Reductions of *E. coli*, echovirus type 12 and bacteriophages in an intermittently operated household-scale slow sand filter

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ABSTRACT

Point-of-use (POU) drinking water treatment technology enables those without access to safe water sources to improve the quality of their water by treating it in the home. One of the most promising emerging POU technologies is the biosand filter (BSF), a household-scale, intermittently operated slow sand filter. Over 500,000 people in developing countries currently use the filters to treat their drinking water. However, despite this successful implementation, there has been almost no systematic, process engineering research to substantiate the effectiveness of the BSF or to optimize its design and operation. The major objectives of this research were to: (1) gain an understanding of the hydraulic flow condition within the filter (2) characterize the ability of the BSF to reduce the concentration of enteric bacteria and viruses in water and (3) gain insight into the key parameters of filter operation and their effects on filter performance. Three 6–8 week microbial challenge experiments are reported herein in which local surface water was seeded with *E. coli*, echovirus type 12 and bacteriophages (MS2 and PRD-1) and charged to the filter daily. Tracer tests indicate that the BSF operated at hydraulic conditions closely resembling plug flow. The performance of the filter in reducing microbial concentrations was highly dependent upon (1) filter ripening over weeks of operation and (2) the daily volume charged to the filter. BSF performance was best when less than one pore volume (18.3-L in the filter design studied) was charged to the filter per day and this has important implications for filter design and operation. Enhanced filter performance due to ripening was generally observed after roughly 30 days. Reductions of *E. coli* B ranged from $0.3\log_{10}$ (50%) to $4\log_{10}$, with geometric mean reductions after at least 30 days of operation of $1.9\log_{10}$. Echovirus 12 reductions were comparable to those for *E. coli* B with a range of $1\log_{10}$ to $>3\log_{10}$ and mean reductions after 30 days of $2.1\log_{10}$. Bacteriophage reductions were much lower, ranging from zero to $1.3\log_{10}$ (95%) with mean reductions of only $0.5\log_{10}$ (70%). These data indicate that virus reduction by BSF may differ substantially depending upon the specific viral agent.

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1.   Introduction

According to the World Health Organization, 1.1 billion people (WHO, 2004) in the developing world lack access to improved sources of drinking water. Point-of-use (POU) drinking water treatment and safe storage technology allows people without access to safe water sources to improve the quality of their water by treating it in the home, thereby taking control of the safety of their drinking water (Sobsey, 2002). One of the most promising emerging POU technologies is the biosand filter (BSF), a household-scale, intermittently operated slow sand filter (SSF).

The BSF consists of a concrete or plastic chamber filled with sand with an elevated discharge tube that allows the filter to maintain a layer of water above the sand surface and prevents dewatering (Fig. 1). The BSF is similar to a conventional SSF in that there is typically no pretreatment or backwashing and operation is simple, being gravity-driven rather than pressure filtration. As in conventional SSFs, the sand bed remains wetted throughout operation and a ripening process occurs, during which a biolayer (or schmutzdecke) forms, head loss increases and performance improves. However, the BSF does not operate continuously but instead, intermittently, wherein a single charge of feed water (typically up to 20 L although multiple daily charges are possible) is made each day. During this charge, the operation is in a declining rate mode of filtration. A portion of the charged water remains in the BSF until the next charge. The time period when water is no longer discharging from the filter is referred to as the idle time.

Head loss in the BSF increases over weeks of operation and filter operation is discontinued when water production (flow rate) is judged subjectively insufficient by the user. The filter is manually cleaned by stirring the upper layer of the media bed. Depending on whether the user has access to alternative sources of water, the unit may be returned to service immediately, though implementers recommended that the filter be flushed for 2 days following cleaning before filtered water is used for drinking.

As many as 500,000 people worldwide rely on the BSF for safe drinking water and there are several reports that have addressed field implementation, user satisfaction, and percentage removal of thermotolerant coliforms or *E. coli* in the field (Murcott, 2002; Earwaker, 2006; Duke et al., 2006; Kaiser et al., 2002; Stauber et al., 2006). However, relatively little has been reported on optimum design and operating conditions of the BSF and on microbial removal under well-controlled laboratory conditions and even less is available in peer-reviewed publications. Two early studies dealt with bacterial removal and BSF performance characteristics (Buzunis, 1995) and with toxicant and parasite removal (Palmateer et al., 1999). These cover laboratory and field investigations that showed only modest reductions ($1–2\log_{10}$) of indicator bacteria. However, Palmateer et al. (1999) demonstrated $3.8\log_{10}$ reduction of oocysts of *Cryptosporidium* sp. and $>5\log_{10}$ reduction of *Giardia lamblia* cysts in the laboratory. More recently, microbial challenge studies with bacteria and viruses under controlled laboratory conditions have been reported (Stauber et al., 2006; Elliott et al., 2006).

