MECHANISMS OF MICROBIAL REDUCTION AND IMPLICATIONS FOR DESIGN AND OPERATION OF THE BIOSAND WATER FILTER

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ABSTRACT

MARK A. ELLIOTT: MECHANISMS OF MICROBIAL REDUCTION AND IMPLICATIONS FOR DESIGN AND OPERATION OF THE BIOSAND WATER FILTER
(Under the direction of Francis A. DiGiano)

The biosand water filter (BSF) is a promising point-of-use (POU) technology for household treatment of drinking water in developing countries. Daily batch operation without filter dewatering leads to holding of a large fraction of the filtered water for idle periods typically up to 24 hr. Despite success in implementation and reduction of diarrheal disease, the mechanisms of microbial reduction and the effects of intermittent operation are largely unknown. Previous studies of filter performance have not led to adequate understanding of the dynamic nature of BSF; treatment efficiency varies both during a single charge and over weeks of operation during the maturation process. Additionally, fecal indicator bacteria have been used almost exclusively to assess BSF performance. Therefore, the impacts of BSF operation and design on waterborne virus reductions were unknown prior to the research reported here. This research was aimed at overcoming these deficiencies in well-controlled laboratory experimentation. The main hypothesis was that idle time increases microbial reductions to produce a dynamic pattern over the filtration cycle (a few hours) and over repeated cycles as the filter matures. The specific objectives were to: (1) relate hydraulic condition to reductions of bacteria and viruses; (2) gain insight into the mechanisms of virus reduction; and (3) accordingly propose changes to design and operation. Reductions of bacteria and viruses increased

over weeks of filter maturation. The highest reductions during a daily cycle were observed during filtration of the filter's pore volume, indicating a positive effect of idle period. A deep-bed maturation process referred to as "media aging" contributed to virus attenuation as shown from measurements at a depth of 30 cm during the idle period. The rate of virus attenuation was first-order and increased through 5 to 10 weeks of operation. An active microbial community appears to be responsible; suppression of microbial activity by addition of sodium azide eliminated virus attenuation during the idle period. Grazing and/or production of microbial exoproducts, including proteolytic enzymes, could be pathways. Modifications to design and operation of the BSF are proposed to take advantage of microbial attenuation processes during idle time.

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LIST OF ABBREVIATIONS

ANCOVA – Analysis of co-variance

BSF – Biosand filter

CC – Cane Creek Reservoir (Carrboro, North Carolina)

CAWST – Centre for Affordable Water and Sanitation Technology (Calgary, Alberta)

cfu – colony-forming units

DI – De-ionized water

DO – Dissolved oxygen

DR – Dominican Republic

MDI – Morrill Dispersion Index

NGO – Non-governmental organization

OWASA – Orange Water and Sewer Authority (Orange County, North Carolina)

PE – Primary effluent

pfu – Plaque-forming units

POU – Point of use

PZC – Point of zero charge

RTD – Residence Time Distribution

SAL – Single agar layer

SODIS – Solar Disinfection

SSF – Slow sand filtration

TDS – Total dissolved solids

TOC – Total organic carbon

UC – Uniformity coefficient (d_{60}/d_{10})

UL – University Lake (Chapel Hill, North Carolina)

UN – United Nations

UNICEF – United Nations International Children's Fund

US – United States of America

USD – US Dollars

USEPA – United States Environmental Protection Agency

WHO – World Health Organization

ZVI – Zero valent iron

LIST OF SYMBOLS

a – Filter parameter comprising all filter characteristics included in the Kozeny equation

A_1 – Cross-sectional area at the free water surface above the filter

A_2 – Cross-sectional area at the exit point of filtered water

b – Filter parameter comprising the ratio A_2/A_1 multiplied by parameter a

$C_t/C_{t=0}$ – Ratio of concentration at time, t , during idle time to concentration at start of idle time

d – diameter of filter media used in the Kozeny equation to estimate S_A/V_G

d_{10} – Effective size of a filter sand sample; the 10^{th} percentile diameter

g – Acceleration due to gravity

h – Elevation at the free water surface above the filter (also z_1)

h_L – Head loss between the free water surface above the filter and the filter outlet

$h_{L,o}$ – Clean bed head loss between the free water surface above the filter and the filter outlet

$h(t)$ – Head loss over a daily filtration cycle at time, t

K – Kozeny empirical filter constant

L – Filter media bed depth

p_1 – Pressure at the free water surface above the filter

p_2 – Pressure at the top of the filter outlet tube

Q – flow rate

v_1 – Velocity at the free water surface above the filter

v_2 – Velocity at the top of the filter outlet tube

$v_{2,o}$ – Velocity at the top of the filter outlet tube at the start of a daily filtration cycle

S_A/V_G – Surface area of media per grain volume, d is diameter of media and v_1 is approach velocity t – time (in various units)

z_1 – Elevation at the free water surface above the filter (also h)

z_2 – Elevation at the top of the filter outlet tube

γ – Density of water

ε – Porosity of the filter media

μ – Dynamic viscosity of water

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CHAPTER 1

INTRODUCTION

1.1 Water and Health in Developing Countries

Diarrhea causes nearly 20% of deaths in children globally, about 1.5 million per year, more than AIDS, malaria and measles combined (UNICEF and WHO, 2009). The fraction of deaths and morbidity attributable to unsafe drinking water in the world today is unknown because the pathogens responsible can also be transmitted by hands, food and fomites, as well as by other routes (Curtis et al., 2000). Nevertheless, drinking water treatment has been shown to provide massive benefits to health, both in emergency alleviation during disease epidemics (Logsdon, 2002; Page et al., 2006) and during the development of the United States and Europe (Cutler and Miller, 2005; Mackenback, 2007).

Although source water protection and centralized treatment are the state-of-the art in the developed countries, nearly one-billion people in other parts of the world have access to neither of these (UN, 2008). Even those drinking water sources classified as "improved" still contain dangerous levels of disease-causing microorganisms. Despite the lofty intentions of the United Nations Millennium Goal to halve the proportion of people without sustained access to improved drinking water sources by 2015, centralized treatment is still decades away in many places. Thus, the challenge is how best to protect health by an interim strategy that is both less costly than centralized treatment and less dependent upon governments.

1.2 Point of Use Treatment as Alternative to Centralized Treatment

A viable alternative to centralized treatment and delivery of water in developing countries is point of use (POU) devices that are installed in the home (Sobsey, 2002). Approximately 19 million people were using POU devices in 2007, with annual growth of 25%. Not included are more than 350 million who boil drinking water as another form of POU treatment (Clasen, 2009).

Many different POU treatment technologies are available and no one technology is ideal for all situations. Chemical disinfectants, solar disinfection, coagulant/disinfectant products and a variety of filters are common examples (Sobsey, 2002). The success of a POU implementation is affected by many factors, including: capital cost, recurring costs, simplicity of use, treatment time, volume-treated, treatment efficiency, durability and aesthetics. Local factors such as availability, supply chain, transience of the population, quality of water sources and cultural acceptability must also be considered if a POU implementation program is to have a sustained impact on a community.

Morbidity from diarrheal disease has been significantly reduced by POU devices (Fewtrell et al., 2005; Clasen et al., 2007). The health impact of a POU intervention will depend not only on the treatment efficiency of the selected technology but also on its acceptability in the home, long-term daily use and durability. Based on all of these criteria, POU filters have been suggested to have more potential than other technologies (Sobsey et al., 2008).

1.3 The Biosand Filter: A Promising POU device

The biosand filter (BSF) is a promising POU technology. As of 2007, over 140,000 units had been installed and this is expected to increase annually by about 25,000 (Clasen, 2009). Its construction is simple, consisting of a concrete or plastic housing filled with gravel and sand that are locally available. The cost of a BSF unit, including construction, training, installation and any subsidies, averages 60 to 90 US dollars (USD) (Jeuland and Whittington, 2009). However, unit costs as low as 10-18 USD have been achieved in some settings (Kaiser and Chang, 1996; Fewster et al., 2004).

Well-constructed units should be durable enough to remain in daily operation for ten or more years (Manz, 2007b). However, breakage or leakage from the interface between the outlet tube and the filter housing have occurred in some plastic models. Long-term sustainability is dependent on BSF models that are durable enough to withstand the blows and jolts that are inevitable over years of use in a home.

Locally made concrete housings hold promise in developing micro-enterprises and economic sustainability. A steel mold is required to make the concrete housing and its cost is often subsidized. However, production costs of individual concrete units are generally low. A circular footprint has lowered the production cost even further by decreasing the mass of cement. As an example, profitable manufacturing and distribution of the circular filter design has continued for five years after the implementers left Kenya (Fewster et al., 2004); cost of materials was under 7 USD per filter.

The BSF is very similar to a slow sand filter (SSF). Both operate by gravity without pretreatment or backwashing and both depend on biological maturation (or ripening) for effective removal of contaminants. During maturation, head loss increases

and filter performance improves. The topmost layer of media traps organic and inorganic particles and dead and living microorganisms. Historically, this layer has been called the schmutzdecke, which means "dirty layer" in German and originates from development of the SSF in Germany in the late 19th century. Use of "schmutzdecke" terminology continues in the literature today although it has also been referred to as the biolayer, filter skin, filter cake or colmation layer. However, maturation is not restricted to the topmost layer of the filter but instead extends into the deeper regions of the media bed (Poynter and Slade, 1977; Wheeler et al., 1988; Lloyd, 1973).

The most important design difference between BSF and SSF is in the pattern of water introduction. The BSF is operated intermittently by introducing a single- or a double charge of water (usually 10- to 20 L) rather than continuously as in the SSF. However, water is not allowed to drain from the filter media such that the media bed remains wetted and, as important, a substantial volume of water is stored for the idle period. Obviously, the filtration rate must decline with the decline in water level above the filter media. The combination of the long idle periods between charges and declining filtration rate during operation produce dynamics in microbial reduction that are unique to the BSF.

The initial filtration rate depends upon the charge volume to diameter of the filter and the particle size of the media. The larger the volume of water for a given diameter, the larger is the available head and thus the higher is the initial filtration rate. Particle size influences filtration rate because it affects the head loss within the filter. The filtration rate is typically about 1 m/hr, which is three-to-13 times higher than the recommended filtration rate for conventional SSF (Pyper and Logsdon, 1991). Based on

typical filter diameters, the corresponding initial flow rates range from 0.4-1.1 L/min. The recent trend is toward lower flow rates (Buzunis, 1995; Stauber, 2007; Manz, 2009a; CAWST, 2009). Repeated daily operation of the BSF produces yet another dynamic in microbial reduction in addition to those produced by the long idle times each day and the declining filtration rate during operation. This is due to the development of the schmutzdecke, as well as deep-bed maturation over weeks of daily operation.

Operation of the BSF is limited by head loss development in the schmutzdecke. Eventually, the head loss is so large that the initial flow rate becomes too low to yield sufficient water for daily needs. At this time, the user performs a simple stirring procedure that disturbs the top of the sand to open up pores that have been clogged or blocked. Sand is not removed intentionally and the standing water containing the suspended schmutzdecke material is discarded.

1.3.1 Health Improvements with Use of BSF

Household implementation of BSF in developing countries has been shown to produce large reductions in diarrheal illness. For example, reductions in diarrheal disease of 40% or greater were documented in studies conducted in the Dominican Republic (DR) (Stauber et al., 2009), Cambodia (Liang, 2007) and Kenya (Tiwari et al., 2009). The reliability and simplicity of the BSF suggest that these reductions can be sustained.

Over 85% of BSFs are still in operation after 0.5-to-8 years of service (Aiken et al., 2010; Duke et al., 2006; Liang, 2007). Far lower levels of sustained use have been reported for other POU technologies (Sobsey et al., 2008). Additionally, cost-benefit

analysis has shown that the BSF compares favorably to other health interventions (Jeuland and Whittington, 2009).

Despite evidence of improved health, sustained use and cost-benefit, the fundamental mechanisms by which BSF removes microbes are still poorly understood. This is because most of the observations of performance have been made at the field level where it is impossible to control operating conditions such as the feed composition, daily charge volume and frequency and method of cleaning. Additionally, fecal indicator bacteria have been used almost exclusively to assess BSF performance. As a result, reduction of viruses and the operational and design features that are important were unknown prior to the research reported here. Viruses indeed should be important to remove because they are a major cause of diarrhea worldwide, accounting for about 40% of cases in developing countries (Ramani and Kang, 2009; Crittenden et al., 2005). Rotavirus infection alone is estimated to cause 6% of deaths worldwide in children under the age of five (Parashar et al., 2003). Along with other waterborne viruses, they are present in surface and ground water and can survive for weeks (Espinosa et al., 2008; Maunula et al., 2005; Lodder et al., 2005).

1.3.2 Goal and Objectives

The goal of this research was to understand the mechanisms responsible for microbial reductions (both bacteria and viruses) in the BSF treatment process in order to improve upon its design and operation. This goal was achieved by conducting microbial challenge experiments with BSF, both in full-scale and in smaller, laboratory-scale units wherein detailed exploration of microbial reduction mechanisms was possible.

The initial set of objectives were to: (1) characterize hydraulic operation; (2) measure the reduction efficiency of *E. coli*, bacteriophages and echovirus 12 under controlled laboratory conditions; (3) gain insight into the effect of filter idle time and daily volume filtered on microbial reductions; and (4) evaluate the impact of filter media properties on microbial reductions. The focus then shifted to understanding reduction of viruses, the most recalcitrant class of pathogens for BSF treatment (Elliott et al., 2008). The objectives were: (1) evaluate the impact of daily volume filtered and idle time on virus reductions; (2) assess the importance of media aging, a deep-bed maturation process, on virus reductions during idle time; and (3) elucidate the role of microbial activity on media aging and subsequently on virus reductions in the BSF.

1.4 Organization of Dissertation

Chapters 3-6 are written in the form of manuscripts. They are preceded by a review of the relevant literature in Chapter 2 and followed in Chapter 7 by conclusions and recommendations for use of the BSF in practice and for modification of design to improve performance. Chapter 3 has already been published (Elliott et al., 2008) and is reprinted here with copyright permission for journal authors. Chapter 4 is a draft manuscript for journal submission having already been published as part of a conference proceedings (Elliott et al., 2009). Chapter 5 is a draft manuscript for journal submission describing virus reductions during idle time and the effects of media aging. Chapter 6 is a draft manuscript for journal submission that explains the role of microbial activity in mediating virus reduction during idle time in the BSF.

A listing of publications and conference presentations to date from the research is provided in Appendix A.

CHAPTER 2

LITERATURE REVIEW

2.1 Drinking Water Treatment at the Point of Use

About one billion people worldwide do not have access to an improved water source (WHO, 2004). Although the goal for developing countries is to develop a centralized treatment and piped delivery system, this will not be achieved for decades. In the meantime, these people must obtain their own drinking water wherever they can, often from contaminated, unsafe ground and surface water sources. Most drink untreated water because it is their only option in the absence of piped water service. Point-of-use (POU) treatment, however, offers an option that can be readily installed. The most common method is to boil drinking water and this method is used by over 350 million people worldwide (Clasen, 2009). However, boiling is expensive, time-consuming and often leads to environmental and indoor air quality problems (Clasen et al., 2008).

Other POU treatment devices offer a viable option to boiling water. They have already been shown effective in reducing diarrheal disease by 30-40% (Fewtrell et al., 2005; Clasen et al., 2007). This finding is at odds with the long standing belief that the most effective interventions are increased water quantity and good practices of sanitation and hygiene (Esrey et al., 1991; Esrey et al., 1985). However, the explanation may be that previous intervention studies have focused on improved water quality at the source rather than at the point of use. In fairness, the relative importance of all intervention

strategies remains widely debated (Clasen and Cairncross, 2004; Schmidt and Cairncross, 2009; Ford, 1999; Curtis et al., 2000; Esrey et al., 1991).

Affordability of POU technologies is a major concern for the poor who live in developing countries. The most common POU technologies reflect the major unit processes of conventional water treatment: coagulation, filtration and disinfection. Typically only one of these processes may be included although some do combine two or more. Detailed description of available POU technologies and their levels of use worldwide can be found elsewhere (Sobsey, 2002; Clasen, 2009; Lantagne et al., 2006).

2.1.1 Health Impact and Sustained Use of POU Technologies

Reductions of both microbiological contamination and diarrheal disease for various POU technologies (chlorine disinfection; solar disinfection (SODIS); combined coagulation-disinfection; ceramic filtration; and intermittent slow sand filtration) have been recently summarized (Sobsey et al., 2008). Typical reductions of microbiological pathogens and surrogate organisms for these technologies are included in Table 2.1; they range from 0.5- to 6-log (all log-reductions are base ten), depending on the technology and the class of pathogen. For example, filtration technologies are credited with greater reductions of protozoan parasites than viruses.

While POU technologies vary in their ability to reduce microbes, epidemiological studies indicate that all of them give about a 30-40% reduction in diarrheal disease (Fewtrell et al., 2005; Clasen et al., 2007). However, the epidemiological studies have had shortcomings, most notably their short-timeframes and lack of blinding (Schmidt and Cairncross, 2009). The bias introduced by these shortcomings may have impacted

Table 2.1 Popular POU technologies: estimated baseline and maximum log microbial reductions.

Treatment Process	Pathogen Group	Baseline LRV[1,2]	Maximum LRV[3]	Factors influencing performance efficacy
Porous ceramic filtration	bacteria	2	6	Pore size/structure, tortuosity, flowrate, filter medium composition, augmentation with silver or other chemical agents (Sobsey, 2002; Brown et al., 2007; Brown, 2007).
	viruses	0.5	4	
	protozoa	4	6	
Biosand filtration(BSF)	bacteria	1	3	Filter maturity, dosing conditions, flowrate, idle time, time between charges, grain size; challenge viral agent (Elliott et al., 2006 and 2008; Stauber et al, 2006; Palmateer et al., 1999).
	viruses	0.5	3	
	protozoa	2	5	
Solar Disinfection (SODIS)	bacteria	3	5.5+	Water oxygenation, sunlight intensity, exposure time, temperature, turbidity and water depth (Sobsey, 2002; Wegelin et al., 1994; Reed, 1997; Kohn and Nelson, 2007; McGuigan et al, 2006).
	viruses	2	4+	
	protozoa	1	3+	
Free chlorine disinfection	bacteria	3	6+	Turbidity and chlorine demand; concentration × contact time (Crittenden et al., 2005; Sobsey, 1989 and 2002).
	viruses	3	6+	
	protozoa[4]	3	5+	
Coagulation/chlorination	bacteria	7	9	Physical removal of chlorine-resistant pathogens by coagulation--flocculation; turbidity; challenge viral agent (Souter et al., 2003; Sobsey, 2002).
	viruses	2-4.5	6	
	protozoa	3	5	

(1) LRV: Log_{10} reductionvalue log10(pretreatment conc)-log10(post-treatment conc).

(2) BaselineLRV: LRV typically expected in actual field practice when done by relatively unskilled persons who apply the treatment to waters of varying quality and where there are minimum facilities or supporting instruments to optimize treatment conditions and practices.

(3) Maximum LRV: LRV possible when treatment is optimized by skilled operators who are supported with instrumentation and other tools to maintain the highest level of performance in waters of predictable and unchanging quality

(4) Minimally effective in reducing concentration of infectious *Cryptosporidium parvum* oocysts.

Adapted from Sobsey et al, 2008.

over half of the reported diarrheal disease reductions in POU studies (Hunter, 2009). Despite debate about interpretation and magnitude of diarrheal reductions by POU devices, the balance of evidence indicates that drinking water treatment in the home can yield sustained positive benefits to health.

Both design and operational considerations could account for differences in the success of POU interventions. They include variations in: treatment efficiency; uptake; use over sustained periods; and durability of the technology. The setting is also very important. For example, the success of a POU technology in a disaster-relief or refugee-camp setting may not predict its suitability for providing safe drinking water over the long term in a household.

Failure to achieve sustained use of POU devices is linked to: high recurring costs;, lack of aesthetic appeal (appearance, taste and odor); burdensome or time-consuming treatment steps; inadequate volume of water treated per day; no local supply chain for consumables or broken parts; and inability to treat locally available water (e.g. filter clogging caused by high turbidity) (Sobsey et al., 2008). A scoring system for the projected sustainability of popular POU technologies is included as Table 2.2. POU filters appear to show more potential for long-term, daily sustained use than other popular POU technologies (Sobsey et al., 2008). Of the available filtration technologies, ceramic candle and ceramic pot filters were reported to be popular, but filter breakage led to high disuse rates (Clasen et al., 2006; Brown et al., 2007, 2008 and 2009).

The sustainability of the biosand filter (BSF) is of particular importance to this research project. In contrast to other POU technologies, BSF installations in the

Table 2.2 Scoring of POU treatment technologies based on sustainability criteria.

Technology	Water Quantity[1]	Water Treatability[2]	Ease of Use[3]	Cost[4]	Need for Supply Chain[5]	Overall Score
Free chlorine liquid	3	1	3	3	1	11
Free chlorine tablets	3	1	3	2	1	10
Coagulation/chlorination	2	3	1	1	1	8
SODIS	1	1	1	3	3	9
Ceramic filters	2	3	2	3	2	12
Biosand filters	3	3	2	2	3	13

(1) Water Quantity: Water quantity produced

3 – high – one dose or one treatment process can be employed to produce 20L per 4 hours

2 – medium – two-four units would be necessary to produce 20L per 4 hours

1 – poor – five or more units are needed to produce 20L per 4 hours (increases user processing time or increases the chances that user will rely on additional untreated sources of water)

(2) Water Treatability: Ability to treat high turbidity/organic matter water

3 – removes turbidity and maintains similar microbial reduction efficacy under poor water quality conditions

2 – does not remove turbidity but still maintains similar microbial reduction efficacy under poor water quality conditions

1 – does not remove turbidity and microbial reduction efficacy is reduced under poor water quality conditions

(3) Ease of use: Maintenance, treatment time and treatment steps

Ease of process:

1 = if treatment is a single step process

0 = if treatment is a multiple step process

Time for water:

1 = if 1 L is treated within 30 min time

0 = otherwise

Maintenance

1 = if no maintenance is required

0 = if technology requires periodic maintenance above cleaning the water storage container

(4) Cost: Cost per L to treat 20L of water a day for one year, including capital and recurring costs (USD)

3 = if $0.001 or less

2 = if $0.001 – 0.01

1 = if > $0.01

(5) Need for Supply Chain: Supply chain requirements for continued use

3 = no supply chain needed for continued use

2 = periodic replacement or replacement parts needed

1 = constant supply chain needed to support continued use

Adapted from Sobsey et al., 2008

Dominican Republic, Haiti and Cambodia were still in regular use in 87-92% of homes six months to eight years after installation (Aiken et al., 2010; Duke et al., 2005; Liang, 2007; Aiken, 2008). In contrast to other POU technologies, no significant decrease in filter use with time since installation was observed (Sobsey et al., 2008; Aiken et al., 2010; Liang, 2007; Aiken, 2008).

User training and education can also be critical components of sustainable POU treatment. However, there have been no systematic attempts to compare the impact of different implementation and training programs on sustained use.

2.2 Description of the Biosand Filter

In addition to high sustained-use, diarrheal reductions of greater than 40% have been reported for the BSF in a variety of settings (Stauber et al., 2009; Tiwari et al., 2009; Aiken et al., 2010; Liang, 2007). Considering its durability, sustained use and proven reductions in diarrheal disease, BSF implementation programs are likely to yield greater sustained health impact than implementation of many other popular POU devices. However, reported microbial reductions in laboratory and field settings have been lower than in some other POU technologies (Table 2.1) and have generally not met USEPA standards for POU treatment devices (Schaub and Gerba, 1991).

2.2.1 History and Implementation

The BSF concept was developed in the late-1980s by Dr. David Manz, of the University of Calgary, Department of Civil Engineering. In essence, it is an adaptation of the slow sand filtration (SSF) process to the household scale. Testing in the laboratory

and the field began in 1991 and the design was refined; the concrete BSF was patented in 1993. A cross-sectional view of a concrete BSF designed with a square footprint is shown in Figure 2.1. The first BSFs to be used in homes were installed in Nicaragua in 1993. Laboratory research on their effectiveness for bacterial reductions was conducted later at the University of Calgary and published in a Master's thesis (Buzunis, 1995). Subsequent BSF designs include circular designs using concrete (Figure 2.2) and plastic housings (Figure 2.3). Concrete designs afford the potential for sustainable local production; whereas plastic designs must be manufactured at a central location but are far lighter and can be stacked for shipping.

Numerous faith-based and other development organizations have installed the BSF in dozens of countries. As of 2007, there were over 140,000 units and this is expected to increase annually by about 25,000 (Clasen, 2009). Although post-disinfection of filtered drinking water is recommended, hundreds of thousands of people are relying solely on the BSF for their drinking water treatment.

2.2.2 Design and Operation

Despite widespread implementation, the BSF process has not been investigated systematically in order to understand how the design and operating parameters affect microbial reductions. Foremost is the absence of information about reductions in viruses given that bacterial reductions have been used almost exclusively to evaluate performance. There are important similarities in reduction of pathogens between the BSF and conventional SSF process (Fox et al., 1994) from which much knowledge can be gained. However, the design and operational parameters of the BSF differ considerably

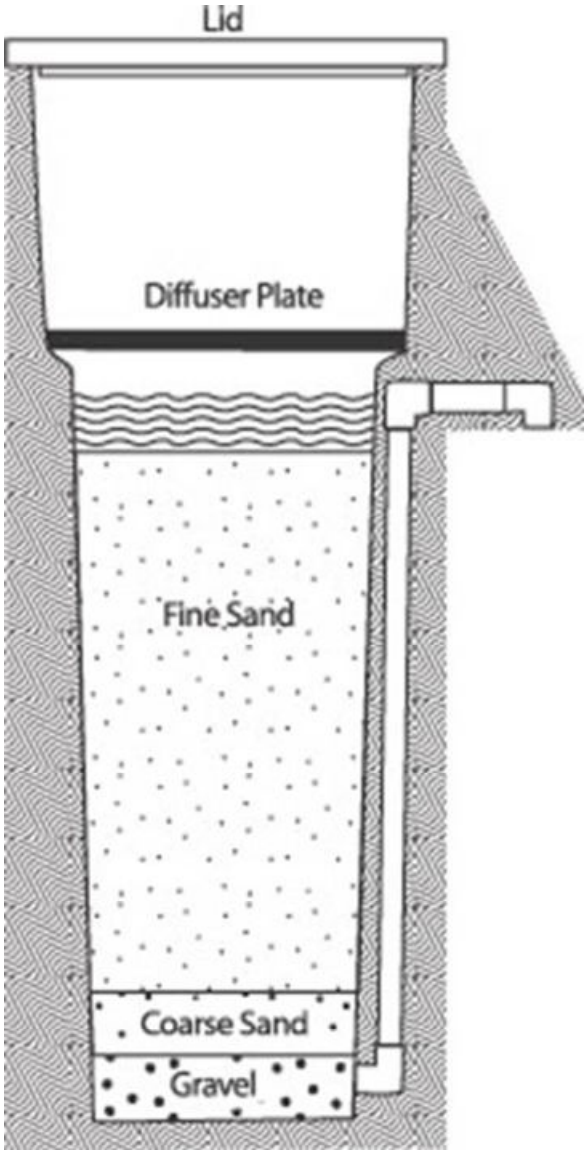

Figure 2.1 Cross-section of a concrete BSF with a square footprint.
Source: CAWST.org

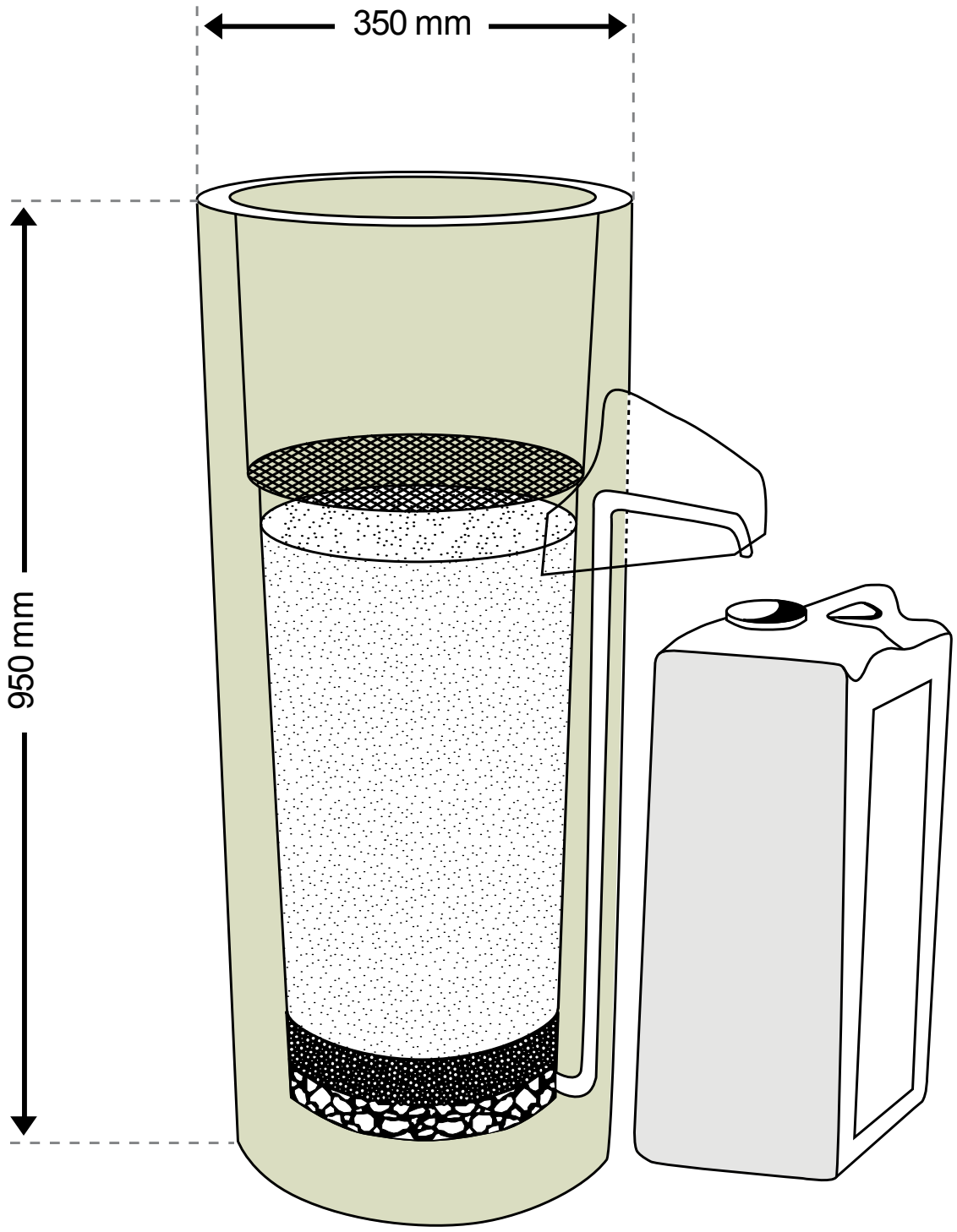

Figure 2.2 Diagram of a concrete BSF with a circular footprint.
Modified from: tearfund.org

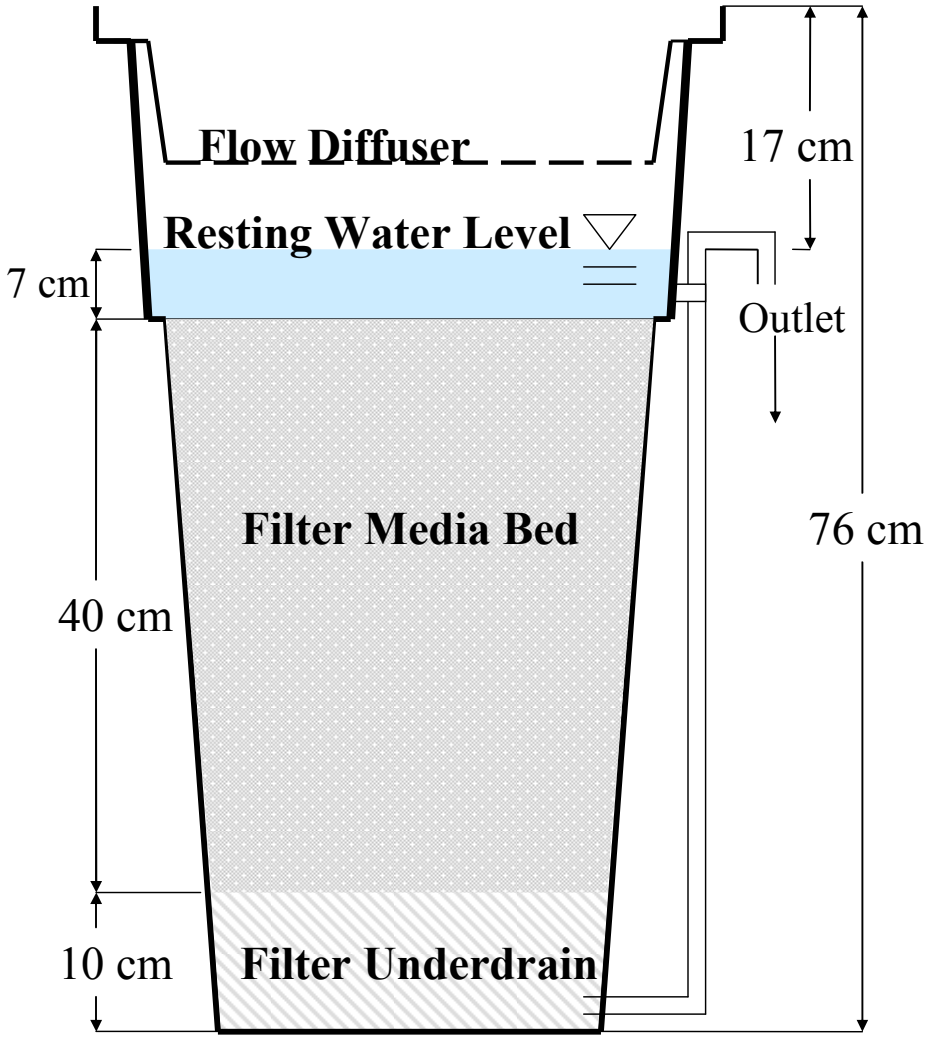

Figure 2.3 Cross-section of a plastic BSF model made by Davnor Water Treatment Technologies, Ltd.

from conventional SSF and therefore deserved far greater attention in systematically controlled experimentation.

