Influence of design and operating conditions on the removal of MS2 bacteriophage by pilot-scale multistage slow sand filtration

William B. Anderson, Jeffrey L. DeLoyde, Michele I. Van Dyke and Peter M. Huck

ABSTRACT

This paper summarizes findings from sixteen MS2 bacteriophage (MS2) challenge tests on biologically mature pilot-scale slow sand filters, conducted at varying water temperatures (< 10° and > 20°C), two hydraulic loading rates (0.1 vs. 0.4 m/h), and two bed depths (0.4 vs. 0.9 m). Few studies have quantified virus removal by slow sand filters at filtration rates on the high end and bed depths on the low end of typical practice, and none report virus removal below 5°C. The conditions investigated are important, because high filtration rates and low bed depths are sometimes seen as ways of making slow sand filtration more cost effective. MS2 removal increased with greater sand depth and warmer water temperature, but decreased at the higher hydraulic loading rate. Average MS2 removals ranged from 0.1 to 0.2 log in the roughing filters and 0.2 to 2.2 log in the slow sand filters. Shedding of MS2 was observed for up to 12 days after seeding was stopped. As a stand-alone process, slow sand filtration (with or without roughing filtration) may not provide adequate virus removal under some conditions and should be combined with a disinfection/inactivation step to provide robust compliance with regulatory requirements and protection of human health.

Key words | drinking water, MS2 phage, slow sand filtration, virus

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INTRODUCTION

Slow sand filtration (SSF) is a robust and sustainable water treatment technology for small communities in developed and developing countries. It is a 200-year-old technology used worldwide to treat microbially impacted surface waters by biological, physico-chemical, and physical removal mechanisms. When this technology incorporates additional features such as roughing filtration, ozone pre-treatment, and slow sand filters in series it is known as multistage slow sand filtration. Roughing filters with gravel media effectively attenuate influent particle and aggregate levels, thereby increasing filter run lengths, decreasing maintenance requirements, and broadening the range of raw water qualities that are suitable for treatment by slow sand filtration (Collins *et al.* 1994).

An important aspect of producing microbiologically safe water is to ensure adequate removal or inactivation of viruses. In cases where slow sand filtration is used as either part of, or the entire treatment process, it is important to have quantitative information on the levels of virus removal that can be expected. In this study we used MS2 bacteriophage as a surrogate for human viruses. MS2 is an icosahedral bacteriophage ~25 nm in diameter that contains single-stranded RNA inside a protein coat (Madigan & Martinko 2006). It has an isoelectric point (IEP) of 3.9 (Schijven & Hassanizadeh 2000) and therefore

doi: 10.2166/aqua.2009.140

a strong negative charge at natural water pH values. This characteristic causes MS2 to be repelled from granular media surfaces, which commonly have a negative charge at pH 7 (Gerba 1984), contributing to poor attachment of MS2 during drinking water filtration. The fact that the IEP of MS2 is lower than that of most human enteric viruses makes it a conservative model for the removal of human viruses (Goyal & Gerba 1979; Bales *et al.* 1993).

Slow sand filtration employs both physico-chemical and biological processes for the removal and inactivation of viruses. Important physical removal mechanisms include attachment to filter media (e.g. Schijven *et al.* 1999, 2003; Dullemont *et al.* 2006) and biofilms (Storey & Ashbolt 2003). The adsorption of viruses to sand grains is typically poor due to the negative surface charges that both exhibit at typical water pH (Schijven & Hassanizadeh 2000). The most significant force preventing virus adsorption to a grain surface is electrostatic repulsion. In practice, the adsorption of negatively-charged viruses onto negatively-charged silica sand in a SSF bed would predominantly involve reversible adsorption to the secondary minimum, which is supported by observations of virus desorption and washout over extended periods of time following seeding experiments (Schijven *et al.* 1999, 2001, 2003; Schijven & Simunek 2002; Dizer *et al.* 2004; Hijnen *et al.* 2004; Dullemont *et al.* 2006).

Factors enhancing virus adsorption to granular media include increased ionic strength (Goyal & Gerba 1979; Lance *et al.* 1982; Bales *et al.* 1991), decreased pH (Schijven & Hassanizadeh 2000), and the presence of multivalent cations (Gerba 1984; Lance & Gerba 1984; Redman *et al.* 1999; Schijven *et al.* 2001; Harvey & Ryan 2004).

The attachment of viruses to biofilms is also important. Biofilms contain many sites for adsorption/attachment of particles, particularly extracellular polymeric substances (EPS), which consist of polysaccharides, proteins, and lipids (Flemming 1995). It has been hypothesized that adsorption of viruses to biofilm constituents is likely the most important removal mechanism not only as viruses become physically restrained but because entrapment facilitates the biologically-mediated mechanisms discussed below (Wheeler *et al.* 1988).

The removal or inactivation of viruses in SSFs can also occur by biologically-mediated mechanisms such as predation and antagonism by other microorganisms (e.g. Quanrud *et al.* 2003; Elliott *et al.* 2006) and extracellular enzymes they produce (e.g. Cliver & Hermann 1972; Ward *et al.* 1986; Deng & Cliver 1992; Nasser *et al.* 2002). There is, however, some specificity of bacterial enzymes and not all enzymes can inactivate all viruses. For example, Nasser *et al.* (2002) demonstrated that the enzyme, protease, was capable of 90% inactivation of coxsackievirus A9 but had no effect on the survival of hepatitis A, poliovirus-1, or MS2 phage (the phage used in this study). On the other hand, there are a variety of bacterial enzymes and they would be present in various combinations and concentrations in SSFs.