The objectives of this research on the BSF were to: (1) characterize its hydraulic operation, (2) measure the reduction efficiency of *E. coli*, bacteriophages and echovirus 12 under controlled laboratory conditions, and (3) gain insight into the key design and operational parameters that affect filter performance.

1.1.   Characteristics of BSF design and operation

As shown in Fig. 1, dewatering of the filter between charges is avoided by a vertical discharge tube that rises from 2- to 7 cm above the height of the filter media. The filtered water passes through the media bed and enters the discharge tube through the underdrain gravel. The discharge tube is screened in the plastic filter used in these experiments but is generally not screened in concrete filters. The elevated outlet allows the media to remain saturated after a charge has been filtered but water is no longer flowing from the outlet. Another unique aspect of the BSF design is to promote uniform drip flow over the sand surface by use of a plastic or sheet metal diffuser with approximately 2-mm diameter holes above the filter media. This diffuser prevents the charge of water from disturbing the biolayer.

The design of the BSF differs significantly from that of the SSF. The maximum recommended filtration rate of the BSF is nearly 15 times greater than for the SSF (1.1 m/h in contrast to a recommended 0.08–0.4 m/h) (Fox et al., 1994). The depth of the BSF sand layer is about 50% less than for the SSF (0.4 m compared to a recommended starting depth of >0.8 m for the SSF with a minimum of 0.5–0.7 m). The range of particle size of the BSF sand is typically broader than in SSF (e.g., the uniformity coefficient may typically exceed 4.0, compared to a recommended value of <3 for the SSF). In addition, the quality of the sand differs because the BSF is constructed with material that is locally available whereas sands used in most
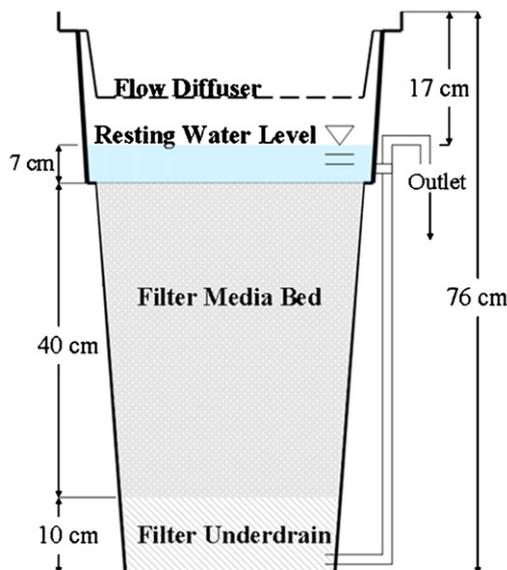

Fig. 1 – A cross-section of the plastic BSF used in these experiments.

SSF are obtained from a commercial source. To provide quality control on local sand selection, the typical procedure is to measure the initial flow rate of a newly loaded filter following a 20-L charge. If the flow rate falls outside the prescribed range based on various filtration models of 0.6–1.0 L/min (0.5–1.1 m/h), the particle size is either too small (flow rate is too low) and the sand requires further washing or too large (flow rate is too high) and thus unacceptable for use.

2.　Methods

2.1.　Filter and media preparation

Plastic, 60-L capacity, filter units were obtained from Davnor Water Treatment Technologies Ltd. (Calgary, Alberta, Canada). Filters contained 5 cm of underdrain gravel, 5 cm of medium-sized support gravel, and 40 cm of sand, the effective size (d_{10}) of which was 0.19–0.22 mm and a uniformity coefficient of 3.5–4.0. All filter media were crushed granite gravel available locally and were sieved according to standard international field procedures for the BSF (Manz, 2007). The initial flow rates in each experiment following the first 20-L charge were approximately 0.9 L/min. Flow rate was measured daily just after the introduction of a charge.

2.2.　Design of microbial challenge studies

Three microbial challenge filtration experiments, with durations of 44–54 days, were conducted consecutively to determine the efficacy of the BSF in reducing the concentration of echovirus 12 and microbial indicators from drinking water and to explore the effects of the daily water charge volume on microbial reductions. The code that gives the single daily charge volume (20 or 40 L) and the percent amendment with pasteurized primary effluent (PE) (0%, 1% or 2.5% PE) for the three filtration experiments is provided in Column 1 of Table 1 along with the duration of each, the drinking water reservoir from which feed water was obtained and the concentration of microbes in the feed water. The choice of a 20-L charge was based on both the maximum volume for the BSF reservoir above the filter material and the lower range of typical daily family usage in developing countries (Sobsey, 2002). The 40-L daily charge volume was intended to represent the higher end of typical water volume for domestic uses by a family in a developing country. Because the reservoir held only 20 L, the second 20-L charge was in increments of less than 5 L as water was filtered and elevation head in the reservoir decreased. In contrast to the instantaneous charge of 20 L, the 40-L charge, therefore, required from 1 to 6 h to charge. This reduced the daily idle period from that observed with the 20-L charge.