The design and operational features of the BSF and conventional SSF are compared in Table 2.3. The range of operational parameters listed for the BSF has evolved through rather limited experience in developing countries. The BSF is similar to conventional SSF in that there is no pretreatment or backwashing. Operation is simple, particularly because filtration is by gravity rather than pressure filtration. As in conventional SSF, the sand bed remains wetted throughout operation and a maturation process occurs, during which a schmutzdecke forms, head loss increases and performance improves. Deep-bed maturation processes, which have been referred to in SSF as media aging, improve performance with very little corresponding head loss (Poynter and Slade, 1977; Wheeler et al., 1988).

Table 2.3 Recommended range of design parameters for conventional SSFs compared to Davnor "60-Liter" plastic BSF and the square-footprint concrete BSF.

		Conventional SSF Guidance[1]			Biosand Filter	
		Ten States Standards	Huisman & Wood	Visscher et al.	Plastic	Concrete
Filtration Rate	m/hr	0.08 - .24	0.1 - 0.4	0.1 - 0.2	0.0-0.9*	0.0-1.2*
Depth of sand						
Initial	m	0.8	1.2	0.8 - 0.9	0.4	0.5
Minimum	m	--	0.7	0.5 - 0.6	0.4	0.5
Depth of supporting media & underdrains	m	0.4 - 0.6	--	0.3 - 0.5	0.13	0.1
Height of filter box above sand surface	m	≥ 0.9	1 - 1.5	1	0.05-0.22*	0.02-0.30*
Freeboard	m	--	0.2 - 0.3	--	0.0-0.17*	0.0-0.28*

[1]From Pyper and Logsdon, 1991

* These parameters vary within this range during one charge due to intermittent operation.

The most important difference between BSF and SSF is the feed flow pattern. The filter is fed intermittently rather than continuously by introducing a single charge of water (perhaps only one per day). The typical charge is 10-to-20 L after which the water level above the filter media declines. The BSF operates, therefore, in the declining rate mode of filtration.

A mathematical model is not available in the literature to describe the decline in flow rate with time in the BSF process after a batch charge is introduced. Instead, a conceptual approach was developed at the outset of this research as a way to understand the flow rate pattern in subsequent BSF experiments. The resulting model is shown in the Appendix. It is based on combining the Bernoulli equation to describe the energy head between the surface of the charge volume above the filter and the filter outlet with the Kozeny equation to describe head loss through the filter media. The final form of the equation (Eq. B-18) is:

$$t = \frac{c}{gA_2}(Q_0 - Q) + a\left[\ln\frac{Q_o}{Q}\right] \qquad (2\text{-}1)$$

where t is time of filtration; c is the ratio of the filter-to-outlet cross-sectional areas ; g is the gravitational constant; A_2 is the cross-sectional area of the outlet; Q_o is the initial daily flow rate; Q is the flow rate at time, t; and a is a constant that combines all the constants in the Kozeny equation:

$$a = K\frac{\mu}{\rho g}\left(\frac{(1-\varepsilon)^2}{\varepsilon^3}\right)\left[\frac{S_A}{V_G}\right]^2 L \qquad (2\text{-}2)$$

where K is the Kozeny constant (empirical value taken from the literature); μ is dynamic viscosity; ε is porosity; L is filter depth; and S_A/V_G is surface area of media per grain volume. The last term can be replaced by $6/d$ for spherical media and $7.5/d$ for angular

media, where d is the particle diameter. None of the manuscripts either published to date or planned for publication as presented subsequent dissertation chapters include this model. This is because the model needs much more testing to show its validity. It is included here and in the Appendix, however, to promote further investigation.

Eq. 2-1 was used to describe a set of flow rate-time data that was collected in a clean BSF (see Figure 2.3 for features) during this research. All the constants in Eq. 2-1 are known physical parameters or can be estimated from filter characteristics. The constant, a (Eq. 2-2), for substitution into Eq. 2-1 required standard assumptions from filtration hydraulics: K was set to 5 (Crittenden et al., 2005); S_A/V_G was calculated for angular media ($7.5/d$) wherein the effective size of the sand, d_{10} (0.22 mm), was used; ε was measured as 0.42 for the particular BSF unit; and L was 40-cm. The resulting value of a was 1216 s. In addition to these parameters, the values of c and A_2 for the BSF design were 457 (dimensionless) and 1.87 cm^2, respectively. The additional assumption was that the head loss accumulated during a charge is small enough to not affect any of these constants. As shown in Figure 2.4, the model appears to fit the data fairly well for a clean filter. However, caution is warranted because the model prediction is extremely sensitive to assumptions about the values of d and ε. More experimental work is necessary to verify the model under different experimental conditions.

The practical value of Eq. 2-1 would be greatly extended if it could be used to predict Q vs. t during each daily charge as the schmutzdecke progressively accumulates. Schmutzdecke development is reflected by the decline in Q_o over repeated daily charges. A crude empirical approach would be to increase the constant, a, in Eq. 2-2 to account for a decrease in ε, an increase in S_A/V_G and/or an increase in K as the schmutzdecke

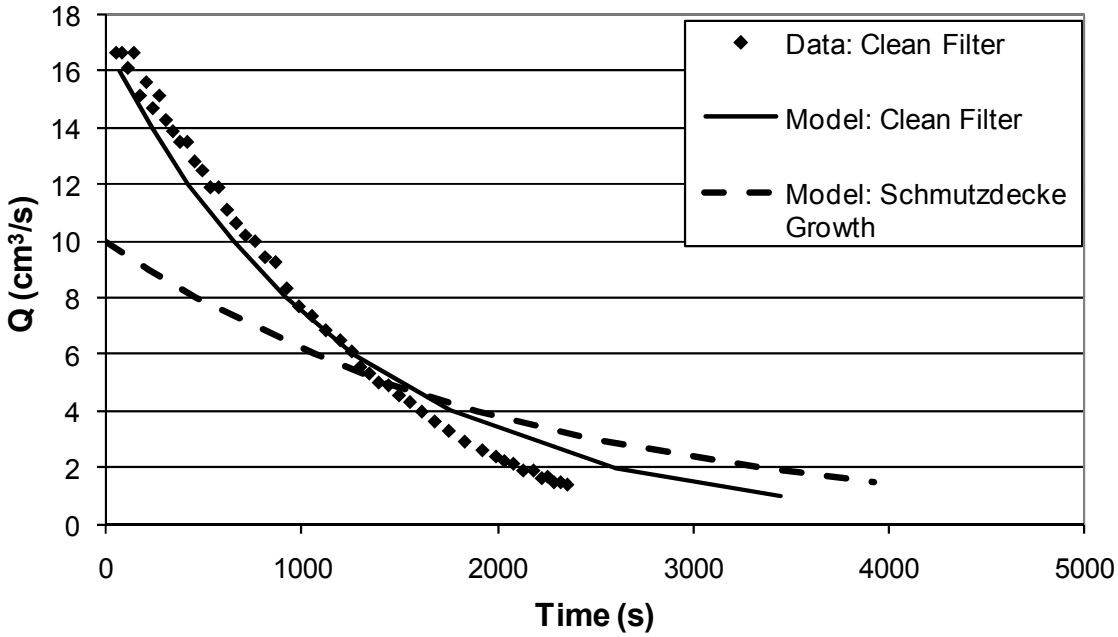

Figure 2.4 Relationship between flow rate and time in BSF. Data and model simulation for a clean BSF; model simulation following schmutzdecke growth.

develops. For illustrative purposes only, Q_o was reduced from 17 to 10 cm^3/s to represent an arbitrary level of schmutzdecke formation and the value of the constant, a, was increased by 70%. The resulting model prediction of Q vs. t for the daily charge corresponding to this initial flow rate and value of constant a is shown in Figure 2-4 as the series "Model: Schmutzdecke Growth." As expected, the filtration time is far longer than for a clean bed. This pattern is consistent with observations in all BSF experiments reported in Chapters 3-5. The approach to modification of Eq. 2-1 to account for an increase in the value of a with filter maturation was not pursued in this research because investigation of mechanisms of microbial reduction was of a much higher priority. Moreover, a more sophisticated model would be needed to account for preferential accumulation of head loss in the topmost layer of the filter. Fitting the current model to filtration data gives a value of a that is depth-averaged whereas a bi-layer model may be

step toward more realistic simulation. A future research area could involve relating the constant, *a*, to schmutzdecke development.

Recommendations for initial flow rates at maximum head vary from 0.4-1.1 L/min, with a trend in recent guidance from implementers toward lower rates (Buzunis, 1995; Stauber, 2007; Manz, 2009a; CAWST, 2009). The maximum filtration rate recommended for BSF is about 1.2 m/h, 3-to-15 times greater than for SSF (Fox et al., 1994; Pyper and Logsdon, 1991). The depth of sand is 0.4 m, which is about 50% less than typical for SSF. Thus, the combination of higher application rate and shorter depth produces a contact time at initiation of each filtration cycle that can be 25 times shorter than in SSF. However, the majority of water filtered in BSF will have a longer residence time in the media bed than in SSF. Residence time increases as the charge is filtered and elevation head declines. Much more important than the increase in residence time during filtration is the storage time of water within the filter during the idle period between charges. Most of a 20-L charge will be retained within the pore volume over this period that typically lasts about 18 hr

Two unique features of the design of the BSF are shown in Figures 2.1 through 2.3. An elevated outlet tube (2- to 7 cm above the height of the filter media) allows the media to remain saturated after a charge has been filtered and the filter remains idle until the next charge. The entry point of the outlet tube is screened in most BSFs made of plastic but is generally not screened in those with a concrete housing. The other unique aspect is a plastic or sheet metal plate (or box-like structure) with 2-mm diameter holes that is located just above the surface of the sand layer and is typically referred to as a

"diffuser." The purpose of the diffuser is to distribute the feed water charge uniformly across the sand surface without disturbing the schmutzdecke.

With repeated daily filtration cycles, the schmutzdecke layer eventually develops to cover the media bed and severely limits the filtration rate. At this time, the user disturbs the top of the sand to open up pores that have been clogged or blocked by the schmutzdecke and accumulated suspended matter. Sand is not removed intentionally. The dirty supernatant water is then removed and the filter placed in service again. Following cleaning, most filter implementers recommend that the BSF be flushed with source water for several days before product water is consumed. This delay is intended to allow for both re-establishment of the schmutzdecke and for flushing of any contaminants mobilized during cleaning. In practice, filtered water is often consumed shortly after cleaning, especially in the absence of other improved drinking water sources.

2.2.3 Microbial Reductions

The majority of research on BSF performance has been conducted in the field by the implementing organization. Filter performance was usually evaluated based on bacterial reductions and turbidity. Sampling methodology is inconsistent across studies and often cannot be determined from reports. Field research on the BSF is recounted in detail elsewhere (CAWST, 2008b; Stauber, 2007).

A summary of microbial reduction or removal achieved in BSF laboratory studies is given in Table 2.4. "Microbial reduction" is used both here and throughout the remaining chapters of the dissertation to describe a decrease in the concentration of culturable or infectious microorganisms during treatment. This decrease could be caused

by sequestration within the filter and/or loss of culturability/infectivity. By contrast, the term "microbial removal" is used for presence/absence assays when all microorganisms are enumerated regardless of culturability/infectivity (e.g., direct counts by microscopy; molecular methods that enumerate DNA, RNA or surface proteins; or assays for a radiolabeled cell component). In this research, the focus is on the effectiveness of BSF treatment to decrease the potential for human infection. Thus, measurements of microbial reduction are a better estimate of effectiveness. The majority of reports deal with reductions of *E. coli*, fecal coliform and total coliform bacteria (Buzunis, 1995; Donnison, 2004; Lee, 2001; Baugartner et al., 2007). A few others deal with removal of protozoan parasites (Palmateer et al., 1999; Koksal et al., 2008; Chuang et al., 2009) and Hepatitis A virus (Sattar, 1998). Protozoan parasite removal (Palmateer et al., 1999) has been reported to exceed the 3-log USEPA standard (Schaub and Gerba, 1991). However, reductions of bacteria and viruses have not met the USEPA reduction standard of 6-log for bacteria and 4-log for viruses (Schaub and Gerba, 1991). Virus treatment was limited to investigations in one setting under a single set of operational conditions. Moreover, as can be seen from Table 2.4, the study designs for evaluating reductions have varied widely. In particular, the microbial reductions achieved prior to biological maturation of the filter have been largely ignored. As a result, definitive conclusions about microbial reductions in the BSF process cannot be made from the published literature.

Table 2.4 Summary of peer-reviewed and grey literature on BSF laboratory studies.

Reference	Summary of Study	Reported Percent Microbial Reductions or Removal
Buzunis, 1995	Tested filter for 2.5 months; dosed daily with environmentally contaminated surface water.	96% (range 99.7-91.1%) for fecal coliforms during sampling on days 10-42.
Sattar, 1998	Filter dosed with 60 L of water with high algal content; dosed for 28 days with 20 L surface water. Hepatitis A virus dosed onto filter; bacteria measured were naturally occurring.	89.8% for total coliforms (n= 4); 87.4% for fecal coliforms (n = 4); 66% for Hepatitis A virus (n=3)
Palmateer et al., 1999	Filters dosed with surface waters for two weeks. Chemicals and microorganisms were dosed. One-time dose of 10^6 *Cryptosporidium* and 10^5 *Giardia* then sampled. Naturally occurring bacteria measured.	>99.999% removal for *Giardia* cysts (n=1); 99.98% *Cryptosporidium sp.* oocysts. 83% reduction for heterotrophic plate counts bacteria (n = 5).
Lee, 2001	Filter dosed for 45 days with 20 L surface water then sampled for two weeks. Naturally occurring bacteria sampled.	99.5% for fecal coliforms in laboratory study (n=5)
Donison, 2004	Filters dosed with 5 L of a 1:10 mix of wastewater to river water for 29 days.	90% *E. coli* in laboratory study (range 52-97%) (n=7)
Baumgartner et al, 2007	Filter dosed with 10 L or 20 L per day. Samples taken at 5-L, 10-L and 20-L filtered. 12-hr and 36-hr idle times examined. *E. coli* and Total coliforms dosed to feed water.	73-84% for total coliforms. Greater bacterial reductions when less volume filtered. E. coli concentrations were too low to observe significant differences.
Koksal et al, 2008	Filter dosed with 20 L per day. Cryptosporidium oocysts spiked for four three-day periods.	>99.999% removal of *Cryptosporidium* oocysts; none were detected in product water. However, spike concentrations in a clean filter bed were only high enough to observe about 99%.
Chuang et al, 2009	Bench-scale filters charged with 1.5 pore volumes per day. 4.5-mm fluorescent microspheres, surrogates for Cryptosporidium oocysts, were spiked for two three-day periods.	99.9% average removal of 4.5-mm microspheres through one-to-two cleaning cycles. Some flushing of microspheres post-cleaning was observed, but always exceeded 99%.

Sources of project summaries: CAWST, 2008b and Stauber, 2007

Adapted from Stauber et al, 2007

2.2.4 Knowledge Gaps between Design/Operation and Performance

Design/operation is linked to performance through better understanding of the microbial mechanism(s) that could account for reductions in bacteria and viruses. The design and operational parameters that are related to microbial processes are: (1) length of time to develop a fully matured (ripened) filter; (2) daily idle time; (3) daily volume charged; (4) cleaning procedure; (5) type of filtration media; and (6) filtration rate. The most important of these are probably idle time and daily volume charged. Idle time typically accounts for greater than 80% of the daily cycle whereupon various microbial attenuation processes are likely to be significant. The importance of daily charge is in relation to the volume of media within the BSF and the idle time effect. That is, the smaller the daily charge relative to the media volume, the larger is the fraction of water stored during the idle time and delivered to the user upon introducing the next charge. The effect of these parameters on microbial reductions has only been addressed in one study of total coliform bacteria (Baumgartner et al., 2007). Total coliform bacteria are of limited use as a surrogate for pathogenic bacteria because of their propensity to multiply in granular media. For all intents, understanding of the effects of idle time and daily charge on bacterial reductions is limited. Even more limiting is understanding of virus reductions. A single virus (Hepatitis A) has been challenged to a mature BSF under only one set of experimental conditions; the reduction was 66% using a 20-L daily charge in a concrete filter with a typical pore volume of 11-15 L (Sattar, 1999).

Reductions of protozoan parasites have been addressed but not in the context of the effects of idle time and daily charge (Palmateer et al., 1999; Koksal et al., 2008; Chuang et al., 2009). The investigations by Koksal et al. and Chuang et al. were

performed at the University of North Carolina using the same full-scale and bench-scale BSF as to be discussed in this research. Protozoan parasite removal has been reported to average more than the 3-log USEPA standard, with typical reductions exceeding 4-log (Palmateer et al., 1999; Koksal et al., 2008). The removal of 4.5-μm diameter fluorescent microspheres was measured between days 20-54 of a bench-scale challenge experiment. Filter cleaning led to temporarily higher concentrations of microspheres in product water, but overall removal still exceeded 3-log (Chuang et al., 2009).

2.2.5 Modification for Arsenic and Fluoride Removal

The BSF has been modified to accomplish removal of arsenic (Ngai, 2002) and fluoride (Hillman, 2007) from groundwater. Arsenic was successfully removed in Nepal by inserting rusted iron nails on top of the diffuser to enable arsenic sorption (Ngai et al., 2007; Ngai, 2002). However, groundwater chemistry has been shown to play a large role in the arsenic sorption capacity of the iron from nails. A versatile, long-term solution may not be achievable (Chiew et al., 2009) although it may still be beneficial for some regions in the short-term. Other POU devices such as the Grainger Prize-winning SONO filter (Hussam and Munir, 2007) are comparably priced and appear to be more versatile. Fluoride removal required addition of bone charcoal, baked lateritic clay, or lateritic clay bricks to the filter media. The fluoride sorption capacity of each candidate medium was quickly exhausted, necessitating frequent replacement that interrupted the filter cycle (Hillman, 2007).

2.2.6 Summary of Laboratory and Field Assessments

The BSF process has been widely adopted and it has continued in operation over long sustained periods. Diarrheal disease reductions have been shown in numerous settings (Stauber et al., 2009; Tiwari et al., 2009; Aiken et al., 2010; Liang, 2007; Duke et al., 2006). However, laboratory investigations have revealed that the USEPA reduction standard of 6-log for bacteria and 4-log for viruses has not been met even though the 3-log standard for protozoan parasites can be met (Schaub and Gerba, 1991). While success shown in field installations justifies continued implementation, more research is needed into possible design modifications to improve reductions. However, these modifications should not be recommended at the sacrifice the convenience, aesthetics and ease of implementation that have made the BSF an effective solution to the problem of unsafe drinking water.

Understanding the mechanisms of microbial reductions is one of the key knowledge gaps that needs to be filled in order improve the BSF design. Despite differences in feed pattern (continuous vs. batch) and application rates (low vs. high) between SSF and BSF, there are strong similarities that offer a logical starting point for insight into the underlying mechanisms of BSF. Toward this end, the next section is devoted to a survey of salient characteristics of the SSF process.

2.3 Slow Sand Filtration

2.3.1 History and Background

Systematic proof of the ability of SSF to protect human health was first demonstrated during the 1892 cholera epidemic that occurred in Hamburg and Altona.

Both cities used the Elbe River as a source of drinking water. The intake for Altona was located downriver from the Hamburg sewer outfalls. Slow sand filtration was used in Altona but not in Hamburg. The much lower death rate in Altona than Hamburg (2.1 vs. 13.4 deaths per thousand) can be attributed to the microbial reductions achieved in the SSF (Manson, 1914).

SSF became the essential component of water treatment in American cities during the early-20th century (Cutler and Miller, 2005). Waterborne disease was rapidly reduced as a result. SSF was later replaced in most US cities by rapid sand filtration (RSF) preceded by coagulation mainly because RSF requires a much smaller footprint than SSF. This meant greater reliance on chemical disinfection before distribution of water to achieve acceptable microbial reductions. Today in the US, SSF is primarily used to treat small-community water supplies. However, it is still used in at least five major European cities. In the UK, 20 to 25% of potable water is treated by SSF (Page et al., 2006).

Typical design and operational parameters of SSF and RSF are compared in Table 2.5. In addition to these parameters, SSF differs from RSF in the following major ways: (1) lack of pretreatment with coagulants or with disinfectants that would interfere with biological activity in the filter; (2) formation of a "dirty skin" (commonly referred to as the schmutzdecke) on top of the sand that is composed of organisms, particles and detritus; and (3) cleaning by scraping or harrowing rather than backwashing (Haarhoff and Cleasby, 1991).

Table 2.5 Typical design parameters of slow and rapid sand filters.

	Slow sand filters[1]	Rapid sand filters[1]
Filtration rate (m/h)	0.1	10
Supernatant water (m)	1.5	1.5
Sand depth (m)	0.8	0.8
Retention time above sand (hr)	15	0.15
Retention time in sand bed (min)	180	2
Cleaning frequency (d)	30-180	1-4

(1) Values should be considered typical and representative, not exact or prescriptive.

Adapted from Haarhoff and Cleasby, 1991

2.3.2 Mechanisms of Microbial Reduction

Both biological and physical/chemical mechanisms have been implicated in reductions of pathogens in SSF (Haarhoff and Cleasby, 1991; Hendricks and Bellamy, 1991). Greater reductions are associated with more biological activity; warmer temperature; smaller diameter of sand grains; longer time in operation; and lower filtration rate (Poynter and Slade, 1977; Wheeler et al., 1988; Haarhoff and Cleasby, 1991; Bellamy et al., 1985a and 1985b; Hendricks and Bellamy, 1991; Hijnen et al. 2004; Pardon and Lloyd, 1994). The effects of these factors on microbial reductions are consistent with the importance of microbial mechanisms. However, physical/chemical filtration mechanisms have also been implicated in the maturation processes (Weber-Shirk, 2002; Weber-Shirk and Chan, 2007). In these studies, naturally occurring chemical species, including aluminum, were shown to enhance particle capture by changing the surface charge or structure of sand grains. In fact, proponents of the physical-chemical mechanism claim it is far more important than the biological mechanism (Weber-Shirk and Dick, 1997a; 1997b).

Maturation, which is also referred to as ripening, is a key characteristic of SSF operation. The term maturation is used here to describe biological and physical/chemical processes that occur over time in SSF and BSF and lead to improved performance; this encompasses both schmutzdecke growth that occurs near the top of the media bed and deep-bed media aging (discussed below). Treatment of all classes of microbial pathogens improves with SSF maturation, despite the diverse physical, chemical and biological properties of bacteria, viruses and parasites. However, the maturation mechanisms for specific microbial pathogens are likely to differ depending on their specific properties.

Schmutzdecke development is the most obvious, but not the only, aspect of SSF maturation. The development of the schmutzdecke leads to improved removal of particles, bacteria and protozoan parasites. The reduction of bacteria has been shown to decrease sharply upon removal of the schmutzdecke thus offering proof of its importance (Hijnen et al., 2004; Dullemont et al., 2006; Unger and Collins, 2008). However, several reports of virus reduction suggest no detrimental effect by removal of the schmutzdecke (Hijnen et al., 2004; DeLoyde, 2007; Poynter and Slade, 1977; Unger and Collins, 2008); the exception is a study wherein removal of the schmutzdecke led to small declines in virus reduction (Dullemont et al. 2006). In another study, the effects of schmutzdecke growth on virus reduction was investigated (Dizer et al. 2004). Although virus reductions increased during growth, the effect of later removal of the schmutzdecke was not included.

Microbial reduction appears to be enhanced by deep-bed filtration. The explanation is a maturation process commonly referred to as “media aging” that occurs below the schmutzdecke. This process would not be disrupted by removal of the

schmutzdecke (Poynter and Slade, 1977; Wheeler et al., 1988; Hijnen et al., 2004; Dullemont et al., 2006). Therefore, it could explain why microbial reductions in aged filters are higher than in new filters even when they are operating at the same head loss (Poynter and Slade, 1977; Windle-Taylor, 1970).

Media aging may be a biological process (Lloyd, 1973). The specific mechanisms could involve microbial predation and adsorption to biofilms (Wheeler et al., 1988). This is evidenced by a decrease in microbial reductions after draining of the filter, as occurs during some methods of cleaning, and by measurements of microbial grazers at depth in SSF (Poynter and Slade, 1977; Hendricks and Bellamy, 1991; Lloyd, 1973).

2.3.3 Lack of Data on Virus Reductions

Despite the importance of viruses as etiologic agents of diarrhea in diverse settings, little research has been done on virus reductions in SSF and the results vary widely. In the US, viruses are responsible for about 80% of enteric disease outbreaks for which the causative agent can be identified (Ryan et al., 2002). In developing countries, pathogenic viruses have been identified in stool samples of over 40% of diarrhea hospitalizations (Ramani and Kang, 2009).

Reductions of waterborne viruses reported in reviews of SSF vary widely [<1-1.5 log (Kool, 1979); <1 to3 log (Rachwal et al., 1996); and 2-4 log (Amy et al., 2006)]. Variability in reductions could be caused by differences in design and operational conditions such as extent of filter maturation and physical-chemical characteristics of challenge viral agents.

2.3.3.1 Mechanisms of Virus Reduction

The effects of design and operational parameters on virus reductions in SSF are listed in Table 2.6. Even less is known about the mechanism of reduction than those to explain reductions of bacteria or protozoa. Parameters that have been linked to an increase in reductions are: decreased filtration rate; increased temperature; increased time-in-use; and increased sand bed depth (DeLoyde, 2007). Increased residence time is another factor although it is simply the quotient of two parameters already mentioned, i.e., sand bed depth/filtration rate. However, there is possibly an independent effect of filtration rate; for example, pore velocity affects collector efficiency (Yao et al., 1971; Qi, 1998).

As noted earlier, removal of the schmutzdecke was shown to have little or no effect on virus reductions (Hijnen et al., 2004; Dullemont et al., 2006; DeLoyde, 2007). Yet, virus reductions increase with time of operation of the SSF. Thus, there is an influence of a maturation process that is not associated with the topmost layer of the filter. Instead, media aging which affects the entire depth is responsible (Poynter and Slade, 1977; Wheeler et al., 1988) (see also Section 2.3.2).

The most probable mechanisms of virus reductions in SSF are summarized in Table 2.7. Adsorption (and desorption) of viruses in SSF has been reported (Dullemont et al., 2006; Hijnen et al., 2004). Direct microbial activity has been implicated through either predation (grazing) or the activity of microbial exoproducts including proteolytic enzymes. Viruses are inactivated by proteolytic enzymes through hydrolysis of the peptide bonds in proteins that comprise the virus capsids.

Table 2.6 Influence of selected design and operational parameters on virus reductions in SSF.

Parameter	Direction of Change in Parameter to Increase Virus Reduction
Filtration rate/pore velocity	Lower filtration rate increases virus reductions (Poynter and Slade, 1977; Windle-Taylor, 1970; DeLoyde, 2007).
Water temperature	Warmer temperature (Poynter and Slade, 1977; Dullemont et al., 2006; DeLoyde, 2007).
Sand depth	Deeper bed (DeLoyde, 2007; Poynter and Slade, 1977).
Time-in-use/ biological maturation	Longer time-in-use. Often stated to be increased biological maturity (Wheeler et al, 1988; Windle-Taylor, 1969; Poynter and Slade, 1977; Dizer et al., 2004; DeLoyde, 2007).
Removal of schmutzdecke	Little or no effect. Small decrease reported following removal of schmutzdecke in some filters (Hijnen et al, 2004; Dullemont et al., 2006; McConnell et al., 1984; Poynter and Slade, 1977; Ellis, 1985; DeLoyde, 2007).

Adapted from DeLoyde, 2007

Table 2.7 Possible mechanisms of virus reduction in slow sand filtration.

Removal Mechanism	Mechanistic Explanation
Predation/grazing on virus particles	Filter feeding protozoa and bacteria can ingest virus particles (Kim and Unno, 1996; Pinheiro et al, 2007; Cliver and Herrman, 1972)
Biological activity, including microbial exoproducts	Non-sterile conditions, and presence of aerobic microorganisms lead to more rapid reductions of infectious viruses (Hurst et al., 1980; Jansons et al., 1989; Quanrud et al., 2003; Herrmann et al, 1974). More specifically, proteolytic enzymes can inactivate viruses (Cliver and Herrmann, 1972; Deng and Cliver, 1992; Nasser et al., 2002; Ward et al., 1986).
Attachment to biofilms	Viruses can become associated with biofilms in drinking water systems (Skraber et al, 2005; Storey and Ashbolt, 2001 and 2003; Wheeler et al, 1988)
Adsorption/attachment to granular media	Viruses undergo reversible adsorption/attachment; long-term detachment has been observed after seeding stops (Schijven and Hassanizadeh, 2000; Dullemont et al., 2006; Hijnen et al., 2004; Dizer et al., 2004)
Sequestration or straining in schmutzdecke	No effect or little effect of schmutzdecke removal on virus concentration in product water (Hijnen et al, 2004; Dullemont et al, 2006; DeLoyde, 2007)

Adapted from DeLoyde, 2007

A deficiency of most research on virus reductions in SSF is the use of infectivity assays to determine concentrations. Infectivity assays do not provide differentiation between mechanisms of inactivation and mechanisms of sorption or sequestration in the sand bed. The use of ^{125}I radio-labelled reovirus is an exception (McConnell et al., 1984). Labelled reovirus was found throughout the media bed with about five-percent recovered in the product water. However, reductions of infectious reovirus in excess of 4-log were observed. In fact, no infectious viruses were recovered in the sand bed or in the product water. Additionally, there was no apparent difference in ^{125}I-labelled reovirus transport between aged sands and clean sands. By contrast, studies in which infectivity assays were used suggest that aged sands were more effective than clean sands in reducing virus concentrations (Poynter and Slade, 1977; Wheeler et al., 1988; Windle-Taylor, 1970). This may indicate that media aging affects virus reduction primarily through inactivation of viruses rather than retention of virus particles in the sand bed.

The general conclusion reached from review of many studies is that virus reduction in SSF is a deep-bed filtration process. Both biological and physical/chemical processes have been cited to explain the treatment of viruses by SSF, but their relative importance is unresolved. Attachment to sand grains and biofilms has been reported to contribute to virus removal and inactivation. However, elimination of viruses by active biological processes, including predation and proteolytic enzyme activity, may be of greater importance (McConnell et al., 1984; Wheeler et al., 1988; Poynter and Slade, 1977). There is indirect evidence that media aging may affect virus reductions primarily through increased inactivation rather than increased sorption or sequestration in the sand bed (McConnell et al., 1984).

2.4 The Relative Importance of Virus Reduction

The motivation for this research is largely premised on possible ways to modify the design and/or operation that would maximize virus reductions in the BSF process. The question of whether viruses are as important as pathogenic bacteria, protozoa and other parasites in drinking water supplies remains open to debate. Bacterial pathogens, including species of *Salmonella* (typhoid fever), *Shigella* (dysentery) and *Vibrio* (cholera), have been traditionally identified as the most important waterborne pathogens. However, many enteric pathogens are now acknowledged to contribute significantly to diarrheal disease. They include bacteria such as enterohemorraghic *Escherichia coli* and *Campylobacter jejuni*; protozoan parasites such as *Cryptosporidium* and *Giardia*; and rotaviruses and caliciviruses (Dennehy, 2005).

The importance of removing viruses by BSF is supported by the fact that viruses have recently been recognized as contributing significantly to the diarrheal disease burden in developing countries. Greater than 40% of patients hospitalized for diarrhea in developing countries are infected with rotavirus or other waterborne viral pathogens (Ramani and Kang, 2009). Rotavirus infection alone accounts for about 6% of deaths worldwide in children under five (Parashar et al., 2003). However, viral pathogens and surrogates are often neglected in performance evaluations of water, sanitation and hygiene technologies designed for use in developing countries. Viruses have been known for a long time to be the most common cause of diarrheal disease in the United States (Dennehy, 2005). They are present and able to maintain infectivity for weeks in surface and ground waters impacted by fecal contamination (Espinosa et al., 2008; Maunula et al., 2005; Lodder et al., 2005; Yates et al., 1985).

CHAPTER 3

REDUCTIONS OF *E. COLI*, ECHOVIRUS TYPE 12 AND BACTERIOPHAGES IN AN INTERMITTENTLY OPERATION 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 six-to-eight 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 (50%) to 4-log, with geometric mean reductions after at least 30 days of operation of 1.9-log. Echovirus 12 reductions were comparable to those for *E. coli* B with a range of 1-log to >3-log and mean reductions after 30 days of 2.1-log. Bacteriophage reductions were much lower, ranging from zero to 1.3-log (95%) with mean reductions of only 0.5-log (70%). These data indicate that virus reduction by the BSF may differ substantially depending upon the specific viral agent.

3.1 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.

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 (Figure 3.1). The BSF is similar to a conventional slow sand filter (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

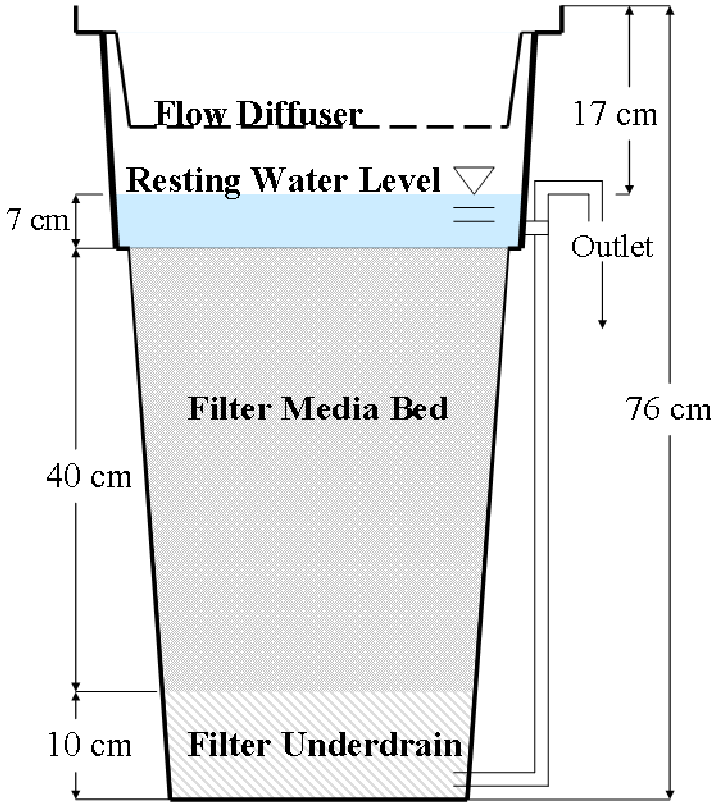

Figure 3.1 Cross-section of the plastic BSF used in these experiments.