Studies have demonstrated that virus removal improves with increased sand depth and lower hydraulic loading rates (e.g. Poynter & Slade 1977). Warmer water temperatures (e.g. Dullemont *et al.* 2006) and increased biological maturity of the SSF (e.g. Poynter & Slade 1977; Dizer *et al.* 2004) are also beneficial by facilitating both biological and physical removal mechanisms. Several studies report that scraping of the Schmutzdecke (the layer on the top of the SSF) does not impact virus removal (Poynter & Slade 1977; Slade 1978; McConnell *et al.* 1984; Hijnen *et al.* 2004; Dullemont *et al.* 2006). Only infrequently has a decrease in MS2 removal following scraping been observed (Dullemont *et al.* 2006). Published literature reviews have credited slow sand filtration with enteric virus removals of 2 to 4 logs (Amy *et al.* 2006) and <1 to 3 logs (Rachwal *et al.* 1996).

The objective of this research was to investigate the virus removal effectiveness of multistage slow sand filtration under conditions of challenging source water characteristics, at the fringe of typical design and operation ranges, and under conditions of extreme cold water.

MATERIALS AND METHODS

Pilot plants

Two pilot-scale multistage slow sand filter systems were used in this study. Both were located inside the intake facility for the Region of Waterloo Mannheim Drinking Water Treatment Plant (Kitchener, Ontario, Canada) and were fed with raw water from the Grand River. The Grand River watershed is heavily impacted by rural and urban activities, with 80% of the watershed used for agriculture

and livestock and 20% occupied by urban centers with a net population of 500,000 people. The Grand River receives secondary and tertiary treated wastewater from 26 sewage treatment plants (servicing 680,000 people), nine of which are upstream of the Mannheim Plant intake. Water quality in the Grand River is highly variable, with turbidity ranging from 1 to 10 NTU about 85% of the time with occasional peaks exceeding 100 NTU. During the pilot system testing, river temperature ranged from 1 to 28°C, total organic carbon from 5 and 8 mg/L, hardness from 160 to 288 mg/L (as $CaCO_3$), conductivity from 402 to 817 (μSiemans/cm), and pH from 7.8 to 8.4.

Pilot plant 1

Pilot plant 1 was provided by MS Filter Inc. (Newmarket, Ontario, Canada) and is a pilot-scale version of a commercially available full-scale multistage filtration system. It was housed in a portable trailer and consisted of two identical trains (1 and 2) that received the same raw water but were operated independently (Figure 1(a)). Each train of pilot 1 included an upflow pre-ozonation column, a down flow secondary ozone contact column, an upflow shallow-bed roughing filter (RF) with a granular activated carbon (GAC) cap, and a downflow shallow-bed slow sand filter (SSF) (Figure 1(a)). Filter and media characteristics are compared with typical applications in Table 1. Ozone was produced onsite by two AZCOZON SNOA-4 ozone generators (AZCO Industries Ltd., Surrey, BC) at an applied ozone dose of 2 to 4 mg/L. Because the ozone dose could not be regulated, it was necessary to turn it off for extended periods during winter months due to issues associated with off-gassing.

The SSF had a maximum sand design depth of 0.45 m which is below that recommended in the literature (~1.0 m) (Table 1). The SSF of pilot 1 was designed for continuous operation at a hydraulic loading rate (HLR) of 0.4 m/h, which is at the high end of the typical design range of 0.05 to 0.4 m/h (Table 1). Sand with an effective size (ES) of 0.35 mm, which is at the upper end of the design range, was used to prevent excessive headloss at the higher HLR.

All columns, valves, and sampling ports were made of polyvinyl chloride (PVC) and tubing was either Teflon® coated or laboratory-grade Tygon® tubing. The exterior of the PVC columns were insulated to prevent changes in water temperature and block light exposure to prevent algal/cyanobacterial growth.

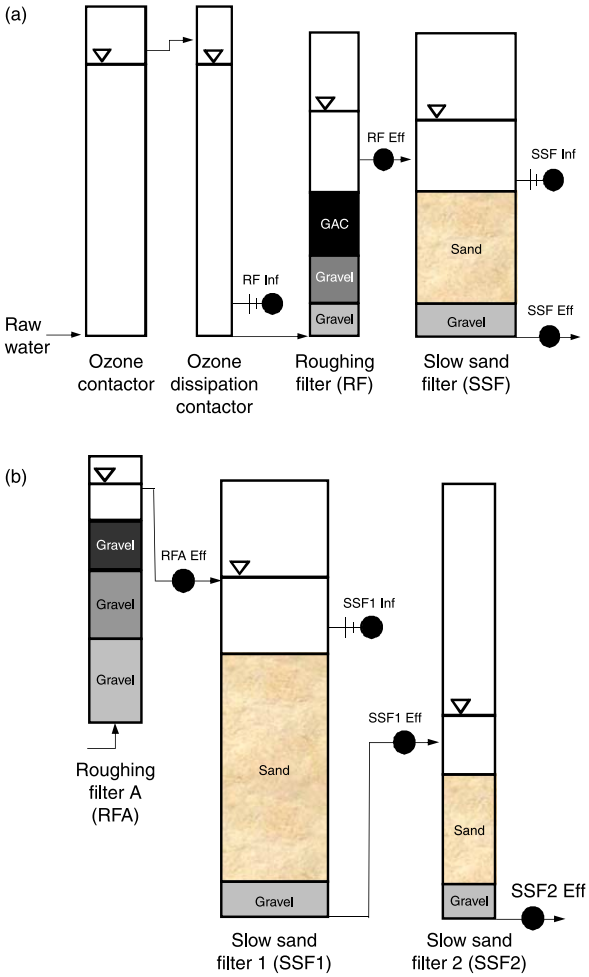

Figure 1 | (a) Pilot Plant 1 and (b) Pilot Plant 2 schematic (not to scale). Filled circles represent influent and effluent sample points.

