limited research has been undertaken on how these materials affect the fate and transport of other contaminants, including pathogens.

A previous study aimed to assess the performance of a sand mound amended with blast furnace slag (Charles et al., 2004). Two sand mounds (138 m<sup>2</sup> × 1 m high) were installed to treat septic tank effluent from four households and two public toilet blocks. Effluent flowed horizontally out from a central trench to the edge of the impermeable liner, with pH increasing with distance travelled due to the high pH of the slag. The horizontal flow path resulted in saturated conditions within the mound. Bacteriophage, viruses that infect bacteria, were used as models for human enteric virus behaviour in the environment. They span the range of shapes, sizes, surface charges and persistence exhibited by many human enteric viruses. Their physical characteristics and tolerance of wastewater treatments make them ideal models to illustrate the behaviour of enteric viruses in groundwater, soil or subsurface environments and for sewage treatment efficacy evaluation. Routine sampling results indicated high removal of thermotolerant coliforms (>3.8 log<sub>10</sub>), however there was significant transport of somatic coliphages present in the sewage with a mean concentration in effluent of 15 pfu (plaque forming units) mL<sup>-1</sup>, a reduction of 1.5 log<sub>10</sub> units. Two bacteriophage were used for field tracer experiments. MS2 is an icosahedral phage with a diameter of 27 nm and an isoelectric point of 3.5 reported to have little or no adsorption in saturated sandy soils at pH 6–8 with low organic carbon content. PRD1 is an icosahedral phage with a diameter of 62 nm and an isoelectric point between 3 and 4. Field tracer experiments resulted in similar transport of PRD1 bacteriophage (2.9 log<sub>10</sub> removal) compared to somatic coliphages, but over 5 log<sub>10</sub> removal of MS2 bacteriophage.

Virus transport and fate in soils is predominantly a function of advection, inactivation, sorption and desorption (Schijven and Hassanizadeh, 2000). While advection and desorption are dependant on the flow conditions, adsorption and inactivation depend on a range of additional factors. Virus adsorption is generally the most important process for attenuation and depends on the pH, soil type, organic matter content, ionic strength and flow rate. Inactivation and irreversible adsorption are required for virus removal. Fate and transport characteristics vary between enteric viruses (Schijven and Hassanizadeh, 2000). Virus inactivation is dependant on the physical characteristics of the different viruses (e.g. surface chemistry and morphology) and environmental factors (e.g.

temperature, microbial activity, pH and ammonia) (Schijven and Hassanizadeh, 2000). Flow rate can affect the contact of viruses with the attachment sites, with increasing velocities reducing contact time and therefore attachment (Schijven and Hassanizadeh, 2000). High ionic strength, such as septic tank effluent, favour virus adsorption; with low ionic strength waters, such as rainfall, able to remobilise attached viruses (Schijven and Hassanizadeh, 2000).

It was hypothesised that inactivation was likely to be the main mechanism for virus removal as the conditions within the mound were generally favourable for virus transport (high pH), but unfavourable for virus survival (up to 25 °C and pH 11.0). Furthermore, ammonia is known to be virucidal above pH 8 (Ward and Ashley, 1977).

This paper reports the results of laboratory experiments undertaken in collaboration with the Sydney Catchment Authority to assess the mechanisms of virus transport in an amended sand mound system. The first set of experiments aimed to investigate the role of inactivation in removal of viruses in the field experiments, under the neutral pH conditions in the influent and high pH conditions in the effluent. The second set of experiments aimed to quantify the transport and fate of viruses in a laboratory column under more adverse conditions for virus removal; specifically high flow rates and low ionic strength.

## 2. Materials and methods

### 2.1. Bacteriophage inactivation in sewage and effluent

Ten glass containers (100 mL) were filled with wastewater from the mound, making five influent–effluent pairs. Physico-chemical analyses (pH, temperature and DO [dissolved oxygen]) were undertaken on two pairs one incubated in the dark at 4 °C, the other at ambient temperature (22 °C). Of the remaining three pairs (Table 1), one pair (SI22 and SE22) were sterilised by autoclaving at 121 °C for 15 min to reduce the influence of microbial activity within the sample. Suspensions of MS2 and PRD1 (10<sup>6</sup> pfu mL<sup>-1</sup>) were added to each of the six containers. One unsterilised pair (I22, E22), with the sterilised pair, were stored in the dark at 22 °C to replicate conditions within the mound (Table 1). The remaining unsterilised pair (I4, E4) were stored at 4 °C providing a temperature control to assist in the identification of the impact of the amending material. The low temperature was also assumed to reduce the influence of microbial activity. All containers were sampled over a 28-day period, which

**Table 1 – Experimental conditions for bacteriophage inactivation in influent and effluent**

Sample code	Sample	pH	Temperature (°C)	Sterilised
I4	Influent	8.1	4	No
I22	Influent	8.1	22	No
SI22	Influent	8.1	22	Yes
E4	Effluent	10.6	4	No
E22	Effluent	10.6	22	No
SE22	Effluent	10.6	22	Yes

corresponded with the time period of the previous field experiments.

Independent duplicate 1 mL aliquots were sampled from each container with separate dilution series for each sample. Three dilutions were analysed for each sample. On three occasions, duplicate analyses of the sample were undertaken. Results are reported as the average of duplicate plates, from the appropriate dilution. Bacteriophage analyses were undertaken by AMS Laboratories, Sydney. The spiked MS2 coliphage (ATCC 15597-B1) was analysed by the method of [Havelaar and Hogeboom \(1984\)](#) using host *Escherichia coli* (Migula) (ATCC 15597) (phage and host supplied by the American Type Culture Collection, Manassas, USA). The spiked PRD1 bacteriophage was analysed by the method for somatic coliphages (ISO 10705-2, 2000) using host *Salmonella typhimurium* L29 (phage and host supplied by Prof. C. Gerba, University of Arizona, Tucson, Arizona). Background concentrations were measured prior to each spike.

