DEVELOPMENT AND EVALUATION OF CHITOSANS AS TRANSFORMATIVE COAGULANTS-FLOCCULANTS TO IMPROVE SAND FILTER DRINKING WATER TREATMENT

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ABSTRACT

Eleanor Holmes: Development and Evaluation of Chitosans as Transformative Coagulant-Flocculants to Improve Sand Filter Drinking Water Treatment
(Under the direction of Mark Sobsey and Lydia Abebe)

The World Health Organization (WHO) reports that 2.1 billion people worldwide lack access to safely-managed water sources. Sand filtration at point-of-use is widely used but does not meet WHO performance targets to reduce virus and bacteria levels in drinking water. This research evaluated chitosan, an organic coagulant-flocculant, to improve microbial and turbidity reductions by sand filtration. Bench-scale 3.9-cm diameter intermittently-operated slow sand column filters with 16-cm sand layers of two different grain sizes were dosed daily over 57-days with microbially-spiked surface water volumes corresponding to daily household use. *E. coli* bacteria and MS2 coliphage virus reductions were quantified biweekly using culture methods. Bacteria and virus reductions were significantly improved at optimal chitosan doses of 10 and 30 mg/L (Wilcoxon Rank-Sum, $p < 0.05$), and achieved $\log_{10}$ reductions meeting 2-star WHO performance levels. Microbial and turbidity reductions generally improved over filter operating time and showed no correlation with water filtration rate.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CI	Confidence interval
CFU/mL	Colony forming unit/milliliter
CWF	Ceramic water filters
CAWST	Centre for Affordable Water and Sanitation Technology
Da	Dalton unit
DAL	Double agar layer
DD	Degree of deacetylation
DOC	Dissolved organic carbon
ECD	Early childhood diarrhea
EPA	Environmental Protection Agency
FIB	Fecal indicator bacteria
GDWQ	Guidelines for Drinking Water Quality
HICs	High Income Countries
INPHWTSS	International Network to Promote Household Water Treatment and Safe Storage
ISSF	Intermittently-operated Slow Sand Filter
LMICs	Low- and Middle-Income Countries
LRV	Log_{10} Reduction Value
MDG	Millennium Development Goal
mg/L	Milligram per liter
MW	Molecular weight
NTU	Nephelometric Turbidity Unit
O&M	Operation and Maintenance

PB	Phosphate buffer
PFU	Plaque-forming units
rpm	Revolutions per minute
SAL	Single agar layer
SDG	Sustainable Development Goal
TDS	Total dissolved solids
TSB	Tryptic Soy Broth
UV	Ultraviolet
UNICEF	United Nations International Children's Emergency Fund
WaSH	Water, sanitation and hygiene
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Background and Significance

1.1.1 Safe Drinking Water

Clean and safe water is vital to lead a healthy, productive life; however, an estimated 2.1 billion people worldwide are at risk of harmful waterborne diseases due to fecal contamination of drinking water sources and supplies (WHO & UNICEF, 2017). Diarrheal diseases associated with water, sanitation and hygiene (WaSH) conditions are responsible for over 840,000 deaths in low and middle income countries (LMICs) each year, of which approximately 360,000 deaths are children under the age of five (WHO, 2014). Waterborne diseases linked to fecal contamination include diarrhea, cholera, dysentery, typhoid, poliomyelitis and other enteroviral diseases, infectious hepatitis due to hepatitis A and E viruses, and respiratory illnesses. The four pathogens attributable to the majority of cases of diarrheal illness in children are rotavirus, Cryptosporidium, enterotoxigenic *Escherichia coli*, and *Shigella* (Kotloff et al., 2013). Possible sources of drinking water contamination include improper human excreta and sewage management, ineffective sewage treatment, agricultural waste runoff, inadequate water treatment systems, deteriorating water distribution and storage systems and poor hygiene practices in water management.

In 2002, the 2015 Millennium Development Goal Target 7c was established and marked the first global effort to expand access to WaSH services, specifically aiming to halve the number of people without access to basic services by 2015, including improved drinking water.

This initial target served as the predecessor for the newly established and better articulated 2030 Sustainable Development Goal 6, which broadly aims to "ensure availability and sustainable management of water and sanitation for all." These international agendas serve to stimulate action and momentum for progress in WaSH coverage and raise the political stakes for the international community to address these persistent and difficult problems. Targets 6.1 and 6.2 of SDG 6 focus on expanding access to safe water services. Water services are characterized by safety, access and availability and fall into five general categories of access and coverage: safely managed, basic, limited, unimproved and surface water. Safely managed services must be improved water sources, contamination-free, accessible on premises and at all times. Improved water sources are piped supplies to homes or public standpipes or non-piped supplies, including protected wells and springs, boreholes, packaged water or rainwater collection systems. Basic services are considered improved sources accessible within a 30-minute round trip. Limited services are improved sources that take greater than 30 minutes to collect water and return home. Unimproved services do not meet any of the aforementioned criteria and include unprotected wells and springs. Finally, the surface water service level is defined as the use of any open body of water as a primary water source. Though 89% of the global population has access to at least basic drinking water services, 844 million people still depend on limited or unimproved drinking water sources for their daily water needs. Within this vulnerable group, over 150 million people rely on untreated surface water sources (WHO & UNICEF, 2017).

Residents in rural areas and in LMICs are particularly susceptible to contracting waterborne diseases due to the lack of improved water services, functional and effective water treatment systems, and inadequate waste collection and management practices. As of 2015, the

global proportion of urban residents with access to safely managed water services exceeded 85%, compared to 55% for rural residents. In the least developed countries, only 53% of the urban population and 25% of the rural population use safely managed services, or 33% of the total population. This is far lower than the estimated 94% of the total population using safely managed drinking water services in North America and Europe. The 2.1 billion people that lack access to safely managed services, including the 1.3 billion people using basic services, are at risk of harmful waterborne diseases due to substantial fecal contamination of both improved and unimproved water sources (WHO & UNICEF, 2017). Furthermore, the proportion of the global population living in urban areas is expected to grow from 55% as of 2018 to 68% in 2050 (DESA & UN, 2018). This shift in population dynamics may put increased strain on outdated and deteriorating water distribution systems in urban areas.

Although access to improved water sources is expanding globally, access alone does not mean the absence of a health risk. Fecal contamination of improved sources or the containers in which the water from these sources is stored is widespread and highly variable between source and location. Therefore, access to improved sources cannot be used as a proxy for access to safe water (Bain et al., 2014; Shaheed, Orgill, Montgomery, Jeuland, & Brown, 2014; Yang et al., 2013). Heitzinger et al. (2015) found that 90% of households studied in rural Peru had access to improved water sources, yet *E. coli* contamination was present in 47% of source water samples and 43% of stored water samples. Onda et al. (2012) reported that in 2010, approximately 1 billion of the 5.8 billion people with access to improved water sources were likely using contaminated water sources.

According to a study by Macy and Quick (2002), approximately 30% of the global diarrheal disease burden may be attributable to drinking contaminated water sources. Recurring

diarrheal disease from drinking contaminated water reduces productivity time for individuals and results in community-wide significant negative economic impacts. It is estimated that 1.5 billion working days would be gained globally with universal basic access to water supply and sanitation with point of use (POU) water treatment (Hutton, Haller, & Bartram, 2007). Recurring WaSH-related illnesses negatively impacts growth and development of children. Globally, children miss over 440 million school days as a result of illness from WaSH related diseases (Moszynski, 2006). Children under 5 would gain 6.8 billion healthy days due to averted cases of diarrhea with improved access to basic water supply and sanitation services coupled with POU treatment (Hutton et al., 2007). In order to promote social and economic development, improvements to WaSH infrastructure, including the provision of high-quality drinking water, are needed.

Concerns over microbially contaminated improved and unimproved water sources illustrate a need for further water treatment to ensure water safety. While large-scale and well-managed treatment systems are a preferred approach to provide access to sources of safe water, these systems require technical expertise and costly resources to build and maintain, therefore they are often inaccessible to rural and disadvantaged populations. International funding is limited so implementation organizations are keen to invest in technologies and systems that are low-cost but highly effective and sustainable at reducing diarrheal disease burdens in these LMIC. The quality of drinking water in the home, formerly not recognized as an important health factor compared to other WaSH interventions, is now considered an important factor when assessing diarrheal disease risk (Clasen, Roberts, Rabie, Schmidt, & Cairncross, 2006; Clasen, Schmidt, Rabie, Roberts, & Cairncross, 2007; Fewtrell et al., 2005). Accessible, affordable, practical and sustainable water treatment options are needed for these vulnerable populations.

1.1.2 Household Water Treatment and Safe Storage

One critical intervention that improves water safety of both improved and unimproved water sources for at-risk populations is household water treatment and safe storage (HWTS). HWTS water treatment technologies are also referred to as point of use (POU) water treatment technologies (Nath, Bloomfield, & Jones, 2006). The WHO Guidelines for Drinking-Water Quality (GDWQ) consider HWTS a priority intervention measure, or recommended interim measure, for those with no or basic access to drinking water services or those with access to unsafe drinking water services in water safety planning (WSP). WSPs are comprehensive risk assessment, monitoring and management approaches that aim to ensure the provision of safe drinking water (WHO, 2017). Various physical, chemical and biological household treatment options are widely implemented, including chlorination, solar disinfection, flocculant/disinfectant powder, slow sand filtration and ceramic filtration (Sobsey, 2002; Sobsey, Stauber, Casanova, Brown, & Elliott, 2008). Yet even with the extensive availability of these POU treatment technology options, millions continue to suffer from preventable waterborne illnesses due to the inability of these technologies to meet highly protective performance criteria supported by the World Health Organization. These criteria were determined by evaluating the tolerable burden of disease, or acceptable risk, associated with exposure to background pathogens in untreated water. The health-based targets are presented in terms of the theoretical maximum number of disability-adjusted life years (DALYs) attributable to waterborne disease per person when using the technology (WHO, 2011). In 2016, the WHO published initial results of performance evaluations based on 1- , 2- and 3-star protective reduction requirements for water treatment technologies. The performance targets are presented in **Table 1.** 3-star or highly protective technologies must achieve $\log_{10}$ reductions for bacteria, viruses and protozoan

cysts greater than 4, 5, and 4, respectively. This would confer a substantial health benefit to users, limiting DALYs attributable to drinking-water diseases to 10^{-6} per person. For 2-star or protective status, the HWT technology much achieve >2, >3 and >2 $\log_{10}$ reductions for bacteria, viruses and protozoan cysts, respectively. DALYs would be limited to 10^{-4} per person using a 2-star POU water treatment technology. In order to be considered a 1-star, interim (minimally protective) technology, protective performance requirements for two of the three pathogens must be met, along with epidemiological evidence of diarrheal disease reduction in credible field studies (WHO, 2016; WHO, 2011). The WHO GDWQ have also established a target level for turbidity in drinking water of < 1 NTU (WHO, 2017).

Table 1. WHO recommended microbiological performance criteria for HWT technology performance classification

Performance classification	**Bacteria** (Log_{10} reduction required)	**Viruses** (Log_{10} reduction required)	**Protozoa** (Log_{10} reduction required)	**Interpretation** (assuming correct and consistent use)
★★★	≥ 4	≥ 5	≥ 4	Comprehensive protection (very high pathogen removal)
★★	≥ 2	≥ 3	≥ 2	Comprehensive protection (high pathogen removal)
★	Meets at least Tier 2 criteria for two classes of pathogens			Targeted protection
-	Fails to meet WHO performance criteria			Little or no protection

Adapted from (WHO, 2016)

POU technologies have generally failed to achieve target reductions in field settings, often achieving only half of the reported maximum $\log_{10}$ reduction performance observed in laboratory studies (Sobsey et al., 2008). The WHO now reports baseline and maximum $\log_{10}$

reduction values (LRVs) for all POU technologies to illustrate the differences between reductions achieved in field use and those achieved in a controlled laboratory setting. Biosand filtration, for example, can achieve a maximum LRV of 3 for viruses; however, in practice only up to a 0.5 $\log_{10}$ reduction is typically observed (WHO, 2011). Despite these shortcomings, epidemiological studies suggest that the reduction in diarrheal disease attributable to POU technologies is between 30-40%, suggesting that these technologies still provide substantial value to users (Clasen et al., 2007; Fewtrell et al., 2005).

Many POU treatment options employing a single treatment barrier do not effectively target all three classes of microbes and therefore do not meet 2-star or 3-star reduction targets specified by the WHO. For example, most household water filtration options, such as biosand and ceramic filters, are inadequate for virus removal and household water chlorination is ineffective in reducing the infectivity of *Cryptosporidium* oocysts. Improvements to the available household water treatment options, especially those that are a single treatment barrier, are needed to further improve drinking water quality and to ultimately save lives. Combining technologies or adding additional steps to treatment processes may substantially improve existing HWT technologies, as long as cost, ease of use and accessibility constraints are managed.

1.1.3 Sand Filtration Technologies

Sand filtration technologies, notably rapid sand filters (RSF), slow sand filters (SSF) and biosand filters (BSF), are commonly used water treatment technologies due to their low manufacturing cost and simple design. Filtration is effective as a drinking water treatment process because as water passes through the small pores in the filter, microbes and particulate organic matter are physically removed. RSFs and SSFs remove contaminants via physical, biological and chemical processes, although RSFs rely primarily on chemical and physical

removal mechanisms. Both types of processes can be used at industrial or community scales as well as at the household level. BSFs are an adaptation of SSFs. Typically used at the household/POU level, BSFs are smaller sand filters that flow intermittently and use a biological layer called the schmutzdecke at the top of the sand column to aid in contaminant removal, analogous to that of a conventional slow sand filter.

Specific pathogen reductions vary depending on the design, materials and operating conditions of the filter device and other performance-related factors. Baseline LRVs for RSFs and SSFs as a standalone treatment mechanism are around 1 to 2-$\log_{10}$, far from the highly protective WHO targets for bacteria, viruses and protozoa of 4, 5, 4, respectively (WHO, 2011). Various studies have shown that SSFs and BSFs are able to extensively remove protozoa from water (Hijnen, Schijven, Bonné, Visser, & Medema, 2004; Palmateer et al., 1999). The maximum LRV achieved by BSFs for both bacterial and viral indicators in lab performance evaluation studies is 3, suggesting significant limitations in the microbial efficacy of this technology to treat water at the household level under typical use conditions (Sobsey et al., 2008).

While some sand filters may achieve the protective requirements specified by the WHO for bacteria and protozoan cysts, most do not and, therefore, only offer users limited protection. Furthermore, virus reductions are often quite low using granular microporous filtration technologies due to their small size relative to the size of the media pores; hence, these filtration methods offer little protection from harmful enteric viruses. In order to meet WHO's 3-star, highly protective requirements, a supplemental treatment should be considered to improve these existing filtration treatment processes.

1.1.4 Chitosan, a Natural Coagulant

Coagulation with chitosan may be an effective addition to existing filtration treatment technologies. Chitosan, a derivative of chitin, is a biodegradable polysaccharide and a byproduct of the crustacean fishing industry. When chitosan is added to water, suspended material including viruses, bacteria and spores are coagulated, the particles then flocculate together during slow mixing and eventually they can be settled out of the water columns. Inorganic coagulants, including ferric sulfate and aluminum sulfate, are commonly used in large water treatment facilities; however, they are highly pH and dose dependent for optimizing performance, which limits their use at the household level (Crittenden, Trussell, Hand, Howe, & Tchobanoglous, 2012). Previous studies have found that pH and dose levels do not significantly affect the efficacy of chitosan as a coagulant (Christensen, Håkonsen, Robertson, & Myrmel, 2016; Fabris, Chow, & Drikas, 2010; Soros, 2015; Soros, Amburgey, Stauber, Sobsey, & Casanova, 2019). Furthermore, chitosan is inexpensive, non-toxic, easy-to-use, naturally occurring and readily available in most places around the world. The accessibility, affordability, and coagulation efficacy of chitosan make it a promising supplemental treatment step to existing water filtration treatment technologies.

The use of chitosan as a coagulant in conjunction with sand filtration technologies could potentially reach the aforementioned 3-star microbe reductions as defined in the international scheme to evaluate POU water treatment technologies (WHO, 2016). It also has the potential to improve and extend the lifespan of currently used SSFs and BSFs. Chitosan may extend the use of sand filters by removing excess organic material and microbes prior to filtration, which would otherwise accumulate in the pores of the filter, lower the flow rate and potentially impact the effectiveness of the filter.

Previous literature that assessed chitosan as a coagulant found that it effectively reduced bacteria and virus concentrations and turbidity in model and natural waters treated by chitosan coagulation followed by microporous ceramic filtration, with no appreciable change in pH (Christensen et al., 2016; Fabris et al., 2010; Soros, 2015; Soros et al., 2019); however, no research is currently available regarding how chitosan coagulation-flocculation may improve microbial and turbidity reductions achieved via intermittently-operated slow sand filtration. Furthermore, the use of chitosan as a supplemental water pre-treatment step for existing intermittently-operated slow sand filtration technologies has also not been assessed with natural waters.

The purpose of this study was to assess the efficacy of chitosan as a coagulant in natural waters, followed by treatment with intermittently-operated slow sand filters. Variables such as optimal dosage of chitosan, sand grain size, and flow rate were evaluated for their potential effect on turbidity and microbe reduction. The reductions of turbidity and the indicator microbes of bacteria and viruses were compared to the WHO household water treatment performance targets.

1.2 Objectives

1. To enhance the performance of intermittently-operated slow sand filtration using coagulation-flocculation sedimentation by chitosan
    a. Improving removal of bacteria and viruses
    b. Improving removal of turbidity
    c. Meeting WHO performance targets
2. To optimize a chitosan dose that achieves maximum reduction values using the proposed dual-treatment barrier to:

a. Maximize removal of bacteria and viruses

b. Maximize removal of turbidity

c. Assess feasibility in household settings

3. To examine the impact of intermittently-operated slow sand filter design parameters on bacteria, viruses and turbidity removal by varying:

    a. Sand grain sizes in filter columns

    b. Filter operating time

CHAPTER 2: REVIEW OF THE LITERATURE

2.1 Point of Use (POU) Water Treatment

In high income countries (HICs), centralized drinking water treatment and source water protection policies are commonplace; however, in LMIC regions this is typically not the case. As of 2015, 69% of the global population has access to piped water systems. In 2017 the WHO/UNICEF Joint Monitoring Program (JMP) report concluded over 800 million people do not even have access to basic drinking water services, of which about 160 million drink untreated surface water (WHO & UNICEF, 2017). Providing centralized, piped water systems to these disadvantaged communities may take decades due to high capital investments, operation and maintenance (O&M) costs and a lack of fee or billing structures in smaller communities. Additionally, in many LMIC countries municipal water supplies still require treatment before consumption and residual chlorine levels may not be sufficient in the distribution systems to maintain high quality drinking water (Reller, Mong, Hoekstra, & Quick, 2001; Weber et al., 1994). In the interim, smaller-scale solutions to clean water access must be developed and implemented that are low-cost and readily understood and adapted by communities.

WaSH control measures, specifically POU treatment technologies, are considered an effective means to provide improved access to clean drinking water and ultimately prevent waterborne diseases (Clasen et al., 2007; Fewtrell et al., 2005; M. Sobsey, 2002; WHO, 2017). POU technologies installed and used in the household and that incorporate safe water storage practices are also commonly referred to as household water treatment and safe storage (HWTS)

technologies. In 2003, when the Third World Water forum in Kyoto recognized the International Network to Promote Household Water Treatment and Safe Storage (INPHWTSS), POU treatment technologies became a much-discussed subject and promising avenue for the international community to address water access needs. In the years since, hundreds of studies have been published evaluating different household-scale water treatment technologies and their health impact in communities, both microbially and economically (Clasen & Cairncross, 2004). Household-scale water treatment can be effective at reducing diarrheal diseases with correct and sustained use, and household water quality is now recognized as a key variable when determining risk of diarrheal disease (Clasen & Cairncross, 2004; Clasen et al., 2006; Fewtrell et al., 2005; Sobsey, Handzel, & Venczel, 2003). A systematic review and meta-analysis of various POU treatment mechanisms by Clasen et al. (2007) reported that POU interventions reduce the prevalence of diarrheal disease in people of all ages. Additionally, interventions to improve water quality in the home were more effective at reducing diarrheal disease than interventions targeting water sources (Clasen et al., 2006). The results from these studies demonstrate a clear benefit to using POU technologies to prevent illness, promote healthy development and increase lifetime productivity.

2.1.1 Role of POU Systems

POU water treatment technologies are commonly used in both HIC and LMIC to treat improved and unimproved water sources. In HIC, POU devices are often used as a second treatment technology to improve aesthetics and taste (Lykins Jr, Goodrich, Clark, & Harrison, 1994). In LMICs, POU treatment is used either as an additional treatment step for contaminated improved water sources or as a standalone treatment method for various water sources. POU water treatment employs many different technologies and methods to improve water quality,

including physical removal via sedimentation and filtration, chemical, UV and solar disinfection and coagulation/flocculation processes. Most available technologies target microbial quality but some POU devices also remove chemical contaminants. As of 2007, 19 million people used POU devices to treat their water worldwide, and an additional 350 million boiled their water as a form of POU treatment (Clasen, 2009).

POU technologies are considered a practical and low-cost means of increasing clean water access, especially to those households at higher risk of consuming contaminated water due to water collection and storage options and practices (Sobsey, 2006). Although not considered an improved means of providing clean water as defined in SDG 6, POU technologies make water safe to drink at the point of consumption and therefore should be considered a valuable tool to reduce diarrheal disease burden (Sobsey, 2002, 2006). POU technologies also offer additional protection for vulnerable populations, including those with HIV/AIDs, children under 5, and the elderly (Gadewar & Fasano, 2005; LULE et al., 2005). POU systems are also valuable in crisis situations where water provision and treatment infrastructure are inadequate, unreliable, nonfunctional or nonexistent, as in refugee camps and in the aftermath of destructive natural and anthropogenic disasters (Kayaga & Reed, 2011).

2.1.2 POU Technologies

POU technologies employ physical and chemical processes to treat water. Physical treatment mechanisms include boiling, heating via fuel or solar energy, ultraviolet (UV) radiation, filtration and sedimentation. Chemical treatment mechanisms include coagulation-flocculation, precipitation, chemical disinfection, adsorption and ion exchange. These methods can be used as standalone treatment systems or used in combination or in series to improve treatment capacity. No single POU system is best suited for all situations because some

treatment technologies are not effective against all pathogens and chemical contaminants. Capital and O&M costs, ease-of-use, durability, volume of water treated, time required to treat water, treatment efficiency over a range of water qualities and aesthetics are all factors that impact the performance and sustainability of a POU system. Cultural acceptability, resource availability and supply chain reliability are important community-based and enabling factors that influence POU device uptake and sustained and effective use (Sobsey et al., 2008).

2.1.3 POU Systems: Treatment Efficiency and Health Impacts

Sobsey et al. (2008) and WHO (2011) reviewed the most commonly used and well-documented POU technologies and reported baseline and maximum LRVs for bacteria, viruses and protozoa as well as diarrheal disease reductions achieved by these systems in controlled studies. These LRVs are documented in **Table 2.** Maximum LRVs are the highest achievable LRV for these systems when tests are performed in a laboratory setting. The baseline LRVs represent a more realistic value obtained from the field by a non-professional user. The baseline LRVs are typically less than half the projected maximum LRV for the specific treatment process and pathogen group. Studied and characterized factors for why these treatment processes achieve varying LRV values are also documented in **Table 2**.

Table 2. Popular POU technologies: estimated baseline and maximum $\log_{10}$ microbial reductions.

Treatment Process	Pathogen Group	Baseline LRV [a,b]	Maximum LRV [c]	Factors influence performance efficacy
Porous ceramic filtration	Bacteria Viruses Protozoa	2 0.5 4	6 4 6	Pore size/structure, tortuosity, flow rate, filter medium composition, augmentation with silver or other chemical agents (Sobsey, 2002; Brown et al., 2007; Brown, 2007)
Biosand filtration (BSF)	Bacteria Viruses Protozoa	1 0.5 2	3 3 5	Filter maturity, dosing conditions, flow rate, idle time, time between charges, grain size; challenge viral agent (Elliot et al., 2006 and 2008; Stauber et al., 2006; Palmateer et al., 1999)
Solar disinfection (SODIS)	Bacteria Viruses Protozoa	3 2 1	5.5+ 4+ 3+	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)
Free chlorine disinfection	Bacteria Viruses Protozoa [d]	3 3 3	6+ 6+ 5+	Turbidity and chlorine demand; concentration x contact time (Crittenden et al., 2005; Sobsey, 1989 and 2002)
Coagulation/ chlorination	Bacteria Viruses Protozoa	7 2-4.5 3	9 6 5	Physical removal of chlorine-resistant pathogens by coagulation-flocculation; turbidity; challenge viral agent (Souter et al., 2003; Sobsey, 2002)

(a) LRV: Log_{10} reduction value: Log_{10}(pretreatment conc.) – Log_{10}(post-treatment conc.)
(b) Baseline LRV: 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
(c) 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
(d) Minimally effective in reducing concentration of infectious *Cryptosporidium parvum* oocysts
Adapted from (Sobsey et al., 2008; WHO, 2011)

Despite the pathogen reduction variation achieved by these POU treatment processes, epidemiological studies have documented considerable diarrheal disease reductions associated

with the use of these POU systems. All five POU processes documented above achieve between a 30-40% reduction in diarrheal disease (Clasen et al., 2007; Fewtrell et al., 2005). Bias may have influenced greater than 50% of reported reductions in these studies, therefore further rigorously designed and conducted epidemiological-microbiological studies are required to fully assess the health impacts associated with POU treatment processes (Hunter, 2009).

In the Sobsey et al. review (2008), a scoring system was applied to the LRVs, reductions in diarrheal disease incidence, and the aforementioned sustainability factors of ease-of-use, cost and supply chain logistics, in order to quantify the sustainability of these technologies. Filtration technologies, specifically ceramic and BSFs, achieved the highest overall scores using the sustainability criteria, largely due to high reports of daily and long-term use; however, high breakage rates were reported for ceramic pot and candle filters. BSFs excelled compared to other POU processes when evaluating use over time, with reported compliance rates exceeding 85% 8 years post-implementation (Sobsey et al., 2008). A more recent study in the Dominican Republic reported compliance rates of 90% after 1 year of implementation (Aiken, Stauber, Ortiz, & Sobsey, 2011). A meta-analysis of BSF use and adoption documented an average 83% compliance rate, ranging from 10-100%, across 25 studies (Nakamoto, Graham, & Gimbel, 2014). Other advantages of using BSFs include absence of recurring costs, limited and simple maintenance procedures and effective performance at a wide range of influent turbidity levels (M. W. Jenkins, Tiwari, & Darby, 2011).

Considering the high sustainability rating for the BSF, focusing on the optimization of intermittently-operated slow sand filter POU technologies and the implementation of these systems may have the greatest sustained health and economic impact; however, reported LRVs for BSFs do not meet the WHO 2- or 3-star performance targets for reductions of bacteria,

viruses and protozoan parasites, suggesting the design and operation of these filters require improvements to achieve greater microbial reductions (Sobsey et al., 2008; WHO, 2011).

2.1.4 Granular Media Filtration

Filtration involves the physical removal of suspended particles in a solution as the solution passes through a porous media. The use of granular medium filtration to treat water dates back to around 2000 BC, when medical records mentioned that filtration through sand clarifies water. As of the 1750s, filtration technologies were commercialized and patented and in the early 1800s countries such as England and Scotland began using centralized water systems employing sand filtration technologies to treat and distribute their water. The Chelsea Water Works Company in London used the first slow sand filter (SSF) in 1829 and by the mid 1800s, sand filtration became popular as correlations between reductions in waterborne disease and the use of sand filtration technologies were recognized. In the 1880s, the United States developed and installed the first rapid sand filter (RSF) system and RSFs are now used in 99% of all centralized water treatment systems (Crittenden et al., 2012).

Granular medium filtration uses a granular material such as sand as the porous medium. Granular media filtration removes sediment, algae, clay, microorganisms and other organic and inorganic particles from water. Granular media filtration is employed at centralized water treatment facilities, community-scale water treatment facilities and even at the household level. Most municipal water treatment and distribution systems use RSF technologies, which typically require a coagulation pretreatment, a uniform porous media size, and a backwashing process. RSF is dependent on depth-filtration to remove particles from water. These particles become trapped throughout the media bed when they collide and adhere to the sand media as they pass through the filter. Even particles much smaller than the pore size in a RSF can be captured via

the mechanisms known to influence removal, including sedimentation, adsorption and interception (pore size exclusion and mechanical straining). At the municipal level, RSFs are continuously operated at a flow rate between 5-15m/h (Crittenden et al., 2012).

Unlike RSFs, SSFs do not require the porous media to be of uniform size, do not always use backwashing as a cleaning mechanism, typically do not require a coagulation pretreatment step and are run at a filtration rate 50 to 100 times slower than RSF systems. Facilitated by non-uniformity of pore sizes and a reduced filtration rate, SSFs trap particulate matter in water in the first few centimeters of the sand bed. A schmutzdecke also develops on the surface of SSF media as the filter matures. The schmutzdecke is a biological filtration layer that assists in both the physical removal and predation/biodegradation of the trapped organic matter. SSF systems are cleaned or regenerated by scraping off the top layer of the media bed. At the municipal level, SSFs operated at a filtration rate of approximately 0.08-0.25 m/h and are operated continuously (Crittenden et al., 2012).