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 two 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) of indicator bacteria. However, Palmateer et al (1999) demonstrated 3.8-log reduction of oocysts of *Cryptosporidium sp.* and >5-log 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.

3.1.1 Characteristics of BSF Design and Operation

As shown in Figure 3.1, 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 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/hr), 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.

3.2 Methods

3.2.1 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 (in North Carolina) 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.

3.2.2 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 experiment coding provided in the left-most column of Table 3.1 gives the single daily charge volume (20 or 40 L) and the percent amendment with pasteurized primary effluent (0, 1 or 2.5 % PE) from Orange Water and Sewer Authority (OWASA, Chapel Hill, NC). Table 3.1 also includes the duration of each experiment, 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.

Table 3.1 Characteristics of filter challenge experiments.

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

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

** CC = Cane Creek Reservoir (Orange County, NC, USA); UL = University Lake (Orange County, NC, USA); LM = Lake Michey (Durham, NC, USA)

In contrast to the instantaneous charge of 20 L, the 40-L charge, therefore, required from 1 to 6 hours 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, N.C. or Lake Michie in Durham, N.C.). 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 to 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 primary effluent (PE) from a local wastewater treatment plant (OWASA, Chapel Hill, NC) was pasteurized and added to feed water in two of the filtration experiments. Amendment with primary effluent and spiking with cultures of microorganisms increased the 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 NTU to 8.96 NTU) and mean total organic carbon (TOC) was 9.1 mg/L (range from 7.5 to 12.6 mg/L).

3.2.3 *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 4ºC 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 -80ºC. Aliquots of each stock were thawed each week, serially diluted ten-fold in phosphate buffered saline and stored at 4ºC 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: 100x Gentamycin/Kanamycin, Nystatin, HEPES buffer and Non-essential amino acids.) at 37ºC, freeze-thawed and chloroform extracted and then enumerated by the plaque technique in cell monolayers. Chloroform extraction was used to purify echovirus stock of macromolecular cell debris. Extracted stock was stored at -80ºC 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).

3.2.4 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 media (sand and gravel in this system). The media bed of a clean, unripened plastic BSF (BioSand Water Filter^{TM}, Davnor, Inc., Calgary, Alberta, Canada), was 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 then replaced with deionized water 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 (±1 cm) and at 13 cm (±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™ Conductivity, Resistivity, and TDS Meter (Cat. No. 09-326-2)).

3.2.5 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) for *E. coli*; 15% (0.07-log) for MS2 ; -18% (none) for PRD-1 and 9.1% (0.04-log) 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 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 Reduction = log(Feed Water Concentration) – log(Filtered Water Concentration) (1)

3.3 Results and Discussion

3.3.1 Tracer Tests

The results of the three tracer tests are presented in Figure 3.2. 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 Figure 3.2 is essentially the same in all three tracer tests. Thus, filter flow rate either in the constant head (two head values) or the

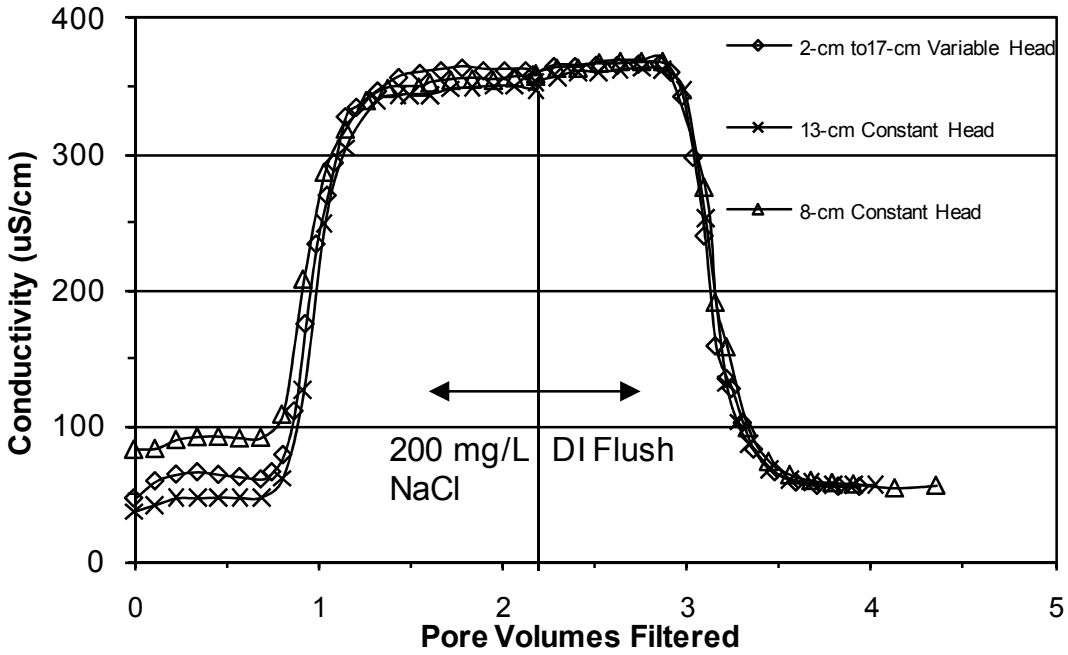

Figure 3.2 Results of three step-input tracer tests conducted with the plastic BSF.

declining head mode did not affect the RTD at the filtration rates tested (0.2 to 1.0 m/hr). 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 (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).

3.3.2 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 (40L-0% PE, 40L-2.5% PE and 20L-1% PE) in Figure 3.3. 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

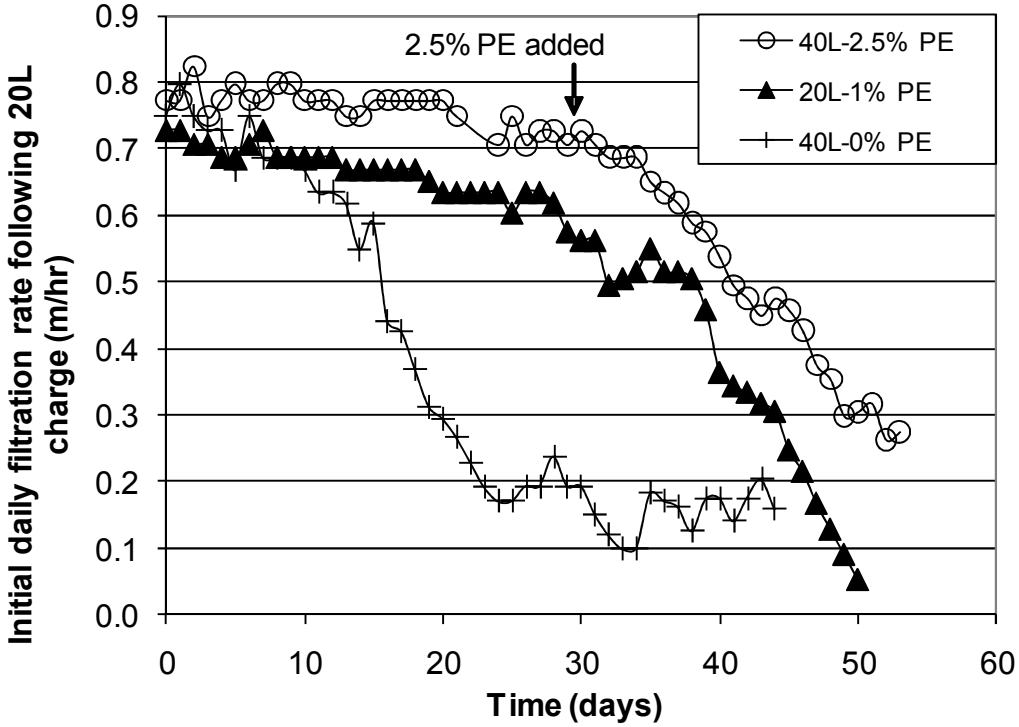

Figure 3.3 Initial daily filtration rate upon 20-L charge of water during three microbial challenge experiments.

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 accelerated 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 slow sand filters (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.

3.3.3 Turbidity Removal

Turbidity decreased through the BSF, producing filtered water turbidities that ranged from 0.65 NTU 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.

3.3.4 Microbial Reductions

Log reductions in *E. coli* during the three BSF experiments are presented in Figure 3.4. 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 reductions in *E. coli* were less for a 40-L than 20-L daily charge of feed water. For example, during the first three weeks, less than 0.6 log (75%) reduction was obtained for the 40-L charge while a 1-1.7 log (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

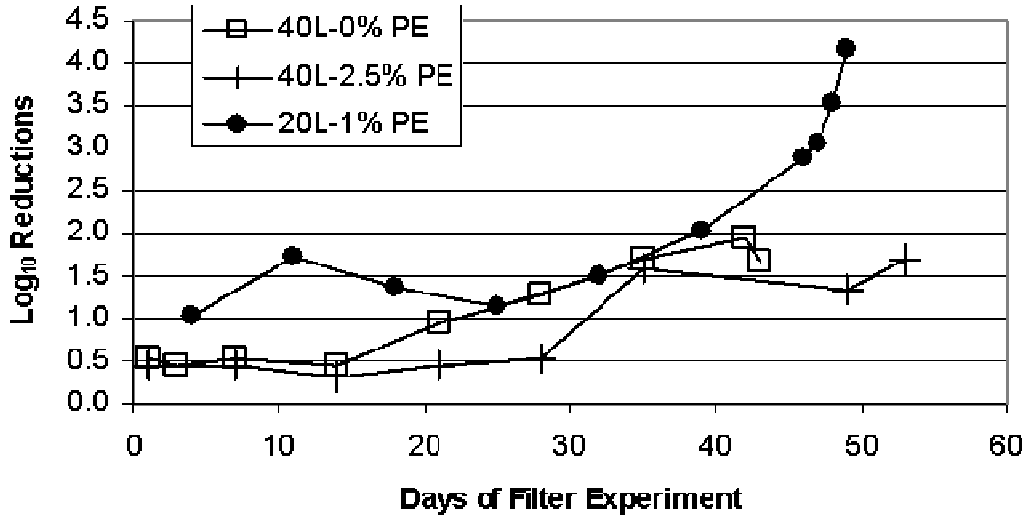

Figure 3.4 *E. coli* reductions over the length of three microbial challenge experiments.

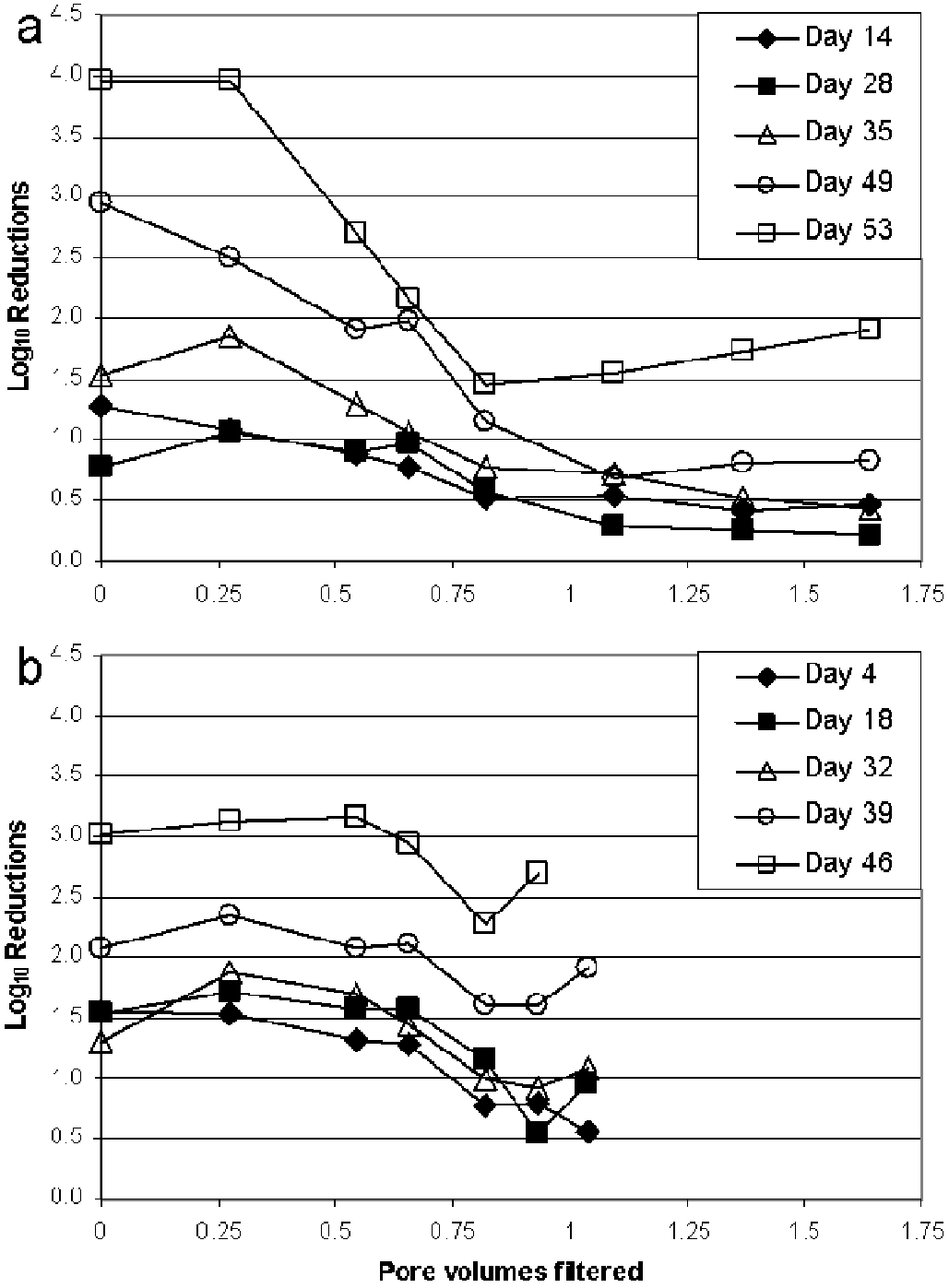

Figure 3.5 (a,b) Reduction in *E. coli* concentration with pore volumes filtered for experiment 40 L-2.5% PE (‘‘a’’ on top) and experiment 20 L-1% PE (‘‘b’’ on bottom). Results from days 7 and 21 of experiment 40 L-2.5% PE and days 11 and 25 of experiment 20 L-1% PE were excluded from the above graphs because they overlay the results of the subsequent sample days.

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 Figures 3.5a and 3.5b, 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 hour idle period. The results also show that the *E. coli* reduction improves with increasing days of filtration due to filter maturation.

As illustrated in Figures 3.5a and 3.5b, 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 Figure 3.5b, 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 Figure 3.6. Reductions in the human enteric virus (echovirus 12) were greater than those for the two bacteriophages

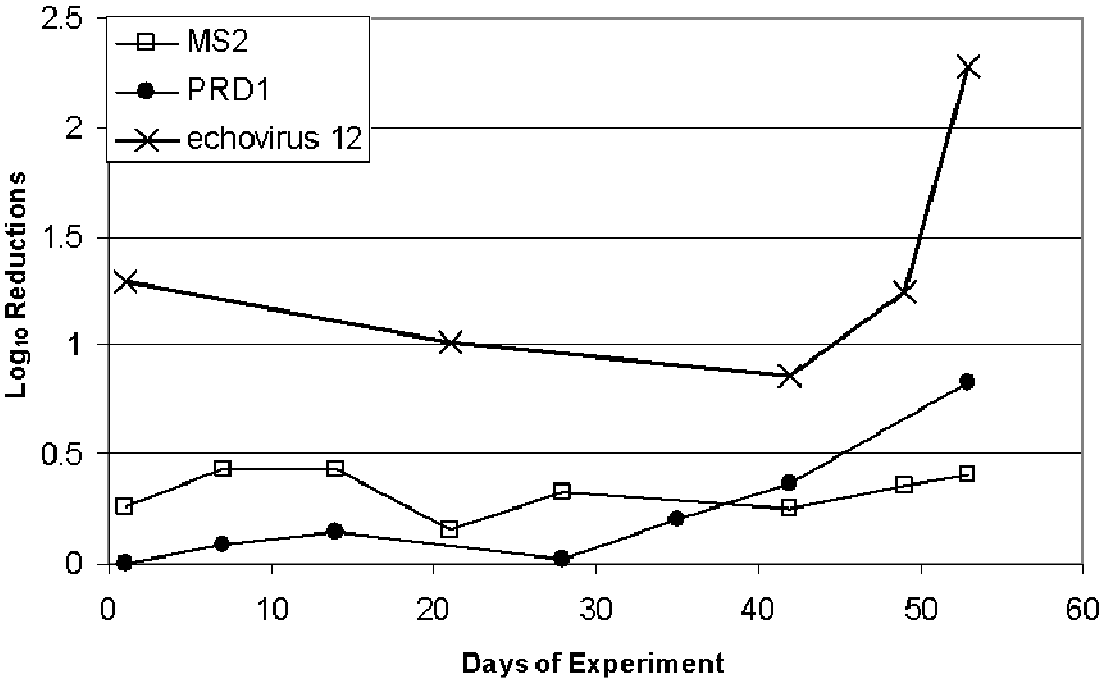

Figure 3.6 Reductions in concentration for three viruses over the length of experiment 40 L-2.5% PE.

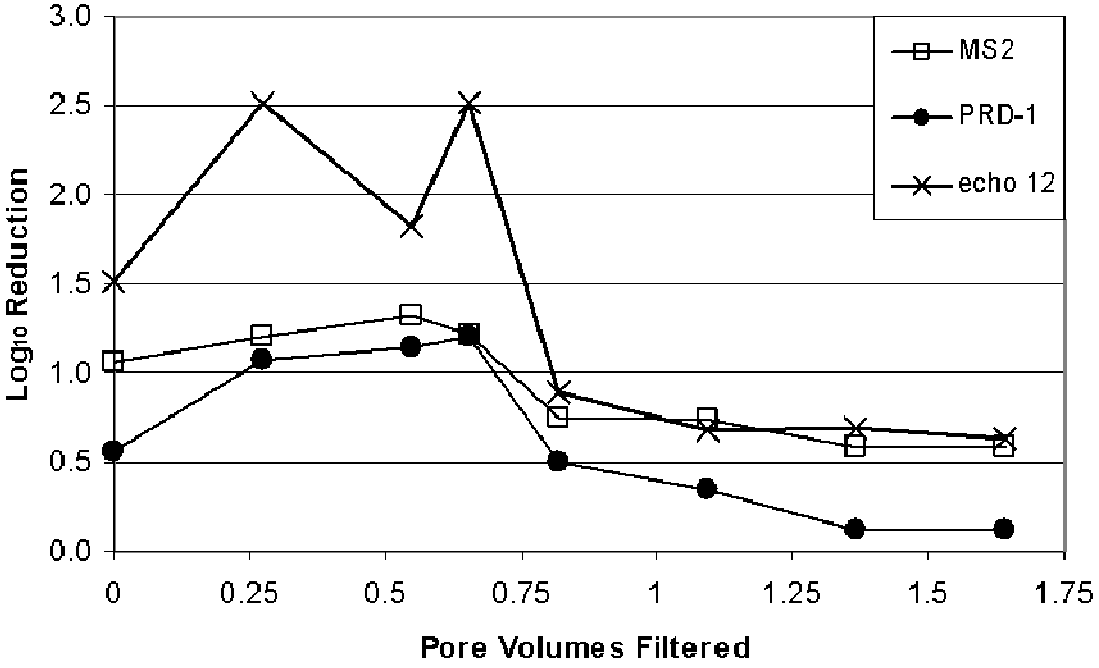

Figure 3.7 Reductions in concentration for three viruses with pore volumes filtered. Data shown is from day 42 of experiment 40 L-2.5% PE.

(MS2 and PRD-1). The reductions of both bacteriophages were uniformly lower than those of *E. coli* (Figures 4 and 5) and imply, not surprisingly, 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 Figure 3.7. This filter had been operated for 42 days during which time ripening was evident by the decline in the initial daily flow rate (Figure 3.3). While the reductions did not steadily decline with pore volumes, mean reductions were higher in grab samples taken when less than 0.7 pore volumes had been filtered (>1-log (90%) for MS2 and PRD-1 and >2-log for echovirus 12). In grab samples taken at greater than 1.1 pore volumes filtered, mean reductions for bacteriophages decreased to less than 0.75 log (82%) and mean reductions decreased to 1.14 log (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* (Figure 3.5).

A larger reduction in echovirus 12 than bacteriophages was observed (Figures 3.6 and 3.7). It is unclear whether this was caused by differences in electrostatic attraction or repulsion between the viruses and the filter media. The isoelectric point for echoviruses is 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 means that the echovirus 12 should have a less negative net charge than bacteriophages at the near neutral pH of the feed solution; thus, there should be less net repulsion by the negatively charged surface of filtration media. However, the surface characteristics of viruses can be altered significantly by the adsorption of soluble

compounds, both in the solution and during stock preparation (Mesquita et al., 2010; Daniels, 1980; Glassman, 1948). Additionally, chloroform was used to remove cell debris prior to storage of echovirus stocks, but was not used for MS2 and PRD-1 (Section 3.2.3). The presence of organic matter can inhibit adsorption of MS2 and PRD-1 in granular media (Blanford et al, 2005; Zhuang and Jin, 2003a; Pieper et al., 1997; Foppen et al., 2006). Therefore, both stock preparation and feed water characteristics may have impacted reductions of challenge viruses. Zeta-potential, measured in the feed water for all challenge viruses, could provide an estimate of both the sign and the magnitude of charge on the viruses in the system. This could yield greater insight into the impact of electrostatic attraction and repulsion on virus reductions.

3.4 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, including physical straining, adsorption and biologically mediated inactivation. 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 smaller media 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. 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. Studies on drinking water quality in BSF-filtered water and reductions of diarrheal disease are needed. 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.

Acknowledgements

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CHAPTER 4:

BENCH-SCALE INVESTIGATION OF BIOSAND HOUSEHOLD DRINKING WATER FILTER PERFORMANCE

Abstract

Six bench-scale biosand filter (BSF) columns were run in parallel to conduct two, eight-week microbial challenge experiments. Three of the columns were loaded with Accusand silica and three with crushed granite for each experiment. Bench-scale experiments provided confirmation that increased schmutzdecke growth, as indicated by decline in filtration rate, is the primary factor causing increased *E. coli* reduction. Reductions of challenge viruses increased modestly with schmutzdecke growth. Filter media type (Accusand silica vs. crushed granite) did not influence reduction of the *E. coli,* the challenge bacterium. The granite media without backwashing was superior to Accusand in reduction of the challenge viruses. However, if the granite media was backwashed first to remove the finest fraction of grains, the virus reductions were not significantly greater than with use of the Accusand media. Backwashing eliminated the very fine grain fraction of granite media. It was postulated that this decreased the surface area available for virus sorption and/or biofilm growth and thus decreased the extent of virus reduction.

4.1 Introduction

The lack of safe drinking water leads to massive health and economic costs in the developing world. Point-of-use (POU) drinking water treatment provides a feasible solution for those without sustainable and affordable access to safe water sources (Sobsey, 2002). A number of technologies, both traditional and commercial, are now available. Many and are being tested in the laboratory and field to determine their efficacy in reducing the concentration of viable pathogens and improving health. One of the most promising POU technologies is the biosand filter (BSF), a household-scale, intermittently operated slow sand filter (SSF).

Household implementation of BSF in developing countries has been shown to produce large reductions in diarrheal illness. For example, a reduction of greater than 40% was achieved in studies conducted in the Dominican Republic (DR) and in Cambodia (Stauber et al., 2009; Liang, 2007). The reliability and simplicity of the BSF suggest that these reductions can be sustained. Survey data in the DR, Cambodia and Haiti, for instance, indicate that >85% of the BSFs are still in service one-to-eight years after their installation (Aiken, 2008; Liang, 2007, Duke et al., 2006). Far lower levels of sustained use have been reported for other POU technologies (Sobsey et al., 2008).

While large reductions in diarrheal illness have been observed consistently in households using the BSF, a wide range of microbial reductions has been reported from measurements in households using BSF in developing countries. Mean *E. coli* and fecal coliform reductions ranged from <1 log to >2log have been reported across numerous field projects (CAWST, 2008; Stauber, 2007). This variability could be due to many factors and is still poorly understood. One possibility is variability in filtration media.

The inventor of the BSF recommends that the ideal choice of media is "crushed rock from a rock quarry" (Manz, 2006). This is an understandable approach to keep the cost of the filters low while also encouraging a locally sustainable microenterprise, but use of local media likely creates variability in filter performance.

Also lacking are mechanistic explanations for microbial reductions through which cause and effect relationships would lead to improved BSF design. The development of a schmutzdecke, similar to that observed in conventional SSF, has been implicated (Elliott et al., 2008). The schmutzdecke is a layer of organic and inorganic particles, living and dead microorganisms localized to the top few cm of the media bed; it is critical to the removal of bacteria and particles in conventional SSF (Hijnen et al., 2004; Dullemont et al., 2006; Unger and Collins, 2008). However, deep-bed filtration also contributes to microbial reductions in SSF (Poynter and Slade, 1977; Wheeler et al., 1988) and is likely to be affected by media characteristics. No study of BSF has yet included the effect of physical and chemical properties of media on microbial reductions.

Protozoan parasites have been shown in controlled laboratory studies of full-scale BSF to be reduced to a greater extent than bacteria and viruses (Palmateer et al., 1999; Koksal et al., 2008). This may be because physical straining and sedimentation mechanisms are more effective for these relatively large microorganisms.

Reductions in bacteria and viruses are reported to vary widely both throughout a daily filtration cycle and over the weeks and months of operation. Factors such as the ratio of daily charge volume to pore storage volume during the idle time (i.e., the time between charges of the daily volume), the extent of schmutzdecke development and the specific characteristics of the challenge organism were implicated (Elliott et al., 2008).

In particular, microbial reduction is a dynamic process that varies throughout the daily filtration cycle and as the filter develops the schmutzdecke. Reductions were greatest at the startup of each daily filtration cycle because microbial attenuation occurs in water that is stored inside the filter during the idle time (Chapters 3, 5 and 6). This water is discharged first following the daily charge as it is displaced by the newly charged water. In fact, this observation may in part explain variability in field measurements of bacterial reductions given that the sampling protocol has not been standardized to account for dynamic performance factors.

Another shortfall in predicting the performance of the BSF is the absence of data to show the reproducibility of microbial reductions at full-scale. Variations in reductions could be due to the inability to reproduce packing of filtration media and to uncertainty in microbial attachment and growth process that in turn affect the time to develop the schmutzdecke. BSF units would need to be operated in parallel to examine reproducibility. However, the typical daily feed to full-scale BSF is 20 to 40 L. Manual operation of parallel full-scale units to examine reproducibility in microbial reductions would thus require unreasonably large volumes of feed waters and, in some cases, stocks of microbial challenge organisms.

The overall goal of this study was to develop a BSF design that could be used to advance understanding of the factors affecting microbial reductions more efficiently than by continued use of full-scale BSF units in the laboratory. This goal was accomplished by reducing the filtration media volume of the full-scale BSF unit while maintaining the same operating characteristics. The result was an experimental system comprising six, small diameter columns that could be operated in parallel with a much smaller daily

charge than the full-scale BSF. Replicate filters are not typically used in studies of SSF and have not been reported for BSF. Without replicates, however, the reproducibility of performance is unknown and thus, conclusions about how microbial reductions are affected by systematic changes in input and operational conditions are not certain. This is especially true in laboratory design of filters because media packing is difficult to reproduce from column to column and thus, head loss development can vary. Small differences in media characteristics in the topmost few centimeters could cause differences in schmutzdecke development. Reproducibility in biological filter performance as measured by microbial reductions is further lessened by variability in maturation rate in the schmutzdecke caused by unavoidable variations in the indigenous microorganisms and particles in the feed water.

The main objectives were to investigate simultaneously the reproducibility of reductions in challenge bacteria and viruses among replicate columns and the effect of media selection (narrowly sieved Accusand silica vs. coarsely screened crushed granite gravel) on microbial reductions. A secondary objective was to validate previous findings from controlled studies using full-scale BSF units (Elliott et al., 2008). To achieve these objectives required that the columns be operated for 7-8 weeks in order to allow the development of a schmutzdecke and the maturation of filter media.

4.2 Materials and Methods

Two bench-scale tests (Column Tests No. 1 and No. 2) were conducted. Each of these lasted eight weeks and included a comparison of microbial reductions in columns filled with Accusand and granite media. The columns were operated to simulate full-

scale BSF. This was accomplished by keeping the idle period between daily charges nearly the same and by reducing the daily charge volume to match the smaller volume of the filter such that the fraction of the daily charged stored within the filter for the idle time would be similar.

4.2.1 Column Design, Preparation and Operation

The design of the filtration system in Column Test No. 1 is shown in Figure 4.1. Six columns were operated in parallel. Three columns were loaded with crushed granite from a local quarry in Pittsboro, NC that was sieved and washed with tap water according to standard international field procedures for the BSF (Manz, 2006). The other three columns were loaded with standard silica (Accusand) (Unimin Corp., Le Sueur, MN). Accusand was selected because of its chemical purity, low organic matter content, and low uniformity coefficient (Schroth et al., 1996) that make it an ideal choice for controlled laboratory studies of filtration. The Accusand was pre-washed by 24-hour exposure to 40% concentrated HCl, followed by a deionized water rinse to pH 5) (Litton and Olson, 1993). Three sieve fractions (U.S. Standard Mesh 30/40, 40/60 and 50/70) were blended together to provide a relatively narrow range of grain size (d_{10}=0.27 mm; d_{60}/d_{10}=1.4) compared to the granite media (d_{10}=0.21 mm; d_{60}/d_{10}=4.0). The underdrain of each column was 8 cm of granite gravel topped with 2 cm of U.S. Standard Mesh 12/20 Accusand.

Sieve analyses of the packed media were conducted following the completion of microbial challenge experiments. The delay was necessary because sieve analyses required destructive sampling of the media.

Elemental analysis of the Accusand and granite filter media was conducted following EPA Method 3051 microwave-assisted strong acid digestion procedure (USEPA, 1994). The acid digestion procedure does not dissolve silicate-based minerals. Therefore, the elements found in the analysis were present in acid-soluble components on the surface of the sand grains and could likely affect the sorption characteristics of the filter media.

The daily charge volume to each filter column in Column Tests No. 1 and No. 2 was selected to ensure that the maximum elevation head and charge -to-pore volume ratio (1.3) were similar to those in full-scale BSF. The feed volume was slightly larger in Column Test No. 2 (450 ml) than in Column Test No. 1 (430 mL) to account for the possible increase in porosity caused by backwashing prior to the start.

The total daily charge volume for all six columns (about 3 L) was distributed to six, 500-mL graduated cylinders so that the challenge organism concentrations were kept about the same in each charge. Each graduated cylinder was hand-mixed before introducing the charge to the column.

The daily charge to each column in Column Test No. 1 had to be introduced in two approximately equal aliquots. Charging the entire volume would have nearly doubled the elevation head of 17 cm used in previously conducted full-scale experiments. The elevation head must be kept the same in order for the initial daily filtration rates both laboratory and full-scale to be similar. An external reservoir (a 250-mL PPE bottle) shown in Figure 4.2 was installed after Column Test No. 1 so that the entire daily charge (450 mL) could be introduced at one time (i.e., 250 mL above the filter media in addition to 200 mL from the bottle) while maintaining the maximum elevation head the same as in

full-scale filters. This modification gave a more consistent pattern of decline in head that was better represented the pattern in full-scale operations.

A schmutzdecke developed and flow rate declined in the filter columns over weeks of daily operation. Therefore, a cleaning procedure similar to that practiced in full-scale BSF (Section 2.2.2) was necessary to restore an adequate filtration rate. Filter operation was discontinued briefly when flow rate decreased to about 10% of the initial value. The schmutzdecke was then scoured by stirring the uppermost 1 cm of the media bed with a sterile pipette. The material suspended by this procedure was drawn into the pipette and discharged to waste. The filter column was then returned to daily operation.

4.2.2 Tracer Tests

Tracer tests were conducted to measure the deviation from plug flow behavior in each of the columns. A step input of 200-mg/L sodium chloride was introduced prior to Column Tests No. 1 and 2. The sodium chloride concentration in the exit stream from each column with volume-filtered following the beginning of the step input was used to calculate the Morrill Dispersion Index (MDI) (Tchobanoglous et al., 2003). The MDI is the ratio of T_{90} to T_{10}, where T_{90} is the time to reach 90% and T_{10} is time to reach 10% of the feed concentration in the exit stream. A reactor that exhibits ideal plug flow would have an MDI of 1.0. An MDI of less than 2.0 is classified as "effective plug flow" by the US EPA (USEPA, 1986).