## 2.2. PRD1 laboratory transport study

Column experiments were undertaken using the amended soil with artificial rain water (ARW, per L of deionised water: 4.07 mg NaNO<sub>3</sub>, 3.24 mg NaCl, 0.35 mg KCl, 1.65 mg CaCl<sub>2</sub> · 2H<sub>2</sub>O, 2.98 mg MgSO<sub>4</sub> · 7H<sub>2</sub>O, 3.41 mg NH<sub>4</sub> · 2SO<sub>4</sub>) ([Davies et al., 2004](#)). The column was constructed from 10.5 cm PVC (internal diameter), capped with a reducer and 5.0 cm internal diameter PVC cap, fitted with a brass nipple (2 cm long, 2 mm internal diameter). The inside of the column was spirally machine roughened to minimise soil–column interface flow and therefore limit edge effects. Silicone tubing (Masterflex HV-96410-16 Precision Silicone (platinum) Tubing, internal diameter 3.1 mm) was fitted to the brass tube. The base of the soil was supported with three layers of wire mesh (pore size 2 mm) overlaid with a 2 cm layer of 2 mm glass beads. Soil slurry was added to a depth of 50 cm during up-flow conditions in small amounts. Regular stirring and shaking aimed to achieve uniform packing. At the top of the column, the interface of the soil and the column was sealed with silicone sealant and a ring of aluminium foil to further limit the short-circuiting of water via the column edges. A soil-free control column was used as a control with the supporting layers of wire mesh and beads, but no soil.

Several pore volumes of ARW were fed (downwards) through the column prior to experimentation to equilibrate the column. Saturated flow conditions were used as flow in

the field is horizontal, and therefore primarily saturated. A flow rate of 1.1 m day<sup>-1</sup> was used. The sodium chloride concentration of the ARW was increased by 2.9 g L<sup>-1</sup> for several pore volumes to enable determination of flow dispersion. The electrical conductivity (EC) was analysed with a Lab Analyser 440 (TPS Pty Ltd, Australia). After completion of the salt tracer, the ARW was inoculated with PRD1 (final concentration 10<sup>4</sup> mL<sup>-1</sup>). PRD1 was grown up in host cells of *Salmonella typhimurium* strain LT2 (ATCC 19585). The column influent was inoculated with PRD1, and the concentration in column effluent was quantified periodically during and after the application of the inoculum to determine the removal of viruses by passage through soil. A minimum of triplicate samples were collected to determine the background and final effluent phage concentrations. Column experiments were undertaken at ambient temperature (approximately 20 °C). At the completion of experiments, the column was destructively sampled to assess percent moisture determination by drying in pre-weighed crucibles at 105 °C for 48 h ([APHA, 1998](#)).

Inactivation in the column material and ARW was studied. Seventy portions sieved amended mound soil (2 g) were weighed into 5 mL polyethylene vials, and an additional 70 vials used with only 2 mL of ARW. Soil was analysed using standard methods ([APHA, 1998](#)) for pH, grain size distribution, carbon content, total and exchangeable iron, bulk density and cation exchange capacity. Phosphorus sorption was analysed using the method from [Rayment and Higginson \(1992\)](#). Stock suspensions of PRD1 were diluted in sterile deionised water such that 0.1 mL of the diluted bacteriophage suspension could be added to achieve approximately 1 × 10<sup>6</sup> virions per vial. Sterilised (autoclaved) ARW was added to the soil microcosms to saturate each of the soils as equally as possible. Control vials of each soil type were left uninoculated to be used for moisture determinations; 0.1 mL of sterile deionised water was added in place of the inoculum. The vials were incubated at 20 °C in the dark. An ibutton™ miniature temperature probe (Maxim/Dallas Semiconductor Corp., Dallas, TX, USA) taped to the inside of the jar was used to record the temperature inside the jar at 120 min intervals throughout the experiment. The soil was then inoculated with PRD1 and triplicate vials were randomly selected and withdrawn periodically from each sealed jar for determination of infectious PRD1. Hence, the microcosms were sampled destructively. In addition, duplicate vials were removed periodically from each jar for percent moisture determination by drying in pre-weighed crucibles at 105 °C for 48 h ([APHA, 1998](#)).

Each 2 g of inoculated soil from sampled vials was washed into a 50-mL Falcon tube using 20 mL 3% (w/v) beef extract solution ([Straub et al., 1992](#)) (pH 9). The soil slurry was then vortexed for 2 min and shaken for 30 min. After further vortexing for a few seconds the slurries were centrifuged for 15 min at 2500g, after which 1 mL of the supernatant was withdrawn by pipette. This was diluted serially in sterile deionised water and assayed by the double agar layer technique ([Adams, 1959](#)). Concentrations of phages were expressed as plaque-forming units per vial.

A recovery control was prepared for each soil type by freshly inoculating 2 g of soil with approximately 1 × 10<sup>6</sup> PRD1

from a stock suspension. The phage was allowed to adsorb to the soil by storing at room temperature for two hours, before being processed as described above. The titre of the stock suspension used to inoculate the recovery controls was also determined. Percent recovery for each soil type was determined as the concentration of phage recovered divided by the concentration inoculated into the soil  $\times 100$ . Phage concentrations in ARW were measured by directly diluting the contents of the microcosm vials with deionised water, and assaying by the double agar layer technique as above.

### 2.3. Statistical analyses

For the inactivation studies, two inactivation models were applied: first-order and biphasic. First-order inactivation was modelled as

$$C = C_0 \text{Exp}(-\mu t), \quad (1)$$

where  $C$  was the concentration of phages,  $C_0$  was the concentration of phages at time = 0,  $t$  was time, and the inactivation rate,  $\mu$ , was considered to be a function of pH, temperature and/or microbial activity. Biphasic inactivation was modelled as

$$C = C_0[\alpha \text{Exp}(-\mu_1 t) + (1 - \alpha)\text{Exp}(-\mu_2 t)], \quad (2)$$

$\alpha$  was the fraction of less stable viruses with a higher inactivation rate coefficient  $\mu_1$ , and  $(1 - \alpha)$  is the fraction of more stable viruses with a lower inactivation rate coefficient  $\mu_2$  (Petterson et al., 2001). The inactivation rate coefficients were derived from the experimental data by fitting the inactivation models with a log-likelihood method previously described by Schijven et al. (2002). Using the likelihood ratios test, the first-order and biphasic inactivation models were compared for each dataset, with significance defined by the  $\chi^2$ -test at  $p < 0.05$  level. Outliers were identified as the residuals from fitting of first-order and biphasic models that were statistical outliers based on boxplot results using SPSS (version 11.5.2.1, SPSS Inc., 2003). They were defined as points where the residual from the fitted inactivation model was more than 1.5 times the inter quartile range above the third quartile. Outliers were excluded from final inactivation rate analyses to improve the statistical comparison of different experimental conditions and prevent the overestimation of the inactivation rate. Outliers only occurred in the initial 2 days of the studies, therefore the remaining data provided information on the longer term behaviour of the viruses. A maximum of one time point within the first 2 days of the study was excluded from five of the twelve studies. Two time points ( $t = 0, 1$ ) were excluded from one study.