Biosand filters (BSF), developed in the 1980s by Dr. David Manz at the University of Calgary, are essentially a household-scale adaptation of SSFs. BSFs may also be described as intermittently operated household-scale slow sand filters (ISSFs). After laboratory and field evaluation and design modifications in the early 1990s, the first BSF was patented and installed in households in Nicaragua (CAWST, 2012). Concrete and plastic variations of the BSF design are still being developed and modified today. BSFs rely heavily on the physical accumulation and biological degradation processes of organic particulate contaminants, including microbes, occurring on and in the sand bed to clarify water; however, physical and chemical treatment processes also contribute to their performance. The sand serves as a microbially active environment where pathogens are retained and subjected to predation by other microorganisms

living in the filter, are physically removed via mechanical trapping by biofilms produced by those microorganisms, adsorb onto the porous media surface or die off during the residence time in the filter (Elliott, DiGiano, & Sobsey, 2011). Unlike RSFs and SSFs, BSFs are intermittently operated. When a batch of water is added to the filter, water that was in contact with the microbial community on and within the filter sand media flows out. Recommended BSF operation involves dosing the BSF once per day to promote the growth of the microbial community within the filter. BSFs can be operated as many as 4 times per day, but an idle time of at least several hours between doses is recommended so a sufficient residence time within the filter can promote microbial attenuation (CAWST, 2012).

2.1.5 Microbial Reductions and Health Impacts Achieved by POU Sand Filtration

Both field and laboratory evaluations of BSF performance report wide ranges of reductions achieved for both bacteria and virus indicators as well as for turbidity. **Table 3** summarizes the effectiveness of BSFs at removing target organisms and turbidity as published in the Center for Affordable Water and Sanitation Technology (CAWST) biosand construction page (2012).

Table 3. Biosand Filter Effectiveness at Removing Target Organisms

Parameter	Effectiveness	Laboratory Results
Bacteria	Effective (>90%)	98.7%[1, 2]
Viruses	Somewhat effective (>80%)	85.9%[3]
Cryptosporidium	Very effective (>99%)	99.88%[4]
Giardia	Very effective (>99%)	>99.99%[4]
Turbidity		87%[3]

Adapted from CAWST 2012. 1. Elliot et al. 2008 2. Young-Rojanschi et al. 2014a 3. Young-Rojanschi et al. 2014b 4. Palmateer et al. 1999

Though BSFs are effective at removing protozoan parasites, they do not meet the 3-star performance targets for HWT technologies as specified by the WHO for bacteria, >4-$\log_{10}$, and viruses, >5-$\log_{10}$ (WHO, 2016). Laboratory study designs evaluating these filters have varied

widely from bench-scale to full-size experiments and subsequent reported reductions are also variable in results. Full-scale evaluations of the 60L BSFs produced by *Davnor Water Treatment Technologies Ltd.* reported average *E. coli* reductions of 94-99% (Stauber et al., 2006). Optimized designs of BSFs achieved reductions between 1.3-1.5-$\log_{10}$ for fecal coliforms and 0.8-$\log_{10}$ for MS2 coliphage in laboratory settings (M. Jenkins et al., 2009). Full-scale evaluations of the Version 10 BSF reported MS2 coliphage reductions exceeding 4-$\log_{10}$ after the first 43 days of filter operation and between 4 to 7-$\log_{10}$ reductions through 294 days of use (Wang et al., 2014).

Studies in field settings typically report lower reduction rates. Fecal indicator bacteria reductions were found to be 84-88% in studies evaluating continued use and health impact of BSFs in the Dominican Republic (Aiken et al., 2011). Plastic BSFs used in Ghana reportedly achieved average *E. coli* reductions of 97% (Stauber, Kominek, Liang, Osman, & Sobsey, 2012). Reduction rates of 83% for *E. coli* were reported in a study evaluating concrete BSFs in the Dominican Republic (Stauber, Ortiz, Loomis, & Sobsey, 2009). Average reductions of 93% for *E. col*i were reported in another BSF evaluation in the Dominican Republic (Stauber et al., 2006). Studies evaluating optimized BSFs filters in Kenya achieved reductions of 1.3-$\log_{10}$ for fecal coliforms (M. Jenkins et al., 2009). Only 80% *E. coli* removal was observed with BSFs used in Nicaragua (Fiore, Minnings, & Fiore, 2010).

Several design and operation characteristics may impact achieved LRVs. Filter ripening time, daily volume charged, idle time between charges, cleaning procedures, sand media composition, filtration rate and deep-bed media aging are all parameters that may affect filtration efficiency (Elliott, Stauber, DiGiano, de Aceituno, & Sobsey, 2015; Elliott, Stauber, Koksal, DiGiano, & Sobsey, 2008; M. Jenkins et al., 2009). Some of these parameters have been

evaluated in bench- and full-scale laboratory studies. Results from several studies have demonstrated that enhanced microbial reductions are a product of improved microbial attenuation when idle time within the filter is optimized. This is achieved by increasing the pore to daily charge volume ratio, reducing daily charge volume, and reducing the operating head (Elliott et al., 2008; M. W. Jenkins et al., 2011; Stauber et al., 2006). Sand grain size has been identified as a critical parameter, with smaller grain sizes improving fecal coliform reductions by 0.4-$\log_{10}$ and MS2 reductions by 0.5-$\log_{10}$ (M. W. Jenkins et al., 2011). Filter sand surface maintenance by scouring can negatively impact reductions of thermotolerant coliforms by upwards of 1-$\log_{10}$, but allowing filters an idle period of 18-24 hours after maintenance overcomes this negative impact (Singer, Skinner, & Cantwell, 2017). While intermittent dosing is a realistic use parameter in household settings, prior research has demonstrated reduced effectiveness of slow sand filtration when operated intermittently (R., Joshi, Dhage, & Tajne, 1980). Recent studies found continuous operation of BSFs achieve significantly higher LRVs compared to intermittently operated BSFs. Microbial reductions are improved by greater than 2-$\log_{10}$ for *E. coli* and 1.5-$\log_{10}$ for viruses (Young-Rojanschi & Madramootoo, 2014). Lower filtration rates have been correlated with improved filter performance for bacteria removal (Napotnik & Jellison, 2014; Singer et al., 2017).

As previously mentioned, positive health impacts have been associated with using BSFs in field settings. The odds of reporting diarrheal disease in households using BSFs in the Dominican Republic was 0.39 times lower than for households not using BSFs (Aiken et al., 2011). In Ghana, the prevalence ratio for diarrhea comparing households that did and did not receive a BSF was 0.40 (Stauber, Kominek, et al., 2012). Consistent use of BSFs was found to

reduce diarrheal disease by greater than 50% in Cambodia (Stauber, Printy, McCarty, Liang, & Sobsey, 2012).

Recent research has explored ways to improve BSF performance. Modifications to existing operation recommendations and alternative sand media have made marginal improvements (M. Jenkins et al., 2009; M. W. Jenkins et al., 2011). Including additional treatment steps within the BSF design to make it a dual barrier water treatment technology have also been investigated. When biosand filtration is followed by UV disinfection as a dual-treatment system, effluent water had *E. coli* concentrations less than 1 CFU/100 mL. This observation was true for both BSFs of small grain size (d = 0.70 mm) and large grain size (d_{max} = 2.0 mm) (Frank, Scheie, Cachro, & Muñoz, 2014). BSFs modified with a layer of zero valent iron as an additional disinfection layer have produced marginal improvements in bacteria and turbidity reductions (Yildiz, 2016). BSFs with a 10-cm thick layer of iron oxide-coated sand media were found to have greater removal performance by at least 1-$\log_{10}$ for fecal coliform and *E. coli* bacteria compared to conventional BSFs (Ahammed & Davra, 2011). Iron oxide-amended bench-scale column BSFs removed 5-$\log_{10}$ MS2 coliphage and greater than 4-$\log_{10}$ rotavirus. Full-scale iron-amended BSFs removed over 4-$\log_{10}$ MS2 coliphage in the first 5 months of use (Bradley, Straub, Maraccini, Markazi, & Nguyen, 2011).

2.1.6 Viral Reduction Limitations of Sand Filtration

Viruses, which are important etiologic agents of diarrhea, infectious hepatitis and other diseases, are particularly difficult to treat with granular media filtration devices due to their small size. Viral pathogens, including adenoviruses, astroviruses, caliciviruses, enteroviruses, Hepatitis A & E, rotaviruses and orthoreoviruses, can be transmitted from person to person through contaminated drinking water (WHO, 2017). Viruses contribute to diarrheal disease

burdens in LMIC. A systematic analysis of 195 countries found that rotavirus was the leading cause of diarrhea in children of all ages (Troeger et al., 2018).

Studies evaluating virus removal with SSFs and BSFs have reported reductions of less than 0.5-$\log_{10}$ to greater than 5-$\log_{10}$ for indicator organisms and enteric viruses (Elliott et al., 2015; Wang et al., 2014). High variability in reported virus reductions is likely attributable to differences in filter design and operation, source water characteristics and indicator organisms. MS2 coliphage is the most common virus indicator organism used in filter evaluations for virus removal. Most studies report reductions between 0.2-1.5-$\log_{10}$ for MS2 coliphage with BSFs (Elliott et al., 2008; M. W. Jenkins et al., 2011; Young-Rojanschi & Madramootoo, 2014). Recent studies of long-term BSF operation have reported LRVs ranging from 3-5 for MS2; however, this level of removal was not achieved until after 3 months of filter operation (Wang et al., 2014).

Higher performance in virus removal has been correlated with lower filtration rates, extended filter operation time, increased sand bed depth, higher temperatures, and longer idle periods between batch doses (DeLoyde, 2007; Elliott et al., 2011). Mechanisms by which viruses are removed in biosand filtration include adsorption to granular media, attachment to biofilms, biological activity, predation, and physical straining. Although the schmutzdecke is important for bacteria removal, studies have not observed an impact on virus removal capacity when the schmutzdecke is disturbed or removed (DeLoyde, 2007; Hijnen et al., 2004). This evidence, combined with observed improvements in virus reductions over filter operation time, suggest that media aging in the filters is responsible for enhanced virus removal (Elliott et al., 2011, 2015). Recent studies have demonstrated positive correlations with virus attenuation and media aging, suggesting that activity of the microbial community is likely the primary

mechanism of virus removal in BSFs (Elliott et al., 2011; Wang et al., 2014). Virus attenuation may be the result of both predation and virus inactivation by proteolytic enzymes (Elliott et al., 2011).

Increasing idle times and decreasing water charge-to-pore volume ratios may improve virus reduction performance for BSFs, but this significantly limits the amount of water a household can treat per day if they only have one POU treatment device. Additionally, it is not reasonable to tell users that water treated with a BSF is not safe to use until appropriate media aging has occurred. Though BSFs are somewhat effective at removing bacteria and turbidity from water, clear limitations still exist with regards to their virus removal capacity. Conventional water treatment systems utilize coagulation-flocculation-sedimentation to capture viruses into larger particles prior to filtration. Only one study has evaluated using coagulation with filtration as a dual treatment barrier in household settings. This group found improved microbial and turbidity reductions after slow sand filtration using a natural coagulant extracted from *Opuntia cochenillifera*; however, they did not evaluate virus removal in the study (Freitas & Sabogal-Paz, 2019). Further coagulant options should be considered and evaluated with ISSFs to improve the removal capacity of this sustainable and accessible technology.

2.2 Coagulation-Flocculation

Conventional water treatment facilities remove inorganic, organic, and colloidal particles as well as dissolved organic matter from water using coagulation and flocculation processes followed by sedimentation and filtration. Chemical coagulants are added to water in order to destabilize the suspended and dissolved particles so they aggregate during flocculation. The formed flocculent particles are removed from water by settling out of solution (gravity sedimentation) or by filtration. Inorganic coagulants, including alum, ferric sulfate and ferric

chloride, hydrolyze when added to water and adsorb to particles. They form lengthy polymeric hydroxy molecules that have positive charges along the chain due to their iron or aluminum moieties. These positive charges make it possible to attract and adsorb to negatively charged particles in water, including viruses and other microbes of health concern. The surface charge or electrical potential of viruses depends on the pH and the dissolved solids types and concentrations in the water. It is also important to note that viruses differ in their surface charge properties, such as their isoelectric point, the pH at which they have zero surface charge and this may influence their adsorption to surfaces and their ability to be coagulated. The extent to the isoelectric point of viruses influences the extent of virus interactions with abiotic surfaces remains uncertain (Dika, Duval, Francius, Perrin, & Gantzer, 2015).

However, it is known that particles such as microbes are destabilized as the coagulants form bridges between particles or neutralize their charge. Particle destabilization can also occur when charged polymers, or organic polyelectrolytes, are added to water. Inorganic coagulants are typically used at municipal water treatment facilities. Flocculation serves to aggregate the destabilized particles into larger particles for removal (Crittenden et al., 2012). The coagulation-flocculation process is considered the most important treatment step to physically remove contaminants (Bellamy, Cleasby, Logsdon, & Allen, 1993; Cleasby, Dharmarajah, Sindt, & Baumann, 1989).

2.2.1 Coagulation Mechanisms

In water treatment practices, coagulants destabilize particles and enhance removal by three primary mechanisms: adsorption and charge neutralization, adsorption and interparticle bridging, and precipitation and enmeshment. Compressing the ionic double layer surface of particulates by increasing ionic strength is also considered a coagulation mechanism; however,

this is not a practical mechanism for drinking water treatment (Crittenden et al., 2012). In the neutral pH range, most colloids and other suspended particles in water have a negative charge. Charge neutralization involves the destabilization of particulates in water when oppositely charged ions or polymers adsorb onto the surface of those particles and neutralize the negative charge. When particles have a neutral charge, they flocculate. This process is dose dependent. If too much ion or polymer is added, the particles may become stable again via charge reversal (Black, Birkner, & Morgan, 1966). Interparticle bridging involves the adsorption of a single polymer chain on multiple particles which creates a bridge between those particles. The optimal dose to maximize interparticle bridging is proportional to the concentration of particles in the water to be treated. These two destabilization mechanisms are the main mechanisms for polymer coagulation. Precipitation and enmeshment and charge neutralization are the primary mechanisms of destabilization and removal when hydrolyzed or pre-hydrolyzed metal salts are added to water. At low doses, when the salts hydrolyze in water they adsorb to the surface of particles and neutralize their charge. At high doses, particulate matter in water is trapped in insoluble precipitates that form as the metal salts bind to the surface of the particles. Precipitation and enmeshment is the predominant mechanism used in water treatment facilities (Crittenden et al., 2012).

2.2.2 Salts and Polymers

Optimized coagulation-flocculation processes are dependent on many factors including the coagulant, the influent water quality and composition of suspended particulates, temperature, pH and dose. Aluminum and ferric ion salts, the most common inorganic coagulants used for water treatment, are pH-dependent and require high doses to be effective; however, they are

equally effective at the same dose for any particulate type. They are less effective at colder temperatures and require rapid, instantaneous mixing times (Crittenden et al., 2012).

The optimization of polymers for coagulation in different settings is complex. Optimal doses, mixing conditions and pH depends on the polymer and the polymer-solution interactions. Nonionic polymers are widely considered useful filter aids and anionic polymers are considered useful flocculant aids, both of which improve the strength of the formed floc. Cationic polymers can be used as primary coagulants but typically only if followed by direct filtration. Synthetic organic polymers are more commonly used in water treatment than natural organic polymers; however, both are typically not used as the primary coagulant for water treatment facilities. Organic polymers are effective at lower doses than inorganic coagulants, and using both together can reduce the amount of inorganic coagulant required by as much as 40-80% (Crittenden et al., 2012).

Health and environmental impacts associated with the chemicals used in water treatment processes is of growing concern. Traditional inorganic coagulants produce toxic residual sludges that may have substantial and persistent adverse environmental effects if not properly disposed (Matilainen, Vepsäläinen, & Sillanpää, 2010). High residuals of the inorganic salts have negative neurological impacts and are considered a risk factor for Alzheimer's (Rondeau, Commenges, Jacqmin-Gadda, & Dartigues, 2000). The use of these coagulants requires additional chemical treatment to limit corrosion in distribution systems (Matilainen et al., 2010). Furthermore, pH and dose control constraints as well as the burden of high solids residuals make inorganic coagulants difficult to optimize for POU treatment. These drawbacks indicate a need for environmentally friendly, readily available, safe and sustainable alternatives to inorganic salts for water treatment by chemical coagulation (Niquette, Monette, Azzouz, & Hausler, 2004).

Natural food- and plant-based polymers that are non-toxic and biodegradable may be promising coagulant alternatives. Extracts from maize, red bean, *Moringa oleifera* and *Strychnos potatorum*, acorns and chestnuts have been evaluated as potential coagulants both for municipal and POU treatment (Babu & Chaudhuri, 2005; Ghebremichael, 2004; Gunaratna, Garcia, Andersson, & Dalhammar, 2007; Šćiban, Klašnja, Antov, & Škrbić, 2009). A Polymer extract from the cactus *Opuntia* spp. achieved average turbidity reductions of 98% (Miller, Fugate, Craver, Smith, & Zimmerman, 2008). Polymers extracted from acorns and nuts reached 70-80% turbidity removal (Šćiban et al., 2009). *S. potatorum* and *M. oleifera*, evaluated as potential coagulants coupled with RSF for household use, significantly reduced turbidity, bacteria and viruses in water. Approximately 2-$\log_{10}$ reduction for bacteria and over 3-$\log_{10}$ reduction for viruses were reported with *S. potatorum* and *M. oleifera*, respectively, followed by direct filtration (Babu & Chaudhuri, 2005). Although some of these plant-based organic coagulants were found to be effective, their access may be limited to the geographic settings in which they grow and are not widely produced and distributed in quantity via commercial suppliers. High operational costs at water treatment facilities may limit natural coagulants from use as the primary coagulant; however, natural coagulants may be more practical in low-resource settings as well as for POU treatment (Niquette et al., 2004).

2.3 Chitosan, A Natural Polymer Coagulant

Coagulation with chitosan, a natural biodegradable polymer, may be an effective addition to existing POU treatment technologies. It is a derivative of chitin, the second most abundant polysaccharide globally (Rinaudo, 2006). Crustacean shells considered waste by the seafood industry are converted to chitin via decalcification and deproteination (Cheung, Ng, Wong, & Chan, 2015). Deacetylation of chitin produces chitosan. Chitosan is a cationic polymer that is

water insoluble but dissolves in most acids. The chitosan polymer chain consists of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine moieties (Cheung et al., 2015). Commercially available chitosan products range in molecular weight and degree of deacetylation, which influence their physical-chemical characteristics. High molecular weight, which also influences the length of the polymer chain, is associated with high viscosity. High degree of deacetylation, which ranges between 40% and 98%, is associated with increased solubility (Mourya & Inamdar, 2008). High degree of deacetylation is also associated with greater numbers of positive charges along the polymer chain. Positive charges accrue as amine groups protonate when chitosan dissolves in water (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Chitosan salts, a product of treating chitosan with acids to modify functional groups on the polymer via cross-linking and grafting, are more soluble in water and are useful in a variety of applications (Cheung et al., 2015).

Chitosan is non-toxic, readily available worldwide, and the US Food and Drug Administration granted it Generally Recognized as Safe (GRAS) status (Kean & Thanou, 2010). Characteristics of chitosan include biocompatibility, low allergenicity, biodegradability and antimicrobial activity (Kumar et al., 2004; Martins et al., 2014). Chitosan has been studied for potential applications in the biomedical, pharmaceutical and dietary supplement, and water and water and wastewater treatment industries (Cheung et al., 2015; Elieh-Ali-Komi & Hamblin, 2016). This research focuses on the use of chitosan as a coagulant for POU drinking water treatment.

2.3.1 Chitosan and Coagulation-Flocculation

When chitosan is added to water, the positive charges along the polymer chain facilitate adsorption interactions with negatively charged suspended particles, metals and dyes (Boamah et

al., 2015; Tran et al., 2015; Vakili et al., 2014). Many modified chitosans are considered effective chelating agents that bind and subsequently remove metals (Boamah et al., 2015). Organic pollutants of health concern, including pesticides, herbicides and polycyclic aromatic hydrocarbons, are readily adsorbed and removed with chitosan (Tran et al., 2015). In addition to its bactericide potential under specific conditions, chitosan also effectively adsorbs to particles in water and enhances turbidity and microbial removal by filtration processes (Abebe, Chen, & Sobsey, 2016; Soros, 2015; Soros et al., 2019).

Interparticle bridging and charge neutralization are the primary coagulation mechanisms associated with chitosan. The long polymeric structure and distributed positive charges allow chitosan to attach and bridge with numerous negatively charged colloidal particles, resulting in coagulation and sedimentation of flocs of destabilized particles (Abebe et al., 2016). As with any other polymer coagulant, factors including dose, pH, mixing conditions and influent water quality impact the particle removal capacity of chitosan.

2.3.2 Chitosan for Colloid Removal

The extent of turbidity removal documented in the literature varies depending on particle and chitosan type. Greater kaolin and bentonite removal is achieved with higher molecular weight chitosans (Chen, Chen, & Wu, 2003; Domard, Rinaudo, & Terrassin, 1989). Greater bentonite removal is also achieved with higher DD of chitosan (Chen et al., 2003). Jar tests using chitosan with model waters exhibited similar trends and found that higher chitosan doses were not associated with increased turbidity removal. Other identified factors that influence turbidity removal with chitosan include clay type and functional groups (Soros et al., 2019). Turbidity reduction by chitosan coagulation and ceramic filtration in natural waters consistently met US EPA standards and WHO performance targets of < 1 NTU (Abebe et al., 2016).

Coagulation-flocculation with alum in conjunction with chitosan resulted in turbidity removals between 74.3 to 98.2% (Bina, Mehdinejad, Nikaeen, & Attar, 2009).

Information regarding the ability of chitosan to remove microorganisms from water via coagulation-flocculation is limited. Strand et al. (2001) documented an inverse relationship with DD and flocculation efficiency: higher DD (99%) required 10 times less chitosan hydrochloride for bacterial flocculation than lower DD (38%). Bina et al. (2009) observed LRVs between 2-4 for *E. coli* when water was treated with alum in conjunction with chitosan. Jar tests using chitosan alone with model waters exhibited $\log_{10}$ reductions of 3-5 for *E. coli* and MS2 at various chitosan dosages (Soros, 2015). Recent evidence published by Abebe et al. (2016) suggests that microbial $\log_{10}$ reductions by chitosan coagulation followed by ceramic filtration range between 4.7 and 7.5 for *E. coli* and between 2.8 and 4.5 for MS2 coliphage using model waters.

Few studies have assessed the use of chitosan coagulation with RSFs and none are currently available that explore chitosan with SSFs and BSFs. *C. parvum* oocyst removal reached 4.2-$\log_{10}$ for water treated with 3 mg/L chitosan followed by rapid sand filtration (Brown & Emelko, 2009). Christensen et al. (2017) assessed coagulation by chitosan combined with RSF, filtration rate = 5.9 m/h, and reported 4.5-5-$\log_{10}$ for *E. coli*, ~2.5-$\log_{10}$ for MS2 and *S. Typhimurium* 28B, and ~3-$\log_{10}$ for *C. parvum*. The same experimental setup resulted in 61% turbidity removal at low doses of 2-6 mg/L chitosan (Christensen et al., 2016).

2.3.3 Chitosan with POU Filtration

Inorganic coagulants, including ferric sulfate and aluminum sulfate, are commonly used in conventional water treatment facilities; however, they are pH and dose dependent for optimum coagulation-flocculation performance, which limits their use at the household level. They are also toxic at high doses, especially aluminum salts, therefore it is unadvised to ask minimally-

trained individuals to oversee dosing and treatment with these coagulants. Furthermore, alum quality varies significantly from source to source and adding excess alum to drinking water makes it salty and unpalatable (Preston, Lantagne, Kotlarz, & Jellison, 2010). Coagulation-flocculation at the POU has not been adopted in part due to the limitations of inorganic coagulants. Few coagulation products are specifically optimized for household use. PUR, which was developed and marketed by Proctor and Gamble, is the most well-known and commercially available POU flocculant-disinfectant product. PUR utilizes iron sulfate as the primary coagulant and calcium hypochlorite as the disinfectant. No natural polymeric coagulants are currently optimized for use in household settings. A coagulant-flocculant system using a natural coagulant that is easily paired with existing filtration technologies is needed to enhance microbial removal performance for POU treatment.

Chitosan is inexpensive, non-toxic, easy-to-use, naturally occurring and readily available in most places around the world. The accessibility, affordability, and efficacy of chitosan make it an ideal supplemental treatment step to existing water treatment technologies. Previous studies have found that pH and dose adjustments do not significantly impact the efficacy of chitosan as a coagulant in water, especially in turbidity removal (Christensen et al., 2016; Fabris et al., 2010; Soros, 2015; Soros et al., 2019). Chitosan has never been evaluated as a credible water treatment product to remove microbes and turbidity in conjunction with intermittently-operated slow sand filtration technologies. Previous studies have determined that modified chitosans effectively remove bacteria and viruses from water both in jar tests, with RSF and with ceramic filtration (Abebe et al., 2016; Christensen et al., 2017; Soros, 2015); however, the performance of chitosan and intermittently-operated slow sand filtration as a dual treatment barrier has not been

evaluated. If high microbial removal over long-term use is achieved and maintained, chitosan may be a simple, affordable and effective means to improve household water filtration devices.

2.4 Summary

There is not an effective granular media filtration system for POU household treatment that removes viruses with sufficient efficacy to meet WHO performance targets for high (3-$\log_{10}$) and very high (5-$\log_{10}$) LRVs. Addressing this problem with traditional inorganic coagulants is not feasible due to the difficulties of precisely controlling coagulant dose and key water quality parameters, such as pH, to optimize performance; however, chitosan may be an attractive alternative because of its aforementioned desirable properties of effective performance at a range of different doses in waters of different quality. Previous studies using chitosan coagulation pretreatment have shown that viruses are more efficiently removed with other filtration technologies such as microporous ceramic filters and RSFs (Abebe et al., 2016; Christensen et al., 2017). Because substantially greater virus reductions are observed when water is pretreated with chitosan before filtration, evaluating chitosan pre-treatment with intermittently-operated slow sand filters or similar simple filters is a logical next step to investigate chitosan coagulation-flocculation for applicability in POU water treatment. This study explores that possibility at lab bench scale as a feasibility and proof of concept study.

CHAPTER 3: METHODS

The bench-scale dual barrier treatment of chitosan coagulation pre-treatment and filtration using small-scale sand filter columns of 16 cm sand depth was evaluated over a period of 57 days. The experiments compared microbial and turbidity reductions of chitosan coagulated water in sand columns filled with two different sands, commercial Accusand and well-characterized rapid silica sand media used by the Orange Water and Sewer Authority in Carrboro, NC. OWASA is a public, non-profit municipal utility servicing southern Orange County, NC. The modified sand filter columns were not specifically designed and operated based on any sand filter preparation and operation guidance. The BSF guidance served as a general reference for how to properly build and charge the filters; however, sand filter depth, water flow rate, maximum head, idle periods between daily charges with microbe-laden test water and amount of water held in the media between daily water charges was not controlled in the design and operation of this bench experiment. The bench experiment served as proof-of-concept to evaluate how chitosan pretreated water impacts the microbial and turbidity reductions achieved by ISSFs. Biological activity was not considered a primary variable of interest in these studies so design and dosing of the sand column filters was not optimized for growth and maintenance of a schmutzdecke and the filters were not operated to simulate a full-scale BSF. The sand columns used in this study were ISSFs with falling head and manual dosing, subjected to weekly cleaning of the top 3 cm of the sand layer to maintain reasonable water flow.

3.1 Overview of Protocol

16 bench-scale, 3.9 cm diameter column sand filters of 16 cm sand depth were designed and constructed. They were intermittently-operated via a single 500 mL daily charge over a 57-day evaluation period. Non-pathogenic bacteria and virus surrogates were used to evaluate the removal of bacteria and viruses from water via chitosan coagulation-flocculation followed by intermittently-operated slow sand filtration. Reductions in turbidity were based on decline in turbidity levels of the natural surface water samples. Natural surface waters were spiked with surrogate microbe stock suspensions on each testing day at target levels that allow for the measurement of greater than 6-$\log_{10}$ removal. Samples of untreated water were taken for initial microbial evaluation, then waters were dosed with target volumes of a 2 g/L stock liquid chitosan solution to achieve designated chitosan concentrations. A 30-minute mixing and settling procedure was commenced to facilitate coagulation-flocculation-sedimentation. Post-chitosan coagulation-flocculation samples were taken prior to dispensing the chitosan-treated water into the bench-scale sand filters. Effluent water samples were taken from the filtrate of each filter. Culture-based assays were used to quantify microbial concentrations in waters for all samples. Log_{10} reductions were calculated between the influent and effluent samples.