Feed water was obtained from the sample taps of local water treatment plants using one of three local water supply reservoirs (Cane Creek Reservoir or University Lake in Chapel Hill, NC or Lake Michie in Durham, NC). None of these source waters receives wastewater discharges. The alkalinity of the source waters was very low with a mean of 26 mg/L and a range of 14.4–48.1 mg/L as $CaCO_3$.

To simulate the presence of wastewater in typical drinking water sources of developing countries and accelerate the ripening process, pasteurized PE from a local wastewater treatment plant (Orange County Water and Sewage Authority (OWASA), Chapel Hill, NC) was pasteurized and added to feed water in two of the filtration experiments. Amendment with PE and spiking with cultures of microorganisms increased the total organic carbon (TOC) of the feed water by up to 50%. The filtration experiment designated as 40 L-2.5% PE was begun without the addition of PE. However, the decision was made to add PE on day 30 in order to accelerate the rate of ripening. The practice was continued in the 20 L-1% PE experiment starting with the first day of filter operation. All feed water samples were allowed to reach room temperature (approximately 20 °C) overnight in an effort to eliminate water temperature as a variable that could impact rates of both ripening and microbial stability.

For each filtration experiment, an aliquot of stock suspensions of the challenge microorganisms was spiked into the daily charge volume to provide the target concentrations listed in Table 1. Following the addition of challenge microbes and PE, mean turbidity was 3.90 NTU (range from 1.86 to 8.96 NTU) and mean TOC was 9.1 mg/L (range from 7.5 to 12.6 mg/L).

2.3.　E. coli, MS2, PRD-1, and Echovirus type 12

A frozen stock of *E. coli* strain B (ATCC No. 11303) was thawed and a culture was grown to log phase in tryptic soy broth, enumerated by spread plating on MacConkey Agar, cooled to 4 °C, and stored for up to 7 days. Daily spike suspensions were prepared by diluting the culture serially and adding an aliquot into feed water immediately prior to charging the filter to achieve the desired concentration.

Stocks of bacteriophages MS2 and PRD-1 were grown, enumerated by the double agar layer procedure USEPA Method 1602 (USEPA, 2001) and stored at −80 °C. Aliquots of each stock were thawed each week, serially diluted ten-fold in phosphate buffered saline and stored at 4 °C for up to 7 days. Aliquots of an appropriate dilution were then dosed into feed water, resulting in the challenge microbe concentrations reported in Table 1.

A stock of echovirus 12 was propagated in monolayers of FRhK-4 cells with maintenance medium (Eagle's MEM with 2% by volume heat-inactivated fetal bovine serum, 0.75% 4 M $MgCl_2$ and 1% of the following: 100 × gentamycin/kanamycin, Nystatin, HEPES buffer and non-essential amino acids) at 37 °C, freeze-thawed and chloroform extracted and then enumerated by the plaque technique in cell monolayers. Extracted stock was stored at −80 °C until needed. Aliquots of enumerated stock were thawed daily, serially diluted, and added into feed water to achieve the desired echovirus 12 concentration for each daily charge. Further details of the procedures used to grow and enumerate stocks of seeded microbes have been reported previously (Elliott et al., 2006; Stauber et al., 2006).

2.4.　Residence time distribution in BSF

The residence time distribution (RTD) of water within the BSF was measured to assess the deviation from ideal plug flow conditions due to dispersion in the flow paths through porous

Table 1 – Characteristics of filter challenge experiments

Experiment coding[a]	Length (days)	Source water[b]	E. coli B $\log_{10}$ (cfu/mL)	MS2 $\log_{10}$ (pfu/mL)	PRD-1 $\log_{10}$ (pfu/mL)	Echovirus 12 $\log_{10}$ (pfu/mL)
40 L-0% PE	43	CC, UL	2.55 ± 0.33	3.10 ± 0.25	3.68 ± 0.18	–
40 L-2.5% PE	54	CC	2.71 ± 0.44	2.74 ± 1.01	3.50 ± 0.36	2.90 ± 0.17
20 L-1% PE	50	LM	2.68 ± 0.37	–	–	2.48 ± 0.62

Microbial concentrations are mean $\log_{10}$ measured concentration per mL and maximum $\log_{10}$ deviation from mean.