Modelling of breakthrough curves was undertaken for the field experiments (Charles et al., 2004) and laboratory experiments. HYDRUS-1D (United States Salinity Laboratory, Riverside) (Šimůnek et al., 1998) is commonly used in modelling micro-organism transport in porous media (Schijven and Šimůnek, 2002; Charles, 2008; Foppen et al., 2007). Conservative tracer breakthrough curves in the column and in the mound were fitted to the convection-dispersion solute transport equation using HYDRUS-1D to calculate porosity and dispersion within the columns. The fit was optimised using the Levenberg–Marquardt non-linear minimisation algo-

rithm for least-squares solutions. The relative concentrations from the phage breakthrough curves ( $C/C_0$ ) were fitted (using log resident concentrations) to the one-site kinetic adsorption equations using HYDRUS-1D. For modelling purposes, inactivation of unattached viruses ( $\mu_u$ ) was assumed to equal the inactivation in ARW, and inactivation of attached viruses ( $\mu_s$ ) was assumed to equal inactivation in the soil–water microcosm. Virus transport coefficients were fitted from the column breakthrough curve to the one-site kinetic adsorption equations, and were compared with the results from the field study results.

## 3. Results

### 3.1. Survival in sewage and effluent

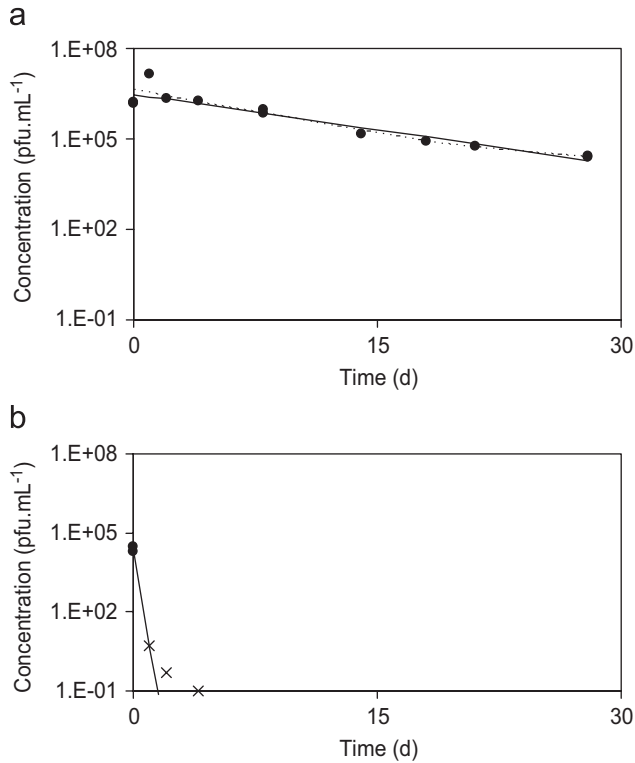
The sample chemistry was generally stable over the duration of the study. Samples had initial pH values of 8.1 and 10.6 for influent and effluent, respectively. Final pH values (32 days) were 7.9 and 7.3 for influent at 4 and 22 °C, respectively, and 10.8 and 10.5 for effluent at 4 and 22 °C, respectively. An initial decrease in dissolved oxygen and slight pH variation was observed over the first 24 h from sampling, with the sample stabilising after such time. Average initial concentrations, as quantified on day zero from the spiked sample containers, of  $10^6$  pfu mL<sup>-1</sup> of MS2 and PRD1 were achieved for all samples except unsterilised effluent at 22 °C where MS2 was  $10^4$  pfu mL<sup>-1</sup>. The average temperatures were 4.7 °C ( $\pm 1.7$  SD) and 22.8 °C ( $\pm 1.3$  SD).

MS2 bacteriophage was very sensitive to the conditions within the mound: high pH, 22 °C, high microbial activity (Fig. 1). Unsterilised effluent exhibited the most rapid inactivation with no phage detectable ( $< 1$  pfu 10 mL<sup>-1</sup>) after 2 days. PRD1 was more stable in the conditions in the mound (Table 2). Both MS2 and PRD1 exhibited an increased inactivation with increased temperature. Outliers were identified in six of the twelve studies which included  $t = 0$  for PRD1 E4 and MS2 I4;  $t = 1$  for PRD1 SI22 and I22;  $t = 2$  for PRD1 SE22; and  $t = 0$  and 1 for MS2 I22 (see Table 1 for sample codes). The biphasic inactivation model (Eq. (2)) was preferred for four of the studies (Table 2) based on the log-likelihood ratios test (significant at the  $\chi^2$  95th percentile). PRD1 had significantly ( $p < 0.05$ ) lower rates of inactivation than MS2 and the first-order model was preferred in all except for the case with highest inactivation, E22 (Fig. 2).

The first-order model inactivation coefficient,  $\mu$ , for MS2 (Table 3) varied significantly between each sample type, except for the sterilised influent/effluent pair. Inactivation was significantly more rapid ( $p < 0.05$ ) in unsterilised effluent than in unsterilised influent, both at 4 and 22 °C. Temperature was a major factor for MS2 inactivation in influent, with  $\mu_{SI22}$  significantly ( $p < 0.05$ ) greater than  $\mu_{I4}$ . The MS2 biphasic models (Table 3) for sterilised influent and effluent were not significantly different, with the data able to be described with all common coefficients. However, both were significantly different from MS2 I22. PRD1 was more robust under the conditions of the study, and hence, comparison of the PRD1 first-order inactivation model coefficients (Table 3) elicited fewer significant factors. Nonetheless, sample type was



significant ( $p < 0.05$ ) for unsterilised samples with inactivation being more rapid in effluent than in influent at 4 and 22 °C. Temperature and microbial activity, separately, were only significant in effluent however combined were significant in influent but not effluent.