3.2 Column Design, Preparation and Operation

The design of the sand filter columns used in the bench-scale experiment are shown in **Figure 1**. A total of 16 columns were operated in parallel for the experiment, of which 8 were loaded with silica sand media used in OWASA's rapid sand filters and 8 others were loaded with standard Accusand silica (Unimin Corp., Le Sueur, MN, USA). These sand conditions are referred to as Accusand and silica sand in the remainder of the report. The sand filter columns were not designed and operated for a specific flow rate. Instead the sand columns outlet tubes

were adjusted in height to decrease/increase the flow rate in an attempt to maintain flow rates among sand conditions within a somewhat uniform range of values. The target filtration rate for the Accusand-filled columns was 0.4-0.6 m/h and the target filtration rate for the silica sand-filled columns was 1.0-1.4 m/h. The target filtration rate for the Accusand columns was determined based on the suggested filtration rate for BSFs, 0.4 m/h (CAWST, 2012). The silica sand filtration rate was a compromise due to limitations in the filter design. Had the effluent tube been moved any higher on the sand column, the falling head would have reached the effluent tube and the flow would cease without filtering over 50 mL of water. This would result in incorrect filtration rate measurements because refilling would bring the filters back to maximum head during measurements and flow would fluctuate.

Accusand was used due to its low organic matter content, chemical purity, and low uniformity coefficient (Schroth, Ahearn, Selker, & Istok, 1996). The Accusand media in the columns was a blend of three sieve fraction sizes (U.S. Standard Mesh 30/40, 40/60, and 50/70). The combination of these sieve fractions provided a smaller average grain size (d10 = 0.24 mm; d60/d10 = 1.40) compared to the silica sand (d10 = 0.50 mm; d60/d10 = 1.40). The recommended range of d10 values, or the effective size range, for BSFs is between 0.15 mm and 0.20 mm in order to achieve a filtration rate of 0.4 m/hr (CAWST, 2012). The Accusand combination used in this experiment had a slightly higher effective size than is recommended, while the silica sand media is greater than double what is recommended for BSFs and ISSFs. Accusand sieve analysis results are presented in **Appendix 1**. A full characterization of the silica sand media, conducted by Pennoni Associates Inc., is presented in **Appendix 2**. A particle size analysis conducted by Trimat Materials Testing, Inc. is presented in **Appendix 3**. Both the silica sand and Accusand media were pre-washed via 24-hour exposure to 10% concentrated HCl, and

subsequently rinsed until the effluent water reached a pH of 5 (Litton & Olson, 1993). The underdrain of each column was 1-3 cm layer of Poly-fil polyester fiber fill.

The daily charge volume of water for the sand column filters, 500mL per filter per day, was determined by comparing the surface area ratio of a full-scale BSF treating 20 L/day to the corresponding dimensions of the sand column filters. A volume of 20 L/day is considered the minimum amount of water required per person per day for basic drinking, hygiene and food preparation needs (Howard & Bartram, 2003). For the bench-scale study, 10L of water was used per day for all 16 filters. The total daily charge volume for each dose (2.5L) was distributed to four 500 mL graduated cylinders so that the challenge organism concentrations were identical for each chitosan dose condition. The additional 0.5 L was included to account for aliquots of sand filter column influent and post-chitosan coagulated-flocculated and settled water that were taken before dosing such treated water into the filters, as well as limit the amount of settled floc in the water added to the sand filters. Four different chitosan doses were evaluated and the daily charge volume of water for each chitosan dose was prepared in separate containers that also received approximately the same concentration of microorganisms. The daily charge volume to the columns was introduced in 50 mL aliquots. Each filter had a standard maximum head volume of 7 cm in order to maintain similar daily filtration rates across all filters. An external reservoir was not used to introduce the total daily charge water volume at one time, so no consistent pattern of decline in head was observed or controlled for in the experiment. Additionally, the filters were not operated like typical BSFs. Weekly scouring of the top 3 cm layer of each sand column may have effectively disrupted any growth of a schmutzdecke. Despite weekly scouring, the flow rate declined in the filter columns over each week of operation and generally over the 57-day dosing period of the experiment. The location of the outlet tube for sand filter column

effluent water was lowered in an attempt to adjust for this flow rate decline in order to maintain similar flow rates over the duration of the study period. The weekly scouring procedure involved: (1) disrupting the top 3 cm of sand in each filter column with a sterile 5-mL pipette for 30 seconds, (2) then adding 50 mL of DI water to the top of the columns in order to suspend the material released from this scoured top layer of the sand bed and (3) then aspirating this resulting mixture from the top of the sand filter column into the pipette and discharging it to waste. This cleaning procedure was repeated twice in succession for each sand column filter, and then the filters were returned back to daily use.

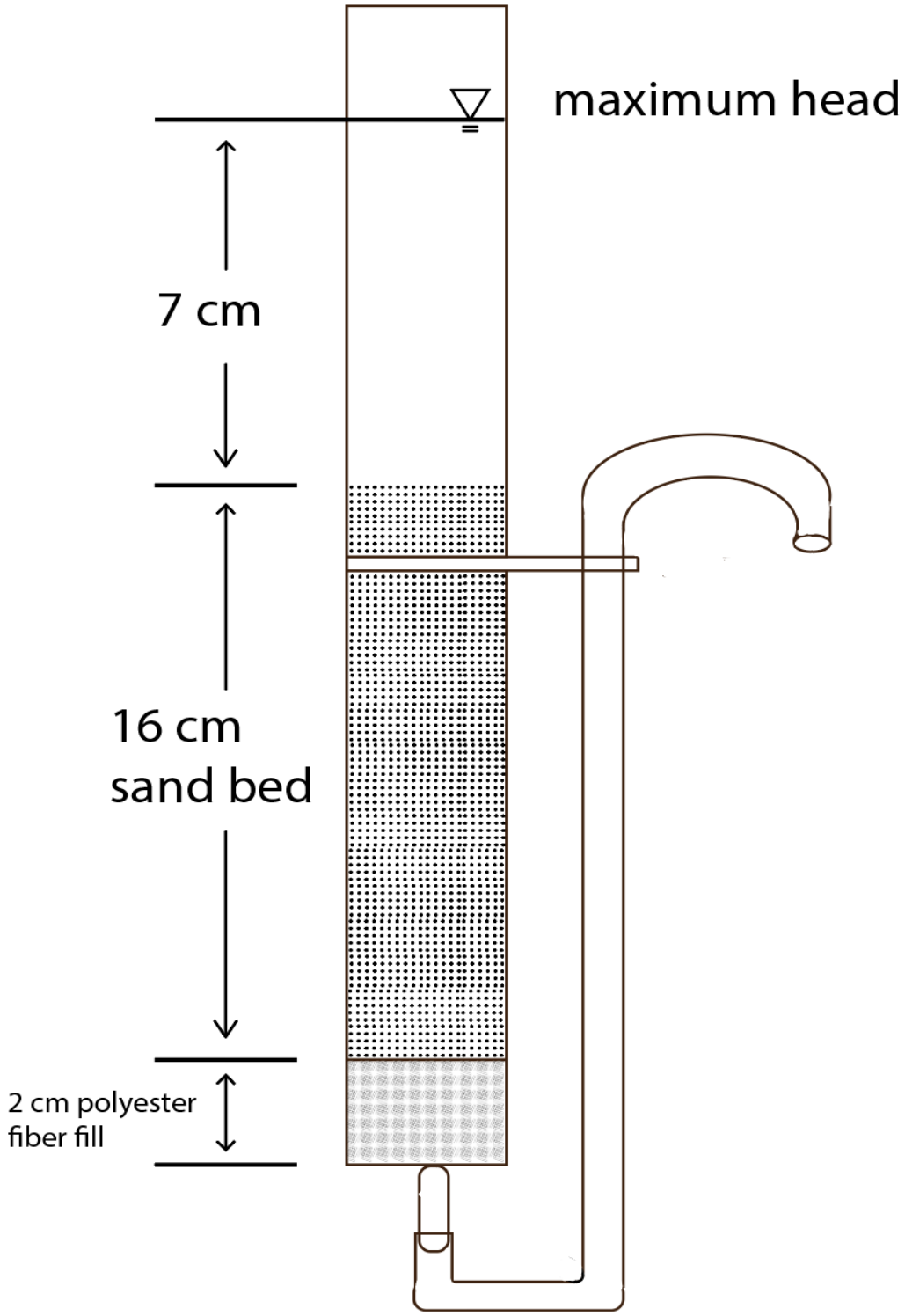


Figure 1. Cross-section of bench-scale columns used in 57-day evaluation

3.3 Challenge Water for Microbial Evaluation

A diagram of the experimental design is presented in **Figure 2**. The challenge water for chitosan coagulation-flocculation-sedimentation and then sand filter column dosing was obtained by periodic surface grab sampling from University Lake in Carrboro, NC. University Lake is one of the protected reservoirs OWASA uses to supply drinking water to the residents of Chapel Hill and Carrboro, NC. University Lake does not receive any identifiable wastewater discharges. The average water quality parameters of University Lake over the study period, October 22nd – December 22nd, 2018, are provided in **Table 4**. Based on longitudinal water quality data provided by OWASA, the fall turnover event for University Lake occurred around the end of November to early December 2018. Water was collected every 1.5 weeks and stored at 4°C for daily use. The day before dosing, the stored water was left out overnight to reach room temperature (about 20°C). Such water storage conditions were intended to achieve a low and relatively consistent water storage temperature over the course of the 57-day experiment and thereby not greatly impact the microbial quality and stability of the test water.

Table 4. Average water quality parameters for University Lake over the 57-day study period (October 24th – December 21st, 2018)

Parameter	Units	Average Values (+/- SD[a])
pH		6.87 (+/- 0.228)
Temperature	°C	9.90 (+/- 3.40)
Specific Conductance	mS/cm	0.088 (+/- 0.011)
Conductivity	uS/cm	62.70 (+/- 10.25)
Dissolved Oxygen %	%	98.06 (+/- 14.79)
Dissolved Oxygen Concentration	mg/L	11.12 (+/- 1.75)
Chlorophyll	ug/L	10.80 (+/- 8.27)
BGA Phycocyanin	Cells/mL	3385.80 (+/- 1298.36)
Fluoride	mg/L	<0.10
Total Coliform	MPN/100 mL	1762.5 (+/- 2363.7)
E. coli	MPN/100 mL	112.15 (+/- 155.78)
TOC	mg/L	7.11 (+/- 1.615)
UV_{254}	cm^{-1}	0.225 (+/- 0.048)
DOC	mg/L	6.04 (+/- 1.43)
Alkalinity	mg/L $CaCO_3$	23.10 (+/- 4.56)

[a]SD = standard deviation

The non-pathogenic test microbes used as bacterial and viral surrogates in this experiment were *E. coli* KO11 (ATCC# 55124) and Male-specific (F+) coliphage (bacteriophage) MS2 (ATCC# 15597-B1), respectively. *E. coli* KO11 was chosen because of its resistance to the antibiotic chloramphenicol, which was added to agar media to select for the bacterium of interest while excluding other interfering or background microorganisms. *E. coli* KO11 is also relatively easy to culture in the laboratory and is a similar size and morphology to common waterborne pathogens such as *Shigella* spp., *Campylobacter* spp., *Salmonella* spp., and *Vibrio* spp. These characteristics make it a representative model organism for the evaluation of bacterial pathogen reductions achieved by chitosan coagulation-flocculation-sedimentation and physical removal by sand column filters.

Bacteriophage MS2 was a suitable virus indicator organism because it shares many common characteristics with noroviruses, enteroviruses and hepatitis A and E viruses with based on shape, size, nucleic acid and outer protein capsid. MS2 is also simple to propagate and store

in the laboratory, not pathogenic, and can be enumerated easily for its infectivity using standardized culture-based assays. Previous research has also determined that MS2 is a conservative estimator of viral reductions achieved via filtration mechanisms as well as coagulation, flocculation, and sedimentation treatments (Kinoshita, Bales, Maguire, & Gerba, 1993; Powelson, Simpson, & Gerba, 1990; Schijven, De Bruin, Hassanizadeh, & De Roda Husman, 2003; Schijven, Hassanizadeh, & De Bruin, 2002).

All microbe stock suspensions were prepared to ensure at least a 6 $\log_{10}$ per 100 mL spiking concentrations in test water so that reductions of 99.9999% or 6 $\log_{10}$ could be measured. A 1 mL volume of frozen suspension of overnight $\log_{10}$ phase growth culture of *E. coli* KO11 was added to 200 mL tryptic soy broth (TSB) with 1% V/V chloramphenicol stock solution (100x stock concentration, 3.4 g/L chloramphenicol dissolved in ethanol, filtered through 0.22-µm-pore-size membrane filter) in a shaker flask. The culture was incubated at 37°C on a shaker table set to 100 rpm for 18-24 hours. The resulting culture was distributed into four 50 mL falcon tubes and centrifuged at 3000 rpm for 15 minutes at 4°C in a Sorvall refrigerated centrifuge with H6000a swing bucket rotor. Approximately 45 mL of the supernatant was decanted and disposed, then the equivalent volume of phosphate buffer (PB) was added (Standard Methods buffer, with 5 mL/L 0.4 M $MgCl_2$ concentrated stock solution) and vortexed. The suspension was centrifuged and washed three times with this buffer composition. The final suspension of *E. coli* was vortexed in PB (Standard Methods buffer, with 5 mL/L 0.4 M $MgCl_2$ concentrated stock solution) until the pellet was completely dispersed in solution. The resulting *E. coli* concentration of this suspension was approximately 10^6 CFU/mL. Each 2.5-liter batch of test water received 15 mL of this concentrated *E. coli* KO11 suspension per day. Washed *E. coli* KO11 cells were prepared each sample day, and leftover washed cells were used on non-

sampling days. A 1 mL volume of propagated and chloroform extracted MS2 bacteriophage stock at a titer of $1x10^{11}$ PFU/mL, stored in -80°C, was added to each 2.5 L batch of challenge water daily.

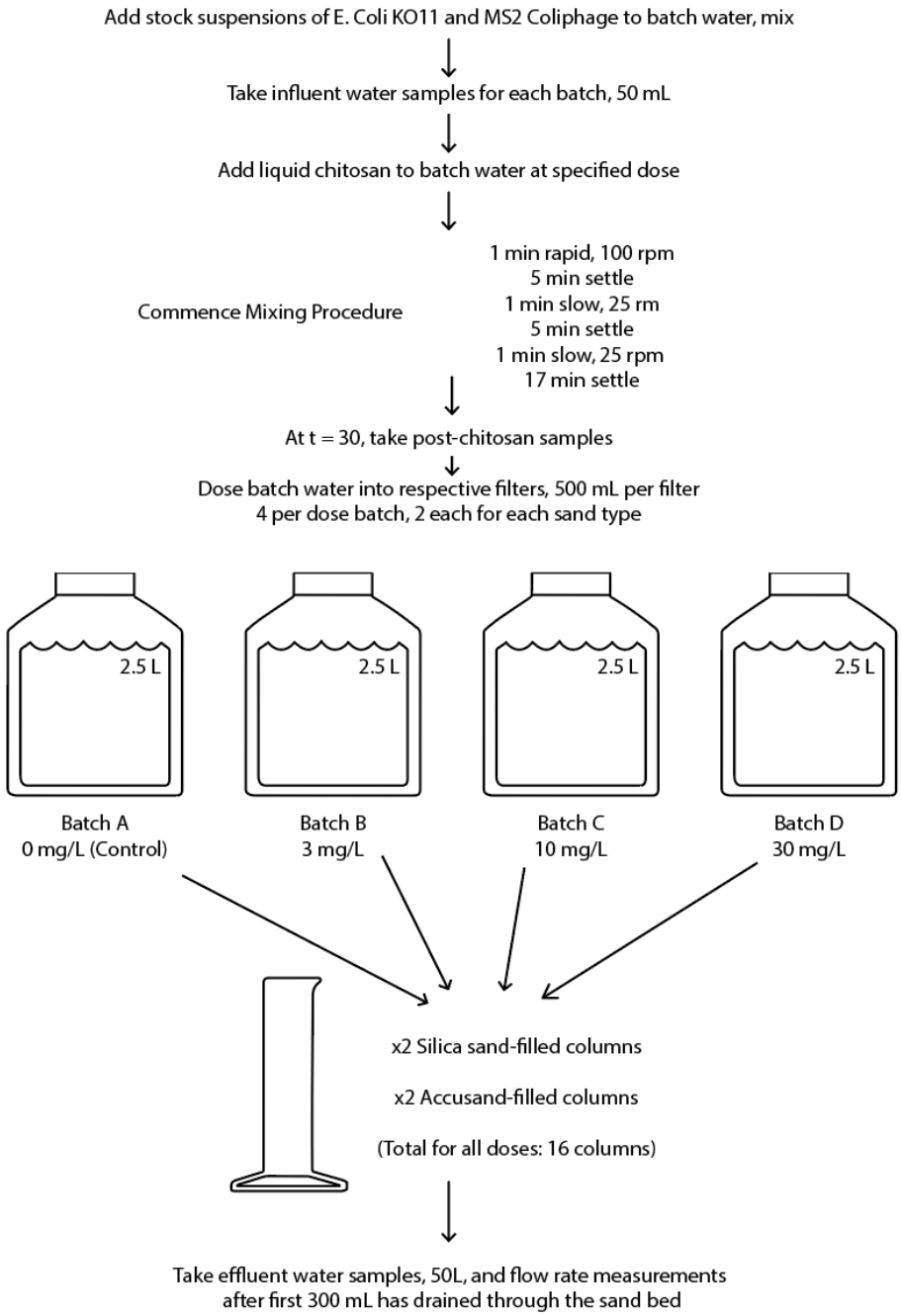


Figure 2. Diagram of experimental design for batch water preparation and dosing into filters

3.4 Chitosan Dosing and Mixing

Chitosan acetate (CH_3COO^-) was selected as the coagulant based on previous work that identified it in a systematic screening as one of the most effective chitosan coagulant types for bacteria and virus reduction (Soros, 2015; Soros et al., 2019) Food grade chitosan was purchased from *Sarchem Laboratories, Inc* in powder form. According to their certificate of analysis, the degree of deacetylation and pH of the food grade chitosan acetate are 90.3% and 4.2, respectively. The full certificate of analysis can be found in **Appendix 4**.

A 2 g/L solution of liquid chitosan was prepared using 2 g chitosan acetate and 1 liter of autoclaved lab grade deionized water. Each batch of test water received an appropriate volume of liquid chitosan solution to achieve the target dose. Three doses of chitosan were tested in the 57-day bench-scale study along with a control condition with no chitosan treatment: 0 mg/L, 3 mg/L, 10 mg/L and 30 mg/L. Duplicate sand column filters were run for each dose for each sand type. Doses were calculated using the dilution equation below:

Concentration (Stock) x Volume (Stock) = Concentration (Sample) x Volume (Sample)

After adding liquid chitosan to the test water, the solutions were rapidly mixed at approximately 100 rpm for 1 minute, then the water was left to settle for 5 minutes. The water was then slowly mixed for 1 minute at approximately 25-30 rpm, left to settle for 5 minutes, and then slow mixed for a final minute before settling for a final 17 minutes. Total coagulation-flocculation-settling time for all test waters was 30 minutes. At 30 minutes, post-chitosan samples were taken for analysis and 500 mL of the challenge water was dosed to each filter.

3.5 Measurement of Flow in Sand Filter Columns

Flow rates for all sand filters were measured twice per week, on sampling days, over the course of the evaluation period. The measure of flow through the filters followed a standard

procedure: the timer was started when the challenge water was dosed into the upper receptacle with a maximum head of 7cm. The time it took for 50 mL of water to flow through the sand filter was recorded. All outlet tubes were adjusted in location to achieve an approximately similar flow rate and flow rate measurement served to ensure these flow rates were comparable for each filter sand column type. Flow rates were not kept consistent between sand types.

3.6 Sampling of Sand Filter Columns

Samples from chitosan coagulation and sand columns filtration were processed twice per week over the 57-day study period, resulting in 17 total challenge experiments. Samples were taken from the pretreated and prefiltered spiked water (influent), the post coagulation treated unfiltered water (post-chitosan), and the post-treated and post sand column filtered water (effluent). These samples were taken for both the test (chitosan treated) and control filters. Dosing occurred in the morning, and on test days, sample processing and assays were performed on the same day. For effluent filtered samples, only 50 mL were taken for analysis. The 50 mL samples were retrieved after at least 250 mL of the daily charge volume had already passed through the filter. This ensured that the water sampled corresponded to the day it was dosed, not any idle water remaining in the filter from the previous day. After all samples were collected and assays were completed, samples were stored in labeled sterile containers at 4°C. Serial 10-fold dilutions for each sample were made with PB (Standard Method buffers, with 5 mL/L 0.4 M $MgCl_2$ concentrated stock solution).

3.7 Turbidity and pH

A Hach turbidimeter was used to measure the turbidity of influent, post-chitosan coagulated and sand filter effluent waters. A Denver Instrument, model 215, pH meter was used to measure the pH of influent, post-chitosan coagulated and sand filter effluent waters. All

samples were thoroughly vortexed before measurements were taken, and turbidity values were recorded for all samples after 1 and 2 minutes in the turbidimeter. Turbidity values for post-chitosan coagulated samples were taken again after a 3-hour settling period. If samples were not processed within 48 hours of the experiment, they were frozen at -20°C to prevent any regrowth in the samples.

3.8 Microbial Methods

Culture based methods were used to quantify microbial concentrations for the influent, post-chitosan coagulated and sand filter effluent samples. The spread plate method was used to quantify *E. coli* KO11 concentrations as colony forming units (CFU) per mL (Eaton, Clesceri, Greenberg, & Franson, 1998). Awesome agar, consisting of 40 g/L tryptic soy agar plus 30 mg/L neutral red and 10 g/L lactose, amended with 1% (V/V) chloramphenicol stock (100x stock concentration, 3.4 g/L chloramphenicol dissolved in ethanol, filtered through 0.22-µm-pore-size membrane filter), was used to limit background organism interference. The double agar layer (DAL) plaque assay method (EPA 1601) on TSA plates with 1% (V/V) streptomycin/ampicillin stock (100x stock concentration, 1.5 g/L ampicillin sodium salt and 1.5 g/L streptomycin sulfate dissolved in deionized water, filtered through 0.22-µm-pore-size membrane filter) and *E. coli* Famp bacteria host was used to quantify MS2 as plaque forming units (PFU)/mL (Environmental Protection Agency, 2001).

3.9 Data Management and Statistical Analysis

All data were recorded and maintained spreadsheets in excel and then further analyzed by statistical methods in RStudio. *E. coli* K011 and MS2 concentrations were calculated using two replicates per sample dilution. Influent to effluent $\log_{10}$ reduction values (LRVs) were calculated using the following equation:

Log reduction = $\log_{10}$(Influent water concentration) - $\log_{10}$(effluent water concentration)

Two separate statistical analyses were performed: a cumulative analysis of all data ignoring time (day of experiment) as a variable, and a binned analysis to assess how filter performance changed with time over the 57-day experiment period. The 17 total experiments over the 57-day study period were grouped into four time bins, as shown in Table 5.

Table 5. Time bin assignments for experiments

Bin	Experiment Numbers in Bins (Intervals) for n = 17 Total Experiment Days
1	1-5 (n = 5)
2	6-9 (n = 4)
3	10-13 (n = 4)
4	14-17 (n = 4)

The data for both analyses did not conform to a Gaussian distribution according to the Kolmogorov-Smirnov test. Non-parametric statistics were used to compare median $\log_{10}$ reductions achieved across chitosan doses and the two sand types. The Kruskal-Wallis test was used to compare $\log_{10}$ reductions achieved by all chitosan doses for the same sand type and to compare all intervals across all doses and stratified by dose. The Wilcoxon rank-sum test was used to compare reductions of bacteria and viruses between chitosan doses for the same sand type, between sand types with the same chitosan dose and between intervals across doses and stratified by dose. The unpaired p-values reported were adjusted for multiple comparisons using the Bonferroni correction. The Spearman Rank Correlation was used to evaluate correlations between microbial $\log_{10}$ reductions, turbidity reductions and sand column effluent filtration rates. This correlation method is well suited for nonparametric data because, rather than measuring a linear association between variables, it measures the monotonic relationship between the variables. An alpha level of 0.05 ($p < 0.05$) was used as the significance level for all statistical tests.

Although the data did not conform to a Gaussian distribution, bar and line graphs were created to visualize differences between filters in terms of average LRVs and changes in LRVs over time. Bar graphs of average LRVs with standard deviation error bars were created in Excel. Line graphs of the LRVs achieved for each filter over time were created in Excel. Box plots of the calculated $\log_{10}$ reductions for both the cumulative and binned analyses were computed in RStudio. The ends of the box represent the 25th and 75th percentiles and the whiskers represent the 5th and 95th percentiles of the data distribution. The horizontal line in the box represents the median value.

CHAPTER 4: RESULTS

4.1 Results and Descriptive Statistics for Microbial and Turbidity Reductions from Water by Chitosan Coagulation and Sand Column Filtration

The average *E. coli* KO11 LRVs for each column over the 57-day duration of operation are summarized in **Figure 3** for columns of both sand types. The average MS2 coliphage LRVs are summarized in **Figure 4**. The average turbidity LRVs for each column are summarized in **Figure 5**. The error bars represent the standard deviation of the average values. The raw LRV data for all filters on each sampling day is presented in **Appendix 4** (*E. coli* KO11), **Appendix 5** (MS2 coliphage) and **Appendix 6** (turbidity). The average LRVs by combined chitosan coagulation and sand filtration ranged from less than 0.5-$\log_{10}$ to greater than 4.5-$\log_{10}$ for *E. coli* KO11 in Accusand columns and from 0.5-$\log_{10}$ to nearly 4-$\log_{10}$ for silica columns (**Figure 3**). The average LRVs reported for Accusand columns were higher than those reported for silica sand columns for all chitosan doses except 3 mg/L, which were both around 1 LRV. Control filters not dosed with chitosan (dose = 0 mg/L) did not exceed a 0.5-$\log_{10}$ *E. coli* KO11 LRV for both sand types. Filters dosed with water coagulated with 10 mg/L chitosan achieved average LRVs of about 4.5-$\log_{10}$ and nearly 4-$\log_{10}$ for Accusand and silica sand columns, respectively. Although greater variability is observed between duplicate filters of each sand type, those dosed with water coagulated with 30 mg/L chitosan reached average LRVs exceeding 3.5-$\log_{10}$ for Accusand-filled columns and greater than 1.5-$\log_{10}$ for silica sand-filled columns.

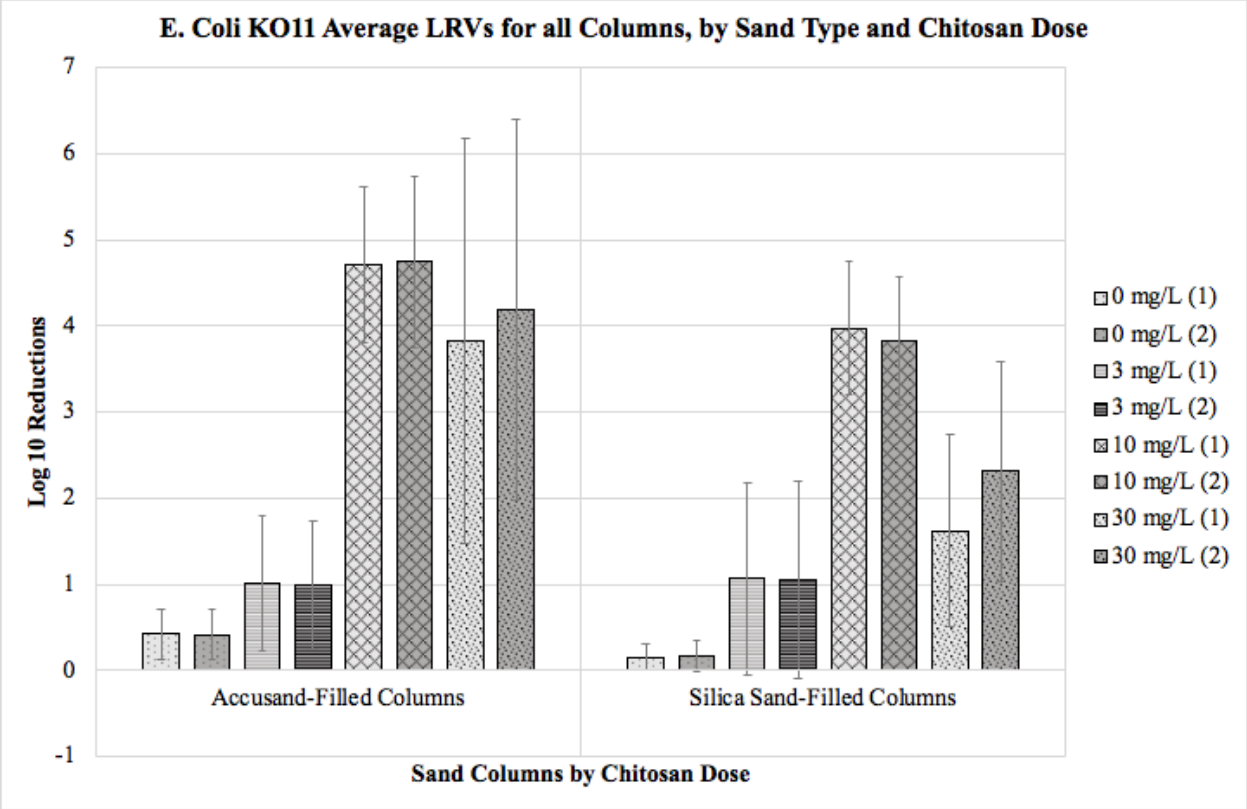


Figure 3. Average Log_{10} Reduction Values with standard deviation error bars for *E. coli* KO11in water treated by chitosan coagulation and Accusand and silica sand column filtration, based on 17 successive samples collected throughout the 57-day experiment period.