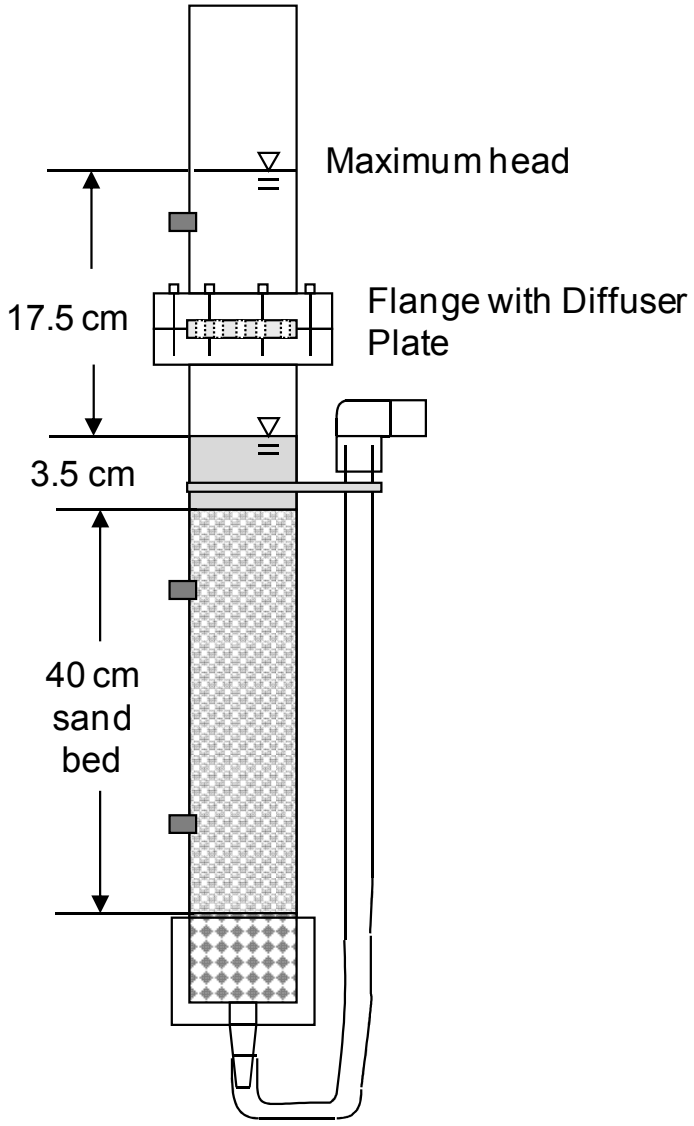

Figure 4.1 Cross-section of bench-scale columns used in Column Test No. 1.

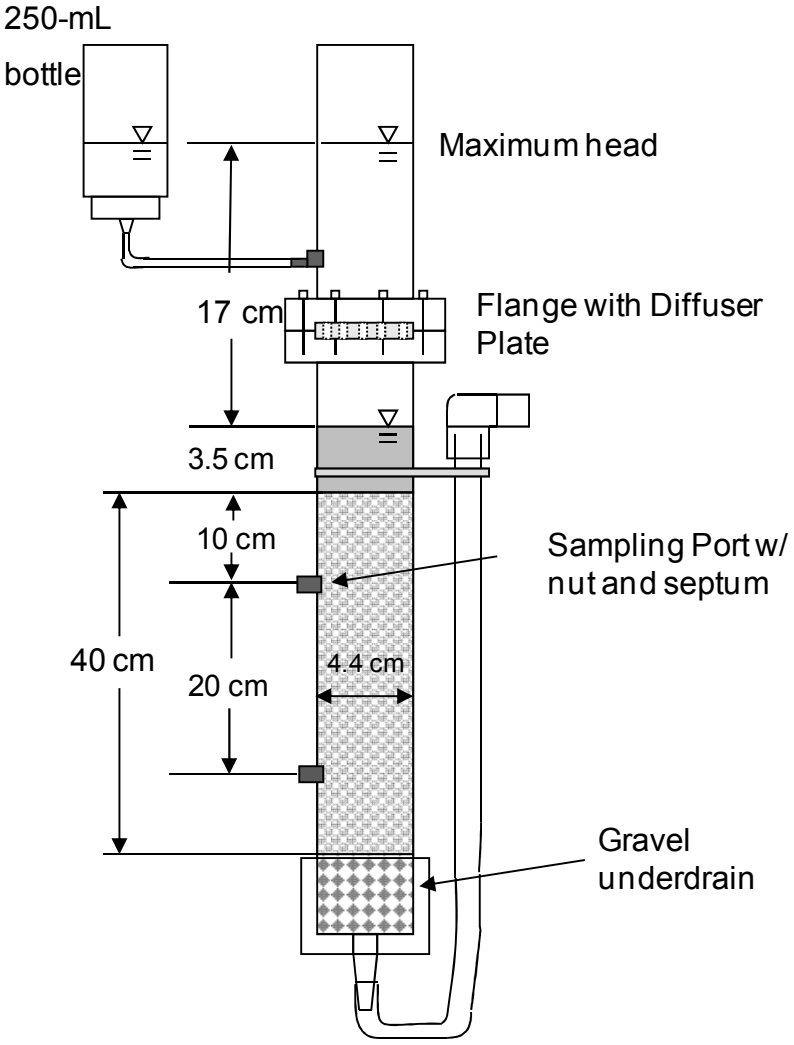

Figure 4.2 Cross-section of bench-scale columns used in Column Test No. 2.

4.2.3 Feed Water for Microbial Challenge Studies

Feed water was obtained from the Cane Creek Reservoir raw water sample taps of the water treatment plant operated by the Orange Water and Sewer Authority (OWASA). Cane Creek Reservoir does not receive wastewater discharges. Sufficient water was collected before each column to be fed daily for the duration of each experiment (54 to 56 days). The total daily charge volume was stored at 4°C until daily use and then allowed to reach room temperature (approximately 20°C) overnight. Thus, water temperature was nearly constant in all experiments and did not influence the rate of ripening nor microbial stability. Stored water for each daily charge was amended with pasteurized primary effluent (PE) from the OWASA wastewater treatment plant in Chapel Hill, NC to simulate the presence of wastewater in typical drinking water sources of developing countries and to accelerate the ripening process. The addition of wastewater increased the total organic carbon (TOC) concentration of the feed water by up to 50%, such that feed TOC was in the range of 7.5-12.5 mg/L. The daily charge was then spiked with cultures of challenge bacteria and viruses to reach concentrations reported in Table 4.1.

To decrease systematic variability between columns, the daily feed water was spiked with PE and challenge microorganisms in one 3-L aliquot and then mixed on a stir plate. Additionally, the order in which the columns were charged (i.e. left-to-right or right-to-left) was alternated each day.

Table 4.1 Characteristics of bench-scale microbial challenge tests. Microbial concentrations are mean log measured concentration per mL and maximum log deviation from the mean.

Experiment Coding	Length (days)	Source water	Columns Backwashed	Pasteurized PE*	*E. coli B* log10 cfu/mL	MS2 log10 pfu/mL	PRD-1 log10 pfu/mL	echovirus 12 log10 pfu/mL
Column Test No. 1	54	Cane Creek	No	1.0%	2.85 ± 1.09	3.45 ± 0.85	-	3.60 ± 1.15
Column Test No. 2	56	Cane Creek	Yes	2.5%	2.79 ± 1.35	2.82 ± 0.34	3.14 ± 0.73	-

* Pasteurized PE = pasteurized primary effluent from OWASA WWTP, Chapel Hill, NC.
** Cane Creek = Cane Creek Reservoir, Carrboro, NC.

4.2.4 Microbial Methods and Virus Characteristics

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). High concentration samples were diluted in phosphate buffered saline and vortexed prior to membrane filtration. MS2 and PRD-1 concentrations were assayed using the single agar layer method on hosts *E. coli* F_{amp} and *Salmonella typhimurium* LT2, respectively (USEPA, 2001). A stock of echovirus 12 was propagated in monolayers of FRhK-4 cells with maintenance medium at 37ºC, freeze-thawed and chloroform extracted and then enumerated by the plaque technique in FRhK-4 cell monolayers (Cromeans et al., 1987). Chloroform extraction was used to purify echovirus stock of macromolecular cell debris. Further details of the procedures used to grow and enumerate stocks of seeded viruses have been reported previously (Elliott et al., 2006 and 2008).

Log reductions in microbe concentration by passage through the BSF were calculated by Eq. 1. All log-reduction values reported are log base 10.

$$\log \text{Reduction} = \log(\text{Feed Water Concentration}) - \log(\text{Filtered Water Concentration}) \quad (1)$$

The focus of this study was on the dynamic behavior of microbial reductions as the filter matured. As such, microbial reductions were measured after collecting the entire volume during each daily filtration cycle. The daily dynamic in microbial reductions is investigated elsewhere (Elliott et al., 2008; Chapters 3, 5 and 6) by collection of grab and composite samples within the daily cycle. The daily charge volumes in Column Tests No. 1 and No. 2 (430-mL and 450-mL, respectively) were approximately 1.3 times greater than the pore volume of the filter.

An 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 virus survival. Mean die-off rates for all viruses after overnight storage were less than 25% per 24 hours for MS2 and PRD-1. Mean die-off for *E. coli* and echovirus 12 were roughly 50% per 24 hours. These were far less than the reductions during filtration and thus not considered significant.

Characteristics of the three challenge viruses are included in Table 4.2. Isoelectric point has been reported to be the most important single parameter for predicting the adsorption of viruses in granular media filter, at least for smaller viruses (Dowd et al., 1998). The isoelectric points listed in Table 4.2 show that the viruses are expected to carry net-negative charges at the pH of the column tests (mean 6.9; range 6.6-7.3). Silica also carries a net-negative charge in this pH range. The fact that MS2 and PRD-1 have much lower isoelectric points than echovirus 12 would suggest that adsorption of MS2 and PRD-1 would be impeded by net electrostatic repulsion to a greater extent. It is possible that the granite media that was also used in this research could offer a more attractive surface due to the presence of Al and Fe oxides as will be

Table 4.2 Characteristics of viruses used in these filter tests.

Virus/Phage	Size (nm)	Isoelectric point	Genetic Material
MS2	26	3.5-3.9	ss-RNA
PRD-1	62	4.2	ds-DNA
Echovirus 12	28-30	5.0-6.4*	ss-RNA

*IEP listed for echovirus 12 is the range of values found in the literature for all echovirus types.

Source: Collins et al, 2004

discussed. However, the isoelectric point can also depend on the characteristics of the solution (Mesquita et al., 2010; Daniels, 1980; Glassman, 1948). For this reason, the net surface charge or the magnitude of charge carried by a virus particle will be specific to each feed water and will also be affected by virus preparation methods. Ideally, zeta potential would be determined for each challenge virus stock in the feed water used in each experiment. This would give an estimate of both the sign and the magnitude of charge on each virus in the relevant feed water throughout the applicable pH range.

4.2.5 Statistical Methods

The data for log reductions of filtered samples did not pass the Kolmogorov-Smirnov test for conformance to a Gaussian distribution. Non-parametric statistics were used instead to compare log reductions. The Mann-Whitney *U* test (also known as the Wilcoxon rank-sum test) was used to compare log reductions of viruses. The resulting p-values are for unpaired, two-tailed tests with significance level α=0.05.

Box plots of log reductions were produced in DeltaGraph version 5.6.4. The whiskers represent the 5th- and 95th-percentiles and the box extends to the 25th- and 75th-percentiles. The median value is represented by a horizontal line through the box and the mean is indicated by a square symbol.

4.3 Results and Discussion

4.3.1 Comparison of Filter Media Characteristics

The inorganic composition of the granite and Accusand media is presented in Table 4.3. Concentrations (mg/Kg) of metals (Ca, Mg, Mn, Al, and Fe) were two orders of magnitude higher on granite than on Accusand. In fact, granite is well known to be composed of only 72% SiO_2, with substantial quantities of Al_2O_3 (14.4%), CaO (1.82%), FeO (1.68%), Fe_2O_3 (1.22%), MgO (0.71%) and MnO (0.05%) (Blatt and Tracy, 1996). The extremely large difference in particular of Al and Fe could have implications for virus attachment. Aluminum and ferric oxyhydroxide surfaces tend to carry a net positive charge at near-neutral pH conditions and thus attract viruses which are negatively charged. The region of pH where surface charge is positive is quantified by the zero-point of charge (PZC) value. The PZC is about 2.0 for SiO_2 in contrast to 9.1 PZC for Al oxides (α-Al_2O_3), 6.7 for γ-Fe_2O_3 and 8.5 for amorphous $Fe(OH)_3$ (Stumm and Morgan, 1996). These differences in PZC would suggest less reduction of viruses in filtration through pure SiO_2 (Accusand) than granite. Whether elemental composition influences microbial reduction is discussed in Sections 4.3.3 and 4.3.4.

Table 4.3 Elemental analysis of the two media used in filter tests.

	Granite (mg/kg)	**Accusand** (mg/kg)
Calcium (Ca)	12,270	90
Magnesium (Mg)	14,875	60
Manganese (Mn)	920	0
Iron (Fe)	23,250	55
Aluminum (Al)	17,030	90

4.3.2 Effects of Backwashing on Grain Size Distribution and Hydraulic Characteristics

Visual inspection of the media packing during Column Test No. 1 revealed considerable variation in the grain size composition of the topmost layer among those columns filled with granite. Differences in grain size were not a major concern from the standpoint of hydraulic performance given that clean-bed head loss and starting filtration rate were similar in all columns. Nevertheless, they could reduce the replication among columns loaded with granite media and could affect particle trapping and microbial attachment near the media surface leading to variability in the rate of schmutzdecke development. Therefore, all six columns were backwashed prior to Column Test No. 2 and before measurement of the grain size distributions. The backwashing procedure consisted of 20-30% expansion for 20 minutes. Although not quantified, visual observation showed removal of a substantial amount of very fine silty material from the granite loaded columns.

The Column Test No. 2 grain size distributions of granite and Accusand media at different filter depths after backwashing are compared in Figure 4.3. The effect of backwashing of the columns filled with granite medium was to cause severe depth stratification of grain sizes. The cumulative distribution showed that 90% of those near the top of the media were < 0.2 mm in diameter compared with < 1.3 mm near the bottom of the media. A very large difference in grain size distributions between granite and Accusand media is still apparent (Figure 4.3) even though the backwash procedure had removed much of the silty fraction from the granite loaded columns. This is to be expected because the granite was crushed and then sieved following the crude procedure

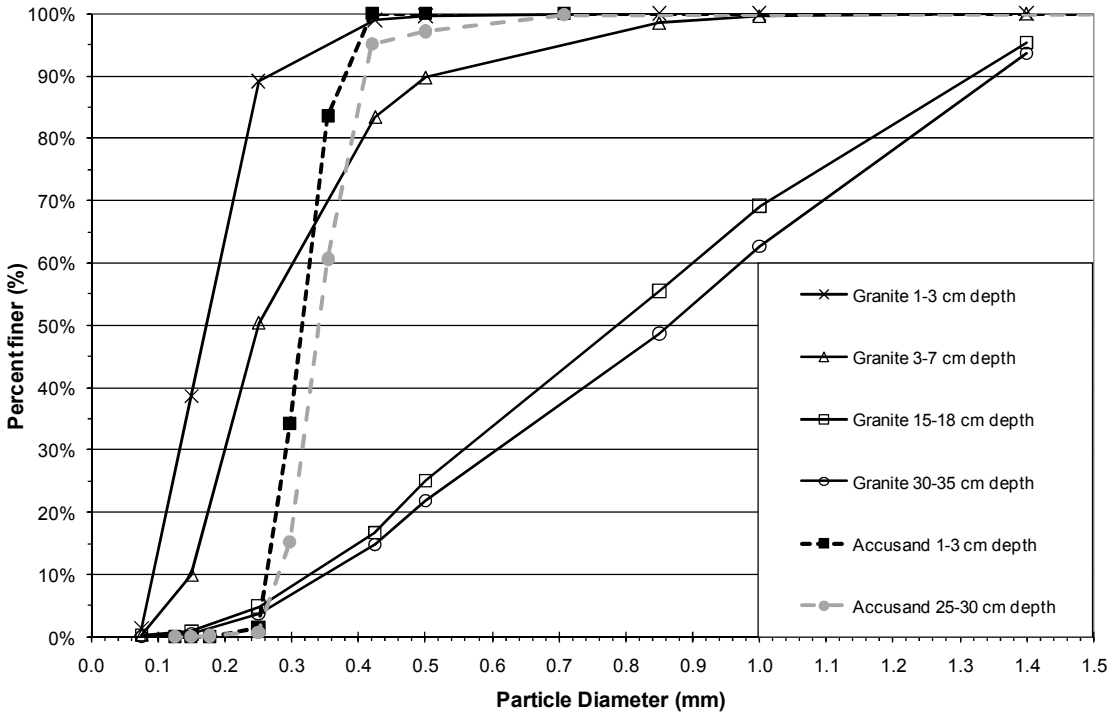

Figure 4.3 Sieve analysis of backwashed Accusand and granite media from Column Test No. 2

recommended for field installation of BSF; in contrast, the Accusand was carefully sieved into narrow size fractions that were then blended together (Schroth et al., 1996).

Besides removing silty fines, backwashing of the granite media changed the hydraulic characteristics as shown by comparison of MDI values in Table 4.7. In Column Test No. 1, the MDI was close to 1.0 in both granite-filled and Accusand-filled columns and this indicates plug flow (i.e., very little longitudinal dispersion). The same was observed from tracer tests on full-scale BSFs (Elliott et al., 2008). However, the MDI obtained for the granite-filled columns after backwashing increased from 1.3 to greater than 2.2 while it remained the same for the Accusand filled columns. The increase in MDI was most likely due to the development of preferential flow paths as the packing configuration shifted from unstratified (not shown) to stratified (Figure 4.3).

Table 4.4: Morrill Dispersion Index for tracer tests conducted prior to Column Tests No. 1 and No. 2.

Replicate Columns in Each Test	Column Test No. 1	Column Test No. 2
Granite #1	1.31	2.24
Granite #2	1.31	2.66
Granite #3	1.29	3.10
Accusand #1	1.16	1.19
Accusand #2	1.22	1.36
Accusand #3	1.16	1.25

4.3.3 Decline in Filtration Rate

The decline in filtration rate during Column Test No. 1 is presented in Figure 4.4a (Accusand media) and 4.4b (granite media). Filter maturation due to growth of the schmutzdecke and particle trapping is expected to increase head loss and thus decrease filtration rate. However, the decline in filtration rate was not the same in each of the three columns with the same media: the decline was far more rapid in Columns A1 and A2 than in Column A3 and in Columns G1 and G3 than in Column G2. In fact, the filtration rate in Columns A3 and G3 increased momentarily rather than decreasing continuously as a result of unintentional disturbance of the schmutzdecke during filter charging. The surface of media bed in columns A1 and A3 and in columns G1 and G3 was intentionally scoured to remove the schmutzdecke when the filtration rate decreased to an unacceptable level. This produced a rapid increase in filtration rate from less than 0.1 m/hr to 0.6-0.9 m/hr.

The decline in filtration rate for each filter column during Column Test No. 2 is presented in Figure 4.5a (Accusand media) and 4.5b (granite media). The reproducibility of filtration rate decline is improved among replicate columns containing the granite

media over that observed in Column Test No. 1 (Figures 4.4b). The improvement could be due to backwashing prior to Column Test No. 2 that removed the fines, narrowed the grain size distributions (Figure 4.3) and made the media size more consistent among the columns. With better control of media size, head loss development, and accompanying filtration rate decline should also be more consistent among the columns. Filtration rates also seem more reproducible among replicate columns containing Accusand than seen in Column Test No.1 (Figure 4.4b). However, this is not explainable by backwashing because the grain size for Accusand was very uniform as received (Figure 4.3).

Despite some variability that is still evident among replicates in Column Test No. 2, the filtration rate decline for columns filled with the granite media appears far more rapid than in those filled with Accusand. The implication is that head loss development was more rapid in the granite than Accusand media, consistent with the smaller size of this media and backwashing that brings all the smallest grain sizes of granite to the surface of filter where particles are efficiently entrapped. By contrast, particle entrapment is probably not as an important a mechanism of head loss development in the columns containing Accusand because of its much larger and more uniform grain size. Instead, head loss development may be caused mainly by growth of schmutzdecke.

The filtration rate decline for columns containing Accusand (Figure 4.5b) appears slower following the scouring of the schmutzdecke. The implication is retarded regrowth of the schmutzdecke compared to startup of the column test. Microorganisms that contribute to schmutzdecke regrowth are derived from the feed water. One possible explanation for retarded schmutzdecke growth, therefore, could be microbial inactivation

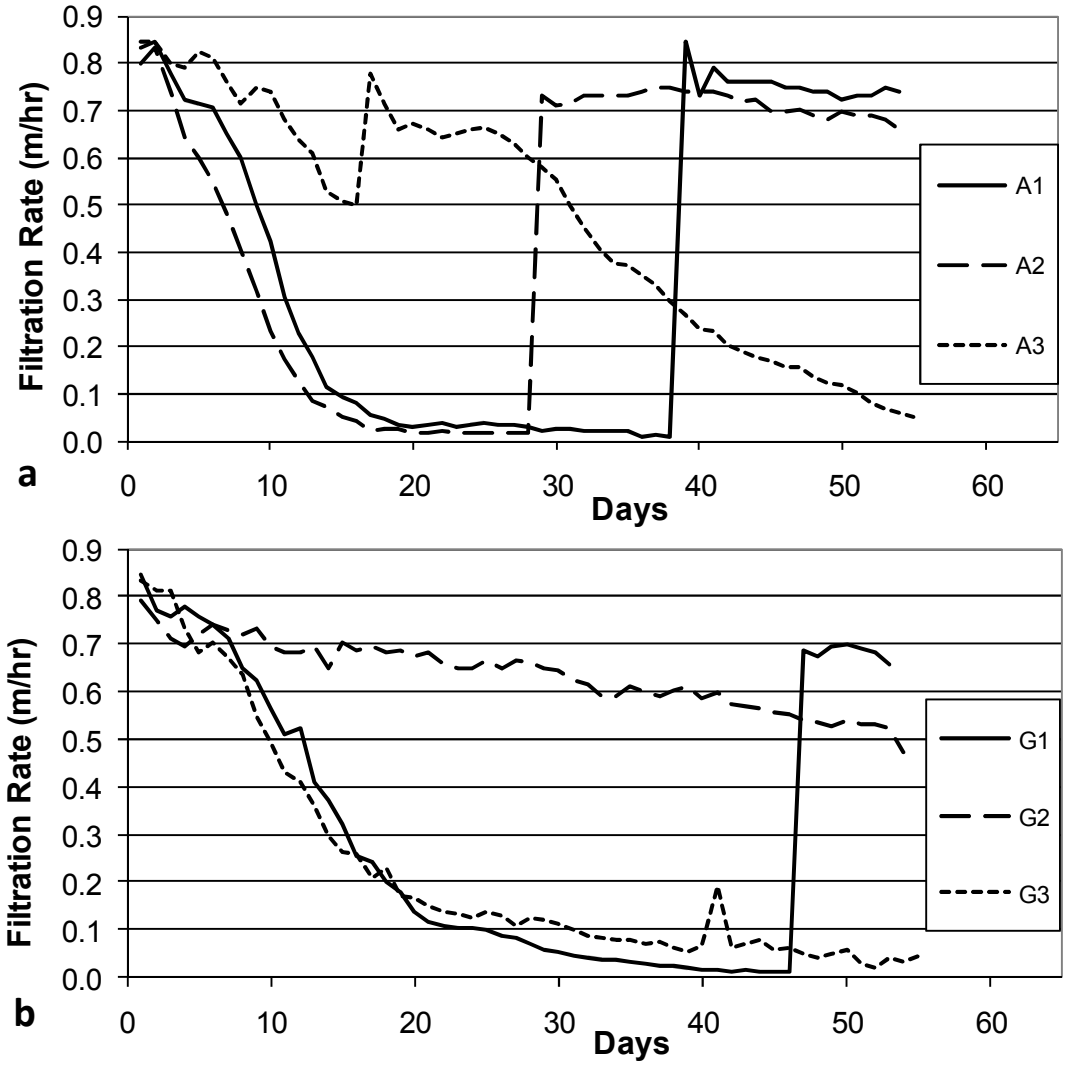

Figure 4.4 (a,b): Initial daily filtration rate during Column Test No. 1. Accusand-loaded filter columns A1-A3 (4.4a) and Granite-loaded filter columns G1-G3 (4.4b).

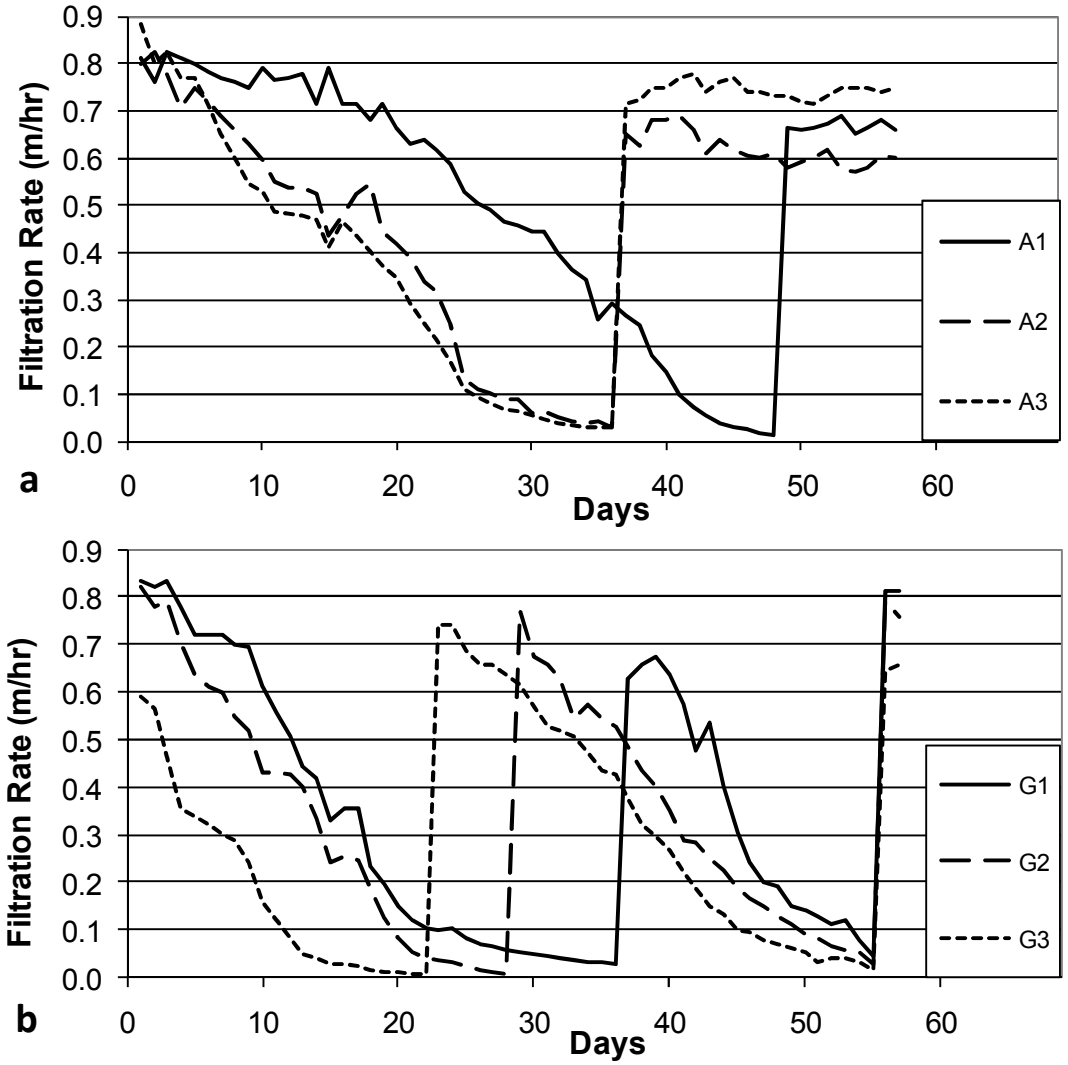

Figure 4.5 (a,b): Initial daily filtration rate during Column Test No. 2. Accusand-loaded filter columns A1-A3 (4.5a) and Granite-loaded filter columns G1-G3 (4.5b).

that occurred during storage of the feed water for many weeks at 4°C prior to the scouring event.

4.3.4 Reductions in Challenge *E. coli*

The *E. coli* reductions from each column over the eight weeks of operation in Column Tests No. 1 and Test No. 2 are summarized in the box and whisker plots shown in Figures 4.6a (Accusand media) and 4.6b (granite media) in Column Test No. 1 and in Figures 4.7a (Accusand media) and 4.7b (granite media) in Column Test No. 2. The reductions ranged widely from less than 1-log to greater than 5-log. The variability in *E. coli* reductions is both within each column over the eight weeks of operation and among replicate columns. Maturation is primarily responsible for variability within each column. Variability among columns containing the same media could be due to differences in head loss development, as indicated by differences in filtration rate decline (Figures 4.4 and 4.5).

The effect of filtration media on *E. coli* reduction can be qualitatively examined by comparing the box and whisker plots in Figures 4.6a and b (Column Test No. 1) and in Figures 4.7a and b (Column Test No. 2). No significant difference was found between the two media types in either column test series. The box and whisker plots show that the range in reductions over each column experiment was similar for the Accusand and granite media despite the fact that the metals content was at least two orders of magnitude higher for granite than the Accusand media (Table 4.3). Metals in the form of iron and aluminum oxide as well as hydroxide coatings have been shown to enhance bacterial reductions (Lukasik et al., 1997; Truesdail et al., 1998). However, the chemical form of

the iron and aluminum on the surface of the granite media is unknown. Additionally, dissolved organic matter commonly found in surface waters has been shown to block the metal oxide and hydroxide sites that can sorb *E. coli* (Foppen et al, 2008). Therefore, enhancement of bacterial reduction by high concentrations of metal oxides and hydroxides on the surface is a possibility. However, this effect is likely to be short-term and sensitive to feed water quality.

The influence of schmutzdecke development on *E. coli* reductions in Column Tests No. 1 and No. 2 is shown in Figures 4.8a and 4.8b, respectively. In these box and whisker plots, the extent of schmutzdecke development is expressed by normalized filtration rate, $Q_I/Q_{I,o}$ where Q_I is the initial daily filtration rate (i.e., upon introduction of the daily charge) for each day and $Q_{I,o}$ is the initial daily filtration rate on the first day of operation. Normalized filtration rates during the eight weeks of operation were classified arbitrarily into three bins as shown in the figures. The largest $Q_I/Q_{I,o}$ bin (values greater than 0.8) represents samples taken during the stages of column operation without a substantial schmutzdecke (the earliest samples and those shortly after schmutzdecke scouring) while the smallest $Q_I/Q_{I,o}$ bin (values less than 0.2) represents stage of column operation when the schmutzdecke is fully formed. Log reductions of *E. coli* increased substantially as $Q_I/Q_{I,o}$ declined which shows the importance of schmutzdecke growth for increasing microbial reductions by (1) physical straining and (2) decreasing flow rate (caused by increased head loss) leading to more efficient depth filtration through longer contact time. Of these two mechanisms, physical straining seems more likely given that the top few centimeters of media surface has been shown to be responsible for enhanced

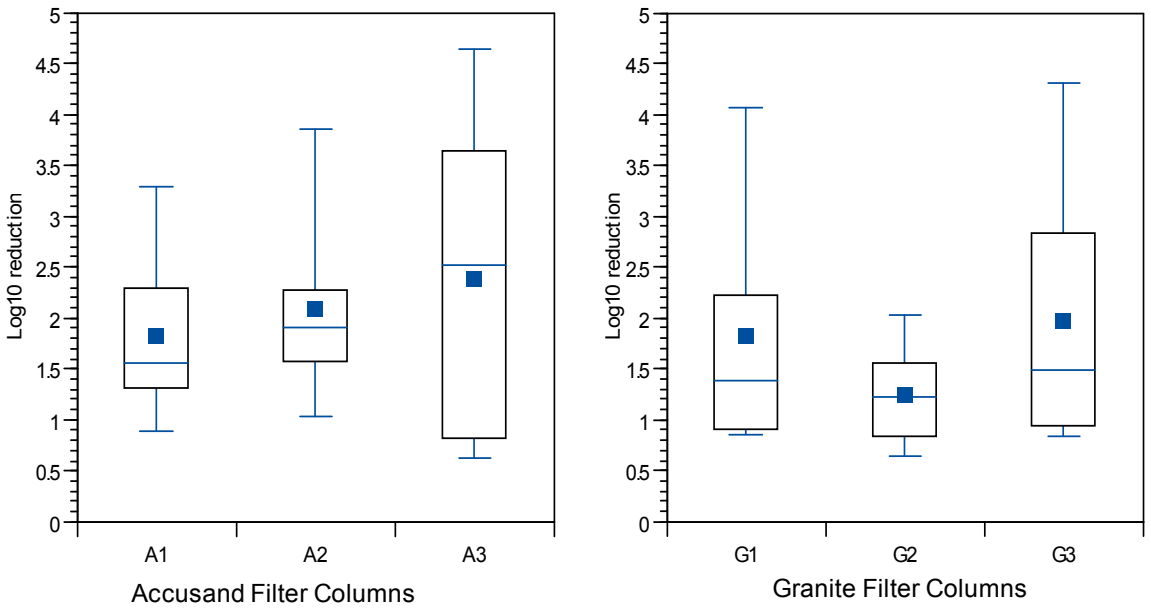

Figure 4.6(a,b): *E. coli* reductions for (a) Accusand-filled columns and (b) granite-filled columns in Column Test No. 1 for samples collected throughout the eight-week experiment.