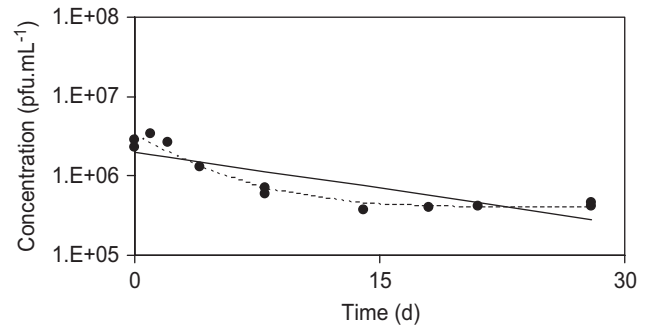
**Fig. 1 – MS2 data (circles), first-order model (continuous line) and biphasic model (dotted line) for (a) influent and (b) effluent at 22 °C.**

**3.2. Survival in amended soil**

The amending material resulted in increased pH and phosphorus sorption capacity as well as in calcium and cation exchange capacity (Table 4). The average temperature within the microcosm jars was 19.5 °C (SD±0.77 °C) for the duration of the experiment (131 days). The moisture content was 17.7±1.0%. The efficiency of the phage recovery method for the soil microcosms was 56±25%, and was not affected by time. Percent recovery was not used to adjust the phage concentrations. PRD1 inactivation (Table 2; Fig. 3) in the soil/water matrix was significantly lower than inactivation in ARW alone. The biphasic model provided a better fit in the ARW only.

**3.3. Transport in columns**

Salt tracer recover was 100%, providing a good fit of the salt tracer breakthrough curve. The porosity used in the model was based on the total porosity of the column (including the



**Fig. 2 – PRD1 data (circles), first-order model (continuous line) and biphasic model (dotted line) effluent at 22 °C.**

**Table 2 – Bacteriophage inactivation rate coefficients and likelihood ratios in sewage, ARW and ARW with amended soil**

Sample	First-order model	Biphasic model		Likelihood ratio test ( $\chi^2_{0.95}$ )		
	$\mu$ (day <sup>-1</sup> )	$\alpha$	$\mu_1$ (day <sup>-1</sup> )	$\mu_2$ (day <sup>-1</sup> )	Log likelihood ratio	Preferred model
MS2						
I4	0.028	0	2.1	0.028	$-1.6 \times 10^{-14}$	First-order
I22	0.18	0.9956	0.23	0	11.4*	Biphasic
SI22	0.52	0.9996	1.23	0.26	40.8*	Biphasic
E4	11.3	Insufficient data			–	(First-order)
E22	> 8.48	Insufficient data			–	(First-order)
SE22	0.44	0.996	1.09	0.26	17.8*	Biphasic
PRD1						
I4	0.0012	0	31.7	0.0012	$-4.4 \times 10^{-12}$	First-order
I22	0.018	0	15.4	0.018	$-1.7 \times 10^{-10}$	First-order
SI22	0.015	0	3.85	0.015	$2.8 \times 10^{-9}$	First-order
E4	0.049	1	0.049	$3.3 \times 10^{-5}$	$-1.6 \times 10^{-6}$	First-order
E22	0.071	0.877	0.287	0	22.3*	Biphasic
SE22	-0.00015	0	3.45	$-2E-04$	$-4.9 \times 10^{-8}$	First-order
ARW	0.0283	0.014	0.161	0.0022	13.2*	Biphasic
ARW+soil	0.0210	0	0.238	0.021	$-4.0 \times 10^{-11}$	First-order

**Table 3 – Comparisons of phage inactivation based on first-order inactivation model**

Samples	Variable <sup>a</sup>	MS2 Likelihood ratios test ( $\chi^2_{0.95}$ )		PRD1 Likelihood ratios test ( $\chi^2_{0.95}$ )	
		Result	Common $\mu$	Result	Common $\mu$
I4 v I22	T, M	$\mu_{I22} > \mu_{I4}$	–	$\mu_{I22} > \mu_{I4}$	–
I4 v SI22	T	$\mu_{SI22} > \mu_{I4}$	–	–	0.00719
I4 v E4	S	$\mu_{E4} > \mu_{I4}$	–	$\mu_{E4} > \mu_{I4}$	–
I4 v E22	T, S, M	$\mu_{E22} > \mu_{I4}$	–	$\mu_{E22} > \mu_{I4}$	–
I4 v SE22	T, S	$\mu_{SE22} > \mu_{I4}$	–	–	0.000824
I22 v SI22	M	$\mu_{SI22} > \mu_{I22}$	–	–	0.0170
I22 v E4	T, S, M	$\mu_{E4} > \mu_{I22}$	–	$\mu_{E4} > \mu_{I22}$	–
I22 v E22	S	$\mu_{E22} > \mu_{I22}$	–	$\mu_{E22} > \mu_{I22}$	–
I22 v SE22	S, M	$\mu_{SE22} > \mu_{I22}$	–	$\mu_{SE22} > \mu_{I22}$	–
SI22 v E4	T, S	$\mu_{E4} > \mu_{SI22}$	–	$\mu_{E4} > \mu_{SI22}$	–
SI22 v E22	S, M	$\mu_{E22} > \mu_{SI22}$	–	$\mu_{E22} > \mu_{SI22}$	–
SI22 v SE22	S	–	0.470 <sup>b</sup>	–	0.0100
E4 v E22	T, M	$(\mu_{E4} > \mu_{E22})^c$	–	–	0.0495
E4 v SE22	T	$\mu_{E4} > \mu_{SE22}$	–	$\mu_{SE22} > \mu_{E4}$	–
E22 v SE22	M	$\mu_{E22} > \mu_{SE22}$	–	$\mu_{SE22} > \mu_{E22}$	–

<sup>a</sup> T = temperature; M = microbial activity; S = sample.

<sup>b</sup> Common biphasic model also.

<sup>c</sup> Limited data.