The average LRVs by combined chitosan coagulation and sand filtration ranged from less than 0.5-log_{10} to nearly 4.5-log_{10} for MS2 coliphage in the Accusand-filled columns and ranged from less than 0.5-log_{10} to nearly 4-log_{10} for the silica sand-filled columns (**Figure 4**). As was observed with *E. coli* KO11 LRVs, the MS2 coliphage LRVs were generally higher for Accusand columns compared to silica sand columns across doses; however, the control filters for both sand types achieved similar MS2 LRVs. Control filters dosed with water receiving no chitosan (dose = 0 mg/L) did not exceed 0.5 LRV for MS2 coliphage for either sand type. Filters dosed with water coagulated with 3 mg/L chitosan achieved on average 1.5-log_{10} and 1.0-log_{10} reductions for MS2 coliphage in Accusand and silica sand columns, respectively. Filters dosed with water coagulated with 10 mg/L chitosan achieved LRVs for MS2 coliphage of ~4.5-log_{10}

for Accusand columns and greater than 3.5-$\log_{10}$ for silica columns. Filters dosed with water treated with 30 mg/L chitosan achieved average MS2 coliphage LRVs of ~4-$\log_{10}$ for Accusand columns and ~2.5-$\log_{10}$ for silica columns.

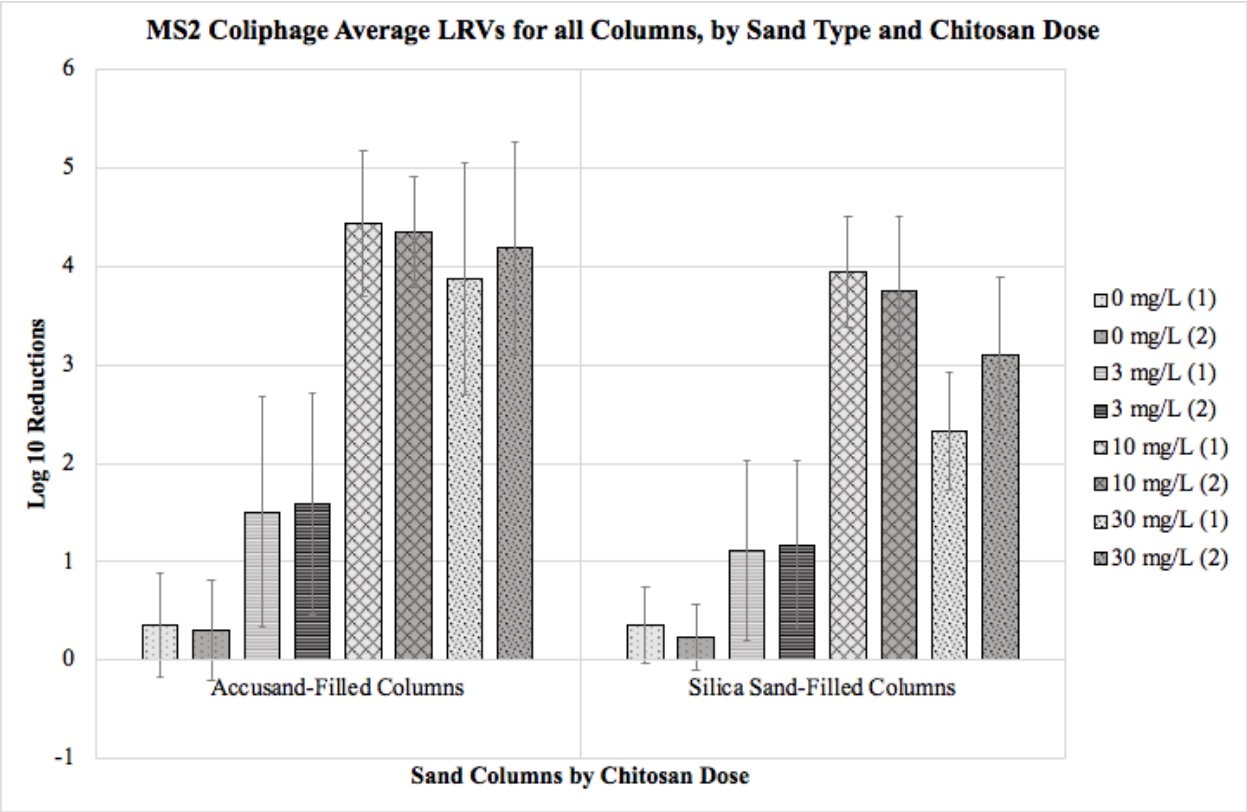


Figure 4. Average Log_{10} Reduction Values with standard deviation error bars for MS2 coliphage in water treated by chitosan coagulation and Accusand and silica sand column filtration, based on 17 successive samples collected throughout the 57-day experiment period.

The turbidity reductions observed for Accusand columns were typically slightly greater than those observed for silica sand columns across doses (**Figure 5**). The turbidity reductions for both sand types ranged from less than 0.2-$\log_{10}$ to about 1-$\log_{10}$. Control filters receiving no chitosan dose (chitosan dose = 0 mg/L) achieved 0.2 LRVs or less in turbidity for both sand types. With a 3 mg/L chitosan dose followed by sand filtration LRVs were about 0.6-$\log_{10}$ for Accusand filters and about 0.5-$\log_{10}$ for silica sand filters. At a 10 mg/L chitosan dose followed by sand filtration LRVs were higher, reaching 1.0-$\log_{10}$ for Accusand filters and about 0.9-$\log_{10}$

for silica sand filters. However, at the highest dose of chitosan tested, 30 mg/l, followed by sand filtration turbidity reductions were lower than at 10 mg/L chitosan dose and similar to the 3 mg/L chitosan dose, with LRVs of about 0.7-$\log_{10}$ for Accusand and around 0.6-$\log_{10}$ for silica sand.

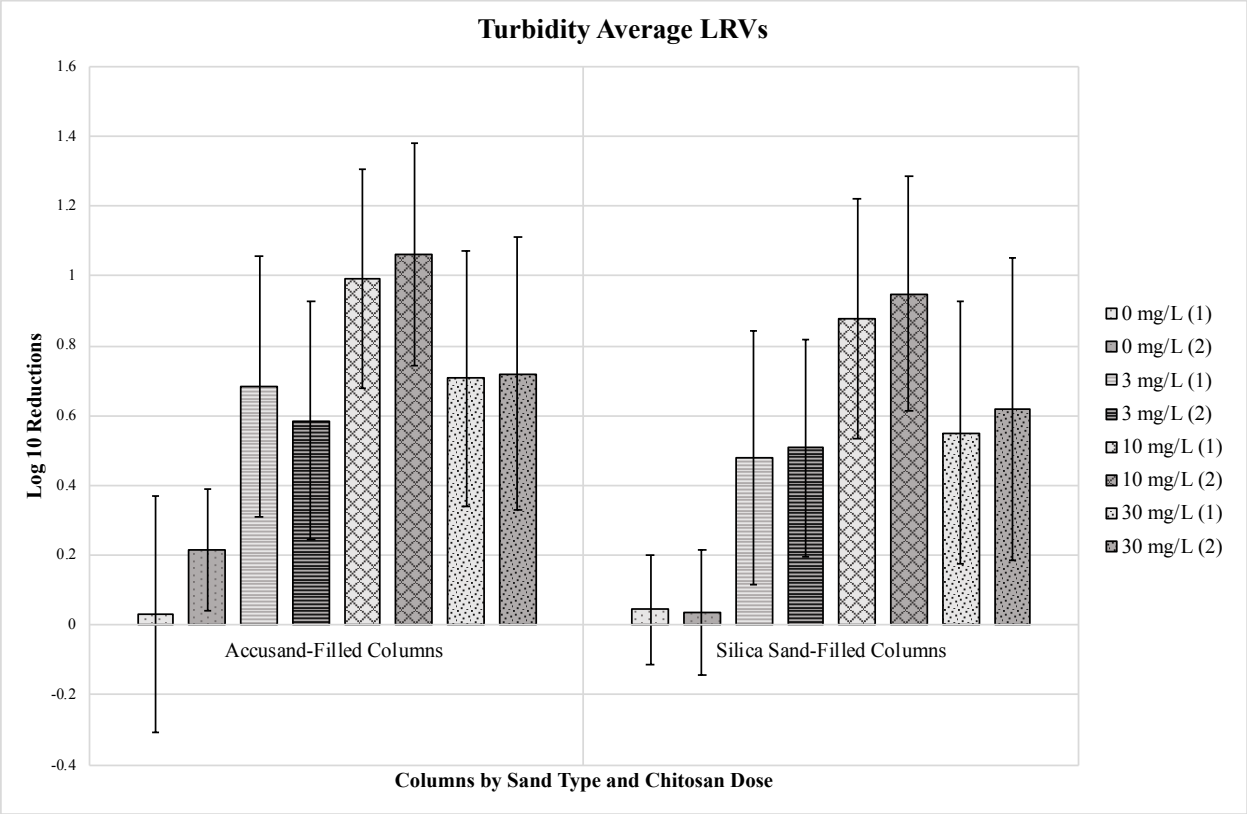


Figure 5. Average Log_{10} Reduction Values with standard deviation error bars for turbidity in water treated by chitosan coagulation and Accusand and silica sand column filtration, based on 17 successive samples collected throughout the 57-day experiment period.

Duplicate filters of same sand type and chitosan dose behaved somewhat differently in observed LRVs over the 57-day experimental period, but the average LRVs achieved between the duplicates were similar and the changes over time were typically correlated. The average differences in LRV values between the duplicate filters are reported in **Table 6** along with the range of differences observed over the 57-day study period. For most conditions, duplicate filters had on average less than 0.5-$\log_{10}$ differences between duplicates for *E. coli* KO11 and MS2 coliphage and less than 0.2-$\log_{10}$ for turbidity. Columns of both sand types dosed with

water coagulated with 30 mg/L chitosan had higher average differences between duplicate filters for *E. coli* KO11 and MS2 coliphage. *E. coli* KO11 LRVs for water coagulated with 30 mg/L chitosan reported maximum differences between duplicate filters exceeding 3-$\log_{10}$ for both Accusand and silica sand columns. MS2 coliphage LRVs for 30 mg/L chitosan followed by silica sand filtration reported an average of about 0.8-$\log_{10}$ difference between the duplicates, with a maximum difference of 1.6-$\log_{10}$. These results suggest that duplicate columns of the same sand type and dose tend to achieve similar LRVs for bacteria, viruses and turbidity. Though substantial differences are observed, especially with filters dosed with 30 mg/L chitosan-coagulated water, the similarities in performance are strong enough to combine the duplicate filter LRVs in subsequent analyses.

Table 6. The average, minimum and maximum differences in LRVs between duplicate filters for each sand type and chitosan dose over the 57-day filter operating time

		E. coli KO11			MS2 Coliphage			Turbidity		
Sand type	Dose (mg/L)	Average	Min.	Max.	Average	Min.	Max.	Average	Min.	Max.
Accusand	0	0.105	0.000	0.356	0.136	0.002	0.363	0.194	0.003	1.024
Accusand	3	0.087	0.012	0.247	0.194	0.024	0.632	0.135	0.029	0.472
Accusand	10	0.340	0.000	1.041	0.328	0.041	0.829	0.152	0.035	0.550
Accusand	30	0.608	0.000	3.395	0.370	0.008	1.322	0.162	0.002	0.449
Silica	0	0.094	0.000	0.321	0.249	0.000	0.698	0.087	0.006	0.304
Silica	3	0.120	0.010	0.293	0.204	0.007	0.509	0.135	0.029	0.472
Silica	10	0.201	0.002	0.662	0.366	0.024	1.031	0.152	0.035	0.550
Silica	30	1.146	0.127	3.046	0.804	0.000	1.600	0.162	0.002	0.449

While **Figure 3**, **Figure 4** and **Figure 5** are useful to visualize and compare average LRVs achieved among the different chitosan doses and sand filter columns, box and whisker plots better present the LRV distributions and their extent of variability observed among these different treatment conditions. In subsequent sections, line graphs are presented that give LRV results achieved over time for each duplicate condition of chitosan dose and filter sand type and

allow visualization of differences in performance between these duplicates. Nonparametric analyses of LRVs are based on average results for duplicate conditions of chitosan dose and sand filter reductions, so only one distribution is presented for each set of conditions for chitosan dose and sand filter type.

4.2 Reductions for Bacterial indicator *E. coli* KO11 by Sand Filter Columns Dosed with Chitosan Coagulated Water

The *E. coli* KO11 LRVs for each individual column over the 57-day study period are displayed in **Figure 6** (Accusand-filled columns) and **Figure 7** (silica sand-filled columns). Variability was observed in LRVs achieved over the 57-day study period at each time point across doses and between duplicate columns of the same sand type and chitosan dose. With the exception of the filters dosed with water coagulated with 30 mg/L chitosan, duplicate filters of same sand type and dose achieved similar LRVs over the 57-day experiment period.

LRVs for control Accusand filter columns (chitosan dose = 0 mg/L) remained below 1-$\log_{10}$ over the course of the experiment. LRVs were initially low for Accusand-filled columns receiving water dosed with 3 and 30 mg/L, hovering around 1-$\log_{10}$. By the latter part of the 57-day experiment period, the LRVs were higher at about 5 to 6-$\log_{10}$ for the columns receiving water dosed with 30 mg/L chitosan and also lower at about 2.5-$\log_{10}$ for the columns receiving water dosed with 3 mg/L chitosan. Substantial variability was observed between days 15 and 27 for the duplicate Accusand columns receiving water dosed with 30 mg/L. Columns receiving waters dosed with 10 mg/L chitosan achieved high LRVs, around 5-$\log_{10}$, at the beginning and end of the study period. There was a substantial decrease in LRVs of about 2 $\log_{10}$ observed between day 27 and nearly day 40 for water dosed with 10 mg/L chitosan. This decline correlates with the fall turnover event in University Lake that occurred over the end of November, early December.

The duplicate silica sand-filled columns dosed with 30 mg/L chitosan-treated water did not give a consistent and monotonic pattern of LRVs and displayed considerable variability in LRVs the middle weeks of the experiment period. This variability occurred around the fall turnover event for University Lake, though the pattern of LRVs over this period is so inconsistent it is unclear how the changing lake water quality parameters factored into filter performance at this dose. However, towards the end of the 57-day dosing period these columns began to achieve similar LRVs of about 2.5 to 3 LRV or more. It is noteworthy LRVs were initially highest at about 4-$\log_{10}$ for the samples dosed with 10 mg/L chitosan and remained generally high throughout the experiment period. LRVs were initially low for silica sand columns dosed with water coagulated with both 3 and 30 mg/L chitosan and LRVs increased over the 57-day course of the experiment. LRV increases by sand columns receiving water with these two chitosan doses occurred more rapidly for the 30 mg/L dose than the 3 mg/L dose and all achieved LRVs of about 3-$\log_{10}$ towards the end of the 57-day experiment period

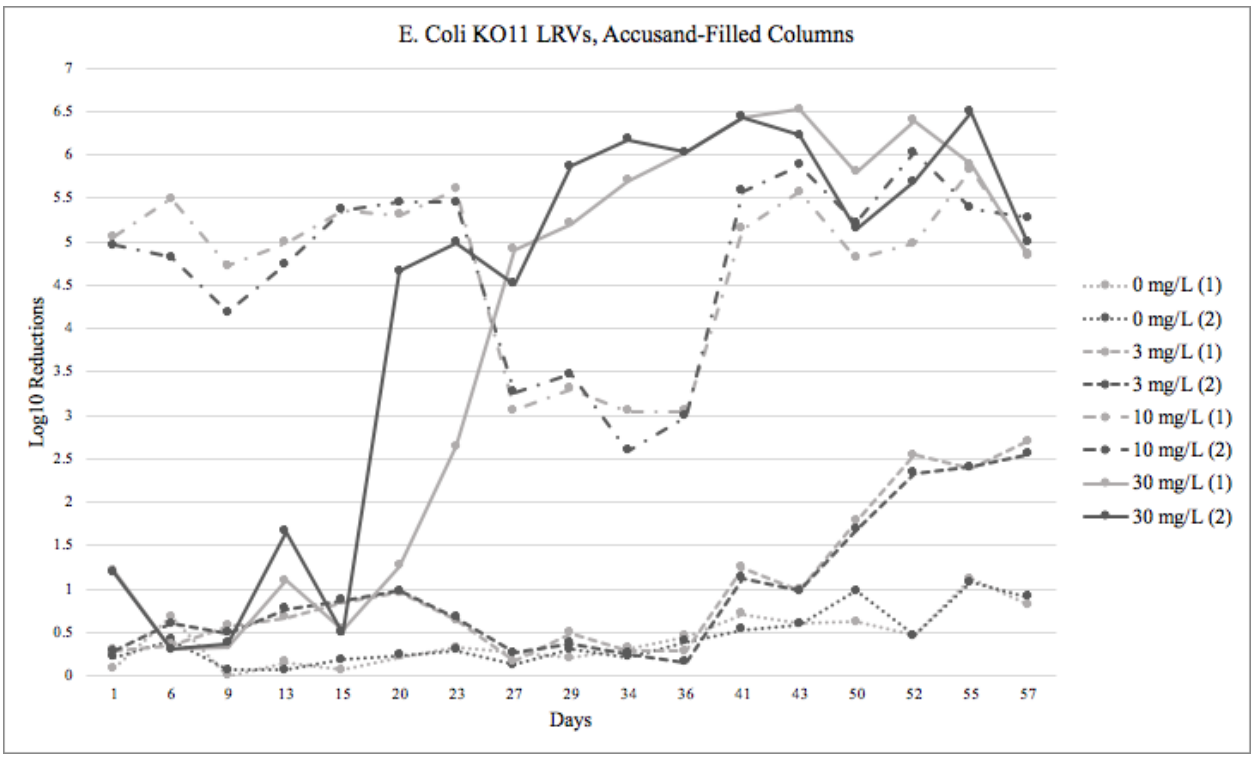


Figure 6. *E. coli* KO11 reductions for Accusand-filled columns for samples collected throughout the 57-day study period, plotted over time (N=17).

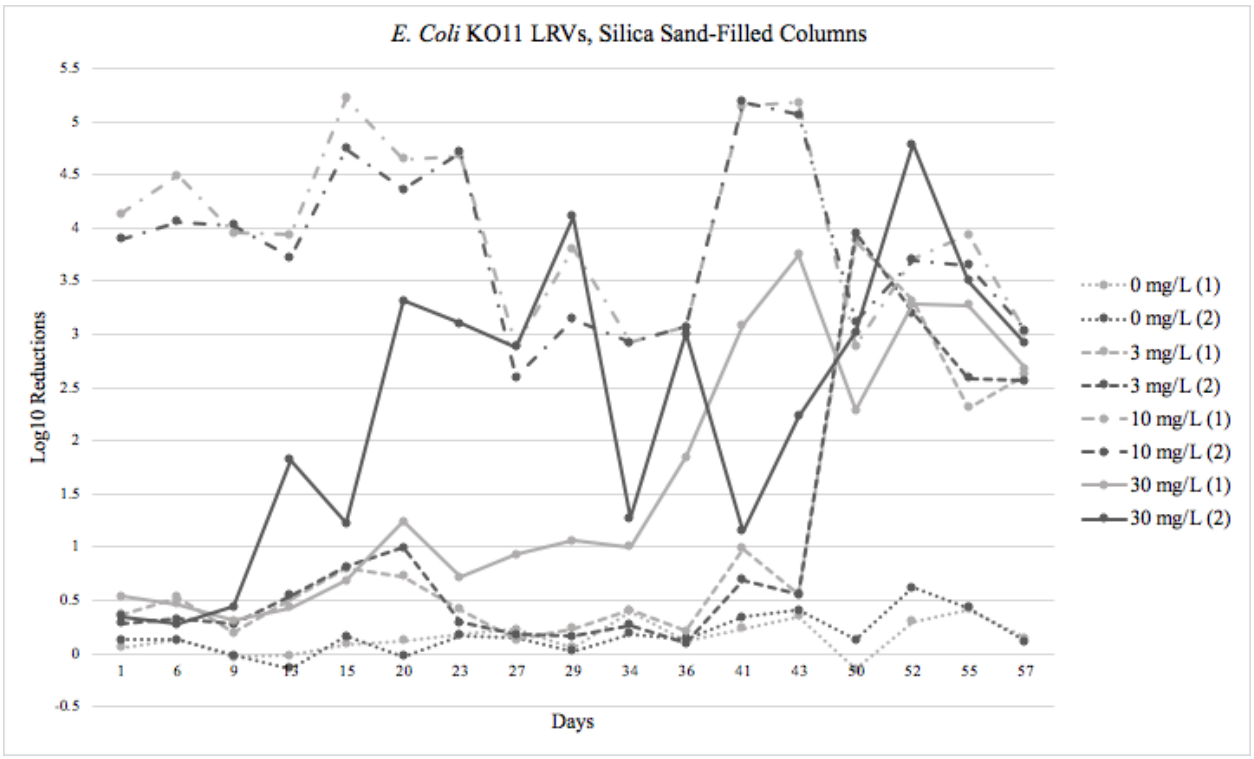


Figure 7. *E. coli* KO11 reductions for silica sand-filled columns for samples collected throughout the 57-day study period, plotted over time (N=17).

Duplicate filters for each dose and sand type generally behaved similarly in LRVs over the experiment period, therefore LRVs computed from the duplicate values were compiled for nonparametric analyses. The *E. coli* KO11 reductions for each chitosan dose and between each sand type over the 57-day study period are summarized in the box and whisker plot shown in **Figure 8**. The LRVs ranged widely among the chitosan doses from less than 0.5-$\log_{10}$ to greater than 6-$\log_{10}$. As with the line graphs in **Figure 6** and **Figure 7**, significant variability was observed within each experimental condition of chitosan dose and sand type. Filters receiving 30 mg/L chitosan-treated water displayed a particularly large range of LRVs for both silica- and Accusand filters, as shown by the large boxes and whiskers.

Examination of **Figure 8** shows differences in LRVs between the four different chitosan dose conditions within the same sand type. For the Accusand columns, substantial differences in LRVs were observed between filters receiving 0 mg/L and 10 mg/L chitosan-treated water, 0 and 30 mg/L chitosan-treated water, 3 and 10 mg/L chitosan-treated water, and 3 and 30 mg/L chitosan-treated water. For the silica sand-filled columns, substantial differences in LRVs were observed among chitosan doses, with the biggest LRV differences and the highest LRV value for filters receiving water with the 10 mg/L chitosan dose compared to all other doses. The medians and ranges of LRVs for each chitosan dose between the silica and Accusand filter columns gave similar patterns of performance, but the Accusand columns consistently achieved higher LRVs than the silica sand columns across all chitosan doses. Nonparametric statistical tests were performed to assess if these differences are statistically significant.

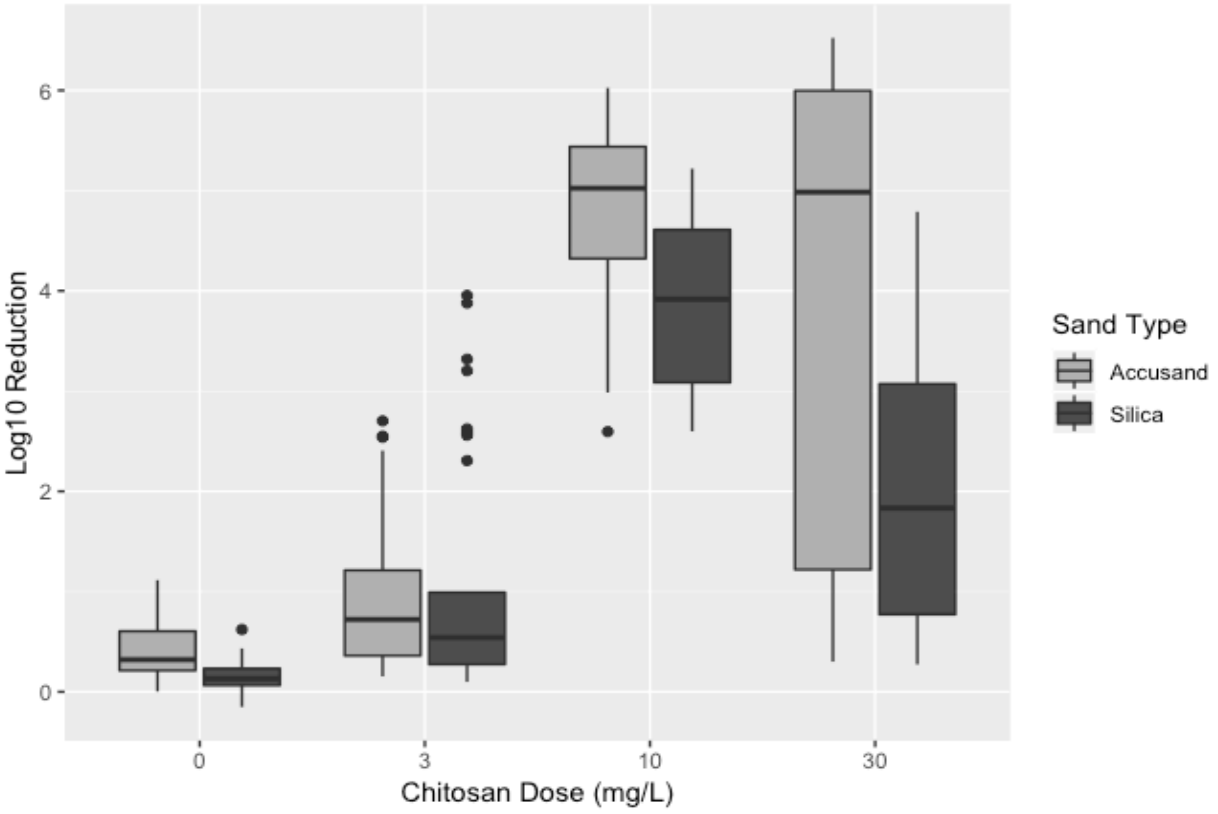


Figure 8. *E. coli* KO11 LRVs among chitosan doses of 0, 3, 10 and 30 mg/L presented cumulatively over the 57-day experiment period for Accusand filter columns and silica sand columns.

The Kruskal-Wallis test was used to assess differences in overall median LRVs of *E. coli* KO11 for each sand type based on chitosan dose and resulting p-values of this statistical analysis are presented in **Table 7**. There were statistically significant differences in bacteria LRVs based on chitosan dose for both Accusand and silica sand columns.

Table 7. Kruskal-Wallis analysis results comparing cumulative median LRVs of *E. coli* KO11 by chitosan dose, stratified by sand type. Reported p-values were adjusted using the Bonferroni correction, m = 2

Sand Type	Comparison	p-value[a]
Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**< 4.40E-16**
silica	Dose (0, 3, 10, 30 mg/L chitosan)	**< 4.40E-16**

[a]Bolded p-values are statistically significant at $p < 0.05$

To evaluate how specific chitosan doses performed relative to one another within the same sand type, overall median LRVs of *E. coli* KO11 by chitosan dose were compared with the Wilcoxon Rank-Sum test. The resulting p-values are presented in **Table 8**. LRVs achieved by all doses of chitosan for both sand types were statistically significantly different than those achieved by the controls (0 mg/L chitosan-treated water). LRVs achieved by columns dosed with 3 mg/L chitosan-treated water were statistically significantly lower than those achieved by 10 mg/L and 30 mg/L chitosan-treated for both sand types. There was not a significant difference in achieved LRVs between 10 mg/L and 30 mg/L chitosan-treated water for the Accusand columns, but the LRVs were significantly different for the silica sand columns.

Table 8. Results of the Wilcoxon Rank-Sum analysis comparing overall median LRVs of *E. coli* KO11 by chitosan dose, stratified by sand type. Reported p-values were adjusted using the Bonferroni correction, m = 6

Sand Type	Dose 1 (mg/L)	Dose 2 (mg/L)	p-value[a]
Accusand	0	3	**3.04E-03***
	0	10	**8.44E-12***
	0	30	**1.45E-08***
	3	10	**9.22E-12***
	3	30	**1.36E-05***
	10	30	1.00*
silica	0	3	**1.23E-06***
	0	10	**8.44E-12***
	0	30	**8.03E-11***
	3	10	**2.37E-12**
	3	30	**3.37E-03**
	10	30	**6.36E-08**

[a]Bolded p-values are statistically significant at $p < 0.05$
*p-values estimated with ties

The Wilcoxon Rank-Sum test was also used to compare overall median LRVs of *E. coli* KO11 between columns receiving water with the same chitosan dose but different sand types. The results are presented in **Table 9**. Statistically significant differences in achieved LRVs were observed between sand types for columns dosed with 0, 10 and 30 mg/L chitosan-treated water.

LRVs achieved by Accusand columns dosed with 3 mg/L chitosan-treated water were not significantly different than those achieved by silica sand columns at the same dose.