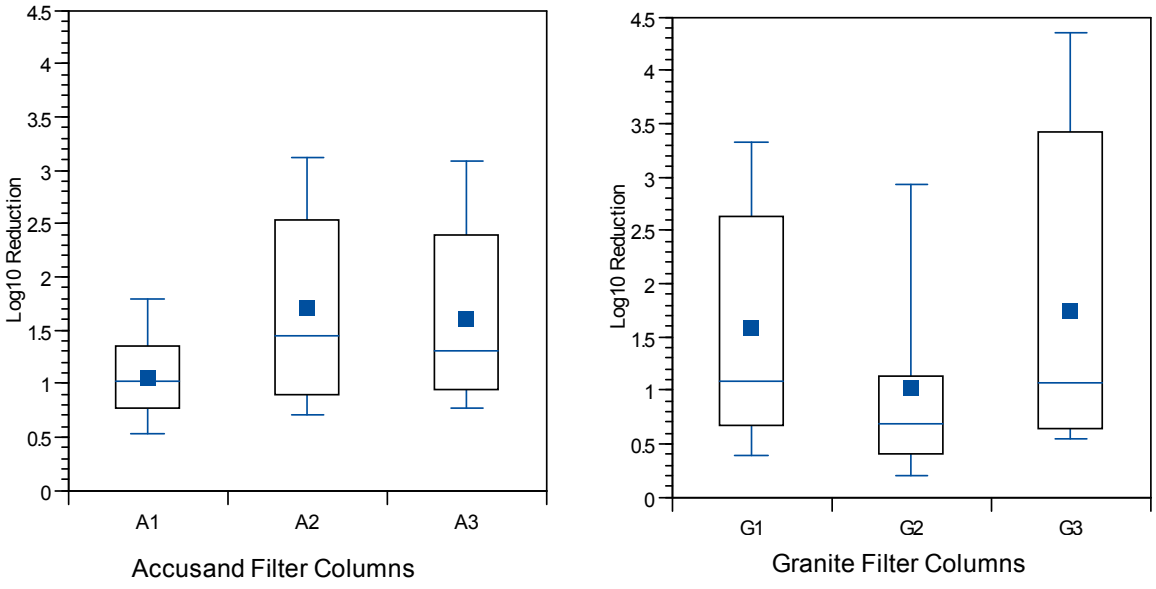

Figure 4.7(a,b): *E. coli* reductions for (a) Accusand-filled columns and (b) granite-filled columns in Column Test No. 2 for samples collected throughout the eight-week experiment.

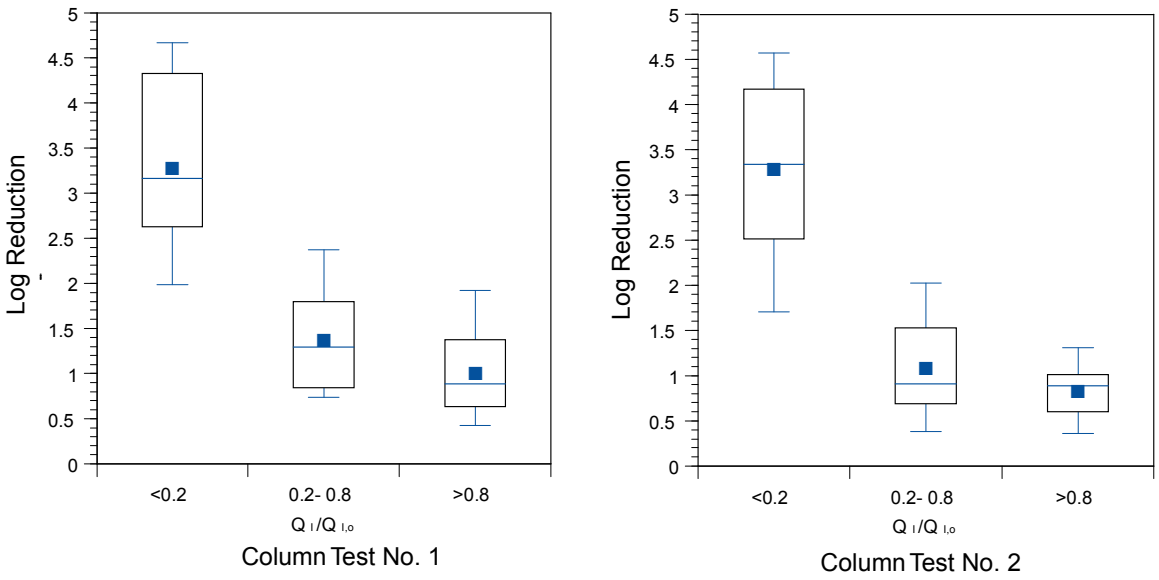

Figure 4.8(a,b): *E. coli* reductions in Column Test No. 1 and Column Test No. 2 organized by bins of normalized filtration rate where Q_I is the initial daily filtration rate on each day of charging the column and $Q_{I,o}$ is the comparable initial filtration rate on the first day.

microbial reductions (Hijnen et al., 2004; Unger and Collins, 2008). However, the same may not be true for viruses.

4.3.5 Reductions in Challenge Viruses

Box and whisker plots of log reduction of MS2 coliphage are given for in Figures 4.9a (Accusand media) and 4.9b (granite media) for Column Test No. 1 in Figures 4.10a (Accusand media) and 4.10b (granite media) for Column Test No. 2. Reductions were generally less than about 1-log. This is approximately four orders of magnitude lower than the maximum *E. coli* reductions. The smaller size of viruses is one explanation for the difference. Variability in reduction was also evident during the eight-weeks of operation of each column. Reproducibility among replicate columns is qualitatively better than observed for *E. coli* but this is probably due to the inability to measure differences within a 1-log reduction range. The extent of virus reductions in these bench-

scale tests were similar to those observed previously in full-scale tests (Elliott et al., 2008).

Less data were available for reductions of PRD-1 coliphage and echovirus 12. The box and whisker plots for log reduction of PRD-1 are given in Figures 4.11a (Accusand) and 4.11b (granite media) for Column Test No. 2 (PRD-1 was not used as a challenge microorganism during Column Test No. 1). Reductions for echovirus 12 in Column Test No. 1 are shown in Figure 4.12 wherein all three columns containing the same media are grouped together because the number of samples from each column was insufficient to warrant a box and whisker analysis (echovirus 12 was not used as a challenge microorganism during Column Test No. 2). The extent of reductions for PRD-1 were similar to those for MS2 whereas those for echovirus 12 were much higher, reaching a reduction of 3.5-log. However, direct comparisons are not possible because the PRD-1 and echovirus 12 reductions were from different column tests (Column Test No. 2 for PRD-1 and Column Test No. 1 for echovirus 12).

The effect of media type on virus reductions was investigated albeit conclusions relative to all three challenge viruses are limited due to a lack of comparable data. Despite the variability shown in Figure 4.9a and b, the Mann-Whitney U-test showed that reductions of MS2 were significantly greater in granite media than Accusand in Column Test No. 1 ($p<0.05$). Echovirus 12 reductions were also far greater in the granite than Accusand media during Column Test No. 1 as shown in Figure 4.10. However, not all of the comparisons support greater virus reductions on granite media in that no significant difference in reductions of MS2 (Figure 4.11a and b) or PRD-1 (Figure 4.12a and b) was found, in Column Test No. 2. Thus, definitive conclusions are not possible.

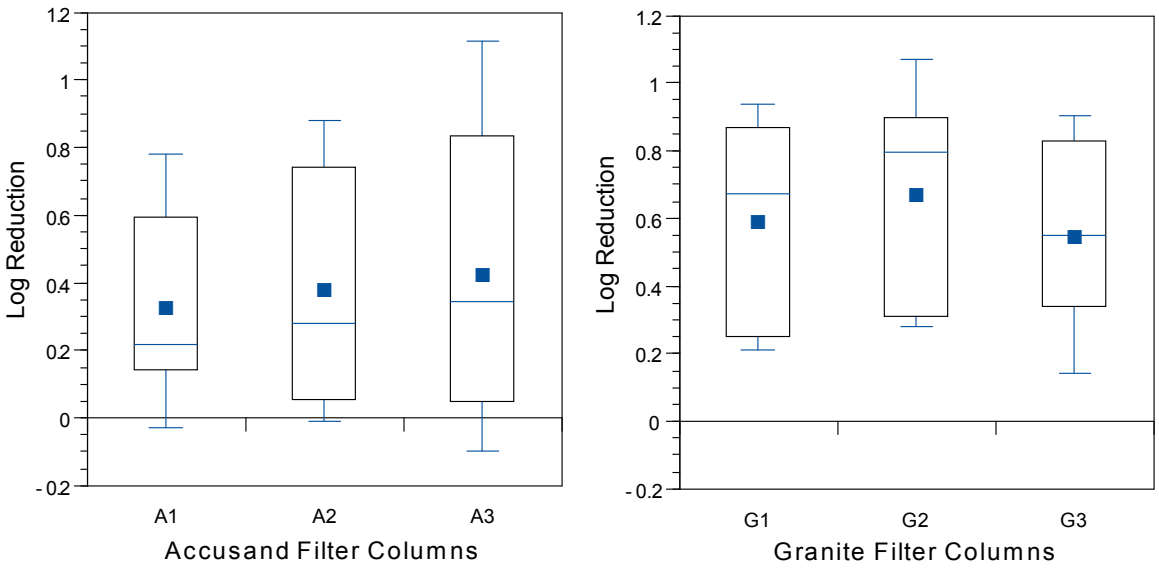

Figure 4.9(a,b): MS2 reductions for (a) Accusand-filled columns and (b) granite-filled columns in Column Test No. 1 for samples collected throughout the eight-week experiment.

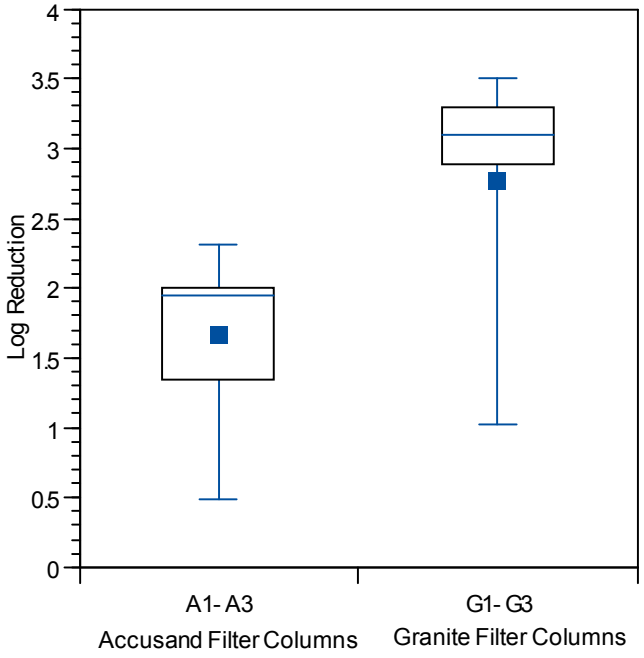

Figure 4.10: Echovirus 12 reductions for Accusand-filled columns and granite-filled columns in Column Test No. 1 for samples collected throughout the eight-week experiment.

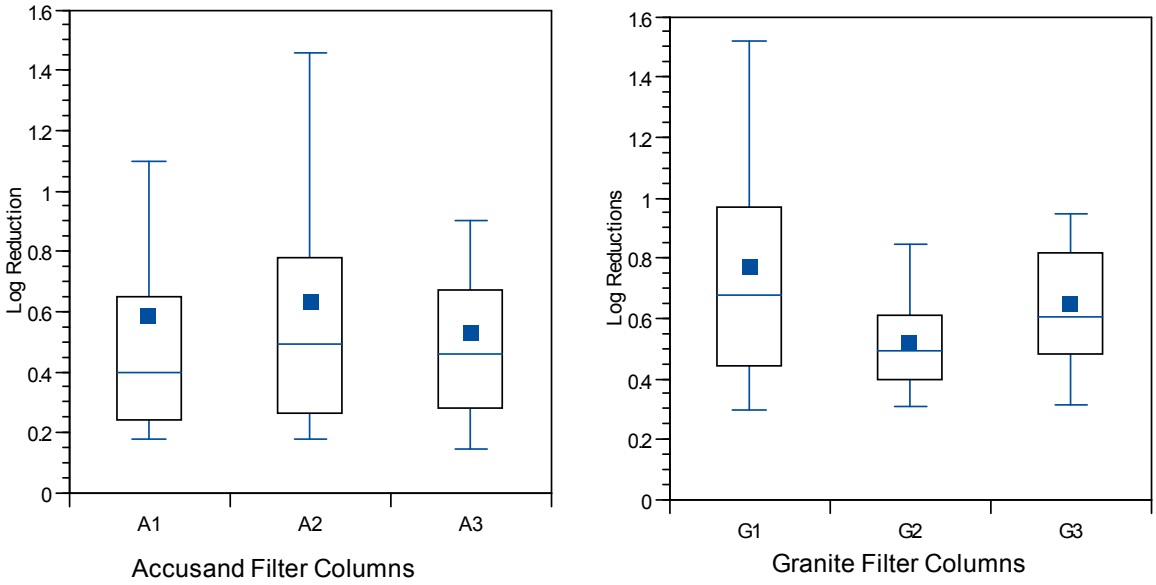

Figure 4.11(a,b): MS2 reductions for (a) Accusand-filled columns and (b) granite-filled columns in Column Test No. 2 for samples collected throughout the eight-week experiment.

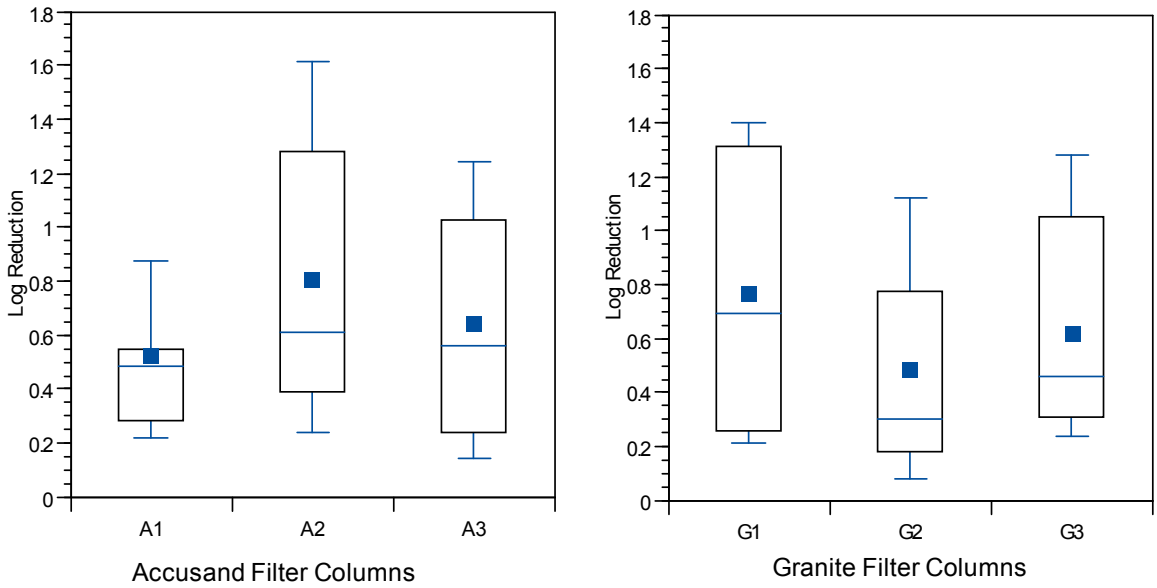

Figure 4.12(a,b): PRD-1 reductions for (a) Accusand-filled columns and (b) granite-filled columns in Column Test No. 2 for samples collected throughout the eight-week experiment.

The initial hypothesis was that virus reductions would be greater in columns containing the granite than Accusand media because of the two-order of magnitude higher concentration of Al and Fe on the surface (Table 4.3) that could have increased virus attachment by creating patches of positive charge on the granite surface (Ryan et al., 2002; Zhuang and Jin, 2003b). These patches of positive charge could attract the viruses that carry a net-negative charge at the near-neutral pH of the feed water (Table 4.2). However, the hypothesis was not supported by virus reductions in Column Test No. 2 wherein no significant difference in reductions of MS2 (Figures 4.11a and 4.11b) nor PRD-1 (Figures 4.12a and 4.12b) were found between columns filled with granite and Accusand. The likely explanation is that the large surface area of fine silty material present in the granite media during Column Test No. 1 was lost during the backwashing procedure prior to Column Test No. 2. This led to postulation that very fine filter material assists in virus reduction by offering a large surface area for capture. Increased surface area could enhance virus reduction by adsorption mechanisms or possibly through biological mechanisms. Mechanisms of virus reduction, depth filtration and maturation are discussed in more detail in Chapters 5 and 6.

The influence of filter maturation on MS2 and PRD-1 reductions was investigated by grouping the data by normalized, initial daily filtration rate ($Q_I/Q_{I,o}$). Insufficient data were available for echovirus 12 reductions to do the same analysis. The results for MS2 and PRD-1 are given in Figures 4.13 and 4.14, respectively. These indicate that greater reductions are associated with lower values of $Q_{I,o}/Q_o$ as had also been noted for *E. coli* reductions (Figure 4.8). Filter maturation is seen again to play a large role in enhancing microbial capture.

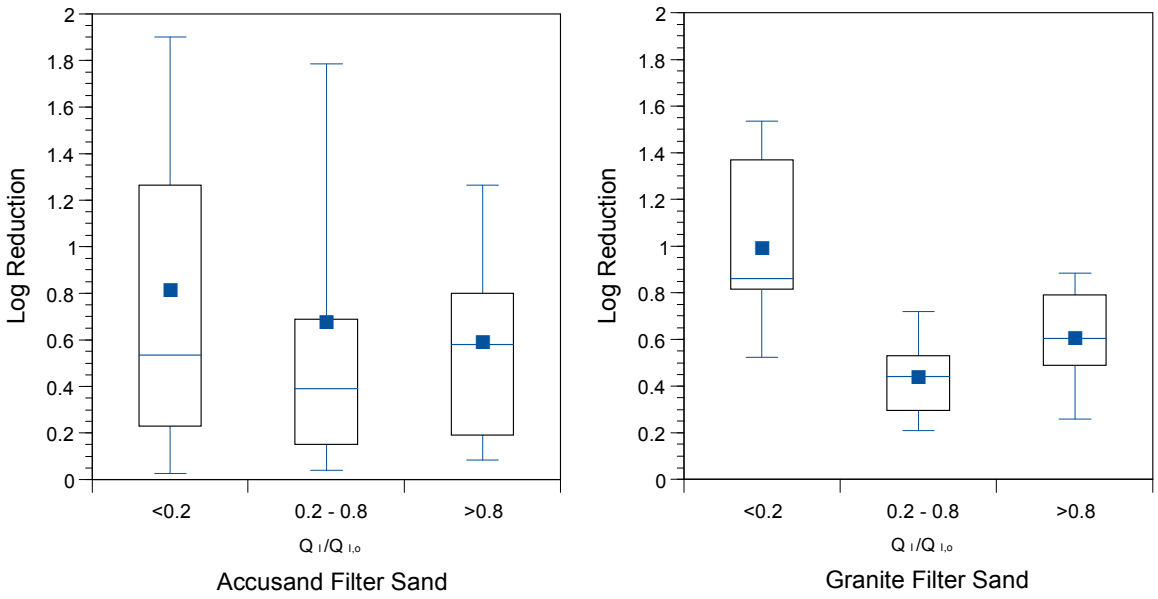

Figure 4.13(a,b): MS2 reductions for (a) Accusand-filled columns and (b) granite-filled columns in Column Tests No. 1 and No. 2 organized by bins of normalized filtration rate where Q_I is the initial daily filtration rate on each day of charging the column and $Q_{I,o}$ is the comparable initial filtration rate on the first day.

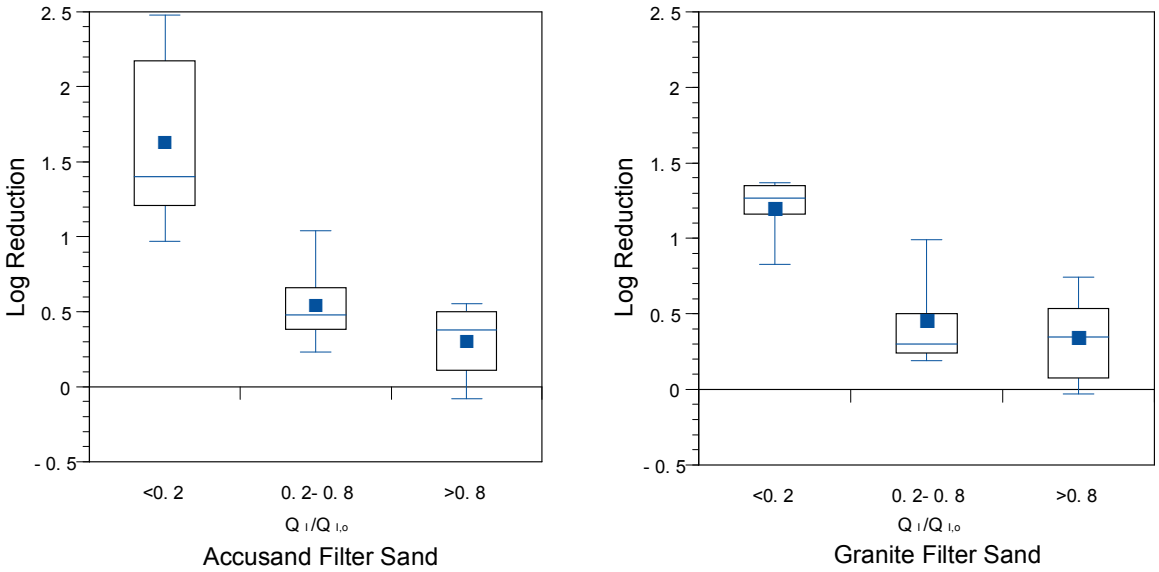

Figure 4.14(a,b): PRD-1 reductions for (a) Accusand-filled columns and (b) granite-filled columns in Column Tests No. 2 organized by bins of normalized filtration rate where Q_I is the initial daily filtration rate on each day of charging the column and $Q_{I,o}$ is the comparable initial filtration rate on the first day.

Questions concerning the mechanism(s) by which virus reductions occur in the BSF process are raised by all of the observations presented in Figures 4.9 to 4.14. Physical straining through the schmutzdecke is unlikely to be an important mechanism of virus reduction because of the small size of viruses. The schmutzdecke could increase viral reductions by decreasing flow rate, leading to more efficient depth filtration. However, schmutzdecke growth also occurs simultaneously with other maturation processes that have been shown to affect depth filtration of viruses in SSF (Poynter and Slade, 1977; Wheeler et al., 1988). These processes will be covered in more detail in Chapters 5 and 6.

4.4 Conclusions

Reproducibility in performance among replicate filters was shown to be a challenging problem in experimental design. The rate of schmutzdecke development, as measured by the decline in filtration rate over eight weeks of experimentation, varied widely across replicate columns (Section 4.3.3) in both Column Tests No. 1 and No. 2. One possible explanation is difficulty in controlling the media size of the topmost layer where the schmutzdecke develops. This was apparent for the granite media because of the large fraction of fines and a very wide grain size distribution. Reproducibility was improved by backwashing to remove these fines. Nonetheless, variation in microbial reductions among replicates of columns packed with the Accusand media, which comprised a very narrow range of grain sizes, was also apparent. Thus, other factors besides variability in grain size are important. Despite the lack of reproducibility, the

mean microbial reductions over the entire course of study were similar for replicate columns in both column tests (Figures 4.9, 4.11 and 4.12).

E. coli reduction was the same using Accusand silica or crushed granite as prepared in the prescribed way for field installation despite the striking differences in inorganic composition (two orders of magnitude higher Al and Fe on granite surface) and grain size distribution (much wider distribution for granite). However, during Column Test No. 1 a greater reduction of viruses was achieved by granite than Accusand filtration. The much higher surface concentrations of Al and Fe on the granite media were suspected to produce a positive surface charge that could attract viruses. This proved to be an unlikely explanation given that virus reductions were not significantly different when using backwashed granite and Accusand media in Column Test No. 2. An alternative explanation is that backwashing eliminated the very fine fraction from the granite media that could have provided a large surface area for virus sorption or enhanced biological activity.

Reductions of *E.* coli, MS2 and PRD-1 increased as filtration rate declined corresponding to the maturation of the schmutzdecke. The most likely mechanism for *E. coli* reduction was physical straining through the schmutzdecke layer. However, increased head loss leads to slower pore velocities that could enhance depth filtration. Because of the small size of viruses, the latter mechanism is thought to be more important for MS2 and PRD-1. Additionally, other maturation processes that influence depth filtration of viruses occur simultaneously with schmutzdecke development. These mechanisms and their effects on virus reductions require further investigation.

CHAPTER 5

EFFECT OF IDLE TIME AND MEDIA AGING ON VIRUS REDUCTIONS IN A HOUSEHOLD-SCALE, SLOW SAND FILTER

Abstract

Virus challenge experiments were conducted at full-scale and bench-scale on an intermittently operated household scale slow sand filter popularly known as the biosand filter (BSF). The effect of daily idle time on the concentration of coliphages PRD-1 and MS2 and enteric virus echovirus 12 was shown both in full-scale and bench-scale testing of BSF loaded with a silica sand medium. Virus reductions were higher for filtered water volumes smaller than the pore volume of the filter in full-scale tests; this water, therefore, had been stored for an idle time that approximated 18 hours prior to release by the next day's charge. A similar result was observed in bench-scale tests. In addition, virus reductions were shown to occur by direction measurements of pore water withdrawn during the daily idle time at depths of 10- and 30-cm. The rate of virus reduction was first-order and increased over 40 to 50 days as the filter matured from repeated daily water charges. The rates ranged from 0.034-log to 0.062-log per hour of idle time compared to only 0.003-log per hour in control samples in which virus spiked water was held for the same period of time but without contact with filter media. Rates of virus reduction during the idle time of newly loaded filters were indistinguishable from those observed in the control samples. Thus, virus reductions were attributed to "aging" of the

filter medium over days-to-weeks of daily filtration. The aging effect was present even at 30-cm depth emphasizing that deep-bed maturation rather than top-layer maturation, historically referred to as schmutzdecke growth, is responsible.

5.1 Introduction

Household-scale, point of use devices could reduce the huge number of diarrheal cases currently attributed to drinking water in developing countries (Clasen et al., 2007). One of the most promising technologies is the intermittently operated slow sand filter popularly known as the biosand filter (BSF).

Household implementation of BSF in developing countries has been shown to produce large reductions in diarrheal illness. For example, a reduction of greater than 40% was observed in studies conducted in the Dominican Republic (DR) (Stauber et al., 2009), Kenya (Tiwari et al., 2009) and Cambodia (Liang, 2007). The reliability and simplicity of the BSF suggest that these reductions can be sustained. Survey data in the DR, Cambodia and Haiti, for instance, indicate that >85% of the BSFs are still in service six months-to-eight years after their installation (Aiken, 2008; Liang, 2007; Duke et al., 2006). Far lower levels of sustained use have been reported for other POU technologies (Sobsey et al., 2008).

Although the BSF has already been implemented widely, a fundamental understanding of the dynamics of microbial reductions is still missing. Explanations obtained from SSF or rapid sand filtration studies do not necessarily apply because the BSF process is operated in a significantly different fashion. In particular, the filtration rate declines to zero as the daily charge is processed and the filter remains idle for many

hours between charges. Moreover, the emphasis in BSF research has been on reductions of enteric bacteria, but pathogenic viruses are responsible for over 40% of diarrhea hospitalizations in the developing world (Ramani and Kang, 2009). The scant laboratory data that are available for the BSF process have generally shown less reduction of viruses than bacteria and protozoan parasites (Elliott et al., 2008; Palmateer et al., 1999; Koksal et al., 2008).

Viral reduction by filtration is also highly strain-dependent. Coliphages MS2 and PRD-1, which are enteric virus surrogates, are known to be treated less efficiently by granular media filtration than most pathogenic viruses (Schijven and Hassanizadeh, 2000). Using a daily charge volume of 1.1-1.3 pore volume, the average reductions in MS2 and PRD-1 over the filtration cycle in BSF laboratory studies was only 0.5-log (70%); by contrast, reductions for PhiX 174 virus and echovirus type 12 were consistently greater (Elliott et al., 2008 and 2009).

Previous work has also suggested that virus and bacterial reductions in the BSF are not constant throughout the filtration cycle (Elliott et al., 2008 and 2009). In particular, the reductions were significantly greater immediately after introduction of the next daily water charge. Understanding the extent to which the length of the idle period between daily charges affects the initial reductions of viruses is a major aim of this research. The hypothesis is that attenuation occurs within the pores of the BSF during the idle period. Attenuation could take place over the entire depth of the filter bed rather than confined to the classic schmutzdecke layer and could be dependent upon the length of the idle time. Additionally, the maturation of the filter over repeated daily charges could also affect the attenuation conditions below the schmutzdecke layer in a process

that will be referred to as "media aging." If attenuation during the idle period is proven to be significant and if media aging enhances virus reductions, maximizing the storage time within the filter could lead to sustained improvement in virus reductions for BSF users worldwide.

5.1.1 Characteristics of the BSF Process

The typical daily charge for a household using the BSF is 20- to 40 L, depending on the number of users relying on the filter for water treatment. Although the BSF process is similar to full-scale slow sand filtration (SSF), the design and operation of the BSF differ significantly from conventional SSF. The maximum filtration rate recommended for BSF is 1.1 m/h which is up to 15-times greater than for SSF (Fox et al., 1994). The filtration rate decreases as the water level declines after a single charge. The depth of sand is 0.4 m, which is about 50% less than for SSF. Thus, the combination of higher application rate and shorter depth produces a contact time at initiation of each filtration cycle that is up to 30 times shorter than in SSF. However, average contact time is typically greater in BSF due to both declining elevation head and extended idle time. In addition, physical-chemical characteristics of the sand are variable among BSF applications because locally available media are used whereas most sands for SSF are obtained from a commercial source and thus more consistent.

Two unique features of the design of the BSF are shown in Figure 5.1. An elevated outlet tube (2- to 7 cm above the height of the filter media) allows the media to remain saturated after a charge has been filtered and the filter remains idle until the next charge. The outlet tube is screened in most BSFs made of plastic but is generally not

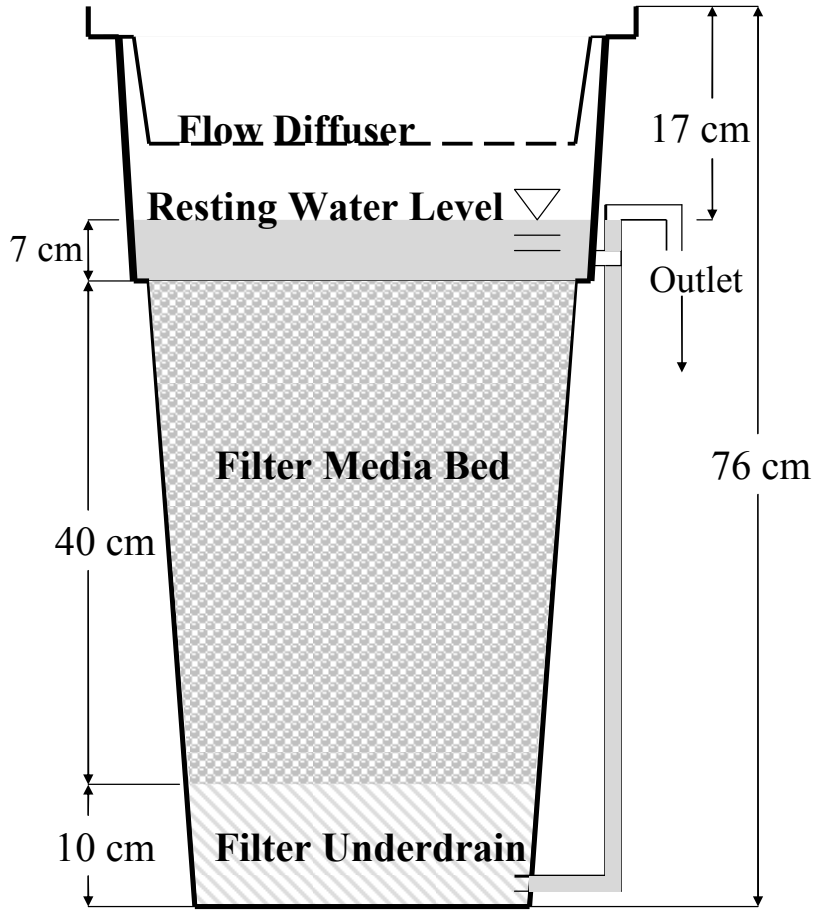

Figure 5.1 Cross-section of the plastic household-scale BSF used in laboratory experiments

screened in those with a concrete exterior. The other unique aspect is a plastic or sheet metal plate called a diffuser with 2-mm diameter holes that rests just above the surface of the sand layer. This plate spreads the feed water charge uniformly across the sand surface without disturbing the schmutzdecke.

5.1.2 Definition of Aged Media

The classic definition of the **s**chmutzdecke is "a layer of material, both deposited and synthesized, on top of the filter bed that causes head loss disproportionate to its

thickness" (Barrett et al., 1991). Ripening, also referred to as maturation, generally describes a physical/chemical and/or biological process that occurs during operation of SSF and BSF, during which a schmutzdecke forms, head loss increases and the removal of incoming particles improves.

The term "aged media" is used here to recognize the possibility that physical/chemical or biologically mediated processes occur during which the surface of all sand grains (even those deep in the filter bed) are modified in such a way that they are more likely to adsorb microorganisms and other colloids. Filters loaded with "clean" sands do not reduce virus concentrations as efficiently as those that have been in operation for a number of months or years (Poynter and Slade, 1978; Windle-Taylor, 1969). This difference in virus reduction efficiency was shown to be retained through the development and removal of the schmutzdecke, indicating that aging is a distinct process from the ripening that occurs at the top of the filter bed (Poynter and Slade, 1978). The time period for aging may be on the order of weeks, months or years. It depends upon biological activity, chemical composition of the source water, temperature and perhaps other factors.

5.2 Materials and Methods

5.2.1 Experiments with Full-scale Plastic BSF

Prior to detailed bench-scale testing of the BSF process, three 6-to-8 week microbial challenge experiments were conducted with full-scale plastic BSFs (Figure 5.1) obtained from Davnor, Inc. (Calgary, Alberta). The daily charge was set at either 20 L or 40 L to simulate the typical application in the household. The pore volume of the filters

was 18.1-18.3 L. Viruses MS2 phage, PRD-1 phage and echovirus type 12, along with bacterium *E. coli* B, were seeded into feed water and assayed for reduction efficiency. Details of virus assay methods and stock preparation can be found Section 5.2.4. The results are covered in more detail in Elliott et al (2006, 2008) and Stauber et al (2006).