**Table 4 – Physicochemical properties of amended soil used for laboratory experiments**

Parameter	Slag	Amended sand	Sand	Sieved column material
Texture	Sand	Loamy sand	Clayey sand	Clayey sand
d <sub>50</sub> (mm)	0.63	0.43	0.38	0.50
pH	9.4	9.2	5.4	8.7
Bulk density (g cm <sup>-3</sup> )	1.6	1.4	1.35	1.68
Electrical conductivity (d m <sup>-1</sup> ) (1:5 water)	1.2	0.5	<0.1	–
Exchange capacity (cmol kg <sup>-1</sup> )				
Aluminium	0	0.03	2.82	0
Calcium	41.55	13.99	0.67	18.4
Cation	42.36	14.59	4.29	18.9
Magnesium	0.36	0.28	0.59	0.3
Phosphorus sorption capacity (kg ha <sup>-1</sup> )	24 000	15 000	1700	–
% Carbon (% dry weight)	0.4	0.4	0.4	0.3

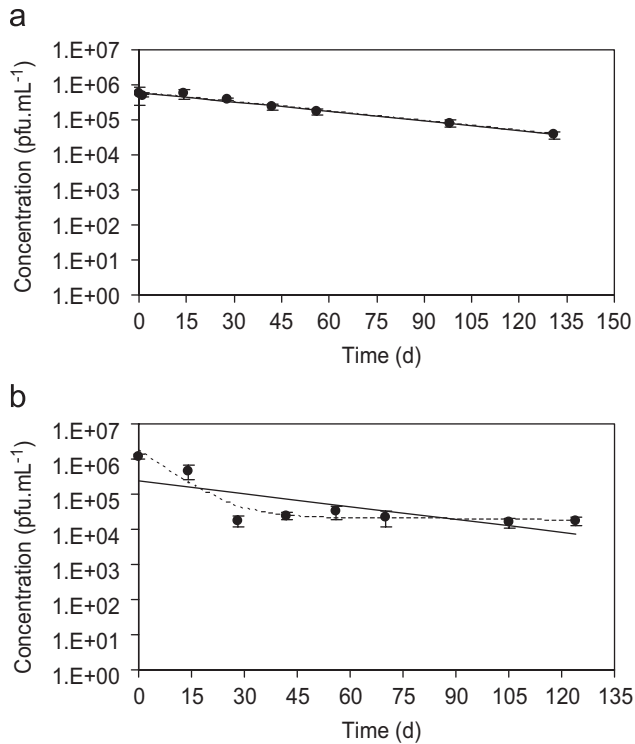
pore space in the cap), with the range calculated from minimum to maximum water content. Therefore the porosity in the model was higher than the water content of the soil. The modelled porosity was  $0.233 \pm 0.021$  (standard error), with a dispersivity  $0.042 \pm 0.015$  m. The values of porosity and dispersivity were then used in the fitting of a one-site kinetic model in HYDRUS-1D to the breakthrough curve data for PRD1, to estimate the attachment and detachment coefficients,  $k_{att}$  and  $k_{det}$ , respectively. Dispersivity and  $\mu_s$  were varied where acceptable fits were not possible fitting just  $k_{att}$  and  $k_{det}$ .

PRD1 breakthrough was rapid, occurring after 0.34 pore volumes (Fig. 4). Over the 50 cm of transport in the columns, there was a  $0.36 \log_{10}$  reduction in PRD1 concentration, calculated as the  $\log_{10}$  of the maximum effluent concentration divided by the maximum influent concentration. The total load recovered of inoculated phage after transport

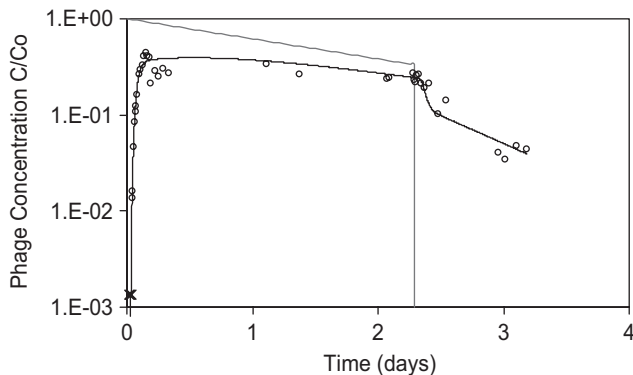
through the column was 59% ( $0.39 \log_{10}$ ), which is comparable to the reduction in concentration. Sampling was stopped prematurely due to difficulties with the inocula. This resulted in limitations for fitting and high standard errors.

Modelling of the virus breakthrough curve resulted in a  $k_{att}$  of  $11.1 \pm 8.24 \text{ day}^{-1}$ ,  $k_{det}$   $0.91 \pm 2.72 \text{ day}^{-1}$  and an  $R^2$  of 0.910. The attached inactivation rate for the model was  $0.831 \pm 1.17 \ln$  units per day. The standard errors for  $k_{att}$  and  $k_{det}$  were high, which needs to be considered in the application of the results to the field scale. Inactivation was observed in the phage inocula (Fig. 4), and was included in the breakthrough curve modelling. Inactivation in the inoculated ARW fed into the columns where full breakthrough curves were obtained resulted in the overestimation of PRD1 removal (Table 2).

Extrapolation of the HYDRUS-1D model from the laboratory column experiments to the field scale predicted a reduction in



**Fig. 3** – PRD1 inactivation data (circles) with error bars indicating standard deviation, linear model (continuous line) and biphasic model (dotted line) for (a) artificial rain water and (b) amended soil.



**Fig. 4** – PRD1 bacteriophage breakthrough curve data (circles), model PRD1 input (grey line) and the fitted one-site kinetic model (solid line).

the concentration of PRD1 of  $0.53 \log_{10}$  over 1.4 m transport ( $0.73 \log_{10}$  over 1.9 m transport), compared with the  $2.9 \log_{10}$  reduction experienced in the field mound studied.

## 4. Discussion

### 4.1. Inactivation

The inactivation rates in this study were comparable to previously reported groundwater studies (Logsdon, 1994; Schijven and Hassanizadeh, 2000). Temperature is considered

one of the most important factors in, and is negatively correlated with, virus survival. In the current study, increasing temperature significantly increased MS2 inactivation in influent, which was consistent with previous reports that MS2 is more stable at temperatures of less than  $10^\circ\text{C}$  (Schijven and Hassanizadeh, 2000; Schaper et al., 2002). For PRD1, which has been reported to remain relatively stable at temperatures up to ambient temperature ( $23^\circ\text{C}$ ) (Schijven and Hassanizadeh, 2000), temperature was only a significant factor in the more adverse high pH conditions of the effluent. Human enteric virus survival has generally been considered to be greatest near neutral pH, yet these viruses are stable within a range of pH 3–10 (Gerba et al., 1996).