Table 9. Results of the Wilcoxon Rank-Sum analysis comparing cumulative median LRVs of *E. coli* KO11 by sand type, stratified by chitosan dose. Reported p-values were adjusted using the Bonferroni correction, m = 4

Dose (mg/L)	Sand Type 1	Sand Type 2	p-value[a]
0	Accusand	silica	**5.06E-04***
3	Accusand	silica	1.00
10	Accusand	silica	**7.88E-04***
30	Accusand	silica	**2.91E-03***

[a]Bolded p-values are statistically significant at $p < 0.05$
*p-values estimated with ties

The influence of the length of filter operation time, which possibly accounts for such phenomena as filter maturation and increased biological activity in the filters, on *E. coli* KO11 LRVs is shown in **Figure 9** and **Figure 10**. In these box and whisker plots, the experiments were binned into 4 time groups of filter operation to observe if there are differences between LRVs achieved at the beginning, early middle, late middle and end of the 57-day experiment period. Both Figure 4.7 and 4.8 present the same data, but Figure 4.7 shows the LRVs for each bin side-by-side for each chitosan dose and separated by sand type. This data presentation helps observe differences between LRVs over time for columns of the same sand type and chitosan dose. Figure 4.8 presents the binned *E. coli* KO11 LRV data paired by sand type and chitosan dose, separated by time bins. This data representation is useful for comparing LRVs of the same chitosan dose and in the same time bin between the two sand types.

LRVs for *E. coli* KO11 increased substantially from time interval 1 to time interval 4 for 0, 3 and 30 mg/L columns. The control columns for both sand types, dosed with untreated challenge water, displayed an approximately linear pattern of increasing LRVs over time. Both silica- and Accusand-filled columns dosed with 3 mg/L chitosan-treated water had consistent

LRV values of approximately 0.5-$\log_{10}$ but displayed a substantial improvement to over 2-$\log_{10}$ in the 4th time quadrant of the study period. Accusand-filled columns dosed with 30 mg/L chitosan-treated water experienced significant stepwise improvement between intervals 1, 2 and 3, then declined slightly in the fourth time interval. For the silica-filled columns of the same dose, substantial LRV improvement was observed from interval 1 to 2, specifically 0.5-$\log_{10}$ to 2-$\log_{10}$, but only gradual LRV increases were observed in subsequent intervals. Both silica and Accusand-filled columns dosed with 10 mg/L chitosan-treated water did not experience substantial increases/declines in achieved LRVs across intervals, reporting LRVs of around 4- to 5-$\log_{10}$ for all intervals. One exception to this pattern was the 4th interval for silica sand-filled columns, which experienced a slight LRV decline to approximately 3.5-$\log_{10}$.

Accusand-filled columns consistently achieved similar or higher LRVs across doses and time intervals compared to silica sand-filled columns. Only one exception to this trend is observed for 3 mg/L chitosan-treated water.

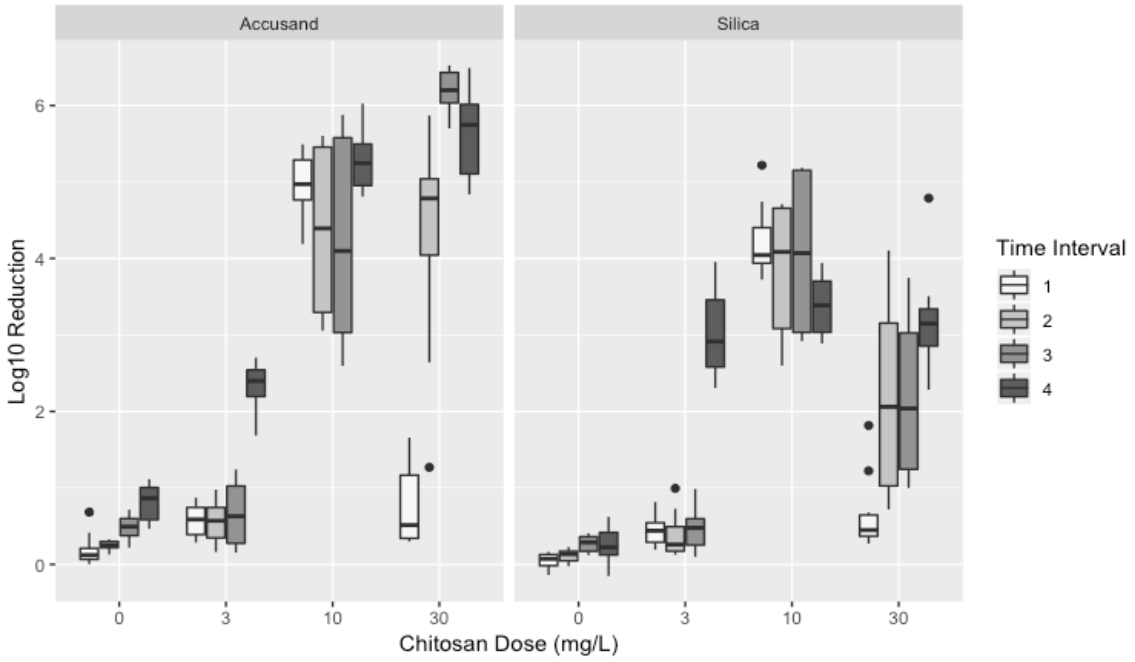


Figure 9. Time binned *E. coli* KO11 LRVs across chitosan doses of 0, 3, 10 and 30 mg/L presented in time intervals over the 57-day experiment period for Accusand filter columns and silica sand columns.

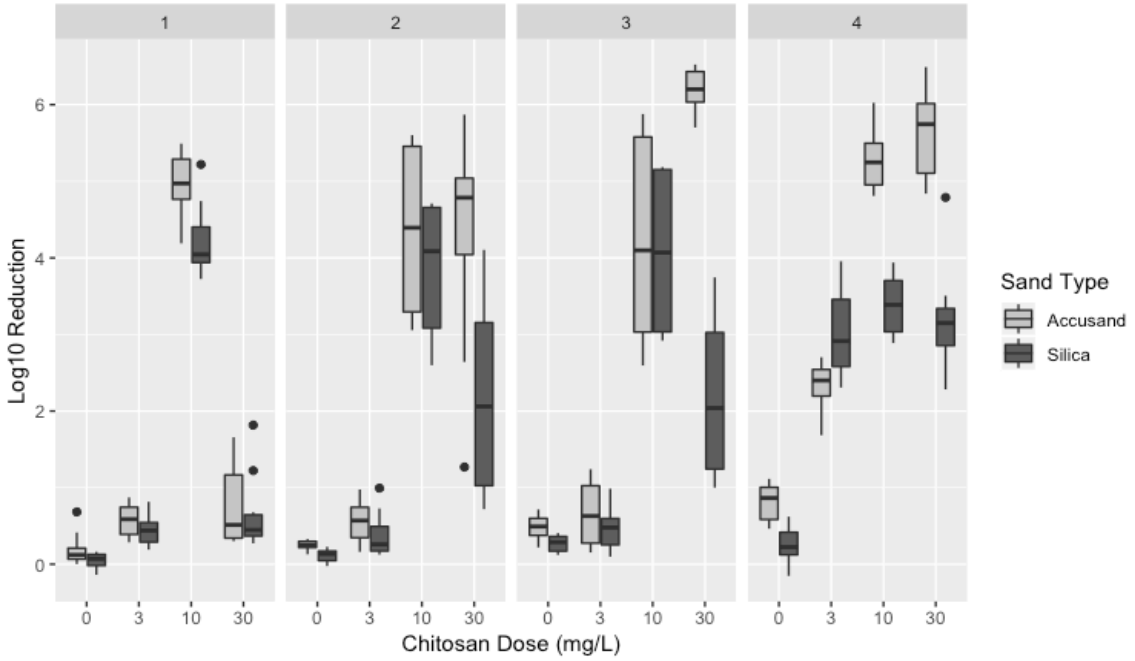


Figure 10. Alternative representation of the time binned *E. coli* KO11 LRVs across chitosan doses of 0, 3, 10 and 30 mg/L presented in time intervals over the 57-day experiment period for Accusand filter columns and silica sand columns.

The Kruskal-Wallis test was used to assess if the differences between binned median LRVs of *E. Coli* KO11 were statistically significant when stratified by time, across all doses within the same sand type. The resulting p-values are presented in **Table 10**. Significant differences in LRVs were observed across intervals for Accusand columns but not for silica columns.

Table 10. Results of the Kruskal-Wallis analysis comparing median LRVs of *E. coli* KO11 by time interval, stratified by chitosan dose and sand type. Reported p-values were adjusted using the Bonferroni correction, m = 2

Sand Type	Dose (mg/L)	Comparison	p-value[a]
Accusand	All doses (0, 3, 10, 30)	Time Interval (1-4)	**6.57E-04**
Silica	All doses (0, 3, 10, 30)	Time Interval (1-4)	0.1474

[a]Bolded p-values are statistically significant at $p < 0.05$

In order to evaluate how Accusand columns, across all doses, performed over time, binned median LRVs of *E. coli* KO11 were compared based on time interval with the Wilcoxon Rank-Sum test for multiple comparisons. This analysis was not run for the silica sand-filled columns because the differences between intervals were not statistically significant (Kruskal-Wallis $p > 0.05$). The resulting p-values are reported in **Table 11**. The results suggest there are significant differences in achieved LRVs for Accusand columns across all doses only between time intervals 1 and 4 ($p < 0.05$).

Table 11. Results of the Wilcoxon Rank-Sum analysis comparing median LRVs of *E. coli* KO11 by time interval, stratified by sand type. Reported p-values were adjusted using the Bonferroni correction, m = 6

Sand Type	Dose (mg/L)	Interval 1	Interval 2	p-value[a]
Accusand	All doses (0, 3, 10, 30)	1	2	1.00*
Accusand	All doses (0, 3, 10, 30)	1	3	0.0616*
Accusand	All doses (0, 3, 10, 30)	1	4	**8.75E-05***
Accusand	All doses (0, 3, 10, 30)	2	3	1.00*
Accusand	All doses (0, 3, 10, 30)	2	4	0.0736*
Accusand	All doses (0, 3, 10, 30)	3	4	1.00*

[a]Bolded p-values are statistically significant at $p < 0.05$
*p-values estimated with ties

The same Kruskal-Wallis analysis conducted in **Table 10** was run again, but for each dose. This serves to evaluate differences in time binned median LRVs based on interval but accounting for different doses and sand types. The adjusted p-values are presented in **Table 12**. These results illustrate that the differences observed when comparing LRVs by interval for all chitosan doses (**Table 10**) are attributable to specific doses. For Accusand-filled columns, 0, 3 and 30 mg/L doses were all significantly different across intervals. For silica sand-filled columns, significant differences between intervals are only observed for chitosan doses of 0 and 30 mg/L.

Table 12. Results of the Kruskal-Wallis analysis comparing median LRVs of *E. coli* KO11 by time interval, stratified by chitosan dose and sand type. Reported p-values were adjusted using the Bonferroni correction, m = 4

Sand Type	Dose (mg/L)	Comparison	p-value
Accusand	0	Time Interval (1-4)	**3.61E-04**
Accusand	3	Time Interval (1-4)	**1.84E-03**
Accusand	10	Time Interval (1-4)	1.00
Accusand	30	Time Interval (1-4)	**2.40E-05**
silica	0	Time Interval (1-4)	**0.0414**
silica	3	Time Interval (1-4)	**1.44E-03**
silica	10	Time Interval (1-4)	0.326
silica	30	Time Interval (1-4)	**1.10E-03**

[a]Bolded p-values are statistically significant at $p < 0.05$

In order to evaluate how columns of the same sand type and chitosan dose performed over time, time binned median LRVs of *E. coli* KO11 were compared based on time interval with the Wilcoxon Rank-Sum test. The resulting p-values are presented in **Table 13**. These tests were only run for doses that were statistically significantly different between time intervals (Kruskal Wallis, $p < 0.05$).

Accusand columns dosed with untreated water experienced statistically significant improvements in LRVs from time interval 1 to 4, but the stepwise improvements between intervals were not always statistically significant. At 3 mg/L, Accusand columns experienced a statistically significant improvement in LRV performance between time interval 3 and 4, but prior to that point had consistent low LRVs of around 0.5-$\log_{10}$. Statistically significant differences were observed between intervals 1 to 2 and 2 to 3 for Accusand columns dosed with 30 mg/L chitosan-treated water, but the declines in LRVs between intervals 3 and 4 were not statistically significant.

For silica sand columns dosed with untreated water, no statistically significant differences in LRV trends over time was observed except between intervals 2 and 3. Silica sand filters receiving water dosed with 3 mg/L chitosan had statistically significantly improved LRVs at

time interval 4 but achieved similar LRVs in the first three intervals. Silica columns dosed with 30 mg/L had statistically significant increases in LRVs from interval 1 to 2 but did not significantly improve over the last three intervals.

Table 13. Results of the Wilcoxon Rank-Sum analysis comparing time binned median LRVs of *E. coli* KO11 for the same chitosan dose and sand type, between time intervals. Reported p-values were adjusted using the Bonferroni correction, m = 6

Dose (mg/L)	Sand Type	Interval 1	Interval 2	p-value[a]
0	Accusand	1	2	0.499
0	Accusand	1	3	**0.0373**
0	Accusand	1	4	**7.05E-03***
0	Accusand	2	3	**0.0420**
0	Accusand	2	4	**5.59E-03***
0	Accusand	3	4	0.108*
3	Accusand	1	2	1.00
3	Accusand	1	3	1.00
3	Accusand	1	4	**2.74E-04**
3	Accusand	2	3	1.00
3	Accusand	2	4	**9.32E-04**
3	Accusand	3	4	**9.32E-04**
30	Accusand	1	2	**5.48E-04**
30	Accusand	1	3	**2.66E-03***
30	Accusand	1	4	**2.74E-04**
30	Accusand	2	3	**8.02E-03***
30	Accusand	2	4	0.124
30	Accusand	3	4	0.394*
0	silica	1	2	1.00*
0	silica	1	3	**0.0130***
0	silica	1	4	0.410*
0	silica	2	3	0.0886
0	silica	2	4	1.41
0	silica	3	4	5.75
3	silica	1	2	1.00
3	silica	1	3	1.00
3	silica	1	4	**2.74E-04**
3	silica	2	3	1.00
3	silica	2	4	**9.32E-04**

3	silica	3	4	**9.32E-04**
30	silica	1	2	**0.0123**
30	silica	1	3	**5.21E-03**
30	silica	1	4	**2.74E-04**
30	silica	2	3	1.00
30	silica	2	4	1.00
30	silica	3	4	0.390

[a]Bolded p-values are statistically significant at $p < 0.05$
*p-values estimated with ties

The Kruskal-Wallis test was used to assess differences in time binned median LRVs of *E. coli* KO11 for each sand type based on dose of chitosan within a single time interval. The resulting p-values are presented in **Table 14**. There are statistically significant differences in LRVs achieved by columns of both sand types and across all intervals based on dose.

Table 14. Results of the Kruskal-Wallis analysis comparing time binned median LRVs of *E. coli* KO11 by chitosan dose, stratified by sand type. Reported p-values were adjusted using the Bonferroni correction, m = 2

Bin	Sand Type	Comparison	p-value[a]
1	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**4.37E-06**
1	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**6.28E-07**
2	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**3.80E-05**
2	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**2.30E-05**
3	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**1.87E-05**
3	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**2.80E-05**
4	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**1.52E-05**
4	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**8.04E-04**

[a]Bolded p-values are statistically significant at $p < 0.05$

To compare how different doses performed within the same time interval, time binned median LRVs of *E. coli* KO11 for each sand type were compared based on dose with the Wilcoxon Rank-Sum test. The resulting p-values are presented in **Table 15**. In interval 1, LRVs achieved by all chitosan doses for both sand types were statistically significantly different than those achieved by the controls (0 mg/L chitosan-treated water). LRVs achieved by Accusand and silica sand columns dosed with 10 mg/L chitosan-treated water were statistically

significantly higher than those dosed with 3 mg/L and 30 mg/L in interval 1. Differences in median LRVs achieved by columns dosed with 3 mg/L and 30 mg/L chitosan-treated water were not statistically significant in interval 1.

In interval 2, LRVs achieved by columns dosed with 10 mg/L and 30 mg/L chitosan-treated water were significantly different than those dosed with untreated and 3 mg/L. This was observed for both sand types. For both Accusand and silica sand columns, the LRVs reported for columns dosed with untreated water were not significantly different than those dosed with 3 mg/L chitosan-treated water. Differences in LRVs between 10 mg/L and 30 mg/L were also not statistically significant. These patterns of differences were the same in interval 3, with one exception. LRVs achieved by Accusand columns dosed with 10 mg/L and 30 mg/L were statistically significant in the 3rd interval.

In interval 4, statistically significant differences in achieved LRVs were observed between Accusand columns dosed with 3, 10 and 30 mg/L chitosan-treated water compared to the columns dosed with uncoagulated water. LRVs for Accusand columns receiving 3 mg/L chitosan-treated water were statistically significantly lower than for 10 mg/L and 30 mg/L chitosan-treated water. There was not a significant difference in performance in interval 4 between columns dosed with 10 mg/L or 30 mg/L chitosan-treated water. silica sand columns dosed with 3, 10 and 30 mg/L all had statistically significantly higher LRVs compared to control filters without chitosan coagulation, but no statistically significant differences in LRVs were observed between these chitosan doses.

Table 15. Results of the Wilcoxon Rank-Sum analysis comparing time binned median LRVs of *E. coli* KO11 by chitosan dose, stratified by sand type. Reported p-values were adjusted using the Bonferroni correction, m = 6

Bin	Sand Type	Dose 1 (mg/L)	Dose 2 (mg/L)	p-value
1	Accusand	0	3	**9.03E-03**

1	Accusand	0	10	**1.09E-03***
1	Accusand	0	30	**9.03E-03**
1	Accusand	3	10	**1.09E-03***
1	Accusand	3	30	1.00
1	Accusand	10	30	**1.09E-03***
1	silica	0	3	**1.09E-03***
1	silica	0	10	**1.09E-03***
1	silica	0	30	**1.09E-03***
1	silica	3	10	**6.50E-05**
1	silica	3	30	1.00
1	silica	10	30	**6.50E-05**
2	Accusand	0	3	0.169
2	Accusand	0	10	**9.32E-04**
2	Accusand	0	30	**9.32E-04**
2	Accusand	3	10	**9.32E-04**
2	Accusand	3	30	**9.32E-04**
2	Accusand	10	30	1.00
2	silica	0	3	0.0625
2	silica	0	10	**9.32E-04**
2	silica	0	30	**9.32E-04**
2	silica	3	10	**9.32E-04**
2	silica	3	30	**6.53E-03**
2	silica	10	30	0.124
3	Accusand	0	3	1.00
3	Accusand	0	10	**9.32E-04**
3	Accusand	0	30	**5.54E-03***
3	Accusand	3	10	**9.32E-04**
3	Accusand	3	30	**5.54E-03***
3	Accusand	10	30	**8.02E-03***
3	silica	0	3	0.963
3	silica	0	10	**9.32E-04**
3	silica	0	30	**9.32E-04**
3	silica	3	10	**9.32E-04**
3	silica	3	30	**9.32E-04**
3	silica	10	30	0.124
4	Accusand	0	3	**5.59E-03***
4	Accusand	0	10	**5.59E-03***

4	Accusand	0	30	**5.59E-03***
4	Accusand	3	10	**9.32E-04**
4	Accusand	3	30	**9.32E-04**
4	Accusand	10	30	1.00
4	silica	0	3	**9.32E-04**
4	silica	0	10	**9.32E-04**
4	silica	0	30	**9.32E-04**
4	silica	3	10	1.00
4	silica	3	30	1.00
4	silica	10	30	1.00

[a]Bolded p-values are statistically significant at $p < 0.05$
*p-values estimated with ties

Time binned median LRVs of *E. coli* KO11 for each dose were compared based on sand type with the Wilcoxon Rank-Sum test in order to evaluate differences in performance between Accusand and silica sand columns within the same time interval. The resulting p-values are presented in **Table 16**. The LRVs between the two sand types when dosed with untreated water were statistically significantly different in time intervals 2, 3 and 4, but not in interval 1. Accusand and silica sand columns dosed with 3 mg/L chitosan-treated water did not have significant differences in median LRVs across all time intervals. Performance between the two sand types at 10 mg/L chitosan-treated water was significantly different in time intervals 1 and 4. At 30 mg/L, no statistically significant difference performance was observed in interval 1 between the two sand types, but Accusand columns performed significantly better than silica sand columns in intervals 2, 3 and 4.

Table 16. Results of the Wilcoxon Rank-Sum Analysis, comparing time binned median LRVs of *E. coli* KO11 by sand type, stratified by chitosan dose. Reported p-values were adjusted using the Bonferroni correction, m = 4

Bin	Dose (mg/L)	Sand Type 1	Sand Type 2	p-value[a]
1	0	Accusand	silica	0.302*
1	3	Accusand	silica	0.870
1	10	Accusand	silica	**0.0113***
1	30	Accusand	silica	1.00
2	0	Accusand	silica	**0.0186**
2	3	Accusand	silica	1.00
2	10	Accusand	silica	1.00
2	30	Accusand	silica	**0.0416**
3	0	Accusand	silica	0.0827
3	3	Accusand	silica	1.00
3	10	Accusand	silica	1.00*
3	30	Accusand	silica	**3.69E-03***
4	0	Accusand	silica	**7.73E-03***
4	3	Accusand	silica	0.0590
4	10	Accusand	silica	**6.22E-04**
4	30	Accusand	silica	**6.22E-04**

[a]Bolded p-values are statistically significant at $p < 0.05$
*p-values estimated with ties

4.3 Reductions for Viral Indicator MS2 Coliphage by Sand Filter Columns Dosed with Chitosan Coagulated Water

The MS2 Coliphage LRVs for each individual sand filter column over the 57-day experiment period are displayed in **Figure 11** for Accusand columns and **Figure 12** for silica columns. Variability was observed in LRVs achieved over the 57-day experiment period at each time point among chitosan doses and between duplicate filter columns of the same sand type and chitosan dose. Duplicate filters of same sand type and dose behaved similarly over the study period; however, the duplicate silica sand columns dosed with 30 mg/L chitosan-treated water had greater observable variability in LRVs throughout the study than observed for other chitosan doses. As was observed with the *E. coli* KO11 reductions, columns receiving water dosed with

10 mg/L chitosan experienced a decline in LRVs during the middle of the study period, which corresponds to when the fall turnover event in University lake occurred. This decline is also observed for the 3 mg/L chitosan dose with both sand types and for 30 mg/L chitosan in silica sand columns.

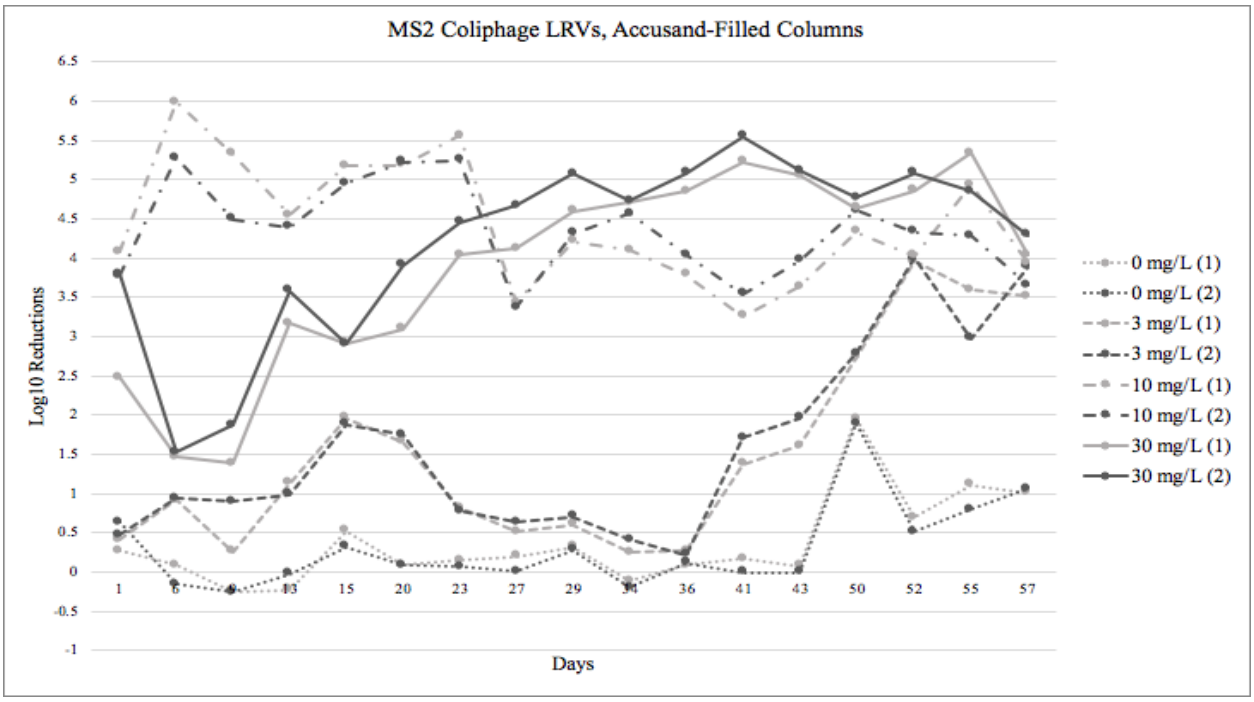


Figure 11. MS2 coliphage reductions for Accusand-filled columns dosed with water coagulated with different chitosan doses for 17 successive samples collected over time throughout the 57-day experiment period.

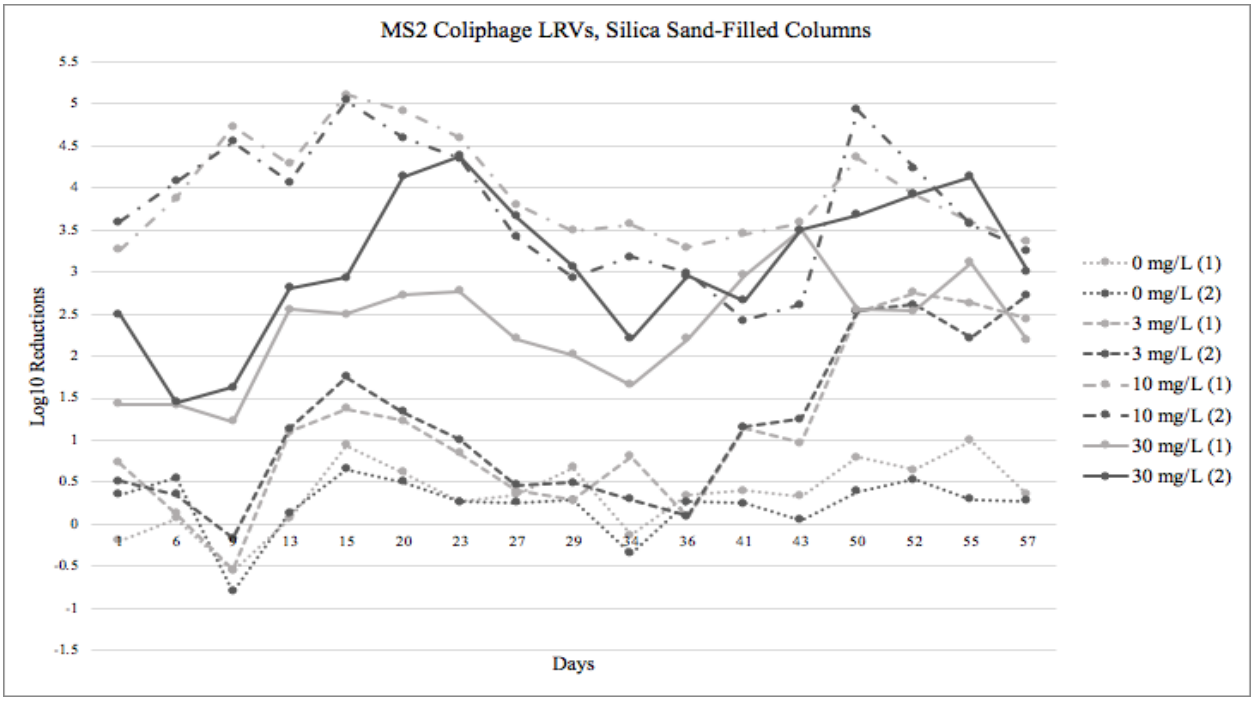


Figure 12. MS2 coliphage reductions for silica sand columns dosed with water coagulated with different chitosan doses for 17 successive samples collected over time throughout the 57-day experiment period.

Because the duplicate sand filters for each chitosan dose and sand type performed similarly in LRV over the study period, the LRVs for the duplicates were compiled for nonparametric analyses. The MS2 coliphage reductions for each chitosan dose and between each sand type over the 57-day experiment period are summarized in the box and whisker plot shown in **Figure 13**. The LRVs range widely among chitosan doses from less than 0.5-$\log_{10}$ to greater than 4-$\log_{10}$. As with the line graphs of LRVs in Figures 4.9 and 4.10, significant variability in LRVs is observed for each condition of chitosan dose and sand type, among these doses and between sand types.

From examination of **Figure 13**, LRV differences are observed among the 4 different chitosan dose conditions within the same sand type. For the Accusand columns, considerable LRV differences were observed between filters receiving 0 mg/L and 10 mg/L chitosan-treated water, 0 and 30 mg/L chitosan-treated water, 3 and 10 mg/L chitosan-treated water, and 3 and 30

mg/L chitosan-treated water. Similar differences in LRVs were observed for the silica sand columns between those same chitosan dose pairs as well as between 10 mg/L and 30 mg/L chitosan doses. As with *E. coli* KO11, MS2 coliphage LRVs for Accusand columns consistently performed similarly or better than the silica sand columns across all chitosan doses. Nonparametric statistical tests were performed to assess if these observed LRV differences were statistically significant.