5.2.2 Experiments with Bench-scale BSF Columns

The need for bench-scale BSF column experiments was motivated by several deficiencies found in full-scale experiments. First, filling the BSF with field-specific media made it impossible to generalize the results because the media characteristics were very difficult to reproduce from batch to batch. Secondly, the large volume of water needed for daily charges (20 to 40 L) and the size of the filters made it impractical to store water and operate many filters in parallel in the laboratory space. Thirdly, study of variation in viral reduction over the filtration depth was not possible without significant modification to the full-scale BSF. To overcome these deficiencies, three BSF columns of small diameter were filled with well-characterized Accusand silica (Unimin Corp., Le Sueur, MN) and were operated in parallel. The daily charge was reduced from 20-40 L in the full-scale design to just 450 mL per column in the bench-scale design and sampling ports were conveniently installed at two different filter depths.

The design of a bench-scale BSF column is shown in Figure 5.2. Columns were constructed of polycarbonate cylinders with inner diameter of 4.4 cm. The daily charge volume (450 mL) maintained hydraulic similitude with full-scale BSFs, both in initial application rate and fraction of charge stored in pore volume during the idle period. Ports were installed at depths of 10- and 30 cm for direct withdrawal of samples from the pores

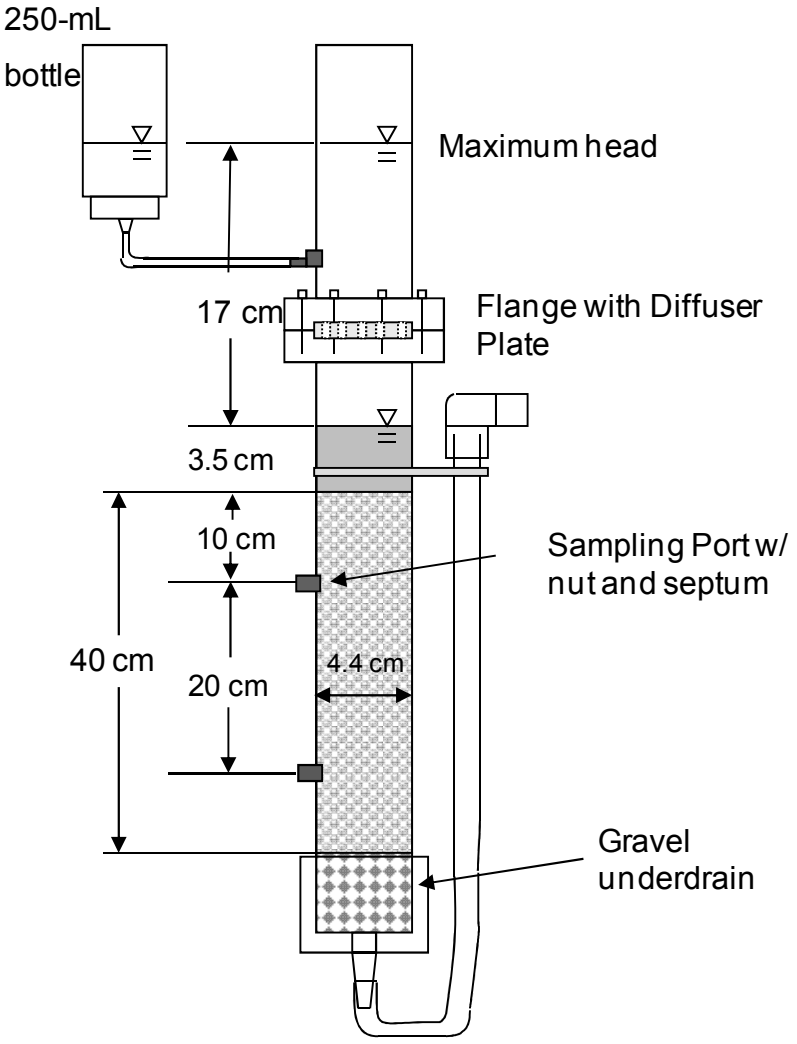

Figure 5.2 Cross-section of bench-scale columns used in Column Test No. 2.

within the filter bed with a polypropylene syringe fitted with a 25-gage beveled needle. By waiting until elevation head declined to zero and taking periodic samples, the sampling ports enabled direct investigation of virus attenuation during the idle period.

Four bench-scale column experiments were conducted. Column Tests No. 1 and No. 2 included three columns operated in parallel for a period of up to 8 weeks. Column Test No. 2 was a replication of Column Test No. 1 with the exception that columns were backwashed prior to operation in an attempt to decrease the column-to-column variability in ripening and head-loss development. Test No. 2 also incorporated direct measurement of idle time effects on viruses in ripened columns via the sampling ports.

Column Test No. 3 consisted of only one column rather than three in parallel. The intent was to serve as a preliminary experiment without replication to determine whether the virus concentration would decline during the idle time in a filter column with clean, freshly loaded Accusand as compared to a fully ripened bed (from Column Test No. 2). In addition, the time required for aging of media to cause an effect of idle time effect was of interest. These results provided insights into the effect of idle time that were deemed important enough to warrant exploration even though replication was not included.

Column Test No. 4 included two columns and was initiated 62 days after the start of Test No. 3. The goal of Column Test No. 4 was to isolate the effect of media aging from any possible experimental artifacts related to storage time of source water or virus stocks. That is, in previous column tests, the storage of source water and/or virus stock for daily feeding over the many weeks could possibly have been a confounding factor in searching for an effect of media aging on virus attenuation. Samples were taken during

the idle periods from Column Test No. 3 and No. 4 on the same date, with the only difference between the two tests being the number of days that the Accusand media had to undergo the aging process.

Slight modifications in column design were made from one column test to another so as to facilitate charge introduction. In Column Test No. 1, less than 60% of the total daily charge could be added at one time without exceeding the 17-cm maximum head used in full-scale experiments. An external reservoir (a 250-mL PPE bottle) shown in Figure 5.2 was installed after Column Test No. 1 so that the entire daily charge (450 mL) could be introduced at one time (i.e., 250 mL above the filter media in addition to 200 mL from the bottle) while maintaining the maximum elevation head the same as in full-scale filters. For Column Tests No. 3 and No. 4 a column with a 5.5-cm diameter above the flow diffuser and the same 4.4-cm diameter below the diffuser was used. The larger diameter reservoir achieved the same objective as the addition of the external reservoir by allowing the entire 450-mL charge to be added without exceeding the maximum elevation head.

The length of operation for Column Test No. 1 and No. 2 was similar to full-scale experiments, i.e., 6 to 8 weeks. This was extended in Column Test No. 3 to 13 weeks. Column Test No. 4 was initiated after 9 weeks of Column Test No. 3 and the two experiments were conducted in parallel for 31 days. Tracer tests with sodium chloride were used prior to each experiment to verify that hydraulic behavior was close to plug flow (Elliott et al., 2008).

Samples were collected for virus assays both during the filtration and idle periods. A virus assay on the entire volume of filtered water collected from a daily charge gave a

composite measure of virus reduction. The first 0.6-to-0.8 pore volumes filtered each day (i.e., the first 200 mL filtered where the pore volume is 300-340 mL or the first 15 L filtered in full-scale BSF experiments where the pore volume is 18.1-18.3 L) were also collected for separate analysis in some cases. Because hydraulic conditions were similar to plug flow, this sample was expected to be composed almost entirely of volume that was stored in the filter from the previous daily charge.

The effect of idle time on attenuation of virus challenge organisms was also measured directly in Column Tests No. 2, 3 and 4. These columns were first charged and filtration continued until an elevation head of nearly zero was reached. Samples were then withdrawn periodically from the sampling ports at 10-cm and 30-cm depths using a 25-gauge beveled needle into a 3-mL sterile plastic syringe (Becton-Dickinson, Franklin Lakes, NJ) throughout the idle time of 18-24 hours. They were immediately refrigerated and analyzed within about 24 hours for MS2 and PRD-1.

5.2.3 Feed water for virus challenge studies

A summary of the experimental design for each of the four BSF column tests is provided in Table 5.1. Feed water was obtained from the raw water sample taps of the water treatment plant operated by the Orange Water and Sewer Authority (OWASA, Carrboro, NC). Two local water supply reservoirs (Cane Creek Reservoir and University Lake) are used by OWASA. Neither of these source waters receives wastewater discharges. The alkalinity of the source waters was very low with a mean of 26 mg/L (range 14.4-48.1 mg/L as $CaCO_3$). Sufficient water was collected before each BSF column test to supply the daily charges for the duration of each experiment (54 to 93

Table 5.1 Characteristics of virus challenge experiments in bench-scale BSF.

Experiment Coding	Length (days)	Source water[1]	Columns Backwashed	Pasteurized PE[2]	MS2 $\log_{10}$ pfu/mL[3]	PRD-1 $\log_{10}$ pfu/mL[3]	echovirus 12 $\log_{10}$ pfu/mL[3]
Column Test No.1	54	Cane Creek	No	1.0%	3.4 ± 0.8	-	3.6 ± 1.2
Column Test No.2	56	Cane Creek	Yes	2.5%	2.8 ± 0.3	3.1 ± 0.7	-
Column Test No.3	93	Univ. Lake	No	2.5%	3.0 ± 0.9	4.0 ± 0.9	-
Column Test No.4	31	Univ. Lake	No	2.5%	3.1 ± 0.6	4.0 ± 0.9	-

(1) Cane Creek = Cane Creek Reservoir, Carrboro, NC; Univ. Lake = University Lake, Chapel Hill, NC

(2) Pasteurized PE = pasteurized primary effluent from OWASA WWTP, Chapel Hill, NC.

(3) Virus concentrations are mean $\log_{10}$ measured concentration per mL and maximum $\log_{10}$ deviation from the mean.

days). The sample volume was stored at 4°C until daily use and then allowed to reach room temperature (approximately 20°C) overnight. Thus, water temperature was nearly constant in all experiments and did not influence the rate of ripening nor microbial stability. Stored water for each daily charge was amended with pasteurized primary effluent (PE) from the OWASA wastewater treatment plant to simulate the presence of wastewater in typical drinking water sources of developing countries and to accelerate the ripening process. The daily charge was then spiked with cultures of challenge viruses MS2 and either echovirus 12 or PRD-1 to reach concentrations in the range of 3- to 4-log pfu/mL. The addition of 1% to 2.5% pasteurized primary effluent and challenge microbe spike solutions increased the TOC of the feed water by up to 50% such that feed TOC was in the range of 7.5-12.5 mg/L.

Virus stocks were diluted and added to feed water daily. Feed water volume consisted of between 1×10^{-5} and 1×10^{-6} volume dilution of the initial virus stock. Details of virus stock preparation are included in Section 5.2.4.

5.2.4 Virus Analyses and Stock Preparation

The BSF columns were operated at room temperature and charged daily with feed water spiked with challenge viruses. An aliquot of feed water used for the previous day was stored at room temperature until microbial samples were analyzed the following day; this served as a control for the effect of time on virus survival. The resulting mean of the die-off rates after overnight storage were considered small (less than 20% over 24 h). Average virus die-off in control samples will be presented alongside die-off rates in filter idle time samples to account for virus inactivation due to time and temperature conditions.

Characteristics of the three challenge viruses are listed in Table 5.2. Isoelectric point has been identified as the best single parameter for predicting virus sorption in granular media (Dowd et al., 1998). The values listed in Table 5.2 show that the viruses are net-negatively charged at the feed water pH, which averaged 6.9 and ranged from 6.6-7.3. Silica also carries a net-negative charge in this pH range. The fact that MS2 and PRD-1 have much lower isoelectric points than echovirus 12 suggests that adsorption of MS2 and PRD-1 would be impeded by net electrostatic repulsion to a greater extent than echovirus 12. However, the isoelectric point can also depend on the characteristics of the solution and, potentially, the medium in which the laboratory virus stock was cultured (Mesquita et al., 2010; Daniels, 1980; Glassman, 1948). For this reason, the net surface charge or the magnitude of charge carried by a virus particle will be specific to each feed water and will also be affected by virus preparation methods. Ideally, zeta potential would be determined for each challenge virus stock in the feed water used in each

experiment. This would give an estimate of both the sign and the magnitude of charge on each virus in the relevant feed water throughout the applicable pH range.

MS2 and PRD-1 concentrations were assayed using the single agar layer method on hosts *E. coli* F_{amp} and *Salmonella typhimurium* LT2, respectively (USEPA, 2001). Stocks of bacteriophages MS2 and PRD-1 were grown, enumerated by USEPA Method 1602 that uses a double agar layer (USEPA, 2001) and stored at -80ºC. Aliquots of each stock were thawed each week, serially diluted ten-fold in phosphate buffered saline and stored at 4ºC for up to 7 days.

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 stock solutions: 100x Gentamycin/Kanamycin, Nystatin, HEPES buffer and Non-essential amino acids) at 37ºC, freeze-thawed and chloroform extracted and then enumerated by the plaque technique in cell monolayers. Chloroform extraction was used to purify echovirus stock of macromolecular cell debris. Extracted stock was stored at -80ºC until needed. Aliquots of enumerated stock were thawed daily, serially diluted and added to feed water

Table 5.2 Characteristics of viruses used in these experiments

Virus/Phage	Size (nm)	Isoelectric point	Genetic Material
MS2	26	3.5-3.9	ss-RNA
PRD-1	62	4.2	ds-DNA
Echovirus 12	28-30	5.0-6.4[1]	ss-RNA

(1) IEP listed for echovirus 12 is the range of values found in the literature for all echovirus types.

Source: Collins et al, 2004

to achieve the desired concentration of echovirus 12 for each daily charge.

Reductions in virus concentration in the BSF were calculated by Equation 1. All log-reduction values reported are log base 10.

$$\log \text{Reduction} = \log(\text{Feed Water Concentration}) - \log(\text{Filtered Water Concentration}) \quad (1)$$

5.2.5 Data Analysis

The data for log reductions of filtered samples did not pass the Kolmogorov-Smirnov test for conformance to a Gaussian distribution. Non-parametric statistics were used instead to compare log reductions. The Mann-Whitney *U* test (also known as the Wilcoxon rank-sum test) was used to compare log reductions of viruses. The resulting p-values are for unpaired, two-tailed tests with significance level α=0.05.

As will be shown in Section 5.3.2, plots of log fraction of virus concentration remaining at idle time, t ($\log C_t/C_{t=0}$) against t were linear thus indicating a first-order reduction rate. Therefore, $C_t/C_{t=0}$ was log-transformed prior to regression analysis using one-way Analysis of Covariance (ANCOVA) for independent samples.

5.3 Results and Discussion

5.3.1 Virus Reduction during Filtration of Daily Charge

Results of virus reductions during a full filtration cycle (1.3 pore volumes) in Column Tests No. 1 and No. 2 are presented in Table 5.3. Reductions of MS2 and PRD-1 were not significantly different, while echovirus 12 reductions were observed to be significantly greater than those of MS2 and PRD-1 ($p<0.0001$). Virus reductions during

Table 5.3 Mean log reductions of viruses in three replicate columns of Column Test No. 1 and No. 2.

Column Test No.	Virus Type	Column 1	Column 2	Column 3	p-value[1]
1	MS2	0.41	0.43	0.49	<0.0001
1	Echovirus 12	1.32	1.51	1.92	
2	MS2	0.59	0.64	0.54	>0.999
2	PRD-1	0.53	0.77	0.58	

(1) P-values of unpaired Mann-Whitney *U*-tests comparing reductions of MS2 to echovirus 12 in Column Test No. 1 and MS2 to PRD-1 in Column Test No. 2

Table 5.4 Mean log reductions of viruses in composite samples collected from the first 0.6-0.8 or 1.1-1.3 pore volume filtered. Data are from a full-scale filter test and Column Test No. 1.

	Pore volumes filtered	Mean $\log_{10}$ reduction	Standard Error	n	p-value[1]
MS2	0.6-0.8	0.87	0.092	47	<0.001
	1.1-1.3	0.54	0.056	59	
echovirus 12	0.6-0.8	2.47	0.118	26	0.07
	1.1-1.3	2.07	0.194	26	

(1) Two-tailed p-value calculated by Mann-Whitney *U* test to compare 0.6-0.8 pore volume and 1.1-1.3 pore volume samples. The differences are considered significant for MS2 and not quite significant for echovirus 12.

a full filtration cycle of greater than one pore volume are generally poor relative to drinking water treatment standards, especially for MS2 and PRD-1.

Virus reductions in composite samples corresponding to filtration of less than one stored volume (0.6-0.8 pore volumes) are compared with reductions in composite samples of the full filtration volume (1.1-1.3 pore volumes) in Table 5.4. The results from the full-scale and column-scale BSF units are included. The 0.6-0.8 pore volume filtered samples represent water that spent 18-24 h of idle time in the filter bed, whereas roughly 10-30% of each 1.1-1.3 pore volume sample was filtered water that did not experience any idle time in the filter bed. The p-values in Table 5.4 indicate that the increase in virus reduction with decreasing volume filtered was significant for MS2 but not for echovirus 12. While conclusions about significance of an effect were therefore

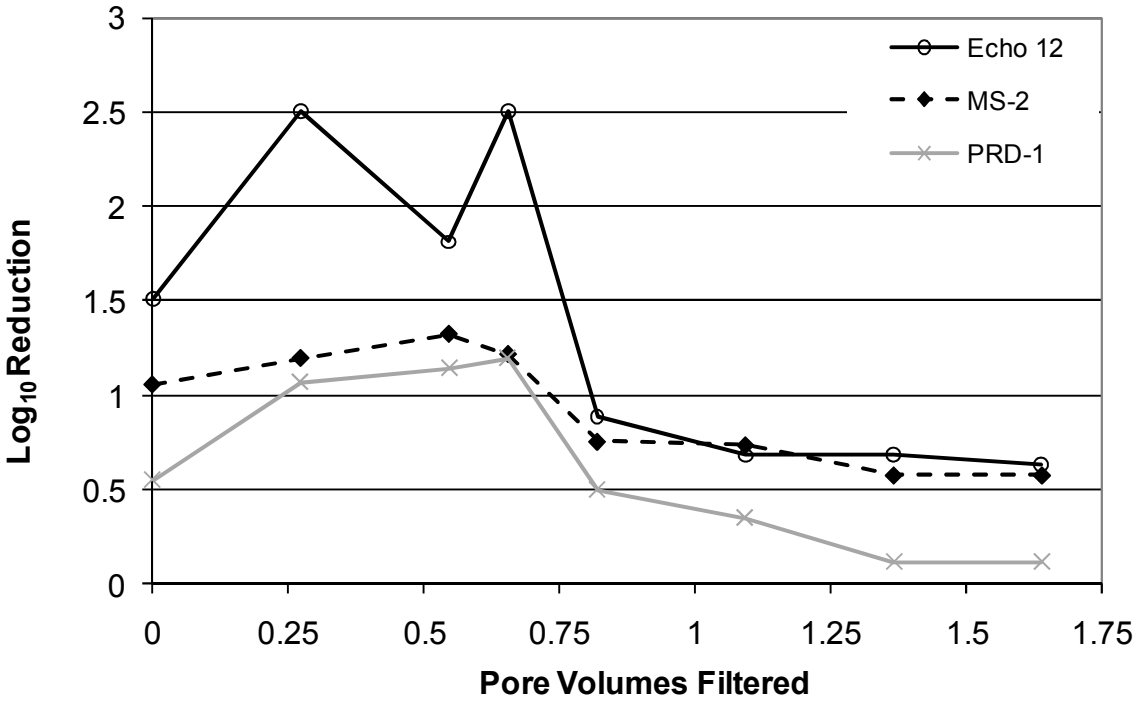

Figure 5.3 Concentrations of viruses in grab samples taken following an 18-hr. idle time. The full-scale plastic filter had a pore volume of 18.3L and had filtered 40 L of feed water daily for 42 days. From Elliott et al., 2008.

limited, further investigation of idle time effects was pursued as detailed below and in Section 5.3.2.

Grab samples of product water were also taken to gain further insight into the effect of idle time on virus attenuation. These data were obtained from a ripened, full-scale BSF on Day 42 of operation. The virus reductions over the course of a daily charge of 40 L are shown in Figure 5.3. The reduction for each of three virus challenges was considerably higher during collection of about the first 15 L of filtered water than later volumes. This volume also roughly corresponds to the pore volume of the filter and thus corresponds to the discharge of water that had been stored in the filter media during the idle time (18 h, in this case) since filtration of the last charge. Because the BSF operates under a condition very close to plug flow (Elliott et al., 2008), the sequence of filtered volumes for which viruses were measured each day also corresponds to the order of displacement (from bottom to top of filter) of stored water from the water charged during the previous day. Idle time was clearly responsible for further attenuation or inactivation of viruses within a filter that had undergone 42 days of ripening or maturation.

The effect of idle time on attenuation of *E. coli* had been documented previously (Elliott et al., 2008). The relatively large size of bacteria had earlier suggested that the effect could be due to sedimentation during the idle time. However, the reduction of viruses during the idle times indicates that other mechanisms must be responsible because their much smaller size (roughly 0.02-0.06 μm, Table 5.2) precludes sedimentation as a major mechanism.

5.3.2 Direct Measurements of Virus Reduction during the Idle Time

The effect of idle time on virus reduction required further investigation because: (1) day-to-day variability in spike microbe concentration can confound interpretation of observed virus reductions in filtered water; (2) even a small amount of longitudinal dispersion would limit deductions about orderly displacement of stored water; and (3) information is still lacking on the progression of virus reduction during the idle time, i.e., within the 18-24 hour period. To address these concerns, bench-scale BSF column tests were conducted with direct measurement of virus reductions during the idle time by withdrawing samples from ports located at 10- and 30 cm depths.

The fraction remaining of the initial MS2 and PRD-1 concentration (rather than log reduction as in Figure 5.3) is plotted logarithmically with the idle time in Figure 5.4.

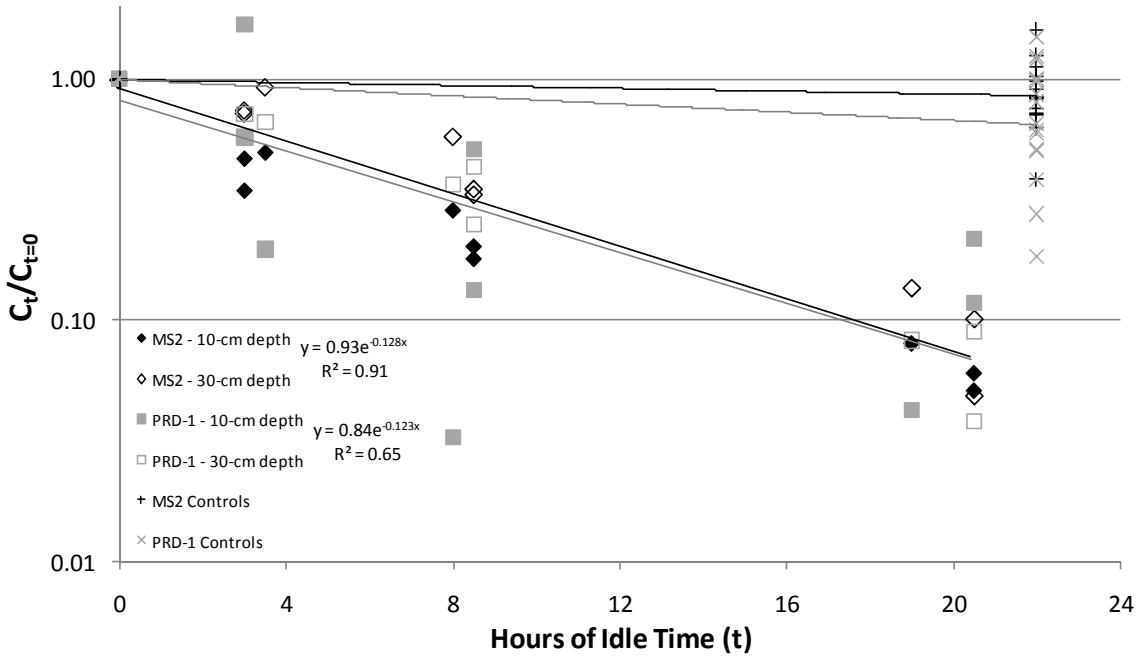

Figure 5.4 MS2 and PRD-1 concentration during filter idle time in Column Test No. 2. Samples were drawn directly from ports at 10-cm and 30-cm depth. Rates of change in virus concentration did not differ significantly with depth, so regressions were run for samples at both depths. Trendline equations and R^2 values were calculated for both depths. Filter columns had been in daily operation for 41-51 days.

These data were obtained on Days 41 and 51 of Column Test No. 2. The initial concentration ($C_{t=0}$) is the virus concentration within the filter bed at the depth of the sample port when the first sample was taken. Also plotted is the fraction of initial concentration for samples stored as control for survival under the same time, temperature and feed water conditions. Both the filter and the control samples produced a linear trend in decline of log fractional concentration, i.e., a first-order rate of decline. The test for homogeneity of regressions reveals that the rate of change in virus concentration was the same at the 10- and 30-cm depths for both MS2 and PRD-1 ($p>0.50$). Therefore, rate data for these two depths were combined for regression analysis. The decrease in fractional concentration from within the filter is greater than in the control (0.056-log - 0.062-log vs. 0.01 - log per hour of idle time; $p<0.0001$ for both MS2 and PRD-1). As shown by these results, even idle times shorter than 8 hours can be beneficial in reduction of infectious viruses. The rates of decline for MS2 and PRD-1 were not significantly different ($p=0.74$).

The average reduction of coliphages MS2 and PRD-1 observed during experiments with a daily BSF charge volume of 1.1-to-1.3 pore volumes was roughly 0.5-log. These coliphages are documented to be more difficult to treat by granular media filtration than most waterborne viral pathogens (Schijven and Hassanizadeh, 2000). The rates of reduction observed during Column Test No. 2 correspond to 1.35-1.49 log reduction in a 24-hour idle time. Thus, reduction of the most recalcitrant waterborne viral pathogens could be greatly improved by limiting filtration in the BSF process to one daily charge of less than one pore volume.

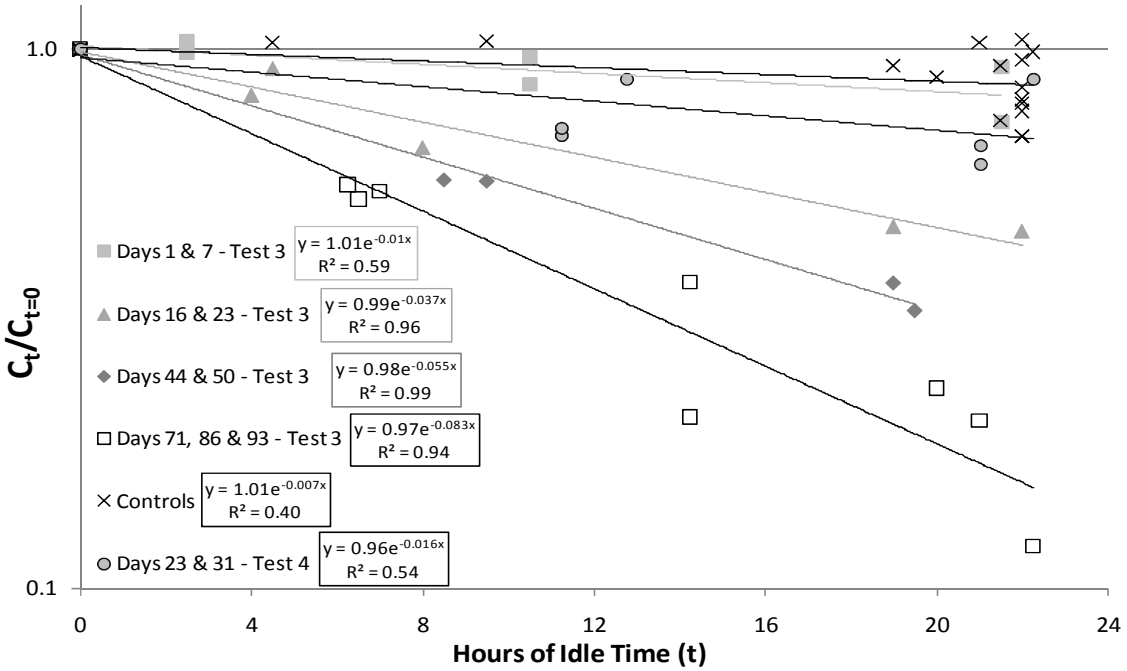

Figure 5.5 Effect of days in operation on PRD-1 concentration during filter idle time in Column Tests No. 3 and No. 4. Samples were drawn directly from sampling port at 30-cm depth.

5.3.2.1 Media Aging Effects during Idle Time

The effect of idle time on virus reduction presented in Figures 3 and 4 is in BSF columns that had been in operation for at least 41 days. The decline in PRD-1 concentration measured at 30-cm depth during selected daily idle times over 93 days of filtration in Column Test No. 3 are shown in Figure 5.5. As in Figure 5.4, log fractional concentration is plotted against idle time to test conformance to a first-order rate of virus reduction. There was no measureable decline in PRD-1 concentration during idle time for the first seven days of operation given that the average rate of decline was not significantly greater than in the controls. However, the rate of PRD-1 attenuation increased in subsequent weeks (Days 16-50) with the highest rates being observed in the final 3-to-4 weeks corresponding to Days 71-93 of filter operation.

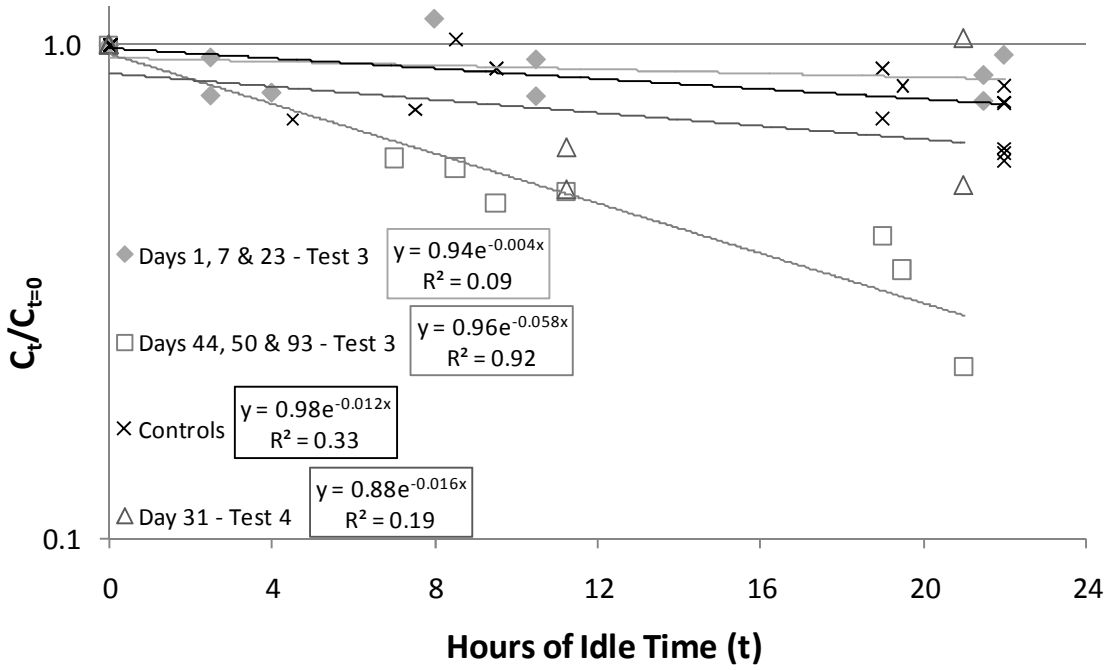

Figure 5.6 Effects of days in operation on MS2 concentration during filter idle time in Column Tests No. 3 and No. 4. Samples were drawn directly from a sampling port at 30-cm depth.

Improvement in virus reductions after months of operation has been observed previously in conventional SSF, even following schmutzdecke removal, indicating that a virus attenuation mechanism may develop over time within the filter bed (Poynter and Slade, 1977). A physical/chemical or biologically mediated aging process has been suggested to occur within the sand bed during SSF operation, imparting a "sticky" coating that is more likely to adsorb colloids and microorganisms (Wheeler et al., 1988). If enhanced virus adsorption is explained by the development of a coating on filter media, the effect would take time to develop.

The companion results for declines in MS2 concentration in Column Test No. 3 are similar to those for PRD-1 as illustrated in Figure 5.6. Less data are available for MS2 because of analytical problems, including inconsistent titer, sample contamination

early in the experiment and contamination of a stock phage solution later in the experiment.

While Column Test No. 2 (Figure 5.4) and Column Test No. 3 (Figures 5 and 6) both demonstrated that idle time influences virus reduction in ripened filters, the magnitude of the effect differed. To make a comparison of virus reduction rates from the slopes of the plots in Figures 5.4-5.6, the filters must have had time to undergo ripening, given that the results from Column Test No. 3 showed an increase in reduction with operating time. Virus reduction rates obtained for filters in operation for 41-51 days in Column Test No. 2 and for 44-93 days in Column Test No. 3 were therefore compared. The reduction rates for MS2 following at least 41 days of aging in Column Test No. 2 and No. 3 were 0.062-log and 0.034-log per hour, respectively. The comparable reduction rates for PRD-1 were 0.056-log and 0.040-log per hour. Both differences were significant ($p<0.0001$ for MS2 and $p<0.01$ for PRD-1). Given the lack of understanding of the mechanism by which "aging" of filter media occurs, it is not surprising that the reduction rates would differ somewhat over the filtration periods selected. Variations in the characteristics of feed water also could have affected the results.

The hypothesis that attenuation of viruses during the idle period increases with media aging, i.e., with more repetition of filtration cycles or more days in operation, was tested more rigorously by controlling for the possibility that concomitantly longer storage time of source water and virus stocks could be an explanation. As explained in the Materials and Methods, Column Test No. 4 was initiated on Day 62 of Column Test No. 3. The storage time of water and virus stocks would thus be the same in both of these experiments by choosing to examine results with a 62-day differential in column

operation time. Therefore, the virus attenuation rates during the idle time periods of Days 62-93 of operation in Column Test No. 3 were compared to those of Days 1-31 in Column Test No. 4. As shown in Figures 5 and 6, the log attenuation rates for PRD-1 and MS2 during idle time were significantly greater in the longer aged media in Column Test No. 3 than in Column Test No. 4 ($p<0.0001$ for PRD-1 and $p<0.05$ for MS2). Thus, the increase in virus attenuation rate during idle time can be attributed to media aging rather than an experimental artifact of longer storage times for water, primary effluent and/or virus stock.