Pilot plant 2

Pilot plant 2 was designed and constructed by Cleary (2005), based on a small full-scale design originally developed by Urfer (2004), and operated continuously for 2.5 years from December 2003 until June 2006. It included a constant head tank, a gravity-fed RF, followed by two SSFs in series (Figure 1(b)). Raw water was pumped into the constant head tank which discharged to roughing filter A (RFA). The upflow RFA was designed according to literature recommended bed depth (1.2 m) and media sizes

Table 1 | Roughing and slow sand filter design parameters for pilot plants 1 and 2

		Pilot 1	Pilot 2	
Roughing filters	**Literature***	**RF**	**RFA**	
Gravel depth (m)				
Total	0.9–1.2	0.6	1.2	
Top layer	0.15–0.20	0.3 (GAC)	0.3	
Middle layer	0.15–0.20	0.2	0.4	
Bottom layer	0.2–0.3	0.1	0.5	
Media diameter (mm)				
Top layer	4–8	0.85–1.2 (GAC)	4.8–9.5	
Middle layer	8–12	2.4–3.4	9.5–12.7	
Bottom layer	12–18	8–12.5	12.7–19.1	
Hydraulic loading rate (m/h)	0.3–1.5	1.5	1.0	
Hydraulic detention time (h)	–	0.3	0.5	
Slow sand filters		**SSF**	**SSF1**	**SSF2**
Sand effective size (mm)	0.15–0.35	0.35	0.37	0.37
Sand uniformity coefficient	Below 3	1.7	1.7	1.7
Bed depth (m)	0.7–1.2	0.45	0.9	0.5
Hydraulic loading rate (m/h)	0.05–0.4	0.1 & 0.4	0.4	0.4
Hydraulic detention time (h)	–	4.0 & 1.0	2.0	1.0
D/d ratio†	>50	900	800	400
L/d ratio‡	>1,000	1,286	2,702	1,351

*Literature recommended values from: Huisman & Wood (1974), Wegelin (1996) and Galvis *et al.* (1998).
†Ratio of column diameter (D) to media diameter (d) (Lang *et al.* 1993).
‡Ratio of bed depth (L) to grain size (d) (Kawamura 1999).
Table adapted from Cleary (2005).

(Collins *et al.* 1994; Wegelin 1996). The first SSF in series (SSF1) had a 0.9 m bed depth, also in accordance with literature recommendations. The column diameter of SSF1 was selected to be similar to that of the SSF columns of pilot 1 in order to achieve a similar HLR at a given flow rate. SSF2 was a smaller diameter column with a maximum sand depth of 0.4 m.

All pilot 2 filter columns were made of clear PVC and covered with polyfoam insulation. All pilot 2 piping was Teflon® tubing (Johnston Industrial Products, Toronto, ON) with stainless steel Swagelok® fittings and valves (Swagelok, Solon, OH).

Filter cleaning and re-sanding

In pilot 1, the upflow RF was cleaned by pumping raw, non-ozonated water into a port at the bottom of the column for ~15 min resulting in a 30 to 40% expansion of the surface GAC media layer. In pilot 2, the upflow RFA was cleaned by closing the effluent valve to increase the water head on the columns to 1.4 m, then rapidly opening the drain valve to flush out the retained solids (repeated twice).

All SSFs were cleaned by allowing water to drain to about 0.05 m below the sand surface, scraping off approximately 0.02 m of sand, and refilling the filter with RF effluent. After several cleanings, when the sand depth reached ~0.3 m for pilot 1 and ~0.6 m for pilot 2 SSF1, clean virgin sand was added. The top 0.03 m and the second layer (~0.05 m deep) of existing mature sand were removed and saved separately. Washed virgin sand was added to the SSF column and backwashed to remove fines. The second and top layers of mature sand were then replaced on top of the virgin sand as described by Barrett *et al.* (1991). Pilot plant 1 SSFs were re-sanded on Sept. 16, 2005, five months

before the MS2 challenge tests began and pilot plant 2 SSF1 was re-sanded on Aug. 2, 2005, about six and half months before the challenge tests. No re-sanding was done during the MS2 challenge phase.

MS2 inoculum preparation and sample enumeration

MS2 phage were prepared based on method ISO 10705-1 (International Organization for Standardization 1995) using MS2 bacteriophage ATCC 15597 B1 with *E. coli* host strain ATCC 15597 (NWRI/AwwaRF 2003). Briefly, the *E. coli* host was grown in tryptone-yeast extract-glucose broth (TYGB) at 37°C to a concentration of approximately 10^8 colony forming units (CFU)/mL. The culture was then inoculated with MS2 at a final concentration of approximately 10^8 plaque forming units (PFU)/mL. Incubation of the culture was continued at 37°C for 24 h. *E. coli* cells were then removed by centrifugation at 10,000 × *g* for 15 min, followed by vacuum filtration of the supernatant through a 0.45 μm filter membrane. The eluant containing MS2 was stored as 4°C, and enumerated as described below. The final concentration of the MS2 stock was 1×10^{11} PFU/mL. To prepare MS2 inoculum solutions for the challenge tests, MS2 stock was diluted in PBW to concentrations that ranged from 5×10^7 to 1×10^8 PFU/mL. The inoculum solutions were transported to the pilot plant at 4°C and used within 14 days.