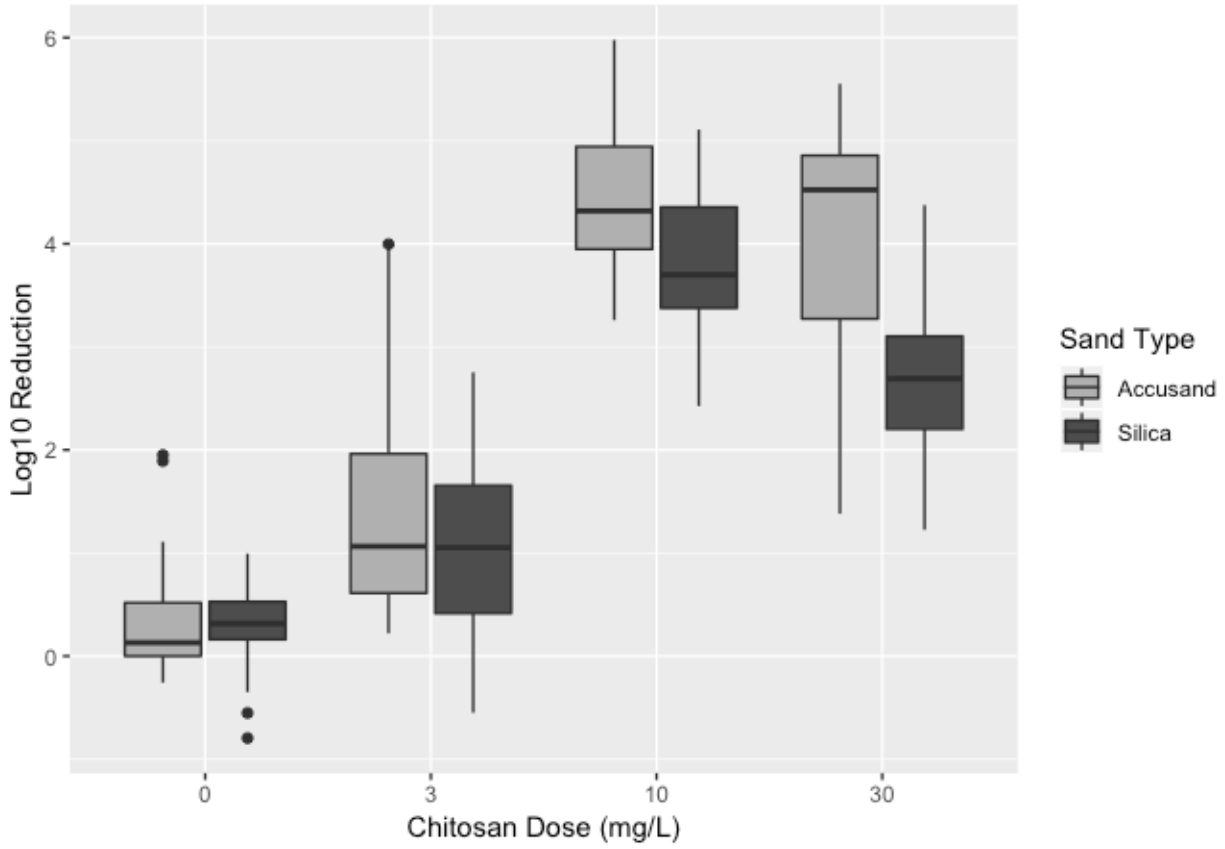


Figure 13. MS2 coliphage LRVs among chitosan doses of 0, 3, 10 and 30 mg/L presented cumulatively over the 57-day experiment period for Accusand filter columns and silica sand columns.

The Kruskal-Wallis test was used to assess differences in cumulative median LRVs of MS2 coliphage for each sand type based on chitosan dose. The resulting p-values are presented

in **Table 17**. There are statistically significant differences in virus LRVs based on dose of chitosan for both Accusand and silica sand filter columns.

Table 17. Kruskal-Wallis analysis results comparing cumulative median LRVs of MS2 coliphage by chitosan dose, stratified by sand type. Reported p-values were adjusted using the Bonferroni correction, m = 2

Sand Type	Comparison	p-value[a]
Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**< 4.40E-16**
silica	Dose (0, 3, 10, 30 mg/L chitosan)	**< 4.40E-16**

[a]Bolded p-values are statistically significant at $p < 0.05$

The Wilcoxon Rank-Sum test was used to compare overall median LRVs of MS2 coliphage based on chitosan dose in order to evaluate how specific doses of chitosan performed relative to one another within the same sand type. The resulting p-values are presented in **Table 18**. LRVs reported for filter columns of both sand types dosed with 3, 10 and 30 mg/L of chitosan were statistically significantly different than those reported for columns dosed with untreated water. Both 10 mg/L and 30 mg/L chitosan doses statistically significantly outperformed the 3 mg/L dose for both sand types. Statistically significant differences in LRVs were observed between silica sand columns receiving 10 mg/L and 30 mg/L chitosan-treated water, but this difference was not significant in Accusand columns.

Table 18. Wilcoxon Rank-Sum analysis results comparing cumulative median LRVs of MS2 coliphage by chitosan dose pairs, stratified by sand type. Reported p-values were adjusted using the Bonferroni correction, m = 6

Sand Type	Dose 1 (mg/L)	Dose 2 (mg/L)	p-value[a]
Accusand	0	3	**3.25E-07**
	0	10	**1.32E-15**
	0	30	**1.32E-15**
	3	10	**2.79E-14**
	3	30	**3.35E-10**
	10	30	1.00
silica	0	3	**1.68E-04***
	0	10	**8.44E-12***
	0	30	**8.44E-12***
	3	10	**1.32E-15**
	3	30	**1.97E-07***
	10	30	**7.37E-06***

[a]Bolded p-values are statistically significant at $p < 0.05$
*p-values estimated with ties

To evaluate differences in LRVs between the two different sand filter column types receiving the same chitosan dose, a Wilcoxon Rank-Sum Test was used to compare median LRVs between sand filter column types for each of the chitosan doses. The results are presented in **Table 19**. There were no significant differences between the LRVs achieved by two different sand column types dosed with untreated water and 3 mg/L chitosan-treated water. LRVs attained with Accusand filter columns were statistically significantly higher for 10 mg/L and 30 mg/L doses of chitosan-treated water than those attained with silica columns.

Table 19. Results of the Wilcoxon Rank-Sum analysis comparing cumulative median LRVs of MS2 coliphage by sand type, stratified by chitosan dose. Reported p-values were adjusted using the Bonferroni correction, m = 4

Dose (mg/L)	Sand Type 1	Sand Type 2	p-value[a]
0	Accusand	silica	1.00*
3	Accusand	silica	0.934
10	Accusand	silica	**0.0138**
30	Accusand	silica	**3.12E-05***

[a]Bolded p-values are statistically significant at $p < 0.05$
*p-values estimated with ties

The influence of duration of time of the experiment, which may account for maturation, increased biological activity and floc accumulation in the filters, on MS2 coliphage LRVs is shown in **Figure 14** and **Figure 15**. These box and whisker plots were created in the same way as were those for the *E. coli* KO11 time binned analysis. **Figure 14** and **Figure 15** present the same data, but **Figure 14** shows the MS2 coliphage LRVs for each time bin side-by-side for each dose, separated by sand type and **Figure 15** presents the time binned LRV data paired by sand filter type and chitosan dose, separated by time bins.

LRVs for MS2 coliphage increased substantially from time interval 1 to time interval 4 for 0, 3 and 30 mg/L columns. The control columns for both sand types, dosed with challenge water not treated with chitosan, achieved their maximum LRVs for MS2 coliphage in the 4th interval. LRVs in intervals 1-3 did not conform to a specific pattern. Both silica- and Accusand columns dosed with 3 mg/L chitosan-treated water had consistent LRV values of approximately 0.5- to 1-$\log_{10}$ initially but displayed a substantial improvement to over 2-$\log_{10}$ and 3-$\log_{10}$ reduction in the 4th time quadrant of the study period, respectively. Accusand-filled columns dosed with 30 mg/L chitosan-treated water gave significant stepwise improvement in LRV between interval 1, 2 and 3, and then declined slightly in the fourth time interval. For the silica-filled columns of the same dose, significant improvement was observed from time interval 1 to 2,

specifically 2-$\log_{10}$ to 3-$\log_{10}$, but was around 3-$\log_{10}$ for subsequent time intervals. Both silica and Accusand-filled columns dosed with 10 mg/L chitosan-treated water gave LRVs of 4 or more in time intervals 1 and 2, then experienced declines to about 3 LRV in time interval 3, but again improved to about 4-LRV in time interval 4.

Accusand filter columns generally achieved similar or higher LRVs across chitosan doses and time intervals compared to silica sand filter columns.

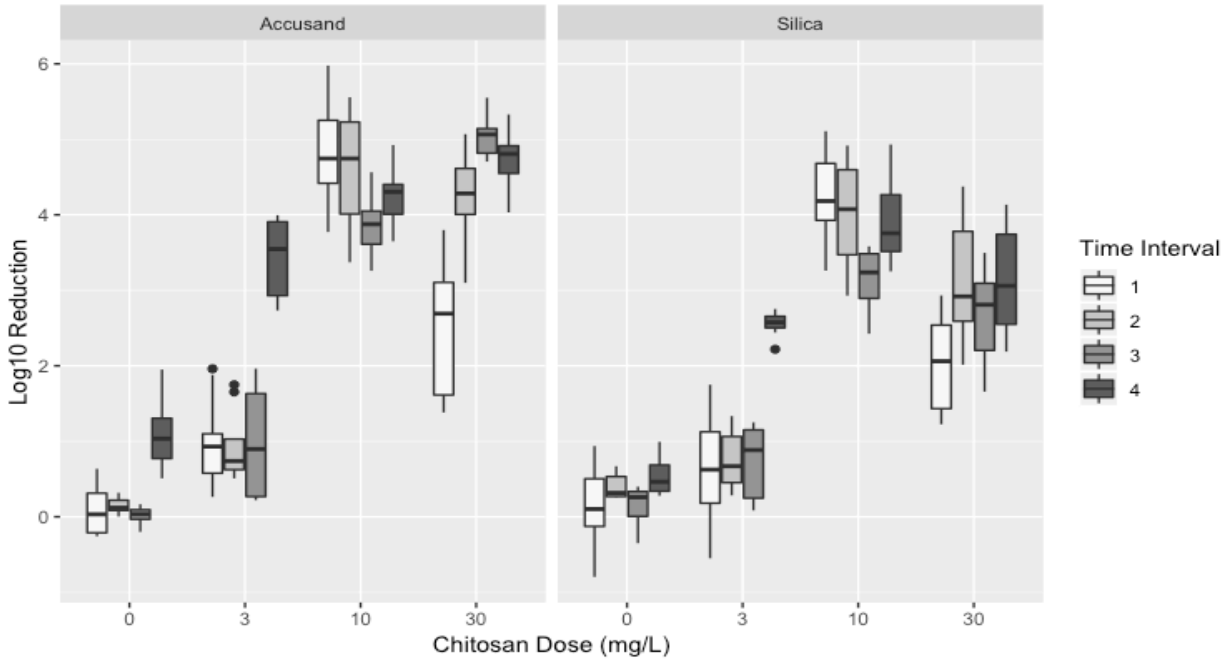


Figure 14. Time binned MS2 coliphage LRVs across chitosan doses of 0, 3, 10 and 30 mg/L presented in time intervals over the 57-day experiment period for Accusand filter columns and silica sand columns.

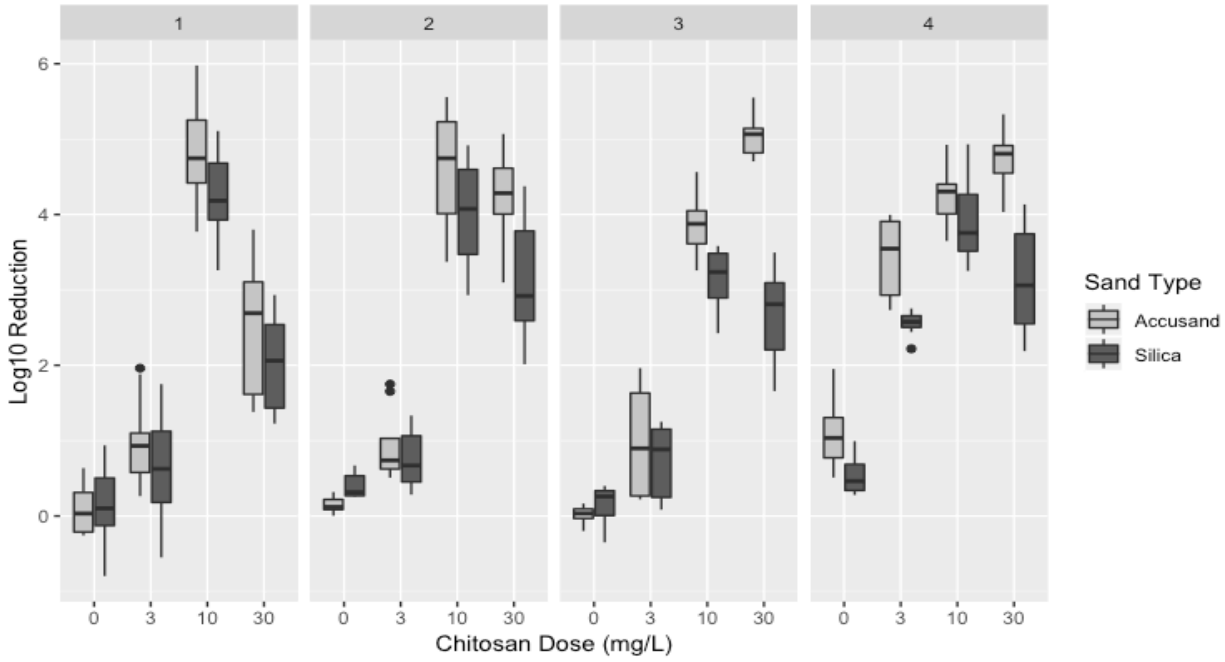


Figure 15. Alternative presentation of time binned MS2 coliphage LRVs across chitosan doses of 0, 3, 10 and 30 mg/L presented in time intervals over the 57-day experiment period for Accusand filter columns and silica sand columns.

The Kruskal-Wallis test was used to assess if the differences between time binned median MS2 coliphage LRVs were statistically significant when stratified by time, across all doses within the same sand type. The resulting p-values are presented in **Table 20**. Significant differences in LRVs were not observed based on interval, across all doses and stratified by sand type. No comparisons were run for specific intervals based on this data.

Table 20. Results of the Kruskal-Wallis analysis comparing median LRVs of MS2 coliphage by time interval, stratified by chitosan dose and sand type. Reported p-values were adjusted using the Bonferroni correction, m = 2

Sand Type	Dose (mg/L)	Comparison	p-value[a]
Accusand	All doses (0, 3, 10, 30)	Time Interval (1-4)	0.114
Silica	All doses (0, 3, 10, 30)	Time Interval (1-4)	0.197

[a]Bolded p-values are statistically significant at $p < 0.05$

The same Kruskal-Wallis analysis conducted in **Table 20** was run again, but for each chitosan dose rather than the cumulative doses considered together. The adjusted p-values are presented in **Table 21**. These results suggest that the despite the previous K-W results (Table 4.14), there are significant differences between intervals for specific doses. For Accusand-filled columns, 0, 3 and 30 mg/L doses were all significantly different across intervals. For silica sand-filled columns, significant differences between intervals are only observed for chitosan doses of 3 and 10 mg/L.

Table 21. Results of the Kruskal-Wallis analysis comparing median LRVs of MS2 coliphage by time interval, stratified by chitosan dose and sand type. Reported p-values were adjusted using the Bonferroni correction, m = 4

Sand Type	Dose (mg/L)	Comparison	p-value[a]
Accusand	0	Time Interval (1-4)	**1.35E-03**
Accusand	3	Time Interval (1-4)	**1.77E-03**
Accusand	10	Time Interval (1-4)	0.120
Accusand	30	Time Interval (1-4)	**7.17E-05**
silica	0	Time Interval (1-4)	0.203
silica	3	Time Interval (1-4)	**1.84E-03**
silica	10	Time Interval (1-4)	**0.0276**
silica	30	Time Interval (1-4)	0.0773

[a]Bolded p-values are statistically significant at $p < 0.05$

In order to evaluate how each column of the same sand type for each separate chitosan dose performed, median LRVs of MS2 coliphage were compared for each pair of time intervals with the Wilcoxon Rank-Sum test. These pairwise comparisons were only run for those doses that were statistically significant in **Table 21**. The resulting p-values are presented in **Table 22**. Accusand columns dosed with untreated water gave statistically significant improvements in LRVs between intervals 3 and 4, but differences in LRV in intervals 1 and 2 were not statistically significant. The same pattern of LRV significance results, significance for time intervals 3 and 4 but not significant for time intervals 1 and 2 also occurred for Accusand column filters dosed with 3 mg/L chitosan-treated water. Statistically significant improvements LRV in

performance were observed for Accusand columns dosed with 30 mg/L chitosan between intervals 1 and 2 and between intervals 2 and 3. The apparent decline in performance from intervals 3 to 4 was not significant.

As was observed with Accusand columns, the silica columns dosed with 3 mg/L chitosan-treated water gave significant increases in LRVs for time intervals 3 to 4 but were not significantly different for time intervals 1 and 2. Columns dosed with 10 mg/L chitosan-treated water had a statistically significant decline in performance between paired intervals 1 and 3 but not between paired intervals 2 and 4.

Table 22. Wilcoxon Rank-Sum analysis results comparing pairs of time binned median LRVs of MS2 coliphage for the same chitosan dose and sand filter column type. Reported p-values were adjusted using the Bonferroni correction, m = 6

Dose (mg/L)	Sand Type	Interval 1	Interval 2	p-value[a]
0	Accusand	1	2	1.00
0	Accusand	1	3	1.00
0	Accusand	1	4	**1.10E-03**
0	Accusand	2	3	0.299
0	Accusand	2	4	**9.32E-04**
0	Accusand	3	4	**9.32E-04**
3	Accusand	1	2	1.00
3	Accusand	1	3	1.00
3	Accusand	1	4	**2.74E-04**
3	Accusand	2	3	1.00
3	Accusand	2	4	**9.32E-04**
3	Accusand	3	4	**9.32E-04**
30	Accusand	1	2	**1.92E-03**
30	Accusand	1	3	**2.74E-04**
30	Accusand	1	4	**2.74E-04**
30	Accusand	2	3	**0.0112**
30	Accusand	2	4	0.498
30	Accusand	3	4	0.963
3	silica	1	2	1.00
3	silica	1	3	1.00
3	silica	1	4	**2.74E-04**

3	silica	2	3	1.00
3	silica	2	4	**9.32E-04**
3	silica	3	4	**9.32E-04**
10	silica	1	2	1.00
10	silica	1	3	**3.29E-03**
10	silica	1	4	1.00
10	silica	2	3	0.169
10	silica	2	4	1.00
10	silica	3	4	0.0625

[a]Bolded p-values are statistically significant at $p < 0.05$
*p-values estimated with ties

To evaluate differences in time binned median LRVs of MS2 coliphage for each sand type based on chitosan dose, Kruskal-Wallis tests were conducted. The resulting p-values are presented in **Table 23**. There are statistically significant differences in LRVs attained by columns of both sand types and across all time intervals based on chitosan dose.

Table 23. Kruskal-Wallis analysis results comparing time binned median LRVs of MS2 coliphage by chitosan dose, stratified by sand filter column type. Reported p-values were adjusted using the Bonferroni correction, m = 2

Bin	Sand Type	Comparison	p-value[a]
1	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**3.63E-07**
1	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**7.68E-07**
2	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**1.52E-05**
2	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**2.77E-05**
3	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**4.29E-06**
3	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**3.30E-05**
4	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**1.75E-05**
4	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**4.63E-05**

[a]Bolded p-values are statistically significant at $p < 0.05$

The Wilcoxon Rank-Sum test was used to compare time-binned LRVs of MS2 coliphage based on chitosan dose in order to evaluate how different chitosan doses performed within the same time interval. The resulting p-values are in **Table 24**. In interval 1, LRVs reported for each set of conditions were statistically significantly different from one another with one exception. LRVs achieved by silica sand filter columns dosed with 0 and 3 mg/L chitosan-treated water were not significantly different. This lack of difference in LRVs for silica sand filter columns at these chitosan doses also remains true for time intervals 2 and 3. In interval 2, LRVs for Accusand filter columns treated with any dose of chitosan were statistically significantly higher than those for Accusand columns dosed with untreated water. Chitosan doses of 10 mg/L and 30 mg/L performed statistically significantly better than the 3 mg/L dose, but no significant differences in LRVs are observed when these two chitosan doses are compared directly. The 10 mg/L and 30 mg/L chitosan doses also had statistically significantly higher LRVs for silica sand filter columns compared to untreated and 3 mg/L chitosan-treated water. LRVs between both types of sand filter columns dosed with 10 and 30 mg/L chitosan-treated water were not statistically significant.

In interval 3, all dose combinations gave statistically significantly different LRVs for Accusand columns. Silica columns dosed with 10 and 30 mg/L chitosan-treated water had statistically significantly higher LRVs than those dosed with untreated water and 3 mg/L chitosan-treated water. No significant differences were observed between LRVs reported for silica columns dosed with water not chitosan-treated compared to 3 mg/L chitosan-treated water, and those dosed with 10 mg/L compared to 30 mg/L. In interval 4, statistically significant differences in achieved LRVs were observed between Accusand columns dosed with 3, 10 and 30 mg/L chitosan-treated water compared to the columns dosed with water not chitosan treated.

LRVs for Accusand columns receiving 3 mg/L chitosan-treated water were statistically significantly lower than for columns 10 mg/L and 30 mg/L. There was not a significant difference in performance in interval 4 between columns dosed with 10 mg/L or 30 mg/L chitosan-treated water. Silica sand columns dosed with 3, 10 and 30 mg/L all had statistically significantly higher LRVs compared to control filters, but no statistically significant differences in LRVs were observed between columns receiving water dosed with 3 and 30 mg/L chitosan and between columns receiving water dosed with 10 and 30 mg/L chitosan. LRVs for silica sand columns receiving 3 mg/L chitosan-treated water were statistically significantly lower than those for 10 mg/L chitosan-treated water.

Table 24. Wilcoxon Rank-Sum analysis results comparing time binned median LRVs of MS2 coliphage by chitosan dose, stratified by sand filter columns type. Reported p-values were adjusted using the Bonferroni correction, m = 6

Bin	Sand Type	Dose 1 (mg/L)	Dose 2 (mg/L)	p-value[a]
1	Accusand	0	3	**4.35E-03**
1	Accusand	0	10	**6.50E-05**
1	Accusand	0	30	**6.50E-05**
1	Accusand	3	10	**6.48E-05**
1	Accusand	3	30	**4.35E-03**
1	Accusand	10	30	**1.30E-04**
1	silica	0	3	0.535
1	silica	0	10	**6.48E-05**
1	silica	0	30	**6.48E-05**
1	silica	3	10	**6.48E-05**
1	silica	3	30	**1.95E-03**
1	silica	10	30	**6.50E-05**
2	Accusand	0	3	**9.32E-04**
2	Accusand	0	10	**9.32E-04**
2	Accusand	0	30	**9.32E-04**
2	Accusand	3	10	**9.32E-04**
2	Accusand	3	30	**9.32E-04**
2	Accusand	10	30	1.00
2	silica	0	3	0.395*

2	silica	0	10	**5.59E-03***
2	silica	0	30	**5.59E-03***
2	silica	3	10	**9.32E-04**
2	silica	3	30	**9.32E-04**
2	silica	10	30	0.299
3	Accusand	0	3	**9.32E-04**
3	Accusand	0	10	**9.32E-04**
3	Accusand	0	30	**9.32E-04**
3	Accusand	3	10	**9.32E-04**
3	Accusand	3	30	**9.32E-04**
3	Accusand	10	30	**9.32E-04**
3	silica	0	3	0.299
3	silica	0	10	**9.32E-04**
3	silica	0	30	**5.59E-03***
3	silica	3	10	**9.32E-04**
3	silica	3	30	**5.59E-03***
3	silica	10	30	0.935*
4	Accusand	0	3	**9.32E-04**
4	Accusand	0	10	**9.32E-04**
4	Accusand	0	30	**9.32E-04**
4	Accusand	3	10	**0.0177**
4	Accusand	3	30	**9.32E-04**
4	Accusand	10	30	0.299
4	silica	0	3	**9.32E-04**
4	silica	0	10	**9.32E-04**
4	silica	0	30	**9.32E-04**
4	silica	3	10	**9.32E-04**
4	silica	3	30	0.782
4	silica	10	30	0.390

[a]Bolded p-values are statistically significant at $p < 0.05$

Comparisons of MS2 coliphage LRVs between each sand column type, silica and Accusand, were statistically compared pairwise for all chitosan doses by the Wilcoxon Rank-Sum test in order to evaluate performance differences within each of the four intervals. The resulting p-values are in **Table 25**. The differences in LRVs attained in time interval 1 by the two sand column types were not statistically significantly different for all 4 chitosan doses. In

time interval 2 the differences in LRVs between Accusand and silica sand filter columns were not significant for chitosan doses of 3, 10 and 30 mg/L but were significant for water not dosed with chitosan. In time interval 3 the differences in LRVs between the two sand filter columns were not statistically significant for water coagulated with chitosan doses of 0 and 3 mg/L but were statistically significant for water treated with chitosan doses of 10 and 30 mg/L. For time interval 4, the differences in LRV between the two sand filter column types were not statistically significant for water dosed with 10 mg/L chitosan but were significant for water dosed with 0, 3 and 30 mg/L chitosan.

When these same data comparing LRV between the two sand filter column types are examined across all 4 time bins by each separate chitosan dose, LRVs for waters not dosed with chitosan were not statistically significant in time intervals 1 and 3 but were statistically significant in time intervals 2 and 4. For sand columns receiving waters dosed with 3 mg/L chitosan, LRVs for the two sand filter columns were not significantly different in time intervals 1, 2 and 3 but were statistically significant in time interval 4. For sand columns receiving waters dosed with 10 mg/L chitosan, LRVs for the two sand filter columns were not significantly different in time intervals 1, 2 and 4 but were statistically significantly different in time interval 3. For sand columns receiving waters dosed with 30 mg/L chitosan, LRVs for the two sand filter columns were not significantly different in time intervals 1 and 2 but were statistically significantly different in time intervals 3 and 4.

Table 25. Wilcoxon Rank-Sum analysis results comparing time binned median LRVs of MS2 coliphage between the two sand column types, stratified by chitosan dose. Reported p-values were adjusted using the Bonferroni correction, m = 4

Bin	Dose (mg/L)	Sand Type 1	Sand Type 2	p-value[a]
1	0	Accusand	silica	1.00
1	3	Accusand	silica	1.00
1	10	Accusand	silica	0.420
1	30	Accusand	silica	0.870
2	0	Accusand	silica	**0.0401***
2	3	Accusand	silica	1.00
2	10	Accusand	silica	1.00
2	30	Accusand	silica	0.0590
3	0	Accusand	silica	0.780
3	3	Accusand	silica	1.00
3	10	Accusand	silica	**0.0186**
3	30	Accusand	silica	**3.72E-03***
4	0	Accusand	silica	**0.0280**
4	3	Accusand	silica	**1.24E-03**
4	10	Accusand	silica	0.642
4	30	Accusand	silica	**1.24E-03**

[a]Bolded p-values are statistically significant at $p < 0.05$
*p-values estimated with ties

4.4 Reductions in Turbidity by Combined Chitosan Coagulation and Sand Column Filtration

The average turbidity values (+/- standard deviation) for both influent and effluent water samples are presented in **Table 26**. Substantial variability in turbidity is observed in the large standard deviation values. This is attributable to changes in turbidity of the lake water over the course of the study. The average influent turbidity level over the course of the study was low at 6 NTU. On average, control Accusand columns (0 mg/L chitosan) reduced turbidity by 1 NTU. Effluent water from control silica columns (0 mg/L chitosan) had average NTU values equal to that of the influent lake water. Both Accusand and silica sand columns produced effluent waters of approximately 2 NTU. Chitosan at 10 mg/L was the only dose to reduce turbidity levels to

less than 1 NTU for both sand types. On average, Accusand and silica sand columns receiving waters dosed with 30 mg/L chitosan had effluent turbidity levels between 1 and 2 NTU.

Table 26. Average turbidity values (+/- standard deviation) for influent and effluent samples

Sample	Turbidity (NTU)
Lake Water	6.34 (+/- 2.86)
Accusand + 0 mg/L	5.35 (+/- 4.96)
Accusand + 3 mg/L	2.11 (+/- 2.18)
Accusand + 10 mg/L	0.628 (+/- 0.431)
Accusand + 30 mg/L	1.11 (+/- 0.507)
Silica + 0 mg/L	6.08 (+/- 2.91)
Silica + 3 mg/L	2.54 (+/- 2.03)
Silica + 10 mg/L	0.807 (+/- 0.536)
Silica + 30 mg/L	1.72 (+/- 1.59)

The turbidity LRVs for each individual sand column over the 57-day experiment period are displayed in **Figure 16** for Accusand columns and **Figure 17** for silica sand columns. Some variability is observed in turbidity LRVs achieved at each time point among the chitosan doses but LRVs between duplicate columns of the same sand filter column type and chitosan dose are similar. As was observed with *E. coli* KO11 and MS2 coliphage, duplicate filters of the same sand type and dose performed similarly over the study period.