Rapid inactivation of MS2 was experienced in unsterilised effluent (pH 10.6) in this study, with  $4.9 \log_{10}$  and  $>3.6 \log_{10}$  reduction in 1 day at 4 and  $22^\circ\text{C}$ , respectively, compared with no detectable inactivation in unsterilised influent (pH 8.1). However, the effluent samples also had the lowest initial concentrations ( $8.6 \times 10^5$  and  $2.5 \times 10^4$  pfu  $\text{mL}^{-1}$ ), compared to a mean initial concentration in the other samples of  $1.8 \times 10^6$  pfu  $\text{mL}^{-1}$ , which may have resulted from rapid inactivation in the time (two hours) between introducing the phage to the sample and sampling that could not be measured by this experiment but assumed given that the same inoculum was used. Such rapid rates of MS2 inactivation have been reported at high pH, with greater than  $4 \log_{10}$  reduction in 2 h at pH 11–11.5 (Logsdon, 1994). While MS2 is more resistant to ammonia than other F-RNA coliphages (Schaper et al., 2002), higher temperatures and ammonia may have a synergistic effect resulting in an increased rate of inactivation. The rapid inactivation of MS2 in the effluent sampled from the mound indicates that inactivation was likely to be a significant removal mechanism, with a  $6 \log_{10}$  reduction predicted in less than 48 h based on the first-order inactivation rate.

PRD1 was more robust under the mound conditions than MS2, with inactivation therefore predicted to account for only  $0.6 \log_{10}$  reduction over 21 days in the mound. Rotavirus is considered to be one of the more resistant human enteric viruses (Höglund et al., 2002). However, PRD1 was more resistant to high pH than has been reported for simian rotavirus, SA11 (Estes et al., 1979), and reovirus (Ward and Ashley, 1977). Inactivation at high pH in wastewater, however, is confounded by the presence of ammonia and its greater virucidal effect on viruses (Ward and Ashley, 1977). For both model viruses examined, pH was the most significant factor (Table 3), with the inactivation rates in effluent consistently greater than those in influent regardless of temperature or sterilisation. Therefore, the ability to modify pH within sewage treatment systems, such as amended mounds, can potentially increase inactivation of human enteric viruses. However, it is important to recognise that the efficacy of pH is dependent on the type of virus.

While pH was assumed to be the main difference between the influent and effluent samples, the transport of the influent through the high pH sand mound would have resulted in changes to the composition of the wastewater that were not quantified here. For example, the effect of microbial activity in effluent may have been less than for influent: temperature was a significant factor in PRD1 survival in influent but not in effluent. Ammonia may have

contributed to the increased inactivation in effluent as it is known to be virucidal above pH 8 as discussed above. While total nitrogen decreased in the sewage system, the proportion of ammonia increased from 89% to 92%, and the calculated concentration (at 25 °C) of free ammonia increased with pH from 5 mgL<sup>-1</sup> in influent (pH 8.1) to 53 mgL<sup>-1</sup> in the effluent (pH 10.6).

The increased inactivation of MS2 in sterilised influent may indicate changes in chemistry during the autoclaving process, as the elimination of microbial activity would generally be expected to decrease the inactivation rate. Similarly the decreased inactivation with increasing temperature experienced in sterilised effluent at 22 °C compared to the unsterilised effluent at 4 °C, where no significant microbial activity was assumed, indicated that autoclaving affected the matrix chemistry. Unfortunately, due to low influent flow at the field site additional samples for chemical analyses were not available.

Outliers were defined for this study by both the first-order and biphasic models. Commonly in inactivation studies, when the second sample is taken shortly after the first, there is an apparent increase in concentration (e.g. Yates et al., 1985). It is hypothesised that this is due to disaggregation in the inactivation media. Inclusion of this first data point value in the determination of the inactivation rate coefficient will underestimate the initial inactivation. Hence, sampling time points were included at 1 and 2 days, providing a more reliable value of the actual virus load at the time of inoculation.

Inactivation rates are quantified to allow the comparison and extrapolation of experimental data sets. These are used for use in management, such as in risk assessment, or in the calculation of the contribution of inactivation to the removal of culturable viruses in experiments. Inactivation has typically been modelled using a first-order model, which assumes a log-first-order decay. However, in many studies, the rate of inactivation decreases over the duration of the study. In these cases, application of the first-order model overestimates the long-term inactivation rate. Alternative inactivation models have been developed to address this issue. Cerf (1977) developed the biphasic model used here, where there are two distinct subpopulations with separate inactivation rates. This model has been employed in the food industry (Geeraerd et al., 2005), and in previous environmental survival of viruses (Pettersen et al., 2001). First-order inactivation was experienced under a variety of conditions including high temperature and high pH, however in all cases first-order inactivation was preferred where  $\mu < 0.05 \text{ day}^{-1}$ . Above this value, the biphasic model was the preferred model (where sufficient data was available). For the four cases where the biphasic model was preferred, the long-term inactivation rates varied from 0.00 to 0.26 day<sup>-1</sup> for both phages. Where the first-order model was preferred, a maximum of 0.6 log<sub>10</sub> reduction was experienced compared to a minimum 0.8 log<sub>10</sub> reduction before the lower of the biphasic inactivation rates dominated. It is therefore conceivable that the biphasic model would become preferable in more scenarios with increased time and inactivation.

The implications of biphasic decay are that over time the inactivation rate decreases, and this long-term inactivation rate is therefore overestimated by a linear model. In the

mound investigated, the timescale for a virus to pass through the system was approximately 20–30 days. Over this timescale, there was limited biphasic behaviour exhibited, and due to the short duration and the nature of the project, there was not sufficient confidence in the biphasic data to extrapolate the results. However, there was evidence that there were small fractions of viruses, in this case 0.4 to 0.04% MS2 and 12% PRD1, that were considerably more robust in these conditions. It has been theorised that these robust viruses may represent a more resistant subpopulation (Pettersen et al., 2001). It is not known how these more robust viruses will behave in the environment when they are released from the sand mound, and therefore how to assess the risks of such enteric viruses as they are transported through the system and potentially ingested.