MS2 bacteriophage were enumerated using a single layer plating technique using tryptone-yeast extract-glucose agar (TYGA), based on ISO standard 10705-1 (International Organization for Standardization 1995). In this method, 1 mL of *E. coli* culture and 1 mL of sample (or sample dilution) containing MS2 were added to 20 mL of TYGA, poured into a sterile Petri dish, and allowed to solidify. The plates were incubated at 37°C for 24 h and examined for plaques. Plaques (clear zones) are produced when the bacteriophage infect and produce an area of lysed *E. coli* cells in the agar. In this method, samples were plated directly or following 10-fold serial dilution in phosphate buffered water (PBW) (0.3 mM KH_2PO_4, 2 mM $MgCl_2{\cdot}H_2O$, pH 7.2) to result in 20–200 plaques per plate. The number of plaques per plate was then multiplied by the dilution factor, and the final result reported as PFU per mL of original sample. Each sample was enumerated in triplicate (calculated relative standard deviations were 10% for influent samples and 13% for effluent samples). Samples were always analyzed within 72 h, and most samples were analyzed within 24 h. Negative controls containing *E. coli* host but no phage were plated before and after sample analysis. Positive controls included testing an MS2 phage solution of known concentration. In addition, 1 mL of uninoculated raw water was tested to ensure there were no phage counts in the river water. Uninoculated raw water was also tested with the positive control, to ensure the raw water would not interfere with the phage assay.

MS2 challenge test protocol

Both pilot plants were fed with untreated Grand River water (water quality details can be found in DeLoyde 2007). A total of 16 MS2 bacteriophage challenge tests were conducted between February and June 2006. Experimental conditions for these tests are provided in Table 2. The water temperature for both the raw river water and SSF influent are provided to account for observed increases attributable to plumbing and process units upstream from the SSFs. The last day of cold water experimentation (test 7b) was March 31, 2006, and the first warm water experiment (test 10) was conducted on May 15, 2006. For the cold water tests the temperature ranged between 3 and 10°C, while for the warm water tests the temperature range was 21 to 24°C. The surface of the slow sand filter of pilot 1 train 1 was scraped to remove schmutzdecke twice during the challenge test period; after tests 2 and 7a. Pilot effluents containing MS2 were diverted to a waste holding tank and periodically taken to a municipal wastewater treatment plant for disposal.

MS2 inoculum was added to the filters at a rate of 1 mL/min for the duration of the sampling period (i.e. step-dose). Measured steady-state MS2 influent challenge levels for both the RF and SSF tests were typically 10^4 to 10^5 PFU/mL (DeLoyde 2007). The bacteriophage were always introduced following the roughing filter at which point no ozone residual remained (except during roughing filter challenges, which are described below). MS2 inoculum was added using a peristaltic pump with Teflon® tubing. During challenge tests, the inoculum bottle was

Table 2 | Detailed experimental conditions for MS2 challenge tests

Test no.	Test date (2006)	Pilot plant and unit tested	HLR* (m/h)	Water temperature (°C)		Sand depth (m)
				Raw	Influent	
1	Feb 14–15	Pilot 1/train 1 (SSF)	0.4	2	4	0.40
2	Feb 27–28	Pilot 1/train 1 (SSF)	0.4	1	3	0.38
3	Mar 6	Pilot 1 roughing filter (RF)	1.5	2	3	–
4	Mar 9	Pilot 1/train 1 (SSF)	0.1	3	7	0.38
5	Mar 20–21	Pilot 1/train 1 (SSF)	0.1	3	6	0.38
6	Mar 21	Pilot 2 roughing filter (RFA)	1.0	5	6	–
7a	Mar 28–29	Pilot 1/train 1 (SSF)	0.1	7	9	0.38
7b†	Mar 30–31	Pilot 1/train 1 (SSF)	0.1	9	10	0.36
8	Mar 29	Pilot 1/train 2 (SSF)	0.4	7	8	0.36
9	Mar 30	Pilot 2-SSF1 & SSF2 in series	0.4	7	8	0.92/0.44‡
10§	May 15–16	Pilot 1/train 1	0.1	16	21	0.36
11	May 18–19	Pilot 1/train 1	0.1	17	22	0.36
12	Jun 2	Pilot 1/train 1	0.4	21	23	0.36
13	Jun 5	Pilot 1/train 1	0.4	20	22	0.36
14	Jun 5	Pilot 2-SSF1 & SSF2 in series	0.4	23	22	0.88/0.44‡
15	Jun 8	Pilot 1/train 1	0.4	22	24	0.36

*Hydraulic loading rate.
†Tests 7a and 7b run under identical conditions but flow was stopped for 30 min to scrape SSF between parts a and b (on Mar. 29) (the SSF was also scraped on Feb. 3 and Feb. 20).
‡First value is sand depth in SSF1, second is sand depth in SSF2 (SSF1 was scraped on Mar. 9, Apr. 6, and Jun. 2; SSF2 was not scraped).
§Tests 1 to 9 were categorized as cold water and tests 10 to 15 as warm water.

continuously stirred using a magnetic stirrer, and held in an insulated container with ice packs. The MS2 inoculum concentration was tested immediately before and after each challenge test; and the phage concentrations did not change significantly during the experiments. For logistical reasons, MS2 seeding was usually initiated the evening before sampling was to begin.