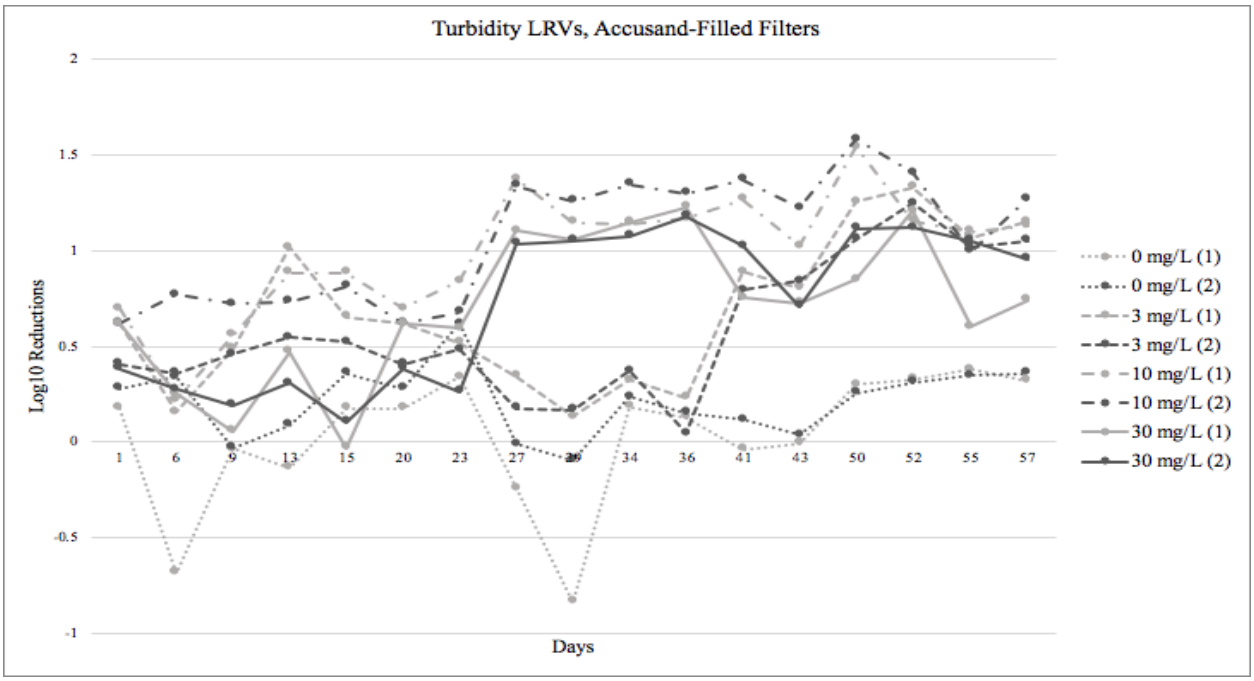


Figure 16. Turbidity reductions for Accusand-filled columns dosed with water coagulated with different chitosan doses for 17 successive samples collected over time throughout the 57-day experiment period.

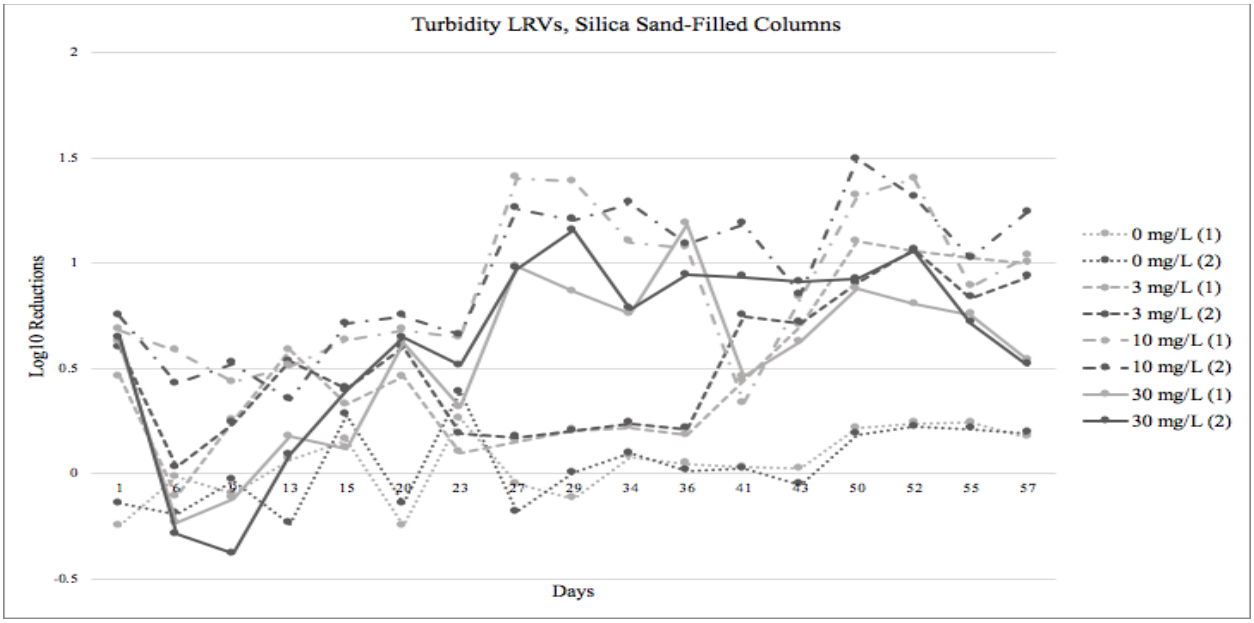


Figure 17. Turbidity reductions for silica sand-filled columns dosed with water coagulated with different chitosan doses for 17 successive samples collected over time throughout the 57-day experiment period.

Because the duplicate filters performed similarly over the study period, the turbidity LRVs for the duplicates were combined for nonparametric analyses. The turbidity reductions for each chitosan dose and between each sand filter column type over the course of the experiment are summarized in the box and whisker plot shown in **Figure 18**. The LRVs vary among chitosan doses from less than 0.25-$\log_{10}$ to around 1-$\log_{10}$. Negative LRV values were sometimes observed and the reasons for them were not explored in this study.

Differences in turbidity LRV between the 4 chitosan dose conditions and between the same doses but for different sand types can be examined in **Figure 18**. Across chitosan doses, both Accusand columns and silica sand columns follow similar LRV patterns. Filters dosed with 10 mg/L chitosan-treated water reported the highest turbidity reductions, followed by chitosan doses 30 and 3 mg/L, respectively. The sand columns dosed with untreated water gave the lowest turbidity LRVs. Accusand filter columns consistently performed better than the silica sand filter columns across chitosan doses. Nonparametric statistical tests were performed to assess if these LRV differences are statistically significant.

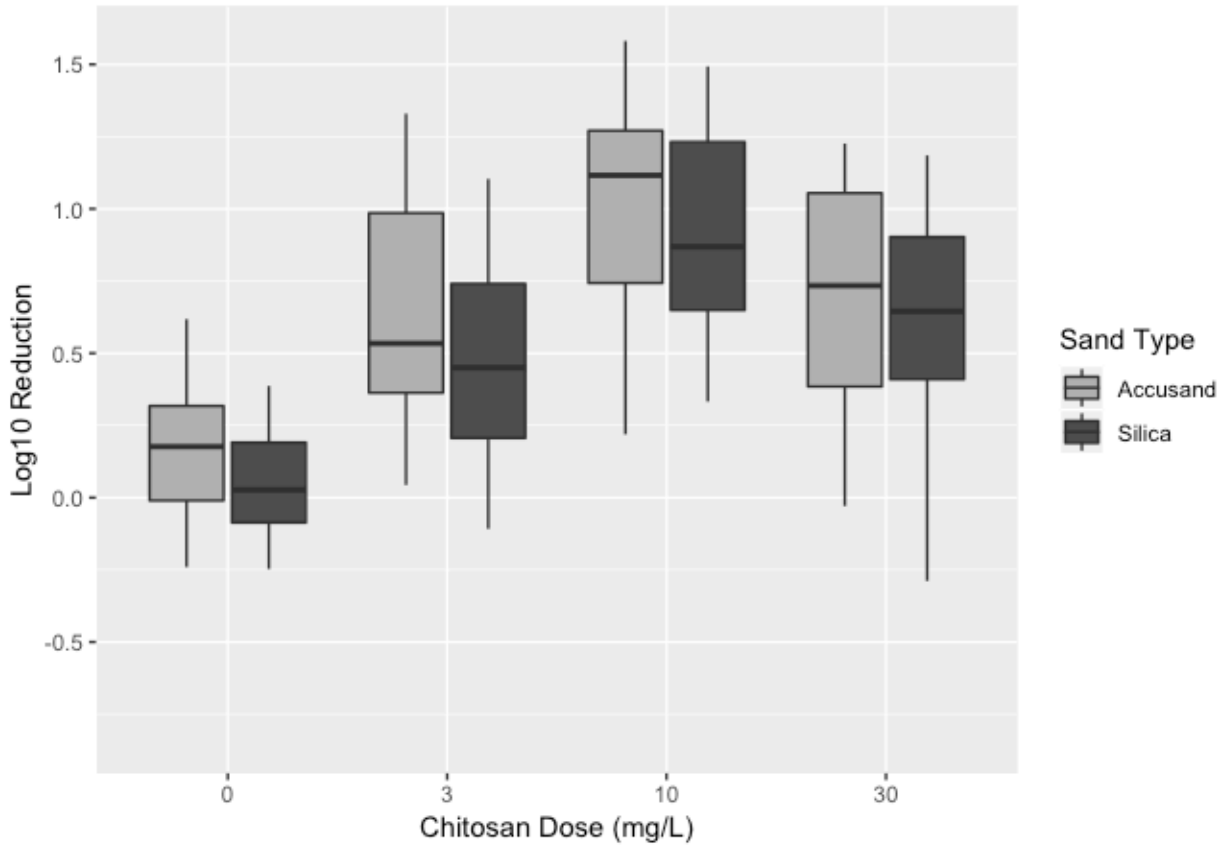


Figure 18. Turbidity LRVs among chitosan doses of 0, 3, 10 and 30 mg/L presented cumulatively over the 57-day experiment period for Accusand filter columns and silica sand columns.

A Kruskal-Wallis analysis was conducted to compare differences in cumulative mean turbidity LRVs based on chitosan dose and sand filter column type and the resulting p-values are depicted in **Table 27**. The results demonstrate that there are significant differences between the turbidity LRVs based on dose of chitosan for filter columns of both sand types.

Table 27. Results of the Kruskal-Wallis analysis comparing cumulative median LRVs of turbidity by chitosan dose, stratified by sand type. Reported p-values were adjusted using the Bonferroni correction, m = 2

Sand Type	Comparison	p-value[a]
Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**5.90E-14**
silica	Dose (0, 3, 10, 30 mg/L chitosan)	**6.80E-14**

[a]Bolded p-values are statistically significant at $p < 0.05$

Cumulative median turbidity LRVs were compared by the Wilcoxon Rank-Sum Test for pairs of specific chitosan doses relative to one another within the same sand type. The resulting p-values are presented in **Table 28**. Turbidity LRVs for Accusand and silica columns dosed with untreated water were statistically significantly lower than those dosed with 3, 10 and 30 mg/L chitosan-treated water. For Accusand filters, 10 mg/L chitosan dose gave statistically significantly higher turbidity LRVs compared to 3 mg/L and 30 mg/L chitosan dose. The differences in turbidity LRVs between 3 mg/L and 30 mg/L chitosan doses were not significant. For silica sand columns, 10 mg/L chitosan dose gave statistically significantly greater turbidity LRVs compared to 3 mg/L chitosan dose. Turbidity LRVs were not significantly between 3 mg/L chitosan dose compared to 30 mg/L and for 10 mg/L chitosan dose compared to 30 mg/L chitosan dose.

Table 28. Wilcoxon Rank-Sum analysis results comparing cumulative median LRVs of turbidity by chitosan dose, stratified by sand filter column type. Reported p-values were adjusted using the Bonferroni correction, m = 6

Sand Type	Dose 1 (mg/L)	Dose 2 (mg/L)	p-value[a]
Accusand	0	3	**3.94E-08**
	0	10	**< 1.32E-15**
	0	30	**4.78E-08**
	3	10	**1.13E-04**
	3	30	1.00
	10	30	**4.97E-03**
silica	0	3	**4.34E-08**
	0	10	**< 1.32E-15**
	0	30	**2.98E-07**
	3	10	**6.35E-05**
	3	30	1.00
	10	30	**0.0198**

[a]Bolded p-values are statistically significant at $p < 0.05$

Cumulative median turbidity LRVs were compared between sand filter column types using the Wilcoxon Rank-Sum Test. The resulting p-values are given in **Table 29**. No

significant differences were observed between the turbidity LRVs achieved by Accusand columns compared to silica columns across all doses of chitosan.

Table 29. Wilcoxon Rank-Sum analysis results comparing cumulative median LRVs of turbidity by sand filter column type, stratified by chitosan dose. Reported p-values were adjusted using the Bonferroni correction, m = 4

Dose (mg/L)	Sand Type 1	Sand Type 2	p-value
0	Accusand	silica	0.0726
3	Accusand	silica	0.560
10	Accusand	silica	0.690
30	Accusand	silica	1.00

[a]Bolded p-values are statistically significant at $p < 0.05$

Time interval binned turbidity LRVs are presented for each type of sand filter in **Figure 19** according to time intervals and in **Figure 20** for each type of sand filter column. No consistent pattern of turbidity LRVs between time intervals is observed across chitosan doses and sand filter column types. Turbidity removal generally improved from time interval 1 to time interval 4, but the extent to which this occurred in each time interval varied among chitosan doses. As with *E. coli* KO11 and MS2 coliphage, the columns that received 10 mg/L chitosan-treated water consistently performed better for turbidity LRV than the other chitosan doses. The next best chitosan dose for turbidity LRVs was 30 mg/L, followed by 3 mg/L and finally water untreated with chitosan. Accusand filter column consistently achieved similar or greater turbidity LRVs across chitosan doses and time intervals compared to Accusand sand filter columns.

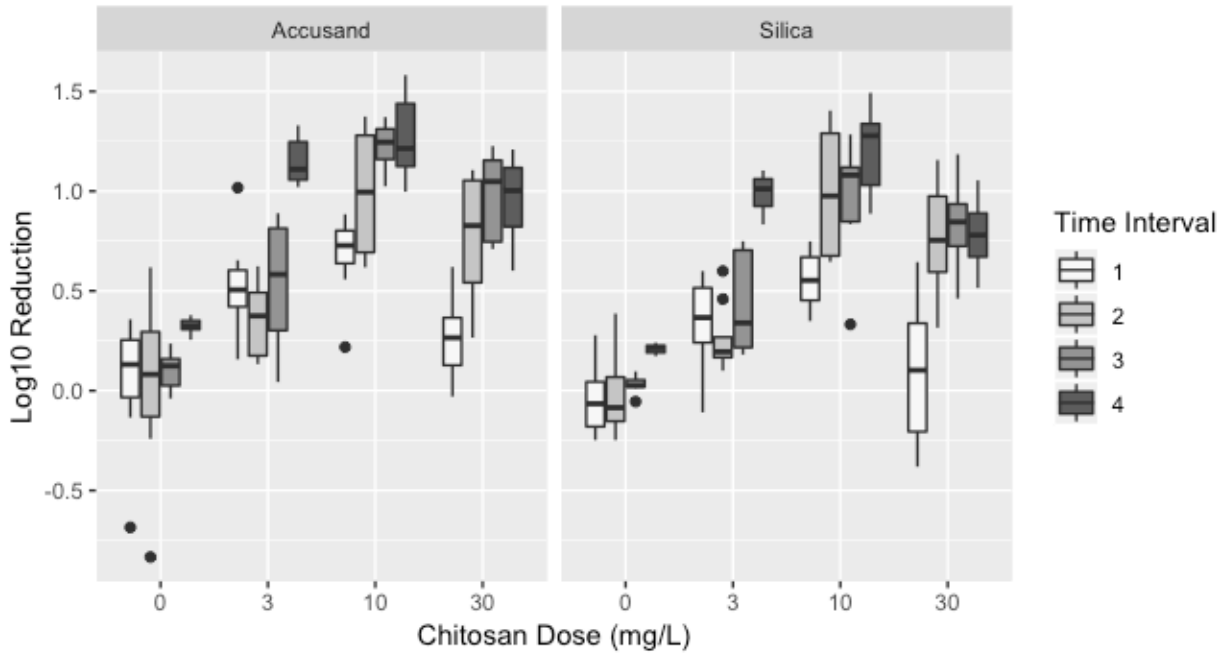


Figure 19. Binned turbidity LRVs across chitosan doses of 0, 3, 10 and 30 mg/L presented in time intervals over the 57-day experiment period for Accusand filter columns and silica sand columns.

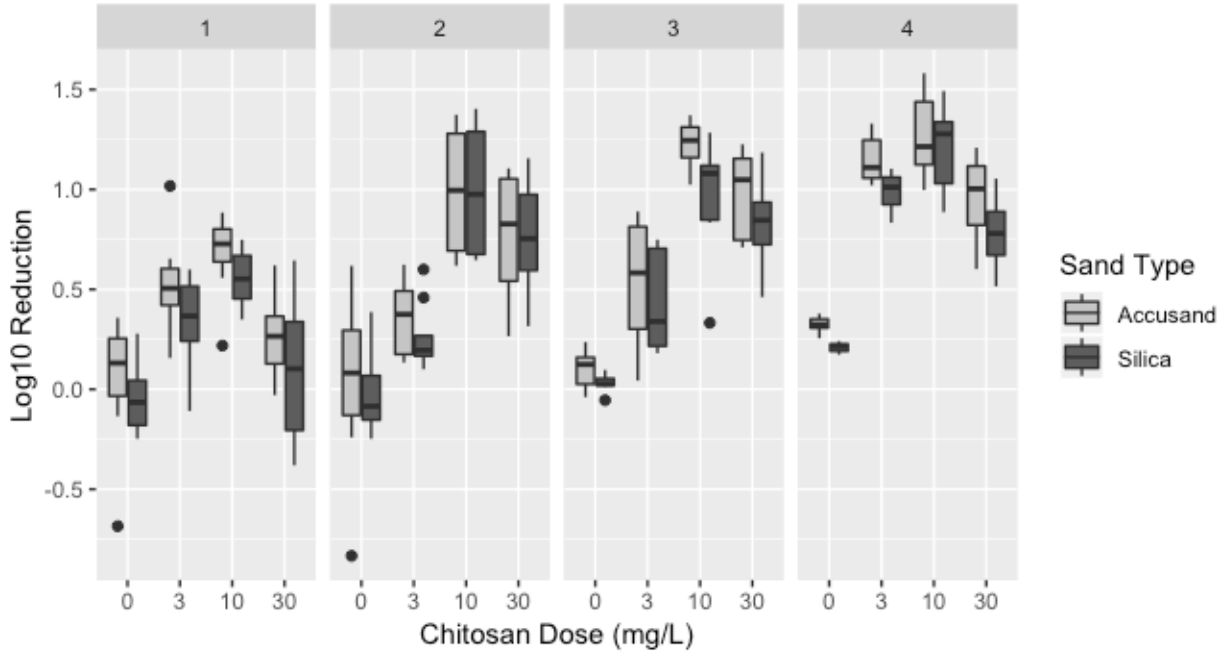


Figure 20. Alternative presentation of binned turbidity LRVs across chitosan doses of 0, 3, 10 and 30 mg/L presented in time intervals over the 57-day experiment period for Accusand filter columns and silica sand columns.

The Kruskal-Wallis test was used to assess if the differences between time binned median turbidity LRVs were statistically significant when stratified by time, across all doses within the same sand type. The resulting p-values are presented in **Table 30**. Significant differences in LRVs were observed based on time interval, across all doses, for both sand types.

Table 30. Results of the Kruskal-Wallis analysis comparing median LRVs of turbidity by time interval, stratified by chitosan dose and sand type. Reported p-values were adjusted using the Bonferroni correction, m = 2

Sand Type	Dose (mg/L)	Comparison	p-value[a]
Accusand	All doses (0, 3, 10, 30)	Time Interval (1-4)	**3.39E-05**
Silica	All doses (0, 3, 10, 30)	Time Interval (1-4)	**9.02E-06**

[a]Bolded p-values are statistically significant at $p < 0.05$

In order to evaluate how both types of sand performed over time, across all doses, binned median LRVs of turbidity were compared based on time interval with the Wilcoxon Rank-Sum test for multiple comparisons. This analysis was not run for both types of sand columns because the differences between intervals were statistically significant (Kruskal-Wallis $p < 0.05$). The results are presented in **Table 31**. The results suggest there are significant differences in achieved LRVs for columns of both sand types across all doses only between time intervals 1 and 3, 1 and 4, and 2 and 4 ($p < 0.05$).

Table 31. Results of the Wilcoxon Rank-Sum analysis comparing median LRVs of turbidity by time interval, stratified by sand type. Reported p-values were adjusted using the Bonferroni correction, m = 6

Sand Type	Dose (mg/L)	Interval 1	Interval 2	p-value[a]
Accusand	All doses (0, 3, 10, 30)	1	2	0.858
Accusand	All doses (0, 3, 10, 30)	1	3	**0.0306**
Accusand	All doses (0, 3, 10, 30)	1	4	**9.98E-07**
Accusand	All doses (0, 3, 10, 30)	2	3	1.00
Accusand	All doses (0, 3, 10, 30)	2	4	**0.0139**
Accusand	All doses (0, 3, 10, 30)	3	4	0.478
Silica	All doses (0, 3, 10, 30)	1	2	0.127
Silica	All doses (0, 3, 10, 30)	1	3	**7.40E-03**
Silica	All doses (0, 3, 10, 30)	1	4	**5.86E-07**
Silica	All doses (0, 3, 10, 30)	2	3	1.00
Silica	All doses (0, 3, 10, 30)	2	4	**0.0443**
Silica	All doses (0, 3, 10, 30)	3	4	0.165

[a]Bolded p-values are statistically significant at $p < 0.05$

The same Kruskal-Wallis analysis conducted in **Table 30** was run again, but for each chitosan dose rather than the cumulative doses considered together. The adjusted p-values are presented in **Table 32**. These results suggest that there are significant differences in LRVs between time intervals for specific doses. With the exception of the control Accusand columns dosed with untreated water, all other dose and sand combinations achieved statistically significantly different turbidity reduction values between time intervals.

Table 32. Results of the Kruskal-Wallis analysis comparing median LRVs of turbidity by time interval, stratified by chitosan dose and sand type. Reported p-values were adjusted using the Bonferroni correction, m = 4

Sand Type	Dose (mg/L)	Comparison	p-value[a]
Accusand	0	Time Interval (1-4)	0.0695
Accusand	3	Time Interval (1-4)	**1.01E-03**
Accusand	10	Time Interval (1-4)	**2.80E-03**
Accusand	30	Time Interval (1-4)	**8.22E-04**
silica	0	Time Interval (1-4)	**0.0359**
silica	3	Time Interval (1-4)	**7.57E-04**
silica	10	Time Interval (1-4)	**3.03E-03**
silica	30	Time Interval (1-4)	**3.48E-03**

[a]Bolded p-values are statistically significant at $p < 0.05$

In order to evaluate how the columns of the same sand type and chitosan dose performed over time, the Wilcoxon Rank-Sum test was used to compare time binned median LRVs of turbidity based on time interval. The resulting p-values are reported in **Table 33**. These tests were run for all conditions except the untreated Accusand columns (Kruskal-Wallis, $p > 0.05$). Silica sand columns receiving untreated water (0 mg/L chitosan) had significantly different LRVs between time intervals 1 and 4 and between time intervals 3 and 4. For Accusand columns receiving water dosed with 3 mg/L chitosan, interval 4 was statistically significantly different than all other intervals, but differences between the first three intervals were not significant. Silica sand columns with 3 mg/L chitosan had significant improved turbidity removal performance in the 4th time interval compared to the earlier time intervals. Turbidity LRVs for Accusand columns receiving water treated with 10 mg/L chitosan experienced a significant decline after interval 1 and did not significantly improve until time interval 4. For silica sand columns at the same chitosan dose, significant improvements in turbidity removal performance were observed between time intervals 1 and 2, but LRVs in subsequent intervals were not statistically different. Accusand columns receiving water dosed with 30 mg/L chitosan achieved low turbidity reductions in the first interval then significantly increased LRVs in

interval 2. Differences in LRVs after that point were not significant. Silica sand columns receiving water dosed with 30 mg/L chitosan followed the same LRV performance pattern as the 10 mg/L chitosan-dosed silica columns.

Table 33. Results of the Wilcoxon Rank-Sum analysis comparing time binned median LRVs of turbidity for the same chitosan dose and sand type, between time intervals. Reported p-values were adjusted using the Bonferroni correction, m = 6

Dose (mg/L)	Sand Type	Interval 1	Interval 2	p-value[a]
3	Accusand	1	2	0.874
3	Accusand	1	3	1.00
3	Accusand	1	4	**2.74E-04**
3	Accusand	2	3	1.00
3	Accusand	2	4	**9.32E-04**
3	Accusand	3	4	**9.32E-04**
10	Accusand	1	2	0.732
10	Accusand	1	3	**2.74E-04**
10	Accusand	1	4	**2.74E-04**
10	Accusand	2	3	1.00
10	Accusand	2	4	0.782
10	Accusand	3	4	1.00
30	Accusand	1	2	**0.0184**
30	Accusand	1	3	**2.74E-04**
30	Accusand	1	4	**5.48E-04**
30	Accusand	2	3	0.782
30	Accusand	2	4	1.00
30	Accusand	3	4	1.00
0	silica	1	2	1.00
0	silica	1	3	1.00
0	silica	1	4	**0.0184**
0	silica	2	3	0.963
0	silica	2	4	0.629
0	silica	3	4	**9.32E-04**
3	Accusand	1	2	1.00
3	Accusand	1	3	1.00
3	Accusand	1	4	**2.74E-04**
3	Accusand	2	3	0.299
3	Accusand	2	4	**9.32E-04**

3	Accusand	3	4	**9.32E-04**
10	Accusand	1	2	**0.0263**
10	Accusand	1	3	**0.0373**
10	Accusand	1	4	**2.74E-04**
10	Accusand	2	3	1.00
10	Accusand	2	4	1.00
10	Accusand	3	4	0.629
30	silica	1	2	**8.23E-03**
30	silica	1	3	**1.92E-03**
30	silica	1	4	**3.29E-03**
30	silica	2	3	1.00
30	silica	2	4	1.00
30	silica	3	4	1.00

[a]Bolded p-values are statistically significant at $p < 0.05$

Kruskal-Wallis analyses were conducted to evaluate differences in time interval binned median turbidity LRVs for each sand filter column type based on chitosan dose. The resulting p-values are presented in **Table 34**. The results demonstrate that there are statistically significant differences between the turbidity LRVs achieved across chitosan doses within the same sand filter column type.

Table 34. Kruskal-Wallis analysis results comparing time binned median LRVs of turbidity by chitosan dose, stratified by sand filter column type. Reported p-values were adjusted using the Bonferroni correction, m = 2

Bin	Sand Type	Comparison	p-value[a]
1	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**6.63E-05**
1	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**5.40E-04**
2	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**3.19E-04**
2	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**4.96E-05**
3	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**4.43E-05**
3	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**4.65E-05**
4	Accusand	Dose (0, 3, 10, 30 mg/L chitosan)	**1.56E-04**
4	silica	Dose (0, 3, 10, 30 mg/L chitosan)	**4.22E-05**

[a]Bolded p-values are statistically significant at $p < 0.05$

The Wilcoxon Rank-Sum test was used to compare time-binned LRVs of turbidity based on chitosan dose in order to evaluate how different chitosan doses performed within the same

time interval. The resulting p-values are in **Table 35**. In time interval 1, turbidity LRVs reported for both sand types were significantly different between chitosan doses of 0 and 3, 0 and 10, and 10 and 30 mg/L. LRVs for Accusand columns in time interval 2 were significantly higher for 10 mg/L and 30 mg/L chitosan doses compared to the control but were not significantly different when directly compared. Filters receiving water treated with 10 mg/L chitosan also performed better than those treated with the 3 mg/L dose, but this was not true when comparing 3 to 30 mg/L. For silica sand columns in interval 2, LRVs at 0 mg/L and 3 mg/L chitosan doses were not significantly different when directly compared, but both were significantly lower than doses of 10 mg/L and 30 mg/L chitosan. Differences in LRVs between 10 mg/L and 30 mg/L were not statistically significant within this interval and sand type.

In interval 3, Accusand filters receiving untreated water (0 mg/L) achieved significantly lower turbidity LRVs compared to all chitosan doses. The 10 mg/L chitosan dose had significantly higher LRVs compared to 3 mg/L and 10 mg/L chitosan doses. All chitosan dose combinations gave statistically significantly different LRVs for silica sand columns in time interval 3 except between doses 10 and 30 mg/L. In interval 4, the control filters (0 mg/L) for both sand types achieved significantly lower LRVs compared to all chitosan doses. When comparing all other doses for both sand types, the turbidity LRVs are not significantly different except between 10 mg/L and 30 mg/L followed by silica sand filtration.