#### 4.2. Column and field studies

Virus inactivation is usually considered to be insignificant in soil-column transport experiments due to the small time-scales involved. However, as breakthrough was monitored for up to six weeks in the test columns, phage inactivation was considered to be influential. Inactivation in soil-water microcosms was assumed to represent phage inactivation in the attached phase,  $\mu_s$ . However, the method does not differentiate between attached and free viruses. The amended soil had a lower rate of removal (attachment) within the column and therefore it is likely they had lower rates of attachment within the microcosms. However, quartz sand has been reported to have a virus sorption capacity of  $2.2 \times 10^{12} \text{ pfu kg}^{-1}$  (Moore et al., 1981) which far exceeds the initial virus load applied ( $10^6 \text{ pfu}$  to  $1.3 \text{ g}^{-1}$  soil dry weight).

Column experiments using repacked soil are commonly used to characterise virus transport in soil (e.g. Sobsey et al., 1995), with results generally considered to be representative of field scale. Column experiments with amended sand (Ho et al., 1991) have demonstrated higher removal of *E. coli* and poliovirus in sand amended with red-mud than unamended sands, with greater than 7 log<sub>10</sub> removal of both organisms in 65 cm columns of amended sand. However, field verification has not been reported, nor virus removal in other media used in decentralised sewage systems. Laboratory studies aimed to provide quantitative data on virus fate and transport in a mound under conditions anticipated to maximise transport: saturated, low retention time, rain water. Previous studies of virus transport in sand have found similarly rapid transport of viruses (McKay et al., 1993; Schijven et al., 2002). Physical heterogeneities, such as roots, macropores and rock fractures, can provide preferential water flow paths (McKay et al., 1993). Soil columns by their nature introduce paths for preferential transport along the soil-column interface, however as repacked column experiments aim to establish the adsorption capacity of the soil and its influence on virus transport and fate, every attempt is made to minimise their influence. The use of rainwater was assumed to provide a worst-case scenario for transport due to low ionic strength resulting in low attachment. However, in sewage there is the potential for viruses to attach to colloids which may decrease inactivation and facilitate transport (Jin and Flury, 2002). Organic matter,



including surfactants in the water have been reported to decrease adsorption due to competition for attachment sites and are also reported to increase desorption of viruses (Schijven and Hassanizadeh, 2000).

Removal within the amended sand column was greater than in natural sandy loams (Charles, 2008) although inactivation in this soil was lower. The amended soil was not preconditioned and therefore was expected to have little microbial activity, and hence low inactivation (Jin and Flury, 2002). Inactivation of PRD1 in the soil-water matrix without preconditioning was lower than in effluent from the soil matrix. Virus removal within the sewage treatment mound system that utilises this soil was greater in the field experiments ( $1.1 \log_{10}$  in 0.7 m) than experienced in the column experiments carried out in the present study ( $0.36 \log_{10}$  in 0.5 m).

The differences between these column experiments and the onsite system scenario that will affect the up-scaling of these results to the field situation are:

- rainwater, as used in the columns, will result in decreased attachment compared to sewage, however colloids/organics in the sewage may also facilitate virus transport and survival;
- heterogeneity in the field may increase travel velocity compared to the repacked columns, thereby decreasing attachment, and may affect removal over the site; and
- lack of microbial activity in the microcosms, if extrapolated, may underestimate inactivation in the field.

#### 4.3. Implications for household systems

The mound system received flows averaging one third of the design flow during the field study. This low hydraulic load was anticipated to result in an over estimation of the performance of the system, with micro-organism inactivation and attachment expected to decrease during increased flows. For a single household system, this degree of under-loading is equivalent to household occupancy rates of two to three people living in a four to five bedroom house, assuming the system is conservatively designed for dual occupancy of each bedroom. Therefore the higher loading rates that would be expected at houses with higher occupancy rates may result in decreased performance of the system.

Extrapolation of the column study model to the field-scale provided a prediction of virus transport in a less well established system and under higher flow rates. At the reduced detention time modelled, 7 days to maximum breakthrough compared to over 20 in the field, the removal was only  $0.73 \log_{10}$  over the 1.9 m width of the system. It is anticipated that this would generally be an underestimate. In an established, well constructed system, the development of a clogging layer would retard virus transport. The high degree of homogeneity in the systems would be expected to increase virus removal, although poor construction could lead to areas without sufficient amending material, and therefore variable pathogen and nutrient removal. However under other condi-

tions, such as prior to clogging layer development and under high flow, this may be representative of transport.

The high pH environment within the mound strongly affected many of the indicator organisms studied. It is not known how long this virucidal high pH may persist, but as it is also important for phosphorus removal it is a key management parameter. Furthermore, high pH is possibly more important than the availability of sorption sites as calcium leaching is expected to result in a decrease in phosphorus sorption.

In the mound system studied, the laboratory estimate of the inactivation rate indicates that inactivation is an important removal mechanism. Therefore these results would not necessarily hold for a similar design with a lower pH. Overall, the amended material sand mound provided greater removal of enteric virus models from sewage than reported for other sewage treatment systems including septic tanks ( $0.6 \log_{10}$ ), aerated treatment units ( $1 \log_{10}$ ), and disinfection systems ( $1\text{--}1.8 \log_{10}$  for MS2) (Charles, 2008).

## 5. Conclusions

- The primary mechanisms for virus removal varied significantly depending on the susceptibility of the individual virus to the system chemistry. MS2 removal, as with thermotolerant coliforms, was rapid in the mound. Inactivation studies for MS2 suggested that inactivation was the primary mechanism a  $6 \log_{10}$  reduction in less than 48 h in effluent. Similarly, removal of PRD1 and somatic coliphages was lower in the mound, and inactivation of PRD1 was lower. These results highlight the necessity for studying a range of organisms when assessing the potential for pathogen transport.
- The application of the amending material to mound systems at the individual household scale requires considerations of the likely flow rates and pH of the system. Considerations for implementing amended soil mound technology should also include the life expectancy of the system and management protocols for decommissioning and/or replacement.

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## REFERENCES

- Adams, M.H., 1959. Bacteriophages. Interscience Publishers Inc, New York.
- APHA, 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.