Influent and effluent sampling was typically initiated 11 to 12 h after MS2 seeding began, well into steady-state conditions. Effluent samples were collected 0.3 to 4 h after their corresponding influent samples, depending on the hydraulic loading rate of the SSF or RF being tested, to account for the tracer-determined hydraulic detention time (HDT) through the filters such that samples from the same 'slug' of water were tested (Table 1). The influent and effluent sample pairs were collected at 0.25 or 0.5 h intervals for 2 to 4.75 h. These data pairs were used to calculate MS2 removal (the number of sample pairs for each experiment is provided in Table 3).

During each challenge test, samples were collected aseptically into sterile 60 mL polypropylene centrifuge tubes (Biologix Research Company, Lenexa, KS). Samples were transported and stored at 4°C immediately after collection. SSF1 influent samples were obtained from a sampling port which was located approximately 0.10 m above the SSF surface in pilot plant 1 and in pilot plant 2 (SSF1) from a similar port located 0.15 m above the sand surface (Figure 1 (a and b)). All SSF effluent samples were collected from the corresponding column effluent discharge tube.

In the roughing filter challenge tests (tests 3 and 6, ozone generator off) the MS2 phage were added before the roughing filters (Figure 1). In test 3 (pilot 1, train 1) MS2 were pumped into the top of the secondary ozone contact column. For test 6 (pilot 2, RFA), MS2 were pumped into the outlet of the constant head tank. For the roughing filter test #3, effluent MS2 sample collection began 1 h following the start of seeding and samples were collected at 0.33 h intervals for 2 h ($n = 6$). For test 6 (with roughing filter A),

Table 3 | Summary of each challenge test showing the average MS2 removal through roughing and slow sand filters

Test no.	HLR (m/h)	Pilot plant details	Average MS2 removal ± standard deviation (%)	Log removal (PFU/mL)	Number of sample pairs analyzed*
Cold water tests (3–10°C)					
Roughing filters					
3	1.5	Pilot 1 (RF)	28.0 ± 5.2	0.1	6
6	1.0	Pilot 2 (RFA)	32.1 ± 14	0.2	5
Slow sand filters					
4	0.1	Pilot 1/train 1 (SSF)	99.3	2.2	1
5	0.1	Pilot 1/train 1 (SSF)	96.9 ± 0.7	1.5	9
7a	0.1	Pilot 1/train 1 (SSF)	99.3 ± 0.1	2.2	8
7b	0.1	Pilot 1/train 1 (SSF)	98.3 ± 0.7	1.8	8
1	0.4	Pilot 1/train 1 (SSF)	58.9 ± 14	0.4	15
2	0.4	Pilot 1/train 1 (SSF)	39.7 ± 14	0.2	14
8	0.4	Pilot 1/train 2 (SSF)	66.6 ± 2.8	0.5	7
9	0.4	Pilot 2 SSF1	91.8 ± 0.9	1.1	6
		Pilot 2 SSF2	65.1 ± 6.4	0.5	4
		SSF1 & 2 combined	97.2 ± 0.8	1.6	
Warm water tests (21–24°C)					
10	0.1	Pilot 1/train 1 (SSF)	99.2 ± 0.2	2.1	8
11	0.1	Pilot 1/train 1 (SSF)	99.1 ± 0.2	2.0	8
12	0.4	Pilot 1/train 1 (SSF)	85.9 ± 1.9	0.9	7
13	0.4	Pilot 1/train 1 (SSF)	90.5 ± 0.8	1.0	7
14	0.4	Pilot 2 SSF1	99.0 ± 0.1	2.0	6
		Pilot 2 SSF2	72.2 ± 4.9	0.6	6
		SSF1 & 2 combined	99.7 ± 0.1	2.6	
15	0.4	Pilot 1/train 1 (SSF)	94.5 ± 1.9	1.3	5

*Influent and effluent pairings based on allowance for appropriate hydraulic detention time.

effluent sampling began 2 h after MS2 seeding had started with samples collected at 0.25 h intervals for 1.5 h ($n = 5$).

Between each challenge test, background influent and effluent MS2 concentrations were determined just prior to the introduction/seeding of the challenge phage (at time 0). The influent MS2 concentration at time 0 never exceeded 33 PFU/mL and, for the most part, MS2 were not detected. The effluent MS2 concentration of the filter being tested at time 0 never exceeded 57 PFU/mL and was typically less than 10 PFU/mL. These concentrations were sufficiently low to be irrelevant when compared to challenge levels.

Phage removal in the challenge tests were expressed as log removals, a shorthand term for $\log_{10}$ removal. This was originally used in reference to the USEPA's Surface Water Treatment Rule (USEPA 1991) and is measured as the common log of the influent phage concentration minus the common log of the effluent phage.