[a] Experiment coding: 20 L = 20 L charged daily, 40 L = 40 L charged daily; x% PE = feed water amended with x% primary effluent charged to the filter daily.

[b] CC = Cane Creek Reservoir (Orange County, NC, USA); UL = University Lake (Orange County, NC, USA); LM = Lake Michey (Durham, NC, USA).

media (sand and gravel in this system). The pores of a clean, unripened plastic BSF (BioSand Water Filter[TM], Davnor, Inc., Calgary, Alberta, Canada), were filled with deionized water (DI) at the start of the tracer test. The tracer test began by removing the DI water above the filter media and charging the filter with a 200 mg/L NaCl solution. The response to this positive step input of tracer was followed. The NaCl feed was replaced with DI so as to produce a negative step input and the response was again followed. This technique provided two independent measures of RTD.

Three separate NaCl tracer tests were conducted to determine the effect of flow rate through the filter on RTD. In two tests, the flow rate was held constant throughout the tracer test by maintaining the head constant at 5 cm ($\pm$1 cm) and at 13 cm ($\pm$1 cm), respectively using peristaltic pumps (Cole Parmer Cat. No. 7553-0 and 7545-0). In a third test, the flow rate was allowed to decline as it would during the daily charge to the BSF; that is, the head was allowed to decrease with time from 17.3 to 2.0 cm. Conductivity of the filtered water was measured as a surrogate for NaCl concentration (Fisherbrand Traceable[TM] Conductivity, Resistivity, and TDS Meter (Cat. No. 09-326-2)).

2.5. Microbial analyses

Filters were charged with feed water spiked with challenge microorganisms daily. Three types of samples were collected at approximately weekly intervals for microbial analysis: (1) aliquots of feed water from the previous and current charge; (2) grab samples of the filtered water taken throughout a daily filtration cycle; (3) a composite sample of the filtered water to measure the daily average concentration of microbes. The aliquot of feed water from the previous charge was stored at room temperature until microbial samples were analyzed the following day in order to serve as a control for the effects of time and temperature on microbial survival. Mean die-off rates for the challenge microbes after overnight storage were: 35% (0.18 $\log_{10}$) for E. coli; 15% (0.07 $\log_{10}$) for MS2; −18% (none) for PRD-1 and 9.1% (0.04 $\log_{10}$) for echovirus type 12. These die-offs were considered small and were disregarded.

E. coli concentrations in water were quantified by membrane filtration on MI agar BBL (Becton-Dickinson, Franklin Lakes, NJ) using USEPA Method 1604 (USEPA, 2002). MS2 and PRD-1 concentrations were assayed using the single agar
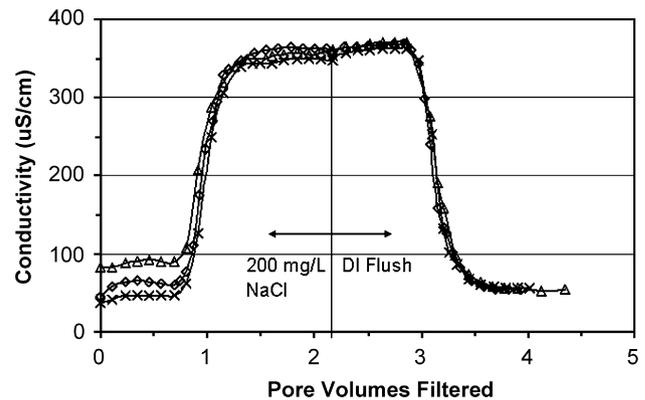

Fig. 2 – Results of three step-input tracer tests conducted with the plastic BSF.

layer method on hosts E. coli F_{amp} and Salmonella typhimurium LT2, respectively (USEPA, 2001). Echovirus 12 samples were inoculated onto confluent monolayers of FRhK-4 cells grown in 60 mm plastic dishes (Beckton-Dickinson, Franklin Lakes, NJ) using the plaque assay adapted from Cromeans et al. (1987). Turbidity and pH were measured using a turbidimeter (Model 2100N, Hach, Loveland, CO) and pH meter (Model 215, Denver Instruments, Denver, CO). Reductions in microbe concentration by passage through the BSF were calculated by

$$\log 10 \; \text{Reduction} = \log 10 \, (\text{Feed Water Concentration}) - \log 10 \, (\text{Filtered Water Concentration}).$$

(1)

3. Results and discussion

3.1. Tracer tests

The results of the three tracer tests are presented in Fig. 2. The ordinate scale is expressed in pore volumes based on an independent estimate of the pore volume (18.3 ± 0.1 L) that was obtained by measuring the water volume needed to fill the filter (sand and underdrain) completely. The corresponding porosity of the media-based measurement of the total bed volume was 44%.