5.3.3 Mechanisms of Media Aging

Mechanistic explanations are needed for virus reduction within the filter bed depth during idle time. Even conventional SSF performance without the added complications of idle time and declining rate filtration characteristic of the BSF process has been notably difficult to describe by a mechanistic model (Campos, 2002). Most investigators believe that conventional SSF performance improves, at least in part, due to biologically mediated processes (Huisman and Wood, 1974; Haarhoff and Cleasby, 1991). However, naturally occurring polymers or metals such as aluminum in the feed water could also be responsible for filter maturation (Weber-Shirk, 2002; Weber-Shirk and Chan, 2007).

Biologically mediated mechanisms that could explain increased virus attenuation include: (1) increased sorption to biologically modified surfaces, including biofilms; (2) grazing of virus particles by bacteria and higher microorganisms; and (3) inactivation of virus particles by anti-viral microbial exoproducts (e.g. proteolytic enzymes). The most

likely abiotic mechanism is increased sorption to modified media surfaces, but mediated by non-biological mechanisms.

Transport of viruses from the interstitial water to the media surface is necessary for virus sorption to occur. Additionally, microbial communities are expected to be concentrated at the media surface. This suggests that the efficacy of mechanisms involving grazing or microbial exoproducts would also be enhanced by virus transport. Brownian diffusion alone provides ample opportunity for virus transport to the media surface during the 18-24 hr idle time (calculations not shown). However, electrostatic repulsion of net-negatively charged viruses and sand surfaces is likely to decrease the frequency of virus-sand contact.

Either virus capture or inactivation could explain the phenomena observed in these BSF investigations. However, the use of infectivity assays to determine the concentration of viruses in these experiments precludes distinguishing between these two mechanisms. Further investigation of mechanisms is warranted. Future experiments will incorporate the inhibition of microbial communities to elucidate the relative importance of biological vs. physical/chemical mechanisms for virus attenuation in BSF.

5.4 Conclusions

Viruses are attenuated during filter idle time within the pores of the BSF filter, but only after some threshold aging period. Both the aging period and the rate of virus attenuation during idle time varied between experiments and viral agents. Both aging period and virus attenuation rate are likely influenced by experimental conditions and properties of the specific viral agent. The implications of the effect of idle time on virus

attenuation are important for BSF practice and design. To maximize microbial reduction, users should be trained and instructed to operate the filters in a manner that maximizes the daily idle time. BSF designers and implementers should be encouraged to design and distribute filters with a smaller upper reservoir to decrease maximum charge volume. The ratio of maximum volume in a single charge to pore volume of the filter media bed should preferably be no greater than 1:1 to maximize virus reductions; moreover, a lower ratio would provide a factor of safety to account for longitudinal dispersion. However, any design changes must account for their effect not only on microbial reductions but also on daily water needs, size and weight of the filter and other factors that affect cost, user satisfaction and sustained use. The improvement in virus reductions as result of media aging in the BSF in periods of zero pore velocity (i.e., during the idle period) is not necessarily unique. Media aging has been shown to improve microbial reductions in constant-flow SSF and it may have the same effect in other filtration technologies that operate either intermittently like the BSF or at low rather than zero pore velocity. Whether this effect is due only to biological filtration or other non-biological mechanisms requires further research.

CHAPTER 6

MICROBIALLY MEDIATED REDUCTION OF VIRUSES DURING IDLE TIME OF A HOUSEHOLD SLOW-SAND FILTER

Abstract

The biosand filter (BSF) is operated intermittently with an 18-24 hr idle time when water is not actively filtered. The role of microbial activity in attenuation of viruses (bacteriophages PRD-1 and MS2) in the media bed during idle time was investigated. Following three weeks of media aging, a first-order rate of virus decline was observed in the pores during the idle time. The rate increased with media aging, reaching a maximum of 0.060 and 0.043 log per hr after 5-6 weeks for MS2 and PRD-1, respectively. Suppression of microbial activity by addition of sodium azide in the feed eliminated media aging, which in turn eliminated the beneficial effect of virus reduction during the idle time. Surface modification by microbial activity was ruled out as an explanation for sorption of virus by discovering that addition of sodium azide after aging the media for seven weeks completely inhibited virus reductions during the idle time. An active microbial community in the media bed was determined to be necessary for media aging. The most likely mechanisms of virus attenuation by the microbial community are grazing by bacteria and higher microorganisms and production of microbial exoproducts including proteolytic enzymes.

6.1 Introduction

A slow sand filter (SSF) that is intermittently operated in declining rate mode is available for household use. Marketed as the "biosand filter (BSF)", this process is intended for purification of water in developing countries. As of 2007, over 140,000 units have been installed and this is expected to increase annually by about 25,000 (Clasen, 2009). The BSF has been highly successful when measured by sustained rates of use and reductions in diarrheal illness (Stauber et al., 2009; Tiwari et al., 2009; Aiken et al., 2010; Sobsey et al., 2008; Liang, 2007). Cost-benefit analysis has shown that the BSF compares favorably to other health interventions (Jeuland and Whittington, 2009). However, gaps exist in knowledge of process performance. While reductions of bacteria and protozoan parasites are reasonably high, virus reductions have not been thoroughly investigated. The mechanisms responsible for virus reductions are likely to be quite different because viruses are one or more orders of magnitude smaller than bacteria and protozoa. Viruses are not effectively captured, therefore, in the slime layer on the surface of media, which is historically referred to as the "schmutzdecke" in the slow sand filtration field.

The classic definition of the schmutzdecke is "a layer of material, both deposited and synthesized, on top of the filter bed that causes head loss disproportionate to its thickness" (Barrett et al., 1991). The removal of the schmutzdecke has been shown to have little or no effect on virus reductions (Dullemont et al., 2006; Hijnen et al., 2004). Instead, viruses penetrate into media where they are captured or inactivated by mechanisms that remain to be elucidated (McConnell et al., 1984; Poynter and Slade, 1977; Chapter 5).

Virus reductions in the BSF have been shown to vary over the daily filtration cycle (Elliott et al., 2008; Stauber et al., 2006; Elliott et al., 2006). The likely explanation is that an attenuation process occurs during the idle period between filtration cycles. A filtration cycle will typically last a few hours, after which the water stands idle. Water stored within the pores of the filter media during this idle time is displaced at the beginning of the next filtration cycle and becomes product water. Once the BSF matured (typically after a few weeks), virus concentrations were found to be lower at the beginning of the filtration cycle, corresponding to production of about one pore volume of water. The attenuation rate of MS2 and PRD-1 during idle time gradually increased over five to ten weeks until it stabilized to a rate of about 1-to-1.5 log per 24 hr (Chapter 5).

A virus attenuation process within the pores is, therefore, believed to occur during the idle time to explain this improved performance. Because the volume stored within the pores represents a substantial fraction of the daily water use, quantifying virus attenuation during the idle time could lead to design improvements. Virus reductions are much greater during idle time than during active filtration. Increasing the length of the idle time, therefore, could significantly improve virus attenuation (Elliott et al., 2008; Chapter 5).

The SSF process, with its similarity to BSF, provides a logical starting point to find explanations for virus reductions. The term "maturation" is commonly used to refer to the processes by which SSF performance improves and/or head loss increases. However, it is not necessarily limited to the formation of the schmutzdecke. Instead, "media aging" (Poynter and Slade, 1977; Wheeler et al., 1988) is used to refer to

physical/chemical or biological processes by which all sand grains (even those deep in the filter bed) are modified. Thus, media aging is a general term to encompass processes that enhance the sorption of colloids and microorganisms during maturation, including development of a microbial community, biofilms and "slime" coatings on sand surfaces (Wheeler et al., 1988; McConnell et al., 1984; Lloyd, 1974).

Virus reductions in SSF were found to be much greater after maturation than in clean filter sands (Poynter and Slade, 1977; Windle-Taylor, 1970). Batch studies with aged SSF media (Wheeler et al., 1988) and activated sludge floc (Gerba et al., 1980; Balluz et al., 1978) have also shown increased virus reductions. These results have been cited as evidence that viruses are adsorbed to biologically modified surfaces. However, microbial activity was not suppressed in these studies in a systematic fashion to draw firm conclusions. To further emphasize the distinction between media aging and schmutzdecke development, virus reductions have been shown to occur throughout the depth of the filter sand (McConnell et al., 1994) and the removal of the schmutzdecke has been shown to have little or no effect on virus reductions (Dullemont et al., 2006; Hijnen et al., 2004).

Although biological activity has been stated as critical to the maturation of SSF (Weber-Shirk and Dick, 1997a; Haarhoff and Cleasby, 1991; Wheeler et al., 1988; Huisman and Wood, 1974), physical-chemical processes could also be responsible. These have been reported to depend upon naturally occurring chemical species in the raw water, including aluminum (Weber-Shirk and Dick, 1997b; Weber-Shirk and Chan, 2007; Weber-Shirk, 2002). Attenuation of microorganisms and particles was shown to be caused by a physical-chemical process. However, viruses are orders of magnitude

smaller than these constituents. Thus, it is unclear whether a physical-chemical maturation process could improve virus capture.

This research is focused on microbial activity as an explanation for media aging and subsequently for enhanced virus reductions in the BSF. By extension, the results could provide insight into virus-reduction in conventional SSF.

6.1.1 Characteristics of the BSF

The typical daily charge of water to the BSF in household use is 20- to 40 L. Although the BSF process is similar to the SSF, the design and operation of the BSF differ significantly. The maximum filtration rate recommended for BSF is 1.1 m/h, which is up to 15-times greater than for SSF (Fox et al., 1994). The filtration rate decreases as the water level declines after a single charge. The depth of sand is 0.4-0.5 m, which is about 50% less than for SSF. Thus, the combination of higher application rate and shorter depth produces a contact time at initiation of each filtration cycle that is up to 30 times shorter than in SSF. However, contact time increases as the charge is filtered and elevation head declines. Differences in particle size and type of sand can also affect performance comparisons between BSF and SSF. The range of particle size for BSF applications is typically broader than for SSF (e.g., the uniformity coefficient may typically exceed 4.0, compared to a recommended value of less than 3 for the SSF). In addition, physical-chemical characteristics of the sand are variable among BSF applications because locally available media are used whereas most sands for SSF are obtained from a commercial source and thus more consistent.

The most important distinction between BSF and conventional SSF with regard to this research is the idle time between filtration of charges to the BSF. The pore velocity is zero during this period, which typically lasts from 18 to 24 hr. Thus, microbial activity within the filter could lead to attenuation of viruses that are stored within the pores during the idle time.

6.2 Materials and Methods

6.2.1 Bench-scale BSF design and virus sampling

The bench-scale configuration of the BSF is shown in Figure 6.1. Four polycarbonate cylinders with a 4.4-cm inner diameter were used to house the filter media. The filters were covered in aluminum foil to prevent algae growth. There are two essential features of the full-scale BSF that were replicated in bench-scale. The first is a diffuser plate, which is formed by 2-mm diameter holes, that distributes the feed water charge uniformly across the sand surface without disturbing the schmutzdecke. The second is an elevated outlet tube (2- to 7 cm above the height of the filter media) that allows the media to remain saturated with water after a charge has been filtered; water stored within the pores is then released at the beginning of the next charge.

The daily charge volume (450 mL) maintained hydraulic similitude with the full-scale BSF, both in initial application rate and fraction of charge stored in pore volume during the idle period. Ports were installed at depths of 10- and 30 cm for direct withdrawal of samples from the pores within the filter bed with a polypropylene syringe fitted with a 25-gage beveled needle into a 3-mL sterile plastic syringe (Becton-Dickinson, Franklin Lakes, NJ). These ports enabled direct investigation of virus

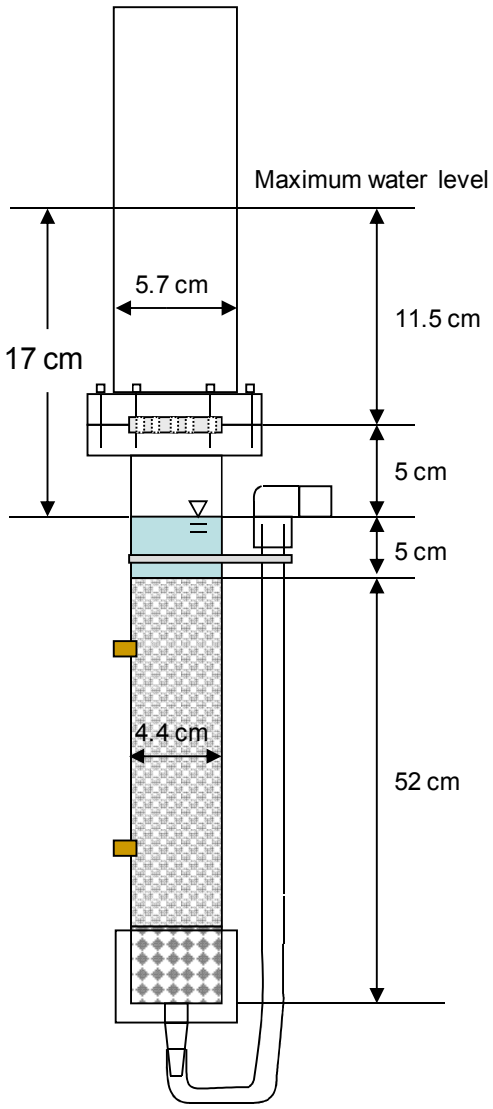

Figure 6.1 Cross-section of the bench-scale BSF columns used in this experiment.

attenuation throughout the idle period. The withdrawn samples were immediately refrigerated and analyzed within about 24 hr for MS2 and PRD-1.

6.2.2 Feed water

Feed water from Cane Creek Reservoir was obtained from the raw water sample taps of the Orange Water and Sewer Authority (OWASA; Carrboro, NC) water treatment plant. Cane Creek Reservoir does not receive wastewater discharges.

Sufficient water was collected and refrigerated for the duration of the 53-day experiment. Feed water was stored at 4 ^{o}C. The daily charge was removed, allowed to reach room temperature (approximately 20°C) overnight before use. It was then amended with pasteurized primary effluent (PE) from the OWASA wastewater treatment plant to simulate the presence of wastewater in typical drinking water sources of developing countries and to accelerate the maturation process. The daily charge was then spiked with cultures of challenge viruses MS2 and PRD-1. Feed water concentrations of MS2 and PRD-1 were 3.63±0.55 and 3.54±0.38 log plaque-forming units (pfu) per mL, respectively. The addition of 2.5% pasteurized PE and challenge microbe spike solutions increased the TOC of the feed water by up to 50% such that feed TOC was in the range of 7.5-12.5 mg/L.

An aliquot of spiked feed water used for the charge to the columns of the previous day was stored at room temperature until microbial samples were analyzed the following day. This served as a control for the effects of time on virus survival. The resulting mean inactivation rates after overnight storage were considered small (less than 10% over

24 h). Virus inactivation in control samples will be presented alongside reduction rates in filter idle time samples.

Feed water volume consisted of between 1×10^{-5} and 1×10^{-6} volume dilution of the initial virus stock. Details of virus stock preparation are included in Section 2.4.

6.2.3 Suppression of microbial activity

The role of microbial activity in virus reductions during the idle period was the focus of experimental investigations. The naturally developed community of microbes was suppressed by daily addition of sodium azide (NaN_3) into the feed water of two of the four bench-scale columns. Sodium azide is a preservative commonly used to reversibly inhibit microbial activity. It blocks the cytochrome system thereby preventing oxidative phosphorylation and subsequent respiration of aerobes and denitrifiers (Weber-Shirk and Dick, 1997a; Forget and Fredette, 1962). Unlike strong oxidizers, including chlorine, sodium azide suspends microbial activity reversibly and is not expected to affect filter sand surfaces or non-living organic matter such as biofilm exopolysaccharides (Weber-Shirk and Dick, 1997a). Most importantly, sodium azide does not affect virus survival. This was confirmed prior to the experiments by exposing MS2 or PRD-1 to sodium azide concentrations up to 50-mM.

The main disadvantage of sodium azide relative to strong oxidizers is its inability to suspend the activity of many anaerobes and facultative microbes. However, it is unlikely that these organisms contribute significantly to biological activity in the sand bed of the BSF. Because dissolved oxygen is expected to be present at 30-cm depth throughout the idle time (Buzunis, 1995), methanogens and sulfate-reducers should be

inhibited. Fermentative organisms could possibly survive, but their slow growth rate and the low organic carbon content makes fermentation unlikely (Weber-Shirk and Dick, 1997b).

The sodium azide concentration in feed water was set at 6-mM (390 mg/L). This is well above the minimum as 3-mM was shown to suppress microbial activity in SSF (Weber-Shirk and Dick, 1997a). However, sodium azide must not suppress growth of *E. coli* Famp and *Salmonella typhimurium* LT-2, the single agar layer (SAL) (USEPA, 2001) phage hosts that are used to enumerate MS2 and PRD-1. In preliminary experiments, growth suppression of the phage hosts occurred at a sodium azide dosage of 0.1 mM. This is well above the final sodium azide concentrations (Section 2.2) in SAL dishes (0.0005-0.05 mM) during the BSF experiment.

6.2.4 Virus Characteristics, Analyses and Stock Preparation

Characteristics of MS2 and PRD-1 are included in Table 6.1. MS2 and PRD-1 are known to be treated less efficiently by granular media filtration than most pathogenic viruses (Schijven and Hassanizadeh, 2000) and are also reported to be insensitive to protease activity (Nasser et al., 2002). Isoelectric point, while not perfect, is the best single predictor of adsorption of viruses in granular media (Dowd et al., 1998). The values listed in Table 6.1 show that the viruses are net-negatively charged at the feed

Table 6.1 Characteristics of viruses used in these experiments.

Virus/Phage	Size (nm)	Isoelectric point	Genetic Material
MS2	26	3.5-3.9	ss-RNA
PRD-1	62	4.2	ds-DNA

Source: Collins et al, 2004

water pH, which averaged 6.9 and ranged from 6.6-7.3. Silica also carries a net-negative charge in this pH range. Therefore, net electrostatic repulsion is expected to impede sorption of both viruses. However, the isoelectric point can also depend on the characteristics of the solution (Mesquita et al., 2010; Daniels, 1980; Glassman, 1948). For this reason, the net surface charge or the magnitude of charge carried by a virus particle will be specific to each feed water and can also be affected by virus preparation methods. Ideally, zeta potential would be determined for each challenge virus stock in the feed water used in each experiment. This would give an estimate of both the sign and the magnitude of charge on each virus in the relevant feed water throughout the applicable pH range.

MS2 and PRD-1 concentrations were assayed using the single agar layer method on hosts *E. coli* F-amp and *Salmonella typhimurium* LT2, respectively (USEPA, 2001). Stocks of bacteriophages MS2 and PRD-1 were grown on their respective hosts in trypticase soy broth, enumerated by USEPA Method 1602 that uses a double agar layer (USEPA, 2001) and stored at -80 ^{o}C. Aliquots of each stock were thawed each week, serially diluted ten-fold in phosphate buffered saline and stored at 4 ^{o}C for up to 7 days.

Reductions in virus concentration in the BSF were calculated by Equation 1. All log-reduction values reported are log base 10.

$$\log \text{Reduction} = \log(\text{Feed Water Concentration}) - \log(\text{Filtered Water Concentration}) \quad (1)$$

6.2.5 Data Analysis

A first-order rate of virus reduction during the idle period was tested by plotting the log transformation of the fractional virus concentration ($\log C_t/C_{t=0}$) against time,

where $C_{t=0}$ is the virus concentration at the beginning of the idle period. The linear regression analysis of these plots was performed with a one-way Analysis of Covariance (ANCOVA) for independent samples. The p-values for unpaired, two-tailed tests with significance level α=0.05 are reported.

6.3 Results and Discussion

6.3.1 Effect of Microbial Suppression on Virus Reductions and Media Aging

The log fractional virus concentrations (log $C_t/C_{t=0}$) are plotted in Figures 2a (MS2) and 2b (PRD-1) against idle time (0-24 hr) after different periods of filter maturation (4-52 days) without addition of sodium azide. Prior to media aging (Days 4 and 8), MS2 and PRD-1 reductions were not significantly different from those in samples taken to control for the effects of time of storage and temperature on virus attenuation (p=0.28 for MS2 and p=0.32 for PRD-1).

The observed rates of virus reduction in Figures 6.2a and 6.2b are first-order as indicated by the linearity of the plots. The first-order rate constant also increased with media aging until about 41 days for MS2 (0.060 log per hr) and 36 days for PRD-1 (0.043 log per hr). These results are similar to previous BSF experiments (Chapter 5).

Enhanced virus reductions due to media aging have also been observed in conventional SSF (Poynter and Slade, 1977; Windle-Taylor, 1970). The mechanisms proposed to explain this effect in aged SSF media include both biological and physical/chemical processes (Poynter and Slade, 1977; Wheeler et al., 1988). However, no direct proof is available of a biological mechanism in previous studies of either SSF or BSF.

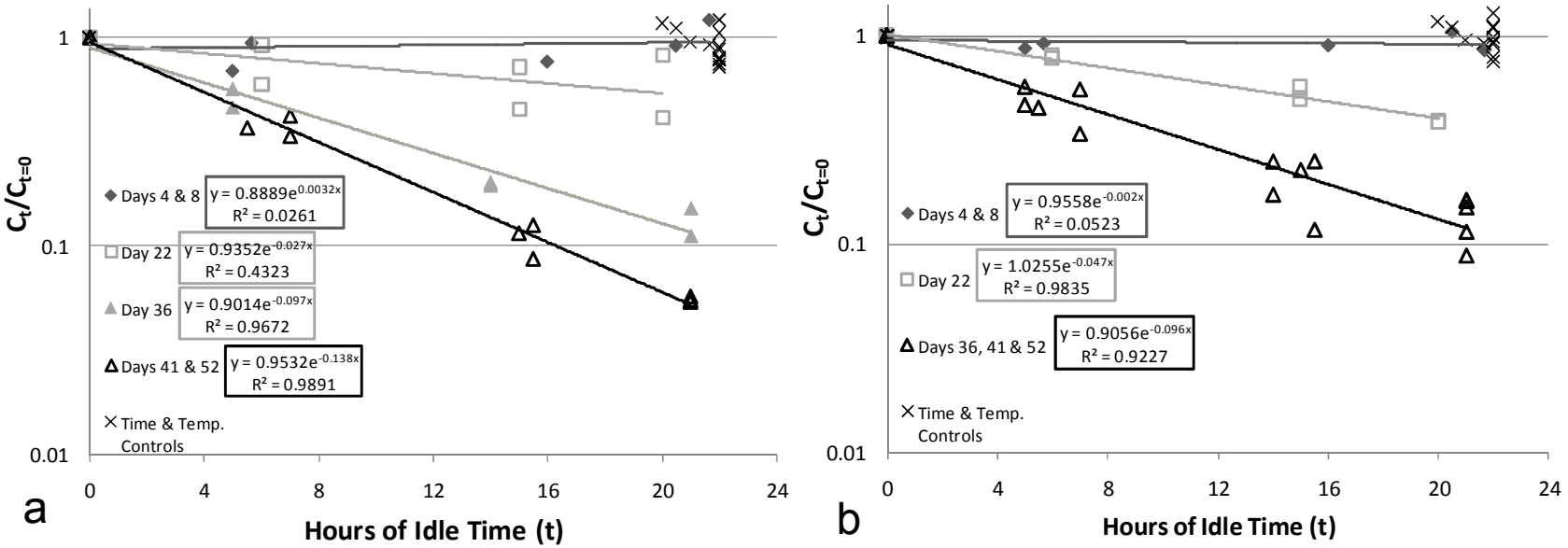

Figure 6.2 (a,b) MS2 (6.2a) and PRD-1 (6.2b) concentration during idle time at 30-cm depth in BSFs. Concentration plotted as fraction of initial (t=0) concentration. Filter maturation was allowed to proceed as in normal BSF operation.

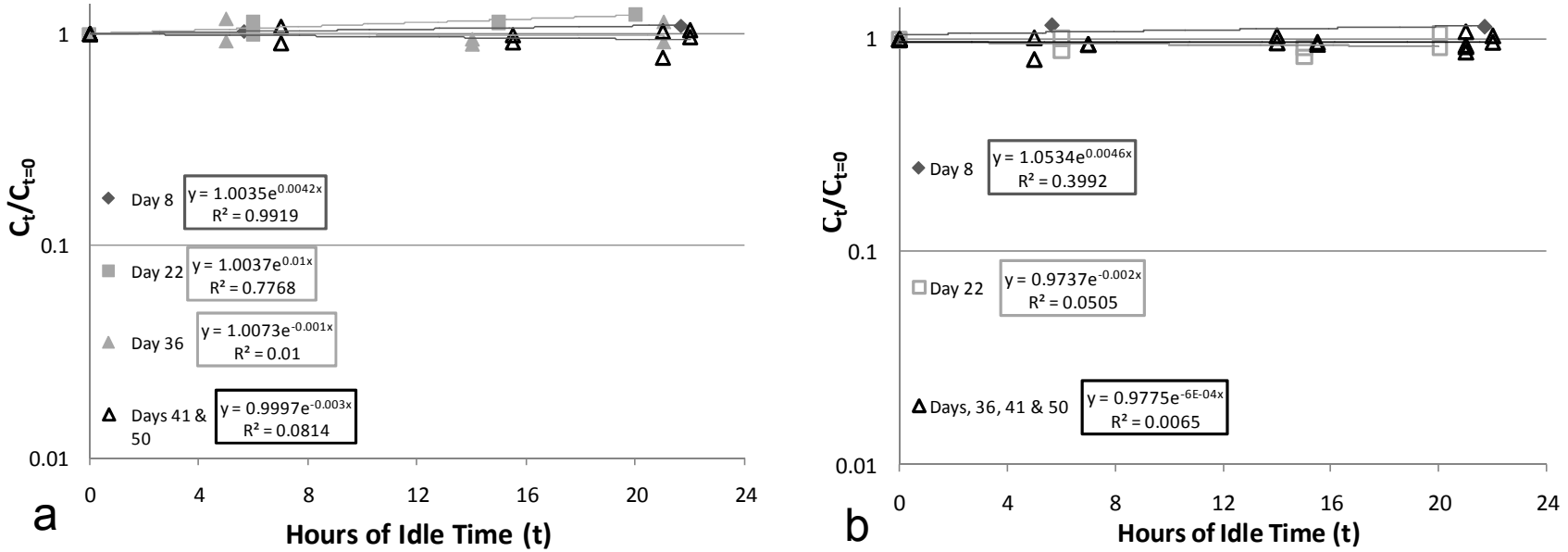

Figure 6.3 (a,b) MS2 (6.3a) and PRD-1 (6.3b) concentration during idle time at 30-cm depth in BSFs. Concentration plotted as fraction of initial (t=0) concentration. Feed water was amended with 6-mM sodium azide daily, suppressing aerobic and denitrifying microorganisms. The scale of the y-axis is the same as in Figure 6.2 to allow for easy comparison.

As shown in Figures 6.3a and 6.3b, virus reductions were completely suppressed during idle time ($p=0.34$ for PRD-1) that were taken repeatedly over the course of the experiment. Virus reductions were significantly greater in columns without (Figure 6.2) than with addition of sodium azide (Figure 6.3) throughout the seven weeks of BSF operation by addition of 6-mM sodium azide to the feed. This is supported by statistical tests that showed no significant change in virus reduction rate over the seven weeks of operation ($p=0.15$ for MS2 and $p=0.35$ for PRD-1) and no significant difference in rates from those in samples used to control for effects of sample storage and temperature ($p=0.14$ for MS2 and $p<0.0001$ for both MS2 and PRD-1). These results strongly suggest that a biological mechanism is responsible for virus reduction during idle time.

6.3.2 Suppression of Microbial Activity after Media Aging

Two categories of mechanisms are possible to explain microbially mediated reduction of viruses. They are described as mechanisms involving: (1) microbially induced modification of media that increases virus sorption; and (2) an active microbial community that either grazes on viruses or generates exoproducts that inactivate or sequester viruses. An experiment was designed to distinguish between these two mechanisms. Two bench-scale BSFs were operated for seven weeks without feeding sodium azide. Microbial activity was then suppressed by addition of sodium azide at 6-mM for three days. The virus reduction rate during the subsequent idle times would not be slowed if modification of media surfaces during the first seven weeks of operation was responsible. Alternatively, a slower rate of virus reduction would occur if an active microbial community was responsible.

Virus reduction rates are shown in Figure 6.4 to be slower during idle times after (Day 50) than before (Day 41) suppression of microbial activity; this was confirmed by statistical testing ($p<0.001$ for both MS2 and PRD-1). Therefore, microbial induced modification of the media surface as a result of aging is not responsible for virus reductions. Instead, the microbial mechanism to explain virus reductions is dependent on the presence an active microbial community. The importance of a microbial community could be inferred from studies of SSF (Hendricks and Bellamy, 1991; Poynter and Slade, 1977) where virus reductions in aged media were decreased at low temperature and after filter dewatering; both of these conditions would adversely affect a microbial community.

The specific mechanism by which the microbial community increased virus reductions could be: (1) grazing on virus particles as a food source; or (2) releasing

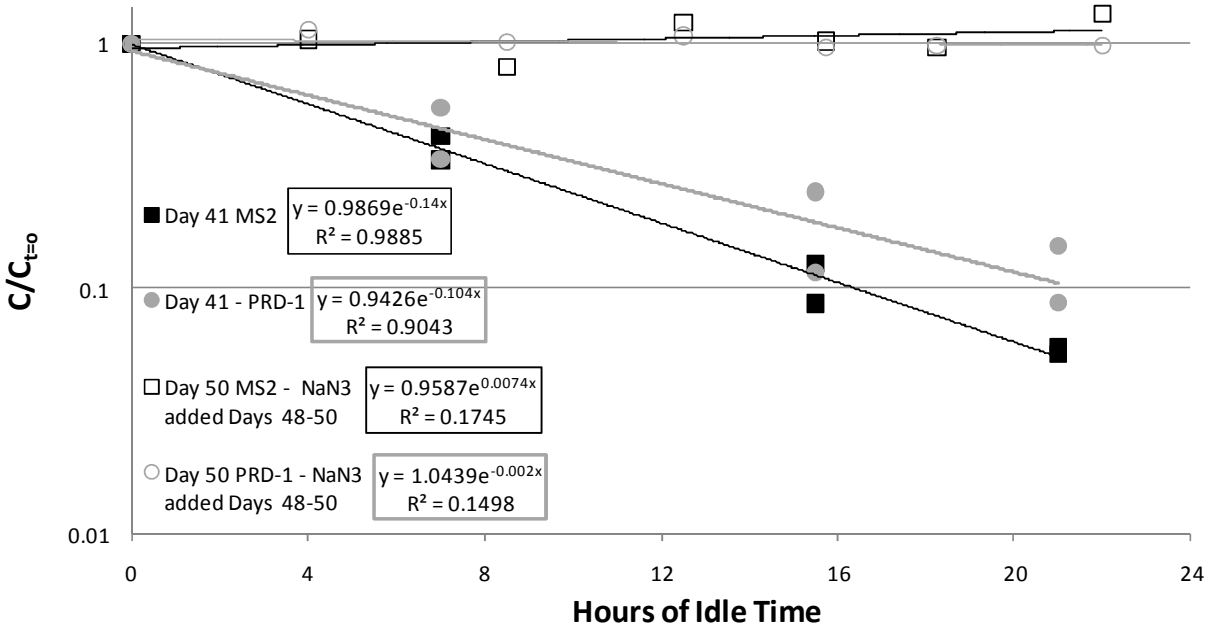

Figure 6.4 – MS2 and PRD-1 concentration at 30-cm depth during idle time. BSFs were allowed to mature through Day 47 and feed water was amended with 6-mM sodium azide was on Days 48-50. Day 41 results are included to contrast virus reductions before and after sodium azide addition.

exoproducts that either inactivate or decrease the infectivity of viruses (Wheeler et al., 1988; Poynter and Slade, 1977; McConnell et al., 1984; Lloyd, 1973). In fact, the first-order rate of virus reductions observed in this research is consistent with findings for virus reduction attributed to both grazers (Kim and Unno, 1996; Pinheiro et al., 2007; Gonzalez and Suttle, 1993; Suttle and Chen, 1992) and microbial exoproducts such as proteolytic enzymes (Walker and Toth, 2000; Northrop, 1964).

Grazers (protozoa and chrysophytes) of bacteria have been found at depth in SSF beds (Weber-Shirk et al., 1997a; Lloyd, 1973). Predation of viruses, however, has not been investigated directly in SSF beds. Nonetheless, grazing by flagellates has been implicated in virus reductions in wastewater (Kim and Unno, 1996) and marine waters (Suttle and Chen, 1992; Gonzalez and Suttle, 1993). Bacteria, including the common biofilm bacterium *Pseudomonas aeruginosa*, have also been documented to use virus capsids as growth substrates (Lipson and Stotzky, 1985; Cliver and Herrmann, 1972; Herrmann et al., 1974).

The alternative microbial pathway, which involves exoproducts, is also feasible. Proteases (also called proteolytic enzymes) are most often associated with virus inactivation. Viruses are inactivated by proteases through hydrolysis of peptide bonds in the protein capsids. However, not all viral agents are equally susceptible to protease-induced inactivation; that is, susceptibility could depend on the combination of specific protease and specific viral strain (Nasser et al., 2002; Cliver and Herrmann, 1972; Northrop, 1964). MS2 and PRD-1 have been reported to be less susceptible to some proteases than some enteric viruses (Nasser et al., 2002).

Differentiation between grazing by microorganisms and protease-induced virus inactivation is difficult to determine experimentally. Proteases are reported to be physically associated with the bacteria that produce them and/or to have very short lifetimes in natural waters (Confer and Logan, 1998; Ward et al., 1986; Rego et al., 1985; Shuval et al., 1971). Therefore, filtration that is intended to remove bacteria and leave soluble proteases is likely to decrease protease concentration inadvertently in the filtered samples.