Statistical analyses were used to compare the average MS2 concentrations when the number of data points for each test was ≥ 7, as it was assumed that means were normally distributed according to the Central Limit Theorem. Only challenge tests conducted on pilot 1 (trains 1 and 2) had $n \geq 7$, except for tests 4 and 15. A modified t-test known as the Smith-Satterthwaite (Montgomery 2003) approximation was used to compare averages for challenge tests. The Smith-Satterthwaite approximation assumes normal distribution and unequal and unknown population variances and is appropriate when 'n' is less than approximately 30.

RESULTS

Hydraulic loading rate

MS2 removal by slow sand filtration increased with decreasing hydraulic loading rate (Table 3). For the cold water SSF tests in Pilot 1 (Figure 2(a)), average MS2 removals at an HLR of 0.4 m/h ranged from only 0.2 to 0.5 log. By reducing the hydraulic loading by a factor of four to 0.1 m/h, average removals were significantly greater at the 1% significance level, ranging from 1.5 to 2.2 log. For warm water experiments (Figure 2(b)), average MS2 removals at an HLR of 0.1 m/h (2.0 and 2.1 log) were significantly greater than those conducted at 0.4 m/h (0.9 to 1.3 log).

Lower HLRs increase detention time, thereby providing greater opportunity for transport/diffusion of virus-sized particles to media and biofilm surfaces for attachment. Lower HLRs also decrease shear, reducing detachment of phage and providing greater opportunity for detached phage to re-attach lower in the filter. In addition, the increased detention time at a lower HLR increases opportunities for the phage to be trapped in biofilm.

Water temperature

Virus removals in slow sand filters have typically been shown to improve as water temperatures increase, due to increased biological activity (Poynter & Slade 1977; Nasser & Oman 1999). Somewhat unexpectedly we did not observe a consistent increase in MS2 removal at warmer temperatures in pilot plant 1 run at an HLR of 0.1 m/h (Figure 3(a)). Although warm water challenge test 10 and 11 removals were significantly higher than cold water tests 5 and 7b (5% significance level), there was no difference between the two warm water challenge tests and cold water tests 4 and 7a (5% significance level).

However, in challenge tests conducted at an HLR of 0.4 m/h, MS2 removal at warm water temperatures (tests 12, 13, and 15) were significantly different (1% significance level) from those done in cold water tests (1, 2, and 8).

One temperature comparison is available for tests run in pilot plant 2 (tests 9 and 14). Both tests were run on SSF1 which has twice the sand depth of the SSF in pilot plant 1 discussed above. At an HLR of 0.4 m/h, the warm water MS2 removal was almost twice that of the cold water test (2.0 vs. 1.1 log) which is consistent with observations made in pilot plant 1 at the same HLR (Figure 4).

Sand depth

Tests 9 and 14 were conducted using SSF1 of pilot plant 2 with a sand depth of 0.88 to 0.92 m, which was approximately twice as deep as the other SSF beds used in this study (Table 1). Both tests were conducted at an HLR of 0.4 m/h. In cold water, MS2 removals in SSF1 were 0.6 to 0.9 log higher than removals in the shallower SSFs tested (Figure 4, tests 1, 2, 8). In warm water, MS2 removals were 0.7 to 1.1 log higher than corresponding removals in shallower SSFs (Figure 4, tests 12, 13, 15).

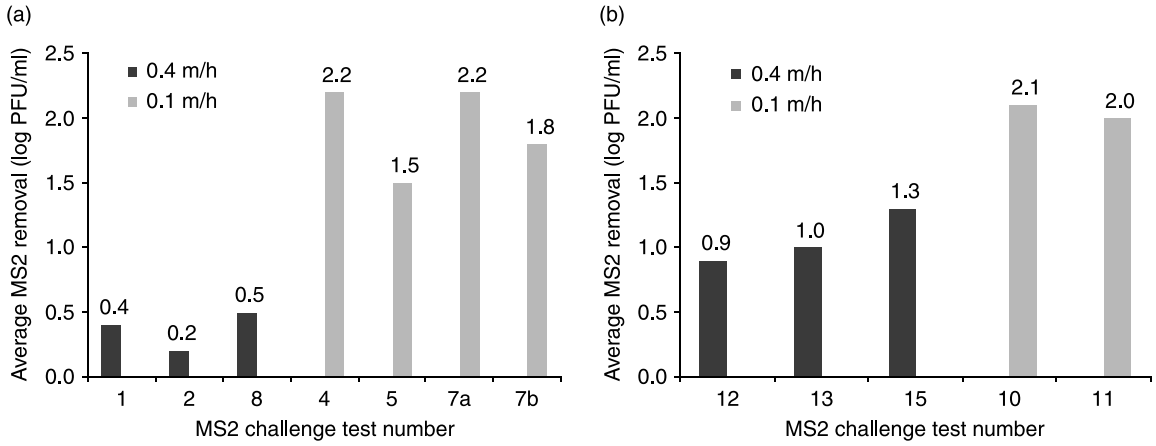

Figure 2 | Average MS2 removal in Pilot Plant 1 slow sand filter at an HLR of 0.1 m/h or 0.4 m/h in (a) cold water and (b) in warm water conditions (Table 2).