The tracer response pattern shown in Fig. 2 is essentially the same in all three tracer tests. Thus, filter flow rate either in the constant head (two head values) or the declining head mode did not affect the RTD at the filtration rates tested (0.2–1.0 m/h). The sharp rise in conductivity to the feed water value occurs in about one pore volume as should be expected of plug flow. The subsequent sharp decline in conductivity to the baseline value after the negative step input is also seen after about one pore volume, again providing evidence of plug flow. These results show a minimal effect of dispersion by tortuous flow paths through the porous media. From the perspective of biolayer development and microbial removal processes, the results suggest the same time is available to all parcels of water that enter the BSF.

The Morrill dispersion index (MDI) was calculated for the two constant head tracer tests by the method demonstrated in Tchobanoglous et al. (2003). The MDI for each of the two tests was approximately 1.3. MDI for an ideal plug flow reactor would be 1.0 and about 22 for a complete-mix reactor. A unit process with an MDI of less than 2.0 is classified as effective plug flow by the USEPA (USEPA, 1986).

3.2. Head-loss development over BSF operating time

The patterns of flow rate decline, and thus head-loss development, after each 20-L charge are compared for the three filter experiments (40 L-0% PE, 40 L-2.5% PE and 20 L-1% PE) in Fig. 3. A decline in flow rate is expected due to head-loss accumulation as the filter ripens or matures. Particle accumulation and biological growth in the top-most layer of the media bed are typically responsible for ripening. On the basis solely of the mass rate of addition of particles, the experiments with the 40-L charge would have been expected to produce more decline in flow rate than the experiment with the 20-L charge. While one of the two 40-L charge experiments did produce the most decline in flow rate, the presence of a brown floc in the feed water from day 14 and to day 25 may have produced an anomalous buildup of head loss and thus the decline in flow rate. The brown floc was observed in water collection vessels and probably sloughed off the raw water supply line during a surge in raw water pumping at the water treatment plant while the feed water was being collected.

The concentration of organic substrate to support microbial colonization on the filters that then leads to head loss is another factor that was not constant among the three experiments because the percent PE was not the same. This may have confounded the expected order of the decline in flow rates. Although the percentages of PE are small, they nevertheless contribute significantly to the DOC of the feed water.

Inherent variability in biomass development, both temporally and spatially, is yet another factor reported for SSFs (Campos et al., 2002), even in the absence of variation in temperature, which was minimal in these experiments. In fact, follow-up investigation not reported herein using small-diameter BSF columns packed with the same media and operated in parallel with the same feed water have also shown large variability in the ripening rate. All of these results suggest that ripening and biofilm development defy easy quantification based on water volume and water quality charged to the filter per day.

3.3. Turbidity removal

Turbidity decreased through the BSF, producing filtered water turbidities that ranged from 0.65 to 2.99 NTU and with a mean of 1.45 NTU (feed turbidity ranged from 1.86 to 8.96 NTU with a mean of 3.90 NTU). Turbidity removal improved as the BSF ripened as indicated by the decrease in mean filtered water turbidity from 1.45 to 0.98 (range 0.65–1.4) NTU after more than 30 days of operation. The trend toward lower filtered water turbidity with time as ripening progressed indicated that filter ripening: (1) enhanced particle straining due to biolayer formation; (2) improved depth filtration by slowing the filtration rate; and/or (3) altered the surface properties of the filtration media.

The removal of turbidity by the BSF was not as high as reported for conventional SSF (Sims and Slezak, 1991). Less effective particle removal can probably be attributed to the higher filtration rates and shorter media bed of the BSF compared to the SSF.
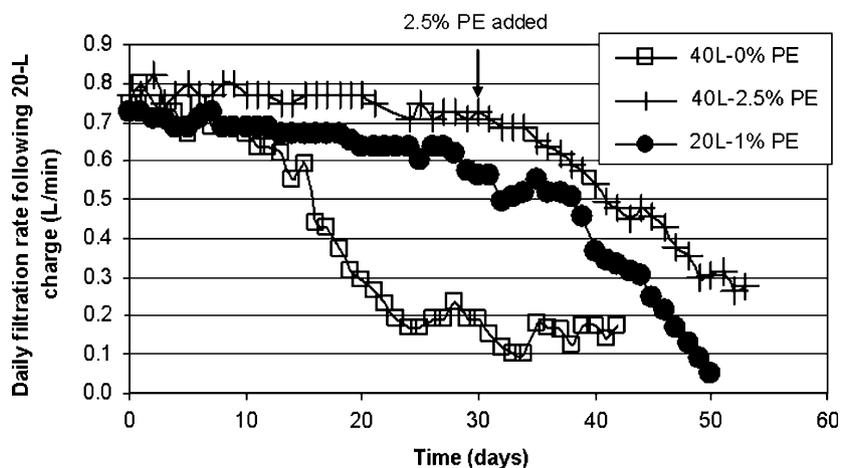