6.3.3 Implications for BSF design and operation

The MS2 and PRD-1 reductions observed in this and in previous studies (Elliott et al., 2008; Chapter 5) fall considerably short of the 4-log reduction stated in the USEPA standard for point of use (POU) devices (Schaub and Gerba, 1991). However, MS2 and PRD-1 are likely to provide worst-case estimates for treatment of viruses. They are more difficult to remove by granular media filtration than most enteric viruses (Schijven and Hassanizadeh, 2000). They appear less susceptible to reduction by protease activity than enteric viruses such as Hepatitis A and Coxsackie A-9 (Nasser et al., 2002). Moreover, reductions of echovirus type 12 (Elliott et al., 2008 and 2009) and phiX 174 (unpublished data) in BSF are consistently 1-3 orders of magnitude greater than MS2 and PRD-1. Therefore, many enteric viral pathogens may be sufficiently reduced to meet the USEPA standard.

Regardless of the virus of concern, this research has shown that virus reductions in the BSF process could be significantly increased by taking greater advantage of microbial processes that occur during the idle time. That is, enlarging the filter volume

or decreasing the maximum charge volume would increase the fraction of water stored during the idle time and subsequently released as product water during the next daily filtration cycle. The optimal design is for the pore volume to be equal to or larger than the maximum charge volume. A factor of safety could be added to account for short-circuiting by either a further lowering of the daily charge volume or an increase in the filter volume.

Beyond design implications, operation of the BSF could be improved based on these research findings. Even if the total daily charge volume has to be larger than the filter volume to meet household water needs, users could be trained to operate the BSF in a way that maximizes the daily idle time. Another important implication is for chlorination practice. Post-disinfection has always been recommended for BSF, but recent guidance suggests that BSF can also be used with chlorinated source water (Manz, 2009). However, media aging has been shown in this study to be synonymous with increased activity of the microbial community which in turn increases virus reduction. Even monthly chlorination of source waters could undermine media aging and thus virus reductions by destroying the microbial community within the filter. In some community water sources in developing countries, periodic chlorination is nevertheless necessary. In these situations, BSF may not be best choice for a POU device.

6.4 CONCLUSIONS

Microbial mediation of virus reductions during the idle period of the BSF process was implied by experimental results in which reductions were completely eliminated by addition of sodium azide to the feed water to suppress microbial activity. The observed

rate of virus reduction was first-order and the rate increased with media aging, consistent with the notion that microbial activity also increases. The microbial mechanism was not related to surface modification but rather to the activity of the microbial community within the filter. The specific pathway could be either by production of microbial exoproducts such as proteolytic enzymes or by grazing of bacteria and higher microorganisms on virus particles as a source of food. Differentiating the importance of these two mechanisms, however, would require further investigation. In this regard, protease inhibitors may be a promising tool for future investigation.

The implications for design and operation of the BSF are significant. Increasing the ratio of the pore volume of the filter to the maximum charge volume would decrease the virus concentrations in product water. Users could also be encouraged to lengthen the idle time in order to achieve the same result. Chlorination of feed water should be discouraged because it would decrease the microbial community responsible for virus attenuation.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Operation of the household BSF process consists of two distinct stages, an active filtration cycle and an idle period when water is held within the pore space. Filtration of the daily batch charge produces a declining rate of filtration over a period of a few hours. The BSF was shown by tracer studies (Chapters 3 and 4) to behave close to a plug flow reactor during the filtration cycle, despite the fact that filtration rate declines over several hours from about 1 m/h to near zero at the end of the cycle. Residence time in the media bed during the filtration cycle can vary from 30 min to 8 hr or more, depending on the elevation head and head loss; however, the idle time between charges typically exceeds 18 hr when a single charge is applied per day. These two scales of dynamics could determine the dominant mechanism of microbial reduction. Microbial reduction was highest at the beginning of filtration cycle, indicating attenuation during the idle time and the mechanism appears to involve an active microbial community. In addition, microbial reductions may occur during the active filtration cycle, perhaps motivated by physical-chemical mechanisms such as adsorption to surfaces of the filtration medium.

The plug flow behavior of the BSF means that water stored within the pores of the filter during the idle time is displaced in a piston-like fashion. This stored water becomes the initial production of filtered water each day. The fact that the stored volume

represents a substantial fraction of the daily water use by the household makes understanding of the mechanism(s) responsible for attenuation of microbes during the idle time very important. Virus attenuation during the idle time, therefore, was the central theme of this research and resulted in recommendations for modifying design and operational strategies that could maximize the public health benefits of this POU technology.

The other time dynamic of great importance is the increase in microbial reduction with days of filter operation. This led to careful evaluation of how a BSF undergoes what is commonly referred to as biological maturation and how this affects attenuation of both bacteria and viruses (Chapters 3-6). While the traditional explanation for improved reduction of bacteria in SSF is development of the schmutzdecke at the surface of the sand, maturation through the bed depth (i.e. media aging) was demonstrated in this research to be very important for virus attenuation. Bacteria are obviously more susceptible than viruses to physical straining through the schmutzdecke because of their larger size. Removal of the schmutzdecke upon filter cleaning to recover head during filtration tests showed its important role in reduction of bacteria; the response was a sharp increase in bacterial concentrations in product water (Chapter 4).

Several mechanisms are possible to explain virus reduction in the BSF. They include sorption within the schmutzdecke, deep-bed sorption to media surface and microbial activity leading to inactivation either by grazers or proteolytic enzymes. Sorption in the schmutzdecke seems an unlikely explanation, given that removal of the schmutzdecke in conventional SSF has been shown in other studies to have little or no effect on viruses. Deep-bed sorption to media surfaces may be important for some

viruses but metals concentration on media surfaces did not appear to be affect viruses with low isoelectric point (Chapter 4). Instead, deep-bed media aging seems a more plausible explanation. This could produce both enhanced sorption of viruses and their inactivation by active microbial communities.

The importance of sorption could depend upon the strength of the net electrostatic attraction or repulsion between the virus and the surface of the media. Echovirus Type 12 and phiX 174 concentrations were reduced much more than MS2 and PRD-1 (Chapters 3-5). Differences in net electrostatic repulsion could be the explanation. Viruses with higher isoelectric point are generally reduced more in filtration through granular media (Dowd et al, 1988). MS2 and PRD-1 have much lower isoelectric points than echovirus 12 and phiX 174, suggesting that adsorption of MS2 and PRD-1 would be impeded by net electrostatic repulsion to a greater extent than for echovirus 12 and phiX 174. However, the isoelectric point can also depend on the characteristics of the solution and the medium in which the laboratory virus stock was cultured (Mesquita et al., 2010; Daniels, 1980; Glassman, 1948). In summary, although the net surface charge on each virus during these experiments could not be determined conclusively, there appears to be a positive correlation between isoelectric point and virus reductions.

The importance of deep-bed media aging was demonstrated by direct measures of virus reduction deep within the filter bed during the idle period. Reductions of MS2 and PRD-1 at bed depths of 10 and 30 cm (Chapters 5 and 6) followed a first-order rate relationship. The reduction rate was measureable after 2 to 3 weeks of media aging and gradually increased over the next 3 to 7 weeks, reaching a rate of 1-to-1.5 log per 24 hr.

Having demonstrated that deep-bed media aging was responsible for virus attenuation, a mechanistic explanation was pursued (Chapter 6). The hypothesis was that a microbial, rather than physical-chemical, process was responsible. Suppression of microbial activity by addition of sodium azide prevented virus attenuation during idle time. Another test of the microbial attenuation hypothesis involved suppressing microbial activity after a long period of filtration. If the dominant attenuation mechanism had been modification of the media surface (by metals or organic material in the feed water, or by-products of microbial growth), then virus attenuation should not have diminished. However, the results showed once again that virus attenuation was completely eliminated. The specific pathway of microbial mediation could be either by production of microbial exoproducts such as proteolytic enzymes, which hydrolyze peptide bonds in the proteins of virus capsids, or by grazing (predation) of bacteria and higher microorganisms on virus particles.

The use of bench-scale filter columns provided experimental advantages over the use of household-scale BSF units (Chapters 4-6). It enabled the simultaneous operation of six filters, including experimental replicates, which would not have been possible with household-scale filters given constraints on budget, labor and laboratory space. Investigation of the mechanisms of virus reduction and the impacts of filter maturation benefited greatly from replication. Statistical power was greatly improved and recommendations regarding BSF design and operation could be made with increased confidence. However, the operation of replicate filters also revealed that reproducibility is a challenging problem in experimental design. The rate of schmutzdecke development, as measured by the decline in filtration rate over eight weeks of experimentation, varied

widely across replicate columns (Chapter 4). One possible explanation is difficulty in controlling the media size of the topmost layer where the schmutzdecke develops. However, experimental designs using replicate filters are uncommon for SSF and have not been reported previously for BSF. Therefore, it is difficult to determine to what extent variability was attributable to flaws in filter preparation and to what extent it is an unavoidable characteristic of the BSF system.

Microbial reductions in the BSF were shown in this and other research to be not as large as in some other POU technologies. However, this research has also revealed that they can be increased. The key is to take maximum advantage of microbial attenuation during the long idle time by holding the charge volume close to the pore volume of the filter. This was shown specifically to be very important for reduction of viruses. Even with current design, the BSF process has been shown in many field studies to improve the health of users while also being readily adopted in a sustainable way and cost effective. However, changes in both design and operation to optimize the idle time effect could further increase microbiological reductions while maintaining the characteristics that make the BSF appealing to users.

7.1.1 Modified Designs to Limit the Charge Volume

The work published early in this research (Stauber et al., 2006; Elliott et al., 2006 and 2008) included the recommendation that the charge volume should ideally be less than the pore volume to maximize the benefit of microbial attenuation during the idle time in production of water from each daily charge. This recommendation has already been implemented in the design of BSF units manufactured by the Centre for Affordable

Water and Sanitation Technology (CAWST), the most prominent technical and training organization for BSF technology. CAWST now uses a steel mold that produces filters with a concrete housing that has a smaller filter box to decrease the maximum charge volume (CAWST, 2008a). As a result, the ratio of the maximum charge volume to the pore volume has been reduced from about 2:1 to 1:1. A further recommendation, however, would be to include a safety factor by reducing the charge volume somewhat more to account for hydraulic dispersion within the filter that mixes charged and stored water; a volume ratio of 0.8-0.9:1 is suggested.

An alternative design recommendation would be to modify the charge -to-pore volume ratio using the U-shaped BSF (UBSF) design shown in Figure 7.1. A vertical inner wall divides the filter housing into two sections. Water charged to the filter box passes downward on the left side of the wall and then passes upward on the right side thereby creating a U-shaped filtration path. The maximum charge volume is restricted to 8-10 L which is about 50% of pore volume. This would ensure that all charged water remains within the filter for a large fraction of the idle time before consumption. Additionally, lengthening the filter bed from 40-50 cm to greater than 110 cm provides more opportunities for capture of microbes and could be especially important for increasing reduction of viruses.

The UBSF requires abandoning concrete in favor of a plastic housing for the filter. While concrete allows for locally sustainable production and micro-enterprise, use of plastic opens the possibility of centralized production and distribution. Plastic also introduces the possibility of further design changes that are not feasible with a concrete housing. In fact, plastic is already being used in commercial designs.

In addition to the potential for higher microbial reductions, the UBSF design offers other advantages. It is more durable than other plastic filter designs because the PVC tube external to the filter housing is no longer needed. This tube is prone to breaking and leaking, as has been documented in BSF installations in the Dominican Republic (Ortiz, 2007). The unfortunate result of leaking filters is less usage and even abandonment of the BSF (Vanderzwaag, 2007).

7.1.2 Modified Design to Incorporate Another Treatment Barrier

The UBSF design would provide for addition of a second treatment barrier. One possibility would be to add a slow-release disinfectant to the chamber just above the filter media on the outlet side. This would provide a polishing step and a disinfectant residual during storage of filter water. Another possibility is to add a sorptive layer of media to increase the removal capacity for viruses as is also included in the design shown in Figure 7.1. The goals would be to improve virus reduction during the early period of filter operation when the beneficial effects of biological maturation are absent and to further augment virus reduction following maturation. Although most sorptive media have been shown to be rapidly exhausted, zero valent iron (ZVI) and the proprietary Composite Iron Matrix (CIM) used in the SONO arsenic filter are possible candidates. A slow surface oxidation process has been identified during which electropositive iron (oxy)hydroxides are continuously generated thus extending the sorptive capacity (You et al., 2005; Hussam and Munir, 2007). Testing of candidate media is still ongoing. One

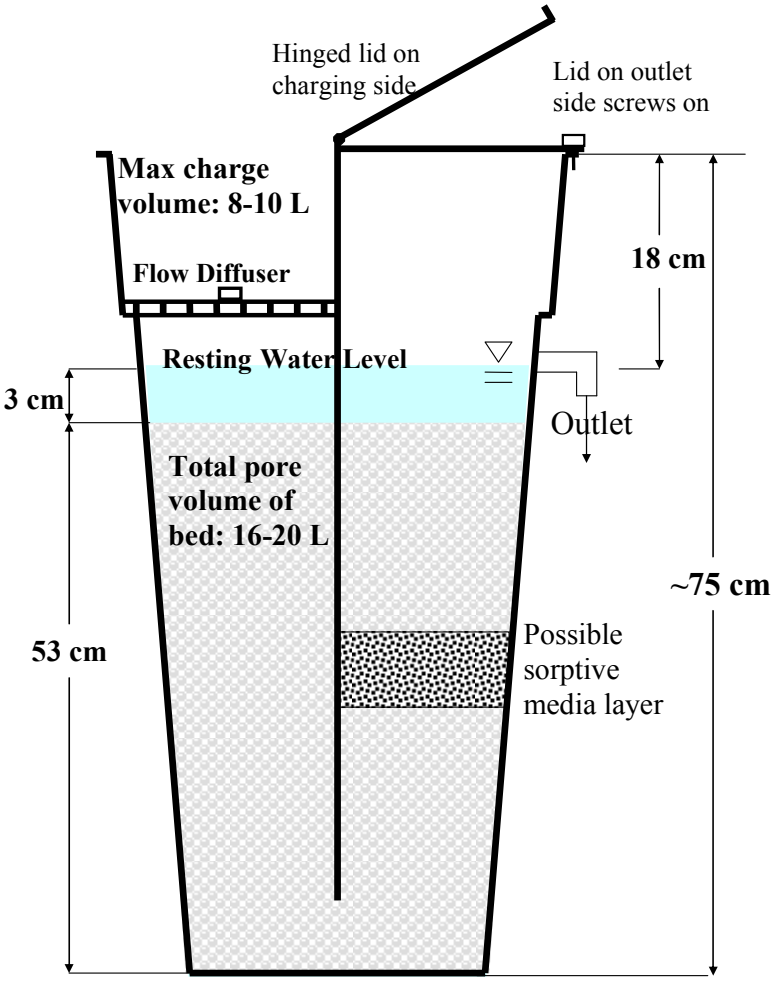

Figure 7.1 Cross-section of the plastic U-shaped BSF used in these experiments.

technical challenge yet to be resolved is filter clogging that is caused by physical expansion of iron hydroxides by surface oxidation processes.

The UBSF design could have limitations primarily related to manufacturing and quality control. A water-tight seal around the baffle is critical to filter performance. Precision manufacturing and a quality food-grade adhesive would be necessary to ensure the integrity of the water-tight seal. The vertical dividing wall would have to be installed near the installation site if shipping required the UBSF units to be stacked.

7.1.3 Operation of the BSF

Beyond design implications, these research findings suggest improvements in operation of the BSF. As discussed earlier in the recommendations, decreasing the daily volume filtered will lead to improved water quality. However, this practice also limits the volume of water produced each day based on current BSF designs. While the volume would be adequate for meeting drinking water needs, it may not suffice for bathing and cleaning. In this case, users should be encouraged to reserve the first water filtered each day for drinking water. As this research has shown, the initially collected volume of filtered water will have the lowest concentration of microbes; thus, health benefits of using the filter will be maximized. Implementation organizations should adopt these findings in their training of BSF users so that filters are operated to give the best possible protection of health.

Chlorination practice is another potential implication for filter operation. Recent guidance suggests that BSF be used even when a centralized piped supply exists because of concern about chlorination being unreliable (Manz, 2009a). However, this would

disrupt the active microbial community in the media bed and lower virus reductions. Even monthly chlorination of source waters could undermine media aging by destroying the microbial community within the filter and thereby lowering virus reductions. In some community water sources in developing countries, periodic chlorination is nevertheless necessary. In these situations, BSF may not be best choice for a POU device.

Post-disinfection of filtered water should be encouraged, especially in newly installed filters that have not undergone the maturation process. This can be facilitated by the UBSF design. Microbial reductions, especially those for virus, can be low in an unripened BSF. Moreover, bacterial reductions are often lowered when large volumes are filtered. The chance of recontamination of filtered water can also be addressed through correct post-disinfection.

7.2 Recommendations

The effects of source water quality and changes in source water quality on microbial reductions in the BSF need to be addressed. The majority of households treating water with BSF will probably have multiple sources of drinking water throughout the year. Significant changes in source water quality (e.g., changing from river water to rainwater) have been reported anecdotally to result in increased turbidity in product water. Although increased turbidity may be thought of mainly as an inconvenience, the health implications could be a more serious concern. Pathogens sorbed to media or particles causing turbidity could also potentially be desorbed from the filter and flushed into the product water in high concentration.

Similar in importance to the effect of source waters on microbial reductions is the effect different viral agents may have on expected reductions. Physical-chemical

characteristics of viruses were found to be important determinants of reduction in this research. However, this investigation was limited to only four viruses. A more diverse selection of viruses, including more human enteric viruses, should be tested. Additionally, virus preparation and storage methods can strongly impact their physical characteristics. Greater consistency in virus preparation methods should be practiced and steps should be introduced to confirm that viruses are dispersed and have not aggregated during storage.

The provision of sufficient dissolved oxygen (DO) to the microbial community may be important for BSF. A DO gradient has been claimed to be established between the atmosphere and an aerobic microbial community on top of the sand (Buzunis, 1995). This theory needs to be investigated, including elucidation of the effect of standing water depth. Additionally, the role of DO at depth should be investigated. Extended idle times could possibly lead to anoxic conditions at depth in microbially active filter media.

Characterization of microbial communities at depth in BSF is needed to understand the specific organisms responsible for grazing and/or production of exoproducts that attenuate viruses. Protozoa and other higher microbes may migrate rapidly into the lower depths of the media bed because of the high pore velocities of BSF. Protein assays for indirect enumeration of microbial communities within the filter media have been unsuccessful.

Mechanisms of microbial reduction require further investigation. Infectivity or culturability assays have been used in all reported studies of BSF performance. These assays do not enable experimental differentiation between microbial inactivation and physical removal during filtration. Fluorescent microspheres could be useful because

they are an inert surrogate for microbes that are similar in size and density. They could provide clear insight into the role of physical removal of bacteria and parasites at various stages of maturation. Enumeration of fluorescent microspheres smaller than bacteria must rely on reading the total fluorescent signal as they cannot be counted efficiently by fluorescence microscopy because of their small size. Alternative means of detecting the presence of virus particles are also available. Methods for fluorescent labeling or radiolabeling of virus particles and subsequent detection have been developed and could be useful (Gitis et al., 2002; Harvey et al., 2002). Molecular methods such as quantitative polymerase chain reaction (qPCR) may not be ideal for filtration experiments lasting many weeks. Nucleic acids of adsorbed viruses can be released even when virus capsids remain adsorbed to the granular media (Harvey et al., 2002; Murray and Laband, 1979). Therefore, detection of fluorescent-labeled or radiolabeled signals on virus capsids appears to be more promising than using molecular methods that enumerate nucleic acids.

The role of the schmutzdecke in microbial reduction needs clarification. Particle removal by straining and adsorption may play a positive role. In addition, predation near the top of the media bed may directly increase microbial reductions. Another effect related to the growth of the schmutzdecke is increase head loss that decreases the filtration rate and increases the residence time as the filtration period lengthens. An increase in residence time provides more time for deep-bed mechanisms to affect microbial reductions. Determining the factors most responsible for microbial reduction, therefore, requires better understanding of the interaction amongst all of these parameters.

The model to describe the decline in flow rate with time (Section 2.2.2; Appendix 2) should be tested further. Data need to be collected at different stages of maturation and the parameter "*a*" fit to those data sets. Additionally, a "bi-layer" model of head loss development that incorporates separate values of *a* for the schmutzdecke and the rest of the media bed should be considered. Such a model could more accurately reflect the accumulation of head loss in the top centimeter of the media bed with little change in head loss at depth.

Accumulation of aerobic endospores, including *Bacillus spp.*, in granular media may enhance removal of other colloids and microorganisms. Preliminary evidence suggests that removal of 1.5-μm and 4.5-μm fluorescent microspheres in granular media was improved by 1-log to 3-log when they were co-injected with *Bacillus subtilis* spores (McLellan et al., 2009; Stimson et al., 2009). The proposed mechanisms are spore-microsphere aggregation and/or co-attachment to mineral surfaces. Aerobic endospores are numerous in natural waters. The possible role of aerobic endospore accumulation in the maturation of BSF (and other biological filters) should be investigated.

Bacterial reductions are highly dependent upon daily charge volume. This research has focused mainly upon the effect of charge volume in relation to the filter volume in virus reductions that appear to depend on active microbial community. Bacterial reductions during idle time, on the other hand, may depend on different mechanisms. Sedimentation is probably an important mechanism for bacteria but not viruses. If so, the rate of bacteria attenuation will likely follow a different pattern than the first-order rate observed for viruses.

Virus reductions during idle time require an active microbial community in the media bed. However, the specific mechanism by which this community attenuates viruses is unknown. The two candidate mechanisms included in Chapter 6 are grazing and activity of proteolytic enzymes. Measuring proteolytic enzyme activity in the bulk solution is problematic because most proteases are physically associated with the bacterial cells that produce them (Confer and Logan, 1998; Ward et al., 1986; Rego et al., 1985; Shuval et al., 1971; Wallenstein and Weintraub, 2008). Inhibition of proteases is a more promising avenue of investigation. Spiking protease inhibitors into feed water could provide insight into the relative contribution of proteases to virus reductions during idle time. However, protease inhibitors are themselves proteins and are biodegradable (Laskowski and Kato, 1980). Therefore, monitoring protease inhibitor concentration during idle time is recommended.

The impact of filter media characteristics on microbial reductions should be investigated further. The size, chemical characteristics and hydrophobicity of filter media may affect filter performance. Smaller media size and the presence of iron and aluminum appeared to enhance virus reductions. Iron-based media that undergo slow oxidation in water show promise, but clogging due to expansive oxidation is an unresolved technical problem. Hydrophobic interactions could possibly be enhanced through the use of media with high organic content. Both MS2 and PRD-1, the most recalcitrant microorganisms tested, are susceptible to adsorption through hydrophobic interactions (Gerba, 1984; Harvey and Ryan, 2004). Batch testing of media to evaluate virus adsorption is recommended, followed by bench-scale filter experiments. Because

waterborne pathogens are subject to different attachment mechanisms, dual media filtration should be explored as an option for the BSF.

APPENDIX A

LISTING OF PUBLICATIONS AND CONFERENCE PRESENTATIONS

Journal articles:

1. Stauber, C.E., M.A. Elliott, F. Koksal, G.M. Ortiz, F.A. DiGiano and M.D. Sobsey. (2006) Characterization of the biosand filter for *E. coli* reductions from household drinking water under controlled laboratory conditions and field use conditions. *Water Sci. Tech.* 54 (3), 1-7.

2. Elliott, M.A., C.E. Stauber, F. Koksal, F.A. DiGiano and M.D. Sobsey (2008) Reductions of *E. coli*, echovirus type 12 and bacteriophages in an intermittently operated household-scale slow sand filter. *Water Res*. 42(10-11):2662-2670.

3. Sobsey, M.D., C.E. Stauber, L. M. Casanova, J.M. Brown, and M.A. Elliott (2008) Point of Use Household Drinking Water Filtration: A Practical, Effective Solution for Providing Sustained Access to Safe Drinking Water in the Developing World. *Environ Sci Technol*. Vol. 42(12):4261–4267. (Publication related to my research, but not directly to dissertation laboratory studies.)

Conference proceedings papers:

1. Elliott, M.A., C.E. Stauber, F. Koksal, K.R. Liang, D.K. Huslage, F.A. DiGiano and M.D. Sobsey. (2006) The operation, flow conditions and microbial reductions of an intermittently operated, household-scale slow sand filter. In *Recent Progress in Slow Sand and Alternative Biofiltration Processes*. Edited by R. Gimbel, N.J.D. Graham and M.R. Collins. International Water Association. London.

2. Elliott, M.A., C.E. Stauber, F. Koksal, K.R. Liang, D.K. Huslage, F.A. DiGiano and M.D. Sobsey (2006) Intermittently operated slow sand filtration for point of use water treatment. *Proceedings of the Carolina Environmental Program's Environmental Symposium: "Safe Drinking Water: Where Science Meets Policy"*. University of North Carolina at Chapel Hill.

3. Elliott, M.A., F.A. DiGiano, A.M. Fabiszewski, P Chuang, L.P. Clark, A Wang and M.D. Sobsey (2009) The effect of idle time on reduction of viruses in an intermittently operated, household-scale slow sand filter. *Proceedings of the Disinfection 2009 Conference*. Water Environment Federation, Atlanta.

4. Stauber, C.E., M.A. Elliott, F.A. DiGiano and M.D. Sobsey (2009) Performance Comparison of the Biosand Filter in Laboratory Studies and a Longitudinal Field Study in Bonao, Dominican Republic. *Proceedings of the Disinfection 2009 Conference.* Water Environment Federation, Atlanta.

5. Elliott, M.A., F.A. DiGiano, A.M. Fabiszewski, P. Chuang, S. Kuhn, M.D. Sobsey (2009) Bench-scale Investigation of Biosand Household Drinking Water Filter Performance. Proceedings of the Carolina Environmental Program's Environmental Symposium: " Sustainable and Safe Drinking Water in Developing and Developed Countries: Where Science Meets Policy". University of North Carolina at Chapel Hill.

Platform Presentations:

1. "Characterization of the Biosand Filter for Microbial Reductions Under Controlled Laboratory and Field Use Conditions." Platform Presentation given at the World Health Organization 3rd Annual Meeting and Research Symposium of the International Network to Promote Household Water Treatment and Safe Storage. Bangkok, Thailand. June 2005.

2. "Characterization of the biosand filter for microbial reductions under controlled laboratory and field use conditions." Platform Presentation given at the International Water Association meeting on Health Related Water Microbiology (WaterMicro 05). Swansea, Wales. September 2005.

3. "Intermittently operated slow sand filtration for point of use water treatment." Platform Presentation at the 'Safe Drinking Water: Where Science Meets Policy' symposium. Chapel Hill, NC. March 2006.

4. "The operation, flow conditions and microbial reductions of an intermittently operated, household-scale slow sand filter." Platform Presentation at the 4th Annual Slow Sand and Alternative Biofiltration Conference. Mulheim an der Ruhr, Germany. May 2006.

5. "Biosand filtration for point of use drinking water treatment." Platform Presentation at the American Water Works Association ACE 2006 conference, San Antonio. June 2006.

6. "Full-scale and bench-scale microbial challenge experiments with the Biosand household drinking water filter." Platform Presentation at the Association of Environmental Engineering and Science Professors: Interactions at the Interface conference, Blacksburg, VA. July 2007.

7. "Treatment of Waterborne Viruses with the Biosand Drinking Water Filter." Platform Presentation at the UNC Safe and Sustainable Drinking Water in Developing and Developed Countries Conference, Chapel Hill. November 2008.

8. "Effect of Idle Time on Virus Concentration in the Biosand Filter." Platform Presentation at the Water Environment Federation Disinfection 2009 Conference, Atlanta. March 2009.

Poster Presentations:

1. "Reduction of Bacteria and Viruses via Household Scale Intermittently Operated Slow Sand Filtration." Poster presentation at the 105th General Meeting of the American Society for Microbiology. Atlanta. June 2005.

2. "Bench-scale investigation of Biosand filter performance against surrogates for bacterial, viral and protozoan pathogens: media type, filter cleaning and idle time." Poster presentation at the Platform Presentation given at the World Health Organization 4th Annual Meeting and Research Symposium of the International Network to Promote Household Water Treatment and Safe Storage, Accra, Ghana. June 2008.

3. "*Cryptosporidium* Oocyst and *E. coli* Reduction from Water by the Biosand Filter." Poster presented by Prof. Mark D. Sobsey at International Water Association World Congress. Vienna, Austria. September 2008.

4. "Effect of sand type and flow rate on biosand filter reductions of *E. coli* and coliphages MS-2 and PRD-1 in water." Poster presented by Anna Fabiszewski at the Carolina Environmental Program's Environmental Symposium: " Sustainable and Safe Drinking Water in Developing and Developed Countries: Where Science Meets Policy". Chapel Hill. November 2008.

5. "Bench-scale investigation of Biosand Filter Performance against a Surrogate for Protozoan Parasites" Poster presented by Prof. Mark D. Sobsey at WHO Network for Household Water Treatment and Safe Storage Research Symposium, Dublin, Ireland, September 2009.

APPENDIX B

DERIVATION OF MODEL FOR FLOW RATE DECLINE WITH DAILY CHARGE IN A CLEAN BSF

Bernoulli Equation

$$\frac{v_1^2}{2g}+\frac{p_1}{\gamma}+z_1=\frac{v_2^2}{2g}+\frac{p_2}{\gamma}+z_2+h_L \qquad \text{(B-1)}$$

Where v_1, p_1 and z_1 are respectively the velocity, pressure and height at the free water surface above the filter (same variables are also shown for position 2 which is at exit of water from filter), g is acceleration of gravity, γ is density and h_L is head loss between positions 1 and 2. At both the free water surface and exit point, p =0. The velocity, v_1, is assumed small compared to v_2 based on the large ratio of the cross-sectional area at surface of filter to cross-sectional area of the exit tube used to collect water. Let z_2 = 0 such that:

$$z_1=\frac{v_2^2}{2g}+h_L \qquad \text{(B-2)}$$

and z_1 is the head that decreases over filtration cycle:

$$z_1=h \qquad \text{(B-3)}$$

Kozeny Equation

$$h_{L,o}=K\frac{\mu}{\rho g}\left(\frac{(1-\varepsilon)^2}{\varepsilon^3}\right)\left[\frac{S_A}{V_G}\right]^2 Lv_1 \qquad \text{(B-4)}$$

Where $h_{L,o}$ is clean-bed head loss, K is the empirical filter constant, μ is dynamic viscosity, ε is porosity, L is filter depth, S_A/V_G is surface area of media per grain volume, d is diameter of media and v_1 is approach velocity

$$h_{L,o} = av_1 \tag{B-5}$$

Where a is the constant comprising all other terms in Eq. B-4.

$$a = K\frac{\mu}{\rho g}\left(\frac{(1-\varepsilon)^2}{\varepsilon^3}\right)\left[\frac{S_A}{V_G}\right]^2 L \tag{B-6}$$

For each daily filtration cycle, a small volume of water (<40 L) enters the filter. The assumption is made that K, ε and/or S_A/V_G do not change due to accumulation of particles during this cycle. Thus "a" remains constant and Eq. (B-6) can be substituted into Eq. (B-2):

$$h(t) = \frac{v_2(t)^2}{2g} + av_1(t) \tag{B-7}$$

where $h(t)$ is the head loss over a daily filtration cycle time, t.

Continuity Principle

$$Q(t) = v_1(t)A_1 = v_2(t)A_2 \tag{B-8}$$

Where $Q(t)$ is flow rate, A_1 is the cross-sectional area at the top of the filter and A_2 is the cross-sectional area at the exit point of filtered water. Solving for $v_1(t)$ gives:

$$v_1(t) = \frac{A_2}{A_1} v_2(t) \tag{B-9}$$

Substituting Eq. (B-9) into Eq. (B-7)

$$h(t) = \frac{v_2(t)^2}{2g} + b v_2(t) \tag{B-10}$$

where

$$b = \frac{A_2}{A_1} a \tag{B-11}$$

Taking the derivative of Eq. (B-10)

$$\frac{dh}{dt} = \frac{v_2}{g}\frac{dv_2}{dt} + b\frac{dv_2}{dt} \tag{B-12}$$

The continuity principle equates flow rate into and out of the filter:

$$-\frac{dh}{dt}(A_1) = v_2 A_2 \tag{B-13}$$

Substituting Eq. (B-13) into Eq. (B-12)

$$-v_2 \frac{A_2}{A_1} = \frac{v_2}{g}\frac{dv_2}{dt} + b\frac{dv_2}{dt} \tag{B-14}$$

Solve for *dt*

$$dt = -\left(\frac{c}{g} dv_2 + cb\frac{dv_2}{v_2}\right) \tag{B-15}$$

Simplifying the product of the constants, *cb* gives:

$$cb = \left(\frac{A_1}{A_2}\right)\frac{A_2}{A_1}a = a \qquad \text{(B-16)}$$

Integrating Eq. B-16 gives:

$$t = \frac{c}{g}\left(v_{2,0} - v_2\right) + a(\ln\frac{v_{2,0}}{v_2}) \qquad \text{(B-17)}$$

where $v_{2,o}$ is the initial exit velocity at start of daily filtration cycle.

NOTE: Eq. B-17 could possibly be applied to successive days of the filtration cycle by accounting for an incremental increase in "*a*" due to continuous development of schmutzdecke. *K* is an empirical constant in the Kozeny equation and generally describes head loss in clean filter. Recognizing that *K*, ε and/or S_A/V_G can change with time due to schmutzdecke development as well as depth penetration of particles, the value of "*a*" will increase with each daily filtration cycle. Correspondingly, $v_{2,o}$ must decrease because "*a*" determines the initial head loss for each filtration cycle which in turn is used to calculate $v_{2,o}$.

Conversion from velocity to flow rate using Eq. B-8

$$t = \frac{c}{gA_2}\left(Q_0 - Q\right) + a\left[\ln\frac{Q_o}{Q}\right] \qquad \text{(B-18)}$$

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