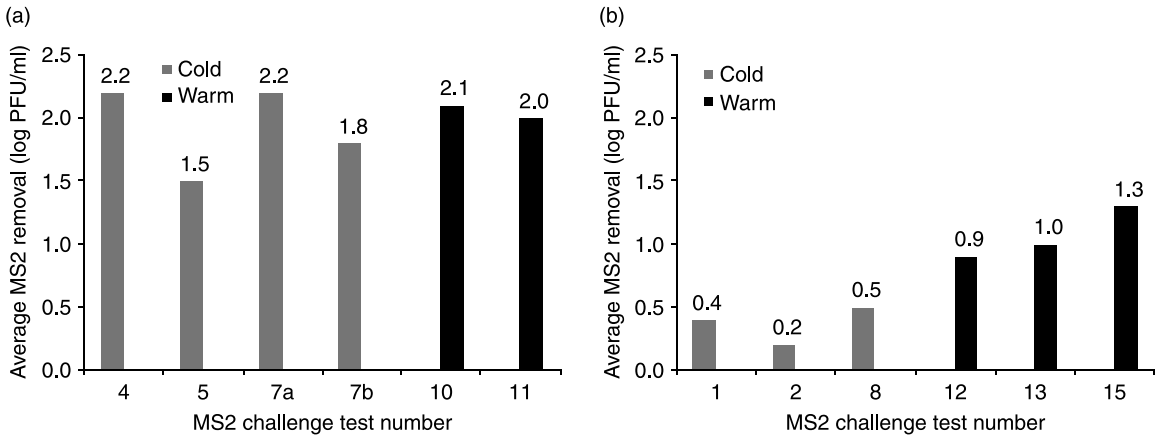

Figure 3 | Average MS2 removals in cold and warm water at HLRs of (a) 0.1 m/h and (b) 0.4 m/h in Pilot Plant 1 slow sand filter.

Thus in both cold and warm water conditions, the double sand depth in SSF1 corresponded to average MS2 log removals that were at least twice as high as in the shallower filters. For a given filtration rate, doubling the sand depth would double the empty bed and actual contact time, facilitating virus removal by biological and physical removal mechanisms. Furthermore, a deeper sand bed would provide additional opportunities for viruses that detached higher in the bed to re-adsorb. This is consistent with other studies that have shown that virus removal is higher with increased SSF bed depth (e.g. Poynter & Slade 1977; Graham *et al.* 1996).

Multistage slow sand filtration

Two slow sand filters in series

In tests 9 and 14 the deep-bed SSF1 of pilot 2 was followed by a shallow-bed SSF2 in series (Figure 5). MS2 phage were seeded into the effluent of roughing filter A for both tests (not between the SSFs). In cold water test 9, a total average MS2 removal of 1.6 log was calculated by adding the average removals in SSF1 (1.1 log) and SSF2 (0.5 log). This combined MS2 log removal was at least a factor of three greater than the average removal for any single SSF tested in cold water at the same hydraulic loading (tests 1, 2, 8). In the warm water test 14, the total average MS2 removal was 2.6 log (2.0 + 0.6). As in the cold water test, the combined MS2 log removal was at least a factor of two greater than the average removal for any single SSF tested under similar conditions (tests 12, 13, and 15). A temperature effect was noted for SSF1 with MS2 log removal in warm water being almost double that in cold water (2.0 vs. 1.1 log) but this could not be confirmed statistically due to the low number of data points ($n = 6$).

Roughing filters

The roughing filters of pilot plant 1 (train 1) and pilot plant 2 provided very little MS2 removal in the cold water conditions tested. Average MS2 removals were 0.1 log in test 3 at 1.5 m/h and 0.2 log in test 6 at 1.0 m/h (Table 3) despite the fact that the roughing filters had been in

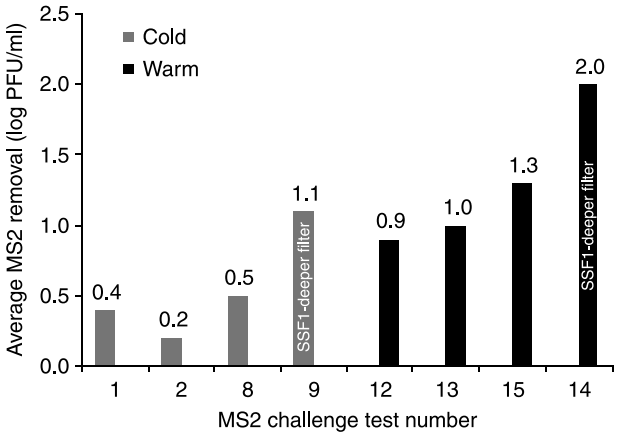

Figure 4 | Average MS2 removal at an HLR of 0.4 m/h-shallow (0.4 m) vs. recommended filter depth (0.9 m) (SSF1, tests 9 & 14). Challenge tests were run under cold and warm water conditions. Challenge tests 1, 2, 8 and 12, 13, 15 were conducted on Pilot Plant 1, and tests 9 and 14 were conducted on Pilot Plant 2.

continuous operation for over two years and were biologically mature. Poor MS2 removal was likely due to increased shear from the relatively high HLRs (1.0 and 1.5 m/h), short hydraulic detention times (20 to 30 min), low biological activity attributable to the cold water temperatures at the time of testing (3 and 6°C), and large media size (minimum 0.85 mm in RF and 4.8 mm in RFA). This is not surprising as the primary function of roughing filters is to protect the slow sand filters from turbidity peaks, rather than to contribute measurably to objectives such as virus removal.