Fig. 3 – Daily filtration rate upon initial 20-L charge of water during three microbial challenge experiments.
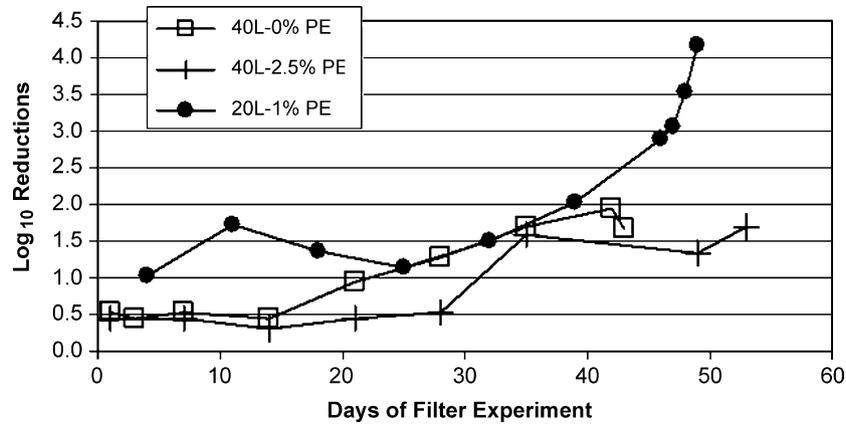
Fig. 4 – E. coli reductions over the length of three microbial challenge experiments.

3.4. Microbial reductions

Log$_{10}$ reductions in E. coli during the three BSF experiments are presented in Fig. 4. The trend in all three filtration experiments is toward increased E. coli reductions with increasing days of filtration. This suggests an impact of ripening caused not only by the maturation of a biofilm but perhaps also by the increase in residence time within the filter as the filtration rate declines due to head-loss buildup.

The log$_{10}$ reductions in E. coli were less for a 40-L than 20-L daily charge of feed water. For example, during the first 3 weeks, less than 0.6 log$_{10}$ (75%) reduction was obtained for the 40-L charge while a 1–1.7 log$_{10}$ (90–98%) reduction was obtained for the 20-L/day charge. The effect of daily charge volume on microbial removal may be explained by the difference in the fraction of the charge water that remains within the filter during the idle period between charges. Given plug flow behavior and a pore volume of 18.3 L, most of the filtered water produced by a 20-L charge originated from the feed water that was introduced on the previous day. By contrast, only 50% of the filtered water (18.3 L) produced by a 40-L charge originated from water stored in the filter bed during the idle period. Thus, the results imply that the reduction in E. coli in water that remains in an idle filter from the charge of the previous day is greater than in subsequent parcels of water that pass through the filter bed following each daily charge of feed water.

The importance of filter idle time on E. coli reduction was explored further by assaying E. coli in grab samples of incremental volumes of filtered water. The results for a series of 40-L charges (40 L-2.5% PE) and 20-L charges (20 L-1% PE) are shown in Figs. 5a and b, respectively. During each daily charge, the progressive time points at which E. coli were measured also correspond to the cumulative pore volumes displaced from the charge water from the previous day. The results show clearly that the E. coli reduction declines with pore volumes displaced on any given day. This is probably due to processes by which microbes entering the filter on the day before are attenuated or inactivated within the filter during the 18–24 h idle period. The results also show that the E. coli

reduction improves with increasing days of filtration due to filter maturation.

As illustrated in Figs. 5a and b, microbial reductions also tend to improve slightly toward the end of each daily filter run. The explanation could relate to a decline in filtration rate with time. When nearly all of the daily charge has been filtered, the elevation head declines to only a few cm and correspondingly the flow rate is only a small fraction of the initial flow rate. Thus, the last parcels of water to leave the filter have remained within the filter for the longest period of time. The effect is particularly evident in Fig. 5b, where the final volumetric samples were taken when elevation head in the filter was about 2 cm.