- Ausland, G., Stevik, T.K., Hanssen, J.F., Kohler, J.C., Jenssen, P.D., 2002. Intermittent filtration of wastewater-removal of fecal coliforms and fecal streptococci. *Water Res.* 36 (14), 3507–3516.
- Cerf, O., 1977. Tailing of survival curves of bacterial spores. *J. Appl. Bacteriol.* 42 (1), 1–19.
- Charles, K.J., 2008. Quantitative microbial risk assessment: a catchment management tool to delineate buffer distances for on-site sewage treatment and disposal systems in Sydney's drinking water catchments. Ph.D., Civil and Environmental Engineering. Sydney, University of New South Wales.
- Charles, K.J., Schijven, J.F., Baker, D., Roser, D.J., Deere, D.A., Ashbolt, N.J., 2004. Transport and fate of nutrients and pathogens during sewage treatment in a mound system. In: *On-site Wastewater Treatment X: Proceedings of the Tenth National Symposium on Decentralised Sewage Treatment and Disposal Systems*. American Society for Agricultural Engineers, Sacramento, USA, pp. 460–469.
- Cheung, K.C., Venkitachalam, T.H., 2006. Kinetic studies on phosphorus sorption by selected soil amendments for septic tank effluent renovation. *Environ. Geochem. Health* 28 (1–2), 121–131.
- Davies, C.M., Ferguson, C.M., Kaucner, C., Altavilla, N., Deere, D.A., Ashbolt, N.J., 2004. Dispersion and transport of *Cryptosporidium* oocysts from fecal pats under simulated rainfall events. *Appl. Environ. Microbiol.* 70 (2), 1151–1159.
- Estes, M.K., Graham, D.Y., Smith, E.M., Gerba, C.P., 1979. Rotavirus stability and inactivation. *J. Gen. Virol.* 43 (2), 403–409.
- Foppen, J.W., van Herwerden, M., Schijven, J., 2007. Measuring and modelling straining of *Escherichia coli* in saturated porous media. *J. Contam. Hydrol.* 93 (1–4), 236–254.
- Geeraerd, A.H., Valdramidis, V.P., Van Impe, J.F., 2005. GInaFit, a freeware tool to assess non-log-linear microbial survivor curves. *Int. J. Food Microbiol.* 102 (1), 95–105.
- Gerba, C.P., Rose, J.B., Haas, C.N., Crabtree, K.D., 1996. Waterborne rotavirus: a risk assessment. *Water Res.* 30 (12), 2929–2940.
- Havelaar, A.H., Hogeboom, W.M., 1984. A method for the enumeration of male-specific bacteriophages. *J. Appl. Bacteriol.* 56, 439–447.
- Ho, G.E., Gibbs, R.A., Mathew, K., 1991. Bacteria and virus removal from secondary effluent in sand and red mud columns. *Water Sci. Technol.* 23 (1–3), 261–270.
- Höglund, C., Ashbolt, N.J., Stenström, T.A., Svensson, L., 2002. Viral persistence in source-separated human urine. *Adv. Environ. Res.* 6 (3), 265–275.
- ISO 10705-2, 2000. *Water Quality-detection and Enumeration of Bacteriophages. Part 2: Enumeration of Somatic Coliphages*. International Organization for Standardization, Geneva.
- Jin, Y., Flury, M., 2002. Fate and transport of viruses in porous media. *Adv. Agron.* 77, 39–102.
- Johansson, L., 1999. Blast furnace slag as phosphorus sorbents-column studies. *Sci. Total Environ.* 229, 89–97.
- Logsdon, G.S., 1994. *The Removal and Disinfection Efficiency of Lime Softening Processes for Giardia and Viruses*. American Water Works Association Research Foundation and American Water Works Association, Denver, CO.
- McKay, L.D., Cherry, J.A., Bales, R.C., Yahya, M.T., Gerba, C.P., 1993. A field example of bacteriophage as tracers of fracture flow. *Environ. Sci. Technol.* 27 (6), 1075–1079.
- Moore, R.S., Taylor, D.H., Sturman, L.S., Reddy, M.M., Fuhs, G.W., 1981. Poliovirus adsorption by 34 minerals and soils. *Appl. Environ. Microbiol.* 42 (6), 963–975.
- Pettersson, S.R., Teunis, P.F.M., Ashbolt, N., 2001. Modeling virus inactivation on salad crops using microbial count data. *Risk Anal.* 21 (6), 1097–1107.
- Rayment, G.E., Higginson, F.R., 1992. *Australian Laboratory Handbook of Soil and Water Chemical Methods*. Reed International Books Australia P/L, trading as Inkata Press, Port Melbourne.
- Sakadevan, K., Bavor, H.J., 1998. Phosphate adsorption characteristics of soils, slags and zeolite to be used as substrates in constructed wetland systems. *Water Res.* 32 (2), 393–399.
- Schaper, M., Duran, A.E., Jofre, J., 2002. Comparative resistance of phage isolates of four genotypes of F-specific RNA bacteriophages to various inactivation processes. *Appl. Environ. Microbiol.* 68 (8), 3702–3707.
- Schijven, J.F., Hassanizadeh, S.M., 2000. Removal of viruses by soil passage: overview of modeling, processes, and parameters. *Critical Reviews in Environ. Sci. Technol.* 30 (1), 49–127.
- Schijven, J.F., Simunek, J., 2002. Kinetic modeling of virus transport at the field scale. *J. Contam. Hydrol.* 55 (1–2), 113.
- Schijven, J.F., Hassanizadeh, S.M., de Bruin, H.A.M., 2002. Column experiments to study nonlinear removal of bacteriophages by passage through saturated dune sand. *J. Contam. Hydrol.* 58, 243–259.
- Šimunek, J., Šejna, M., van Genuchten, M.T., 1998. *The HYDRUS-1D Software Package for Simulating the One-dimensional Movement of Water, Heat and Multiple Solutes in Variably Saturated Media*. International Ground Water Modelling Centre, Colorado School of Mines, Golden, CO.
- Sobsey, M.D., Hall, R.M., Hazard, R.L., 1995. Comparative reductions of hepatitis A virus, enteroviruses and coliphage MS2 in miniature soil columns. *Water Sci. Technol.* 31 (5–6), 203–209.
- Straub, T.M., Pepper, I.L., Gerba, C.P., 1992. Persistence of viruses in desert soils amended with anaerobically digested sewage sludge. *Appl. Environ. Microbiol.* 58, 636–641.
- USEPA, 2002. *On-site Wastewater Treatment Systems Manual*. US EPA, Washington.
- Ward, R.L., Ashley, C.S., 1977. Identification of the virucidal agent in wastewater sludge. *Appl. Environ. Microbiol.* 33 (4), 860–864.
- Yates, M.V., Gerba, C.P., Kelley, L.M., 1985. Virus persistence in groundwater. *Appl. Environ. Microbiol.* 49 (4), 778–781.