MS2 shedding

Release of MS2 from the slow sand filters (shedding/tailing) was observed up to 12 days following the termination of MS2 seeding (Table 4). This may have been attributable to reversible attachment or the retardation of MS2 adsorbed to, or entrapped within, microflocs or biofilm in interstitial spaces in the filter. This confirms observations of others in studies employing slow sand filters (Dizer *et al.* 2004; Dullemont *et al.* 2006) and sand columns (Bales *et al.* 1993; Schijven *et al.* 1999; Schijven & Simunek 2002; Hijnen *et al.* 2005).

The MS2 challenge levels used in this study were more representative of a spike and like most pathogens in water, a specific virus may or may not be present at any given point of time. Typically, peaks are observed following rainfall events, spring thaw, sewage plant discharges due to process failures or outbreak conditions in the community, etc. As such, shedding following a peak of a specific virus may be higher than 'typical' or 'average' concentrations (before the peak). This shedding could expose consumers for longer periods to higher than normal concentrations.

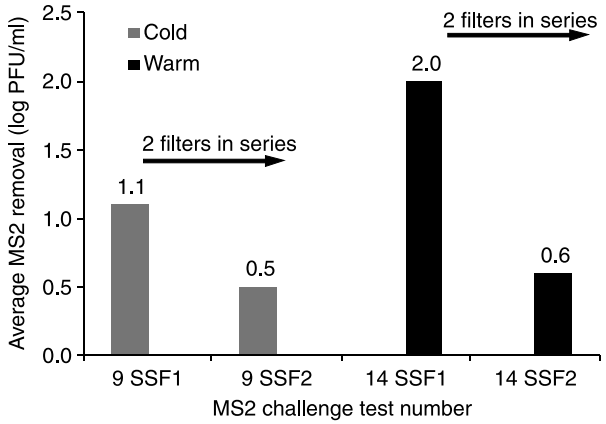

Figure 5 | Average MS2 removal using two slow sand filters in series (SSF1 was ~0.9 m deep and SSF2 was ~0.45 m deep). Experiments were conducted in pilot plant 2 at an HLR of 0.4 m/h under cold and warm water conditions.

Table 4 | MS2 shedding from Pilot Plant 1 slow sand filter (SSF)

Test no.	Effluent concentration during MS2 addition (PFU/mL)	Elapsed time following cessation of MS2 addition (d)	Average MS2 SSF influent concentration (PFU/mL)	Average MS2 SSF effluent concentration (PFU/mL)
1	10^4	4.9	1	20
		5.0	1	29
		9.1	1	23
		12	–	12
2	10^4	3.1	0	119
		6.3	–	49
4	10^3	3.9	19	20
		6.9	1	14
		10	1	6
5	10^4	6.2	33	57
7b	10^4	44	0	0
8	10^4	1.0	3	122
		2.0	0	57
		47	0	0
10	10^3	1.4	5	52
11	10^3	13	0	0
12	10^4	2.3	9	5
13	10^4	2.1	0	5

CONCLUSIONS

A total of 16 MS2 bacteriophage challenge tests were conducted between February and June 2006 in two multistage slow sand filter pilot plants in southern Ontario, Canada. Comparisons were made at two hydraulic loading rates (0.1 and 0.4 m/h), in two water temperature ranges (<10°C and >20°C), and at two sand depths (0.36–0.40 m and 0.88–0.92 m). The pilot plants were operated using untreated river water, which is considered challenging for this technology and is a reason why a roughing filter was included upstream of the slow sand filter in each pilot plant.

The following general conclusions can be drawn:

- Average removals of MS2 ranged from 0.2 to 2.2 log in the slow sand filters and 0.1 to 0.2 log in the roughing filters under the conditions tested.
- Virus removal increased with greater sand depth and warmer water temperature, but decreased at higher hydraulic loading rates. Multiple slow sand filters in series can improve virus removal.
- MS2 phage may continue to be released from slow sand filters following the disappearance of the phage in the influent water, as demonstrated by shedding of previously entrapped/adsorbed phage for up to 12 days after seeding was stopped.

Based primarily on the modest MS2 bacteriophage removals seen in this pilot scale study, and observations of shedding, it is reasonable to conclude that viruses could pass through full-scale SSFs and into the filter effluent, depending on the influent virus concentration, water temperature, and slow sand filter plant design and operation. Therefore slow sand filtration, even when preceded by roughing filtration or a second slow sand filter in series, may not be suitable as a stand-alone system to ensure virus-free treated drinking water. Systems designed and operated under the conditions discussed here should be combined with a disinfection/inactivation step to ensure robust compliance with regulatory requirements and protection of human health.

To optimize virus removal, SSFs should be designed with bed depths as recommended in the literature (>1 m) and be operated at the lower range of hydraulic loading rates recommended in the literature (~ 0.1 m/h), especially in cold water conditions (<10°C).

ACKNOWLEDGEMENTS

The authors would like to acknowledge Robert LeCraw, Michael Galan, Ernie Selvadurai, and Brian Jobb from MS Filter, Inc. for the use of their pilot system and invaluable operation and design instruction. We would also like to thank the Region of Waterloo, in particular Lane Stevens and Olga Vrentzos, for their support and access to facilities. Special thanks to Shawn Cleary and Souleymane Ndiongue who provided valuable guidance on the operation of the pilot systems and to Vanessa Morton and Bridget Schmidt for their assistance in the lab.

Funding for this project was provided by a Natural Sciences and Engineering Research Council of Canada grant and scholarship, and our Industrial Research Chair in Water Treatment partners (listed at: http://www.civil.uwaterloo.ca/watertreatment/).

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First received 13 May 2009; accepted in revised form 10 September 2009