The log reductions in virus challenges are presented in Fig. 6. Reductions in the human enteric virus (echovirus 12) were greater than those for the two bacteriophages (MS2 and PRD-1). The reductions of both bacteriophages were uniformly lower than those of E. coli (Figs. 4 and 5) and imply that fecal bacteria such as E. coli may not be a good indicator of reduction of some viruses by the BSF. However, the reductions of echovirus 12 were comparable to those for E. coli.

The effect of the filter idle time and volume filtered on bacteriophage and human enteric virus reductions is shown in Fig. 7. This filter had been operated for 42 days during which time ripening was evident by the decline in the initial daily flow rate (Fig. 3). While the reductions did not steadily decline with pore volumes, mean reductions were higher in grab samples taken when <0.7 pore volumes had been filtered (>1 log$_{10}$ (90%) for MS2 and PRD-1 and >2 log$_{10}$ for echovirus 12). In grab samples taken at >1.1 pore volumes filtered, mean reductions for bacteriophages decreased to less than 0.75 log$_{10}$ (82%) and mean reductions decreased to 1.14 log$_{10}$ (93%) for echovirus 12. These results lend further support to the concept of an attenuation mechanism produced by residence time within the filter during the idle period as was suggested by reductions in E. coli (Fig. 5).

A larger reduction in echovirus 12 than bacteriophages, as observed in Figs. 6 and 7, may have been caused by stronger sorption to the filter media. The isoelectric points of echoviruses are higher (5.0–6.4) than for MS2 (3.5–3.9) and PRD-1 (4.2) (Collins et al., 2004). A higher isoelectric point
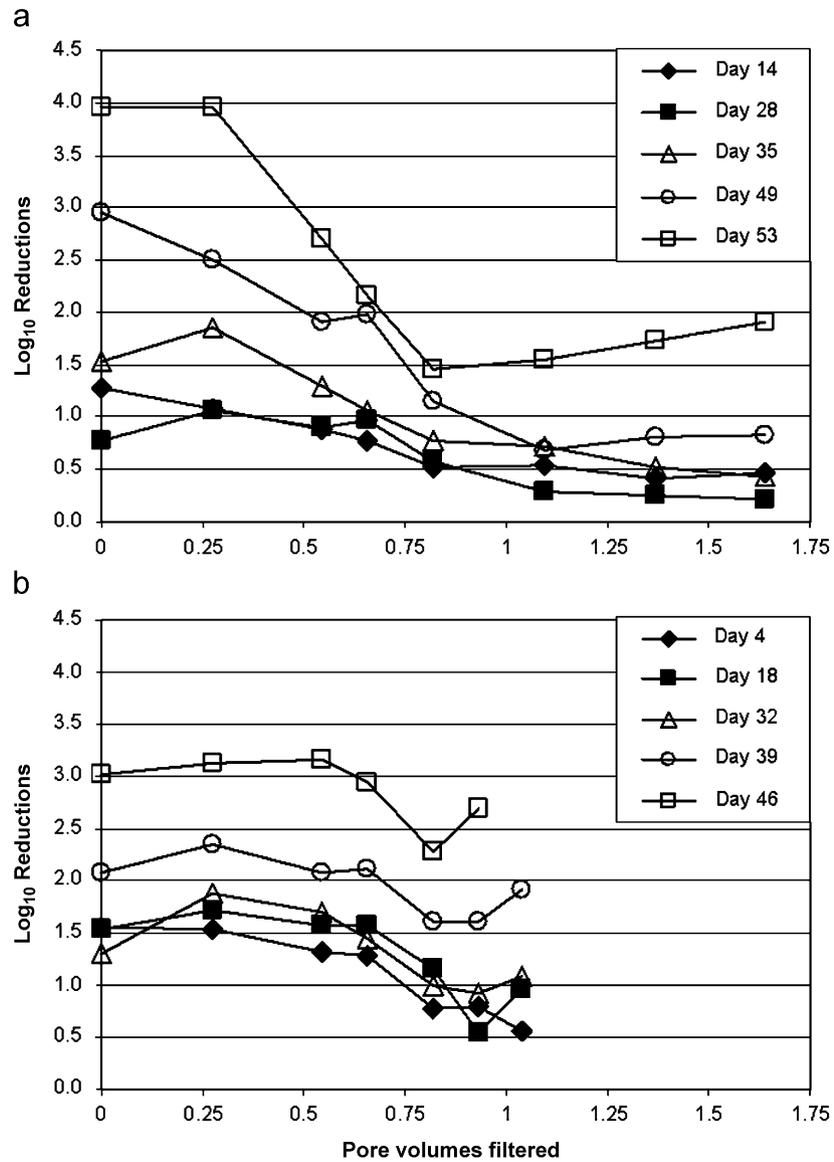
a

b

Pore volumes filtered

Fig. 5 – (a,b) Reduction in *E. coli* concentration with pore volumes filtered for experiment 40 L-2.5% PE ("a" on top) and experiment 20 L-1% PE ("b" on bottom). Results from days 7 and 21 of experiment 40 L-2.5% PE and days 11 and 25 of experiment 20 L-1% PE were excluded from the above graphs because they overlay the results of the subsequent sample days.
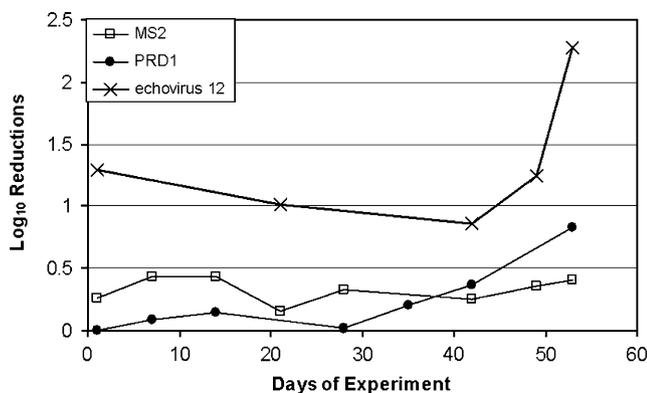

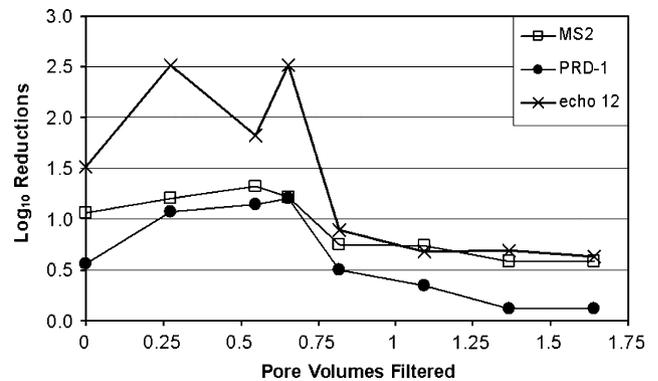Fig. 6 – Reductions in concentration for three viruses over the length of experiment 40 L-2.5% PE.

Fig. 7 – Reductions in concentration for three viruses with pore volumes filtered. Data shown is from day 42 of experiment 40 L-2.5% PE.

means that the echovirus 12 is less negatively charged than bacteriophage at near-neutral pH of the feed solution; thus, there is less repulsion by the negatively charged surface of filtration media. The mechanisms of virus removal or inactivation in the BSF, however, require further investigation.

4.   Conclusions

Reductions of bacteria, viruses, and turbidity by the BSF tend to be lower than those demonstrated for traditional SSF (Fox et al., 1994). However, they can increase substantially with repeated charges and time in use as filters matured (ripened) and increased retention time of water in the filter bed. Differences in the reductions for different microbes, particularly for viruses, suggest multiple mechanisms of attenuation. The increase in microbial attenuation that occurs over repeated charges and time in use is related to filter maturation and needs systematic study. These findings raise questions about the assurance of safe water provided to users of the BSF during the early stages of operating the BSF before ripening. Whether or not the media, for example, could be modified to provide an adsorptive function to compensate for lower initial microbial reduction due to the lack of ripening remains for future research.

The results from these microbial challenge studies indicate that reductions could be increased by increasing the retention time of water in the filter. This could be accomplished by: (1) introducing a daily charge volume that is smaller than the filter bed pore volume; (2) designing a filter in which the reservoir volume is less than the pore volume; (3) constructing a flow rate control device that would reduce the flow rate; (4) using a smaller medium to increase head loss; and/or (5) encouraging users to allow a longer time interval between the introduction of each charge of water.

More research under well-controlled conditions is needed to understand the mechanism(s) that are responsible for microbial attenuation in the BSF so as to optimize its design and operation of the BSF. This research has shown that the idle period is very important, which has implications for selection of charge volume, frequency of charge introduction and duration of charge filtration.

Whether the observed microbial reductions by the BSF are sufficient to provide microbiologically safe drinking water without addition of a disinfectant remains to be shown by epidemiological study of microbial heath risks such as reductions of diarrheal disease from BSF-filtered waters and by comparison of the observed microbial reductions to those required by drinking water regulations in specific countries or regions. Also of practical importance is whether microbial reductions observed in this study can be generalized to BSFs in household use globally and, correspondingly, the extent to which microbial reductions achieved by the BSF correlate with reductions in household waterborne illnesses, such as diarrhea.

Acknowledgments

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