Table 35. Wilcoxon Rank-Sum analysis results comparing time binned median LRVs of turbidity by chitosan dose, stratified by sand filter columns type. Reported p-values were adjusted using the Bonferroni correction, m = 6

Bin	Sand Type	Dose 1 (mg/L)	Dose 2 (mg/L)	p-value[a]
1	Accusand	0	3	**1.23E-03**
1	Accusand	0	10	**4.55E-04**
1	Accusand	0	30	0.631
1	Accusand	3	10	0.213
1	Accusand	3	30	0.0690

1	Accusand	10	30	**4.35E-03**
1	silica	0	3	**0.0125**
1	silica	0	10	**6.50E-05**
1	silica	0	30	1.00
1	silica	3	10	0.173
1	silica	3	30	0.993
1	silica	10	30	**0.0312**
2	Accusand	0	3	0.498
2	Accusand	0	10	**9.32E-04**
2	Accusand	0	30	**0.0177**
2	Accusand	3	10	**1.86E-03**
2	Accusand	3	30	0.169
2	Accusand	10	30	0.782
2	silica	0	3	0.228
2	silica	0	10	**9.32E-04**
2	silica	0	30	**1.86E-03**
2	silica	3	10	**9.32E-04**
2	silica	3	30	**6.53E-03**
2	silica	10	30	0.629
3	Accusand	0	3	**0.0280**
3	Accusand	0	10	**9.32E-04**
3	Accusand	0	30	**9.32E-04**
3	Accusand	3	10	**9.32E-04**
3	Accusand	3	30	0.228
3	Accusand	10	30	0.124
3	silica	0	3	**9.32E-04**
3	silica	0	10	**9.32E-04**
3	silica	0	30	**9.32E-04**
3	silica	3	10	**0.0112**
3	silica	3	30	**0.0280**
3	silica	10	30	1.00
4	Accusand	0	3	**9.32E-04**
4	Accusand	0	10	**9.32E-04**
4	Accusand	0	30	**9.32E-04**
4	Accusand	3	10	1.00
4	Accusand	3	30	0.390
4	Accusand	10	30	0.0886

4	silica	0	3	**9.32E-04**
4	silica	0	10	**9.32E-04**
4	silica	0	30	**9.32E-04**
4	silica	3	10	0.390
4	silica	3	30	0.0625
4	silica	10	30	**0.0112**

[a]Bolded p-values are statistically significant at $p < 0.05$

Comparisons of turbidity LRVs between each sand column type, Accusand and silica, were statistically compared pairwise for all chitosan doses by the Wilcoxon Rank-Sum test. This was used to evaluate performance differences within each of the time intervals between sand types. The resulting p-values are in **Table 36**. Of all comparisons, only the LRVs for the control filters (0 mg/L chitosan) in time interval 4 were statistically significantly different between sand types. This suggests that both sand types, despite differences in sand particle size and filtration rates, were able to remove turbidity equally well when receiving identical influent waters.

Table 36. Wilcoxon Rank-Sum analysis results comparing binned median LRVs of turbidity between the two sand column types, stratified by chitosan dose. Reported p-values were adjusted using the Bonferroni correction, m = 4

Bin	Dose (mg/L)	Sand Type 1	Sand Type 2	p-value[a]
1	0	Accusand	silica	0.492
1	3	Accusand	silica	0.492
1	10	Accusand	silica	0.173
1	30	Accusand	silica	1.00
2	0	Accusand	silica	1.00
2	3	Accusand	silica	1.00
2	10	Accusand	silica	1.00
2	30	Accusand	silica	1.00
3	0	Accusand	silica	0.522
3	3	Accusand	silica	1.00
3	10	Accusand	silica	0.113
3	30	Accusand	silica	1.00
4	0	Accusand	silica	**6.22E-04**
4	3	Accusand	silica	0.113
4	10	Accusand	silica	1.00
4	30	Accusand	silica	0.420

[a]Bolded p-values are statistically significant at $p < 0.05$

4.5 Impact of Chitosan on pH

Previous research has shown that chitosan has limited impact on water pH regardless of chitosan dose (Christensen et al., 2016; Soros, 2015; Soros et al., 2019). These findings were supported by the pH results of this research. The % change in pH is represented in **Figure 21**. The raw % decrease in pH values for all filters on each sampling day are presented in **Appendix 8**. In most cases only limited changes in pH, +/- 5%, were observed. Outliers are also observed that extended to greater than 30% change. Outliers may be explained by instrument error.

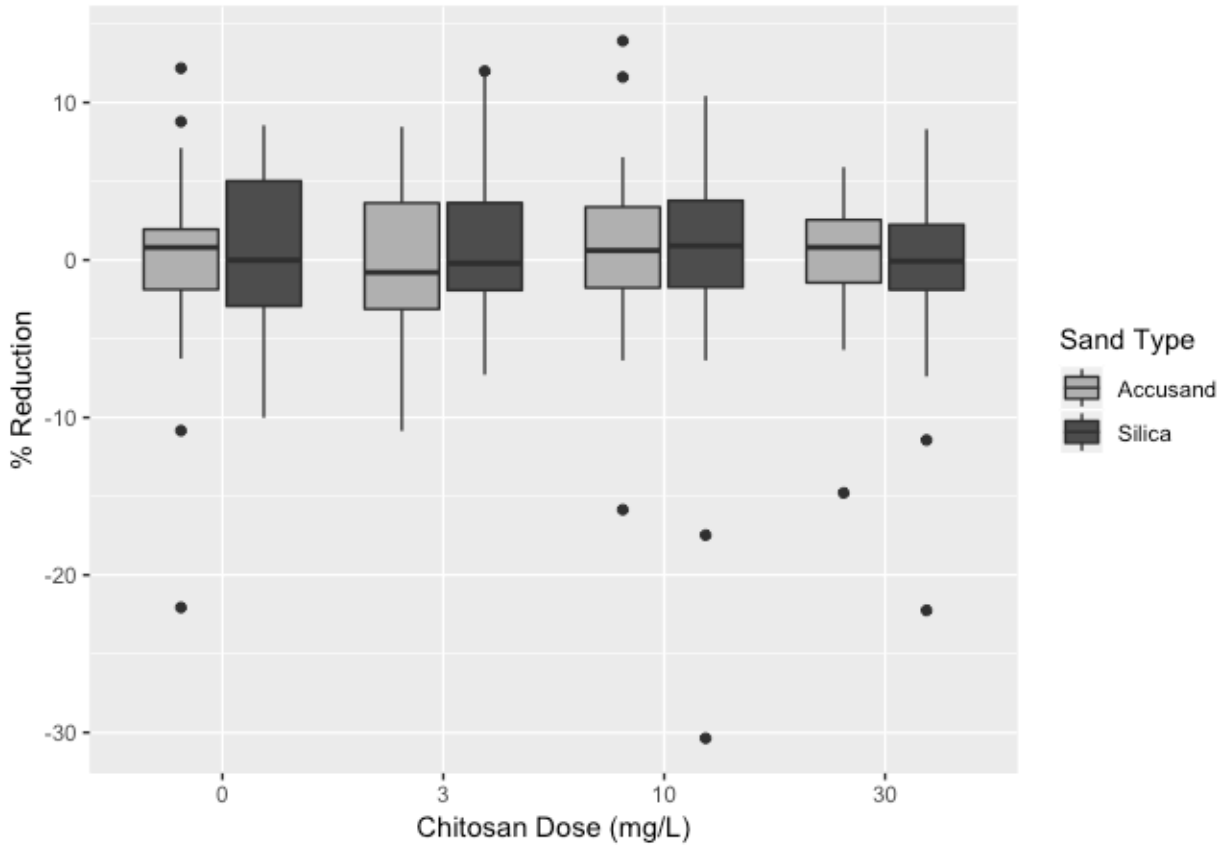


Figure 21. Percent reduction in pH across chitosan doses of 0, 3, 10 and 30 mg/L presented cumulatively over the 57-day experiment period for Accusand columns and silica sand columns.

4.6 Flow Rate Changes in Sand Filter Columns Over the Experiment Period

The sand filters in this study were not run like typical biosand filter systems that promote growth of the schmutzdecke and require idle time within the filter to enhance microbial reductions. The filters in this study were operated intermittently, were subjected to weekly scouring, and the daily volume dosed into the filters greatly exceeded the pore-volume within the sand bed. These conditions, as well as the fact that there no measurable parameter was used as a proxy to estimating filter maturation over time, meant that the impact of flow rate or filtration rate on the removal capacity of the sand filter columns was not directly studied.

Flow rate measurements were conducted on each sampling day except on sampling day 2. These measurements were taken by measuring the duration of time for the first 50 mL of water to pass through the filter after the filter was filled to the maximum head of 7 cm. When maximally full, the flow rate was at the maximum rate. As the head declined, the flow rate declined because the pressure forcing the water through the filter declined. This decline in flow rate varied for each filter and was not directly measured in this study. Flow rates were converted to filtration rates because filtration rates do not depend on the sand bed surface area. The filtration rate is a measure of flow rate per square meter of the surface area of the sand filter column. The average filtration rates over the course of the experiment, with standard deviation error bars, are presented in **Figure 22**. The raw filtration rate values for all filters on each sampling day are presented in **Appendix 9**. CAWST (2012) recommends biosand filters operate at a maximum filtration rate of 0.4 m/hr. The silica sand filter columns in this experiment had an average filtration rate of 1.2 m/hr (+/- 0.18), while the Accusand filter columns had an average filtration rate of 0.6 m/hr (+/- 0.07).

The filtration rates for each column with the same sand type were adjusted to remain approximately equal over the duration of the study. Because this adjustment was achieved by moving the outlet tube up and down the length of the column, there was still substantial variability in flow rate observed over time. Additionally, despite weekly sand filter column scouring procedures for the top 3 cm of the filter column, filters receiving water treated with any dose of chitosan did experience flow rate decline over time. Occasionally over the course of the study, the flow rate of the filters declined to such an extent that moving the outlet tube to well below the column bed still did not sufficiently increase the flow rate of the column back to the target flow rate range. When this occurred, more rigorous scouring procedures were used to

disturb the sand bed to about 10 cm down the length of the column. After this more rigorous scouring procedure, the flow rates could be successfully adjusted back to the target range for each sand type.

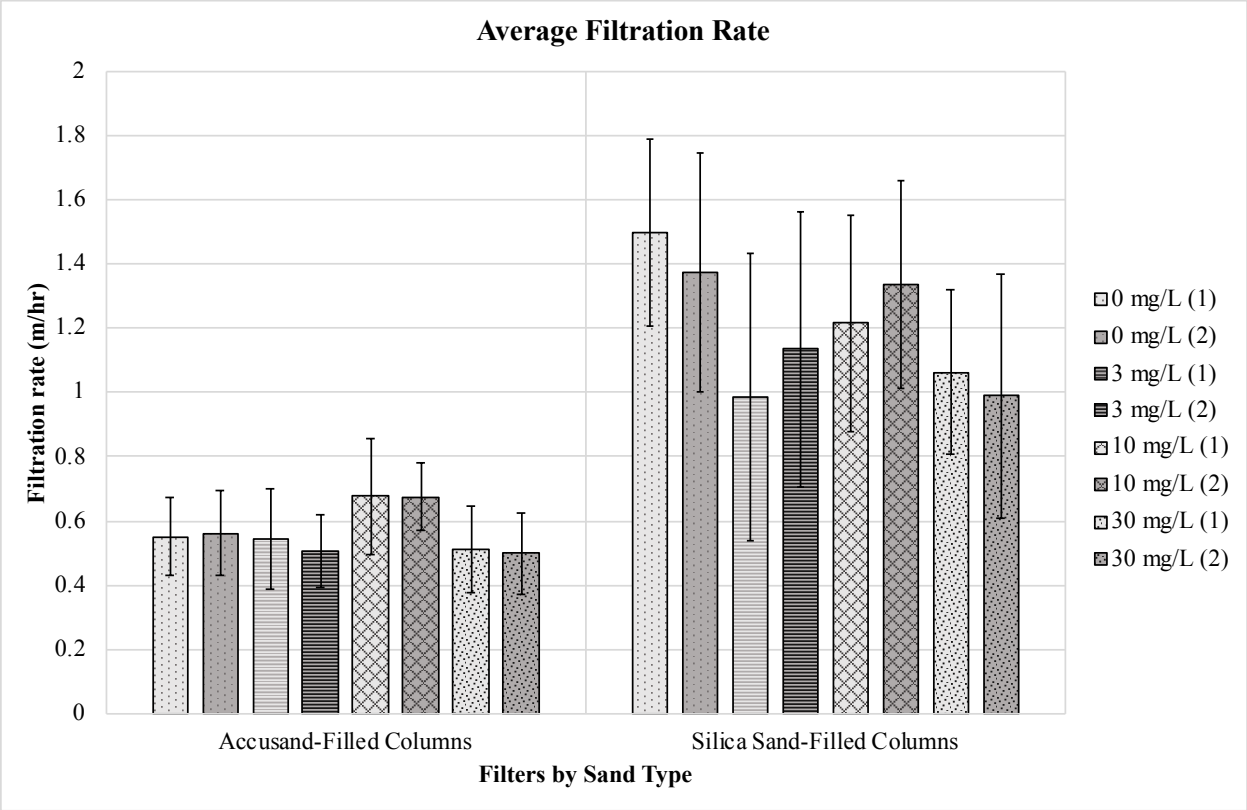


Figure 22. Average filtration rate, with standard deviation error bars, for Accusand filter columns and silica sand filter columns receiving water with different chitosan doses for 16 successive samples collected throughout the 57-day experiment period

Previous studies have reported improved removal of target indicator organisms and turbidity with filtration technologies at lower filtration rates (Napotnik & Jellison, 2014; Singer et al., 2017). It is possible that variability in filtration rate in this study may account for variability in LRVs for bacteria, viruses and turbidity. A Spearman's rank-order correlation test was used in order to evaluate if there was an association between flow rate of the sand filter columns and LRVs for *E. coli* KO11, MS2 coliphage and turbidity. The correlation matrix is presented in **Table 37**. **Figure 23** displays the correlation matrix between all of the variables

and is color coded with blue and red-white to indicate strong and weak associations, respectively. A very strong monotonic relationship is observed between *E. coli* KO11 and MS2 coliphage LRVs, as well as between both of these variables and turbidity LRVs. This was expected based on the matching patterns in LRVs achieved by columns of the same sand type and dose across these variables. Very weak associations between filtration rate and LRVs for these variables are observed, suggesting no monotonic relationship between filtration rate and LRVs of bacteria, viruses and turbidity.

Table 37. Correlation matrix produced by the Spearman's rank-order correlation test to compare LRVs and Filtration Rates

	E. coli KO11 LRVs	MS2 Coliphage LRVs	Turbidity LRVs	Filtration Rates
E. coli KO11 LRVs	1.000	0.906	0.767	-0.099
MS2 Coliphage LRVs	0.906	1.000	0.727	-0.071
Turbidity LRVs	0.767	0.727	1.000	-0.068
Filtration Rates	-0.099	-0.071	-0.068	1.000

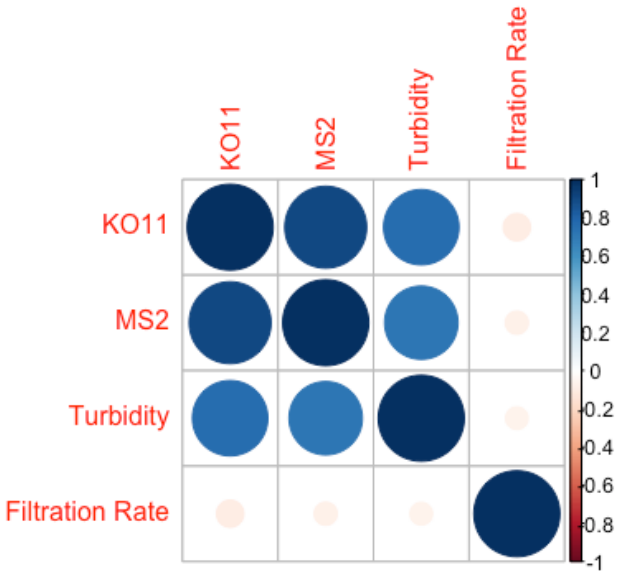


Figure 23. Matrix graphical display of strengths of Spearman rank-order correlations for LRVs of *E. coli* KO11, MS2 coliphage, turbidity and flow rate.

A chart of this correlation matrix including numeric correlation values is presented in **Figure 24**. The distribution of values for each variable is shown on the diagonal. Below the diagonal are bivariate scatter plots with fitted lines, and above the diagonal is the correlation value, rho, with significance levels indicated by stars. Based on these results, there is not sufficient evidence to suggest there is a correlation between filtration rate and LRVs for bacteria, viruses and turbidity. There is strong evidence supporting associations between LRVs for bacteria compared to viruses, bacteria compared to turbidity, and viruses compared to turbidity.

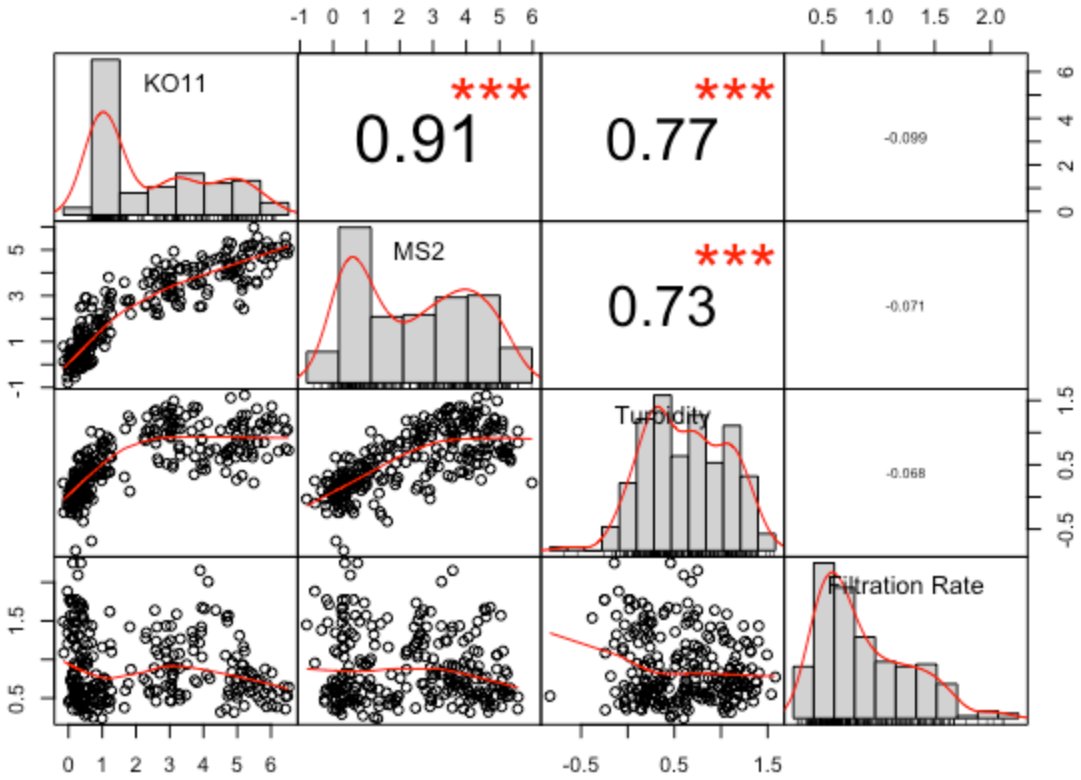


Figure 24. Chart display of the Spearman Rank correlation matrix and correlation values for LRVs of *E. coli* KO11, MS2 coliphage, turbidity and flow rate with p-values. Significance levels are shown as symbols: 0 = "***", 0.001 = "**", 0.01 = "*" , 0.05 = "."

CHAPTER 5: DISCUSSION

5.1 Performance Efficacy of Chitosan Coagulation and Sand Filtration

The results of this study demonstrate that the LRVs for indicator bacteria and viruses achieved using small scale, shallow bed sand column models of intermittently-operated slow sand filtration systems can be significantly improved for bacteria, virus and turbidity removal by pre-treating challenge waters with chitosan salts. The small-scale filters dosed with water treated with 3, 10 and 30 mg/L chitosan demonstrated significantly improved LRVs for both bacteria and viruses compared to those dosed with uncoagulated water (Kruskal-Wallis, $p < 0.05$). Chitosan at 10 mg/L and 30 mg/L doses with Accusand columns consistently produced significantly higher LRVs for bacteria and viruses, both reaching about 4.0 to 4.5-$\log_{10}$, than the 3 mg/L dose of about 1.0 to 1.5-$\log_{10}$. Silica columns receiving water dosed with 10 mg/L chitosan achieved greater than 3.5-$\log_{10}$ reductions for both bacteria and viruses compared to about 2 to 2.5-$\log_{10}$ and about 1-$\log_{10}$ reductions for 30 mg/L and 3 mg/L chitosan doses, respectively. Silica and Accusand filter columns dosed with 10 mg/L chitosan typically reported greater LRVs for bacteria and viruses compared to 30 mg/L, although this trend was not consistent over the entire study period (Wilcoxon rank-sum, $p < 0.05$). The maximum average LRVs of *E. coli* KO11 and MS2 coliphage were achieved with a dose of 10 mg/L of chitosan acetate and Accusand miniature column filtration, reaching 4.75 (+/- 0.99) and 4.43 (+/- 0.74) $\log_{10}$, respectively. By comparison, Accusand filtration without chitosans achieved maximum average LRVs of 0.42 (+/- 0.29) for bacteria and 0.36 (+/- 0.53) for viruses. The average reported reductions at 10 mg/L and 30 mg/L chitosan doses with Accusand filter columns met

the 2-star protective LRV targets set by the WHO for HWT technologies, exceeding the 3-$\log_{10}$ reduction level for viruses and the 2-$\log_{10}$ reduction level for bacteria. These performance targets were also met for 10 mg/L silica sand filter columns in the first half of the study; however, cumulative median LRVs for MS2 coliphage eventually were below the 4-$\log_{10}$ target by the end of the 57-day study period.

Pretreatment with chitosan salts followed by miniature column sand filtration as a model ISSF also improved turbidity reductions. All three doses of chitosan tested significantly improved turbidity reductions compared to filtration alone; however, the 10 mg/L chitosan dose was the only dose to achieve <1 NTU for average effluent turbidity with both sand filter column types. Filtration alone without chitosan coagulation pre-treatment produced average filtrate water turbidity levels between 4-6 NTU. The 10 mg/L chitosan pretreatment followed by sand filtration achieved on average maximum and minimum filtrate water turbidites of 0.90 NTU (+/- 0.67) and 0.56 NTU (+/- 0.27), respectively. This chitosan dose coupled with filtration met the 1 NTU level recommended by the WHO GDWQ for turbidity. In terms of turbidity LRVs, all three chitosan doses followed by small scale sand column filtration exceeded an average of 0.4-$\log_{10}$ for turbidity reduction, with 10 mg/L exceeding 0.8-$\log_{10}$ for both sand types. By comparison, filtration alone achieved on average < 0.25-$\log_{10}$ turbidity removal. The addition of chitosan to challenge waters did not significantly change pH, even at the highest chitosan dose.

Generally, Accusand filter columns performed better than silica sand filter columns for all performance indicators used in the study. This was expected based on the smaller pore sizes and slower filtration rates of this sand. Although both were silica-based sands, the comparisons between the two sand types are made with caution because they were operated with different experimental conditions. Differences in median LRVs achieved by the two sand types studied,

when considered cumulatively, typically increased with increasing chitosan dose. Filters receiving untreated water or water treated with 3 mg/L chitosan reported median LRVs within <0.5-$\log_{10}$ between Accusand and silica sand media for both bacteria and viruses. These differences in average median LRVs for bacteria and viruses between sand types increased to 0.5-1 and 2-3 for 10 mg/L and 30 mg/L chitosan doses, respectively. The analyses of performance results for the successive time intervals over the 57-day experiment frequently gave higher LRVs for Accusand than silica sand across time periods and chitosan doses, but exceptions to this trend are observed for some chitosan doses and time intervals. The differences in median LRVs for turbidity do not increase with increasing chitosan dose, but median LRVs for turbidity were always greater with Accusand sand columns than with silica sand columns. However, these LRV differences between sand types were not significantly different (Wilcoxon rank-sum, $p < 0.05$). Filtration rate decline was not investigated specifically in this study. Differences in the rate of media aging for each sand type as a function of operating time and reduction of contaminants also was not evaluated.

Removal efficiency did not correlate with sand particle size, as is observed with the achieved LRVs for bacteria compared to viruses. This may be because coagulation increases the effective size of these microorganisms as they floc together. Differences between *E. coli* KO11 and MS2 coliphage isoelectric points may also account for similarities in removal efficiency between the surrogate microbes. The isoelectric point for MS2 coliphage is between 3.5-3.9 compared to 5.6 for unmodified *E. coli* cells (Collins et al., 2004; Sherbet & Lakshmi, 1973). The pH of the challenge water used in this experiment was 6.87 (+/- 0.228). Because *E. coli* KO11 has a higher isoelectric point, it should have a less negative net charge than MS2 coliphage at neutral pH. With a more negative net charge, MS2 coliphage and cationic chitosan

polymers may have had a greater electrostatic attraction compared to *E. coli* KO11 and cationic chitosan polymers. Previous studies have documented MS2 adsorbing to positively charged membranes at a higher concentration than other commonly used virus surrogates with higher relative isoelectric points (Dika et al., 2015). The enhanced electrostatic attractive forces between the polymers and microbes would likely have to exceed the negative electrostatic forces between the microbes and the sand grains because existing evidence suggests higher isoelectric points for viruses are associated with improved removal via granular filtration compared to those with lower isoelectric points (Dowd, Pillai, Wang, & Corapcioglu, 1998). These explanations for why bacteria and virus removal are comparable are only speculative, as many other experimental conditions can also impact electrokinetic properties, electrostatic forces, surface charges and adsorption.

Results from the time-binned analysis demonstrate that the influence of time on filter performance was not consistent across chitosan doses or sand types. However, improvements in contaminant reduction performance over time were observed for the majority of conditions. Reported LRVs for bacteria and viruses in the last time interval were statistically significantly better than those in the 1st time interval for untreated, 3 mg/L and 30 mg/L chitosan-treated water. This pattern was observed for columns of both sand types. The 10 mg/L chitosan dose followed by sand filtration achieved high bacteria and virus LRVs in all time intervals, although their magnitudes varied substantially in each bin. In some cases, the 1st time interval had greater reported LRVs for bacteria and viruses than the last time interval, suggesting a decline in filter performance over time.

The fall turnover event occurred for University Lake towards the end of November, early December 2018. This correlates with time intervals 2 and 3 as defined in this investigation.

Declines in filter performance for filters of both sand types receiving water dosed with 10 mg/L chitosan were correlated with the approximate dates of the turnover event. Differences in LRVs were more apparent for *E. coli* KO11 than MS2 coliphage. LRVs for 3 mg/L chitosan dose also decreased in these time intervals, but the differences were not significant (Wilcoxon rank-sum, $p > 0.5$). At 30 mg/L chitosan dose, there was consistently improved LRV performance over these time intervals for both sand types.

Previous studies have associated filter maturation or media aging to improved microbial reductions (Elliott et al., 2011, 2015). Typically, filtration rate is used as a proxy to indicate media aging. Filtration rates in this study were kept within a target range for each sand type, therefore media aging effects in filtration rate and performance were not directly investigated. Separating LRVs into time intervals may provide indirect insight into how media aging may impact filter performance. However, because media aging was not directly evaluated as an experimental variable, potential impacts are only speculative. The results suggest that when water is treated with an optimal chitosan dose for the influent water quality, in this case 10 mg/L chitosan, substantially improved reductions for bacteria and viruses are achieved independent of filter operating time and filter maturation. At non-optimal chitosan doses and for untreated water, media aging is correlated with increased LRVs. However, variability in performance across time intervals suggests improvements in reductions occur at different rates for different chitosan doses and sand filter column types. Additionally, the extent to which filter maturation enhances microbial reductions are likely chitosan dose-dependent and influenced by sand type. Declines in LRVs over time may indicate a plateau-effect in terms of the extent to which media aging may improve filter performance, or it may indicate that media aging is less important an

indicator of LRVs compared to other experimental parameters such as surface water quality, chitosan dose or type of sand.

The filtration rate for each column was variable over the study period, but filtration rates were not correlated with LRVs for bacteria, viruses or turbidity (Spearman Rank Correlation coefficient $-0.1 < \text{rho} < 0.1$). The Accusand filters and silica sand filters gave average filtration rates of 0.6 m/hr (+/- 0.07) and 1.2 m/hr (+/- 0.18), respectively. These filtration rates are somewhat faster than the recommended filtration rate of 0.4 m/hr for BSFs (CAWST, 2012), although much slower than the filtration rates of rapid sand filters. These results suggest that filtration rates may be increased over those of the BSF while maintaining high LRVs for bacteria and viruses when using chitosan coagulation-flocculation prior to sand filtration. Such increased filtration rates would increase the volume of water treated per day per household. Furthermore, performance in terms of LRVs for bacteria and viruses may be further enhanced if columns were operated at the recommended filtration rate for BSFs.

Small diameter (3.9 cm), shallow sand bed bench-scale filters were advantageous for study design because all 16 filters could be operated simultaneously and duplicate filters could be used to helped improve data representativeness and statistical power. Replicate columns of the same sand type and chitosan dose allowed conclusions about the extent to which chitosan coagulation-flocculation improves sand filtration performance to be made with higher confidence. Limitations in costs and labor were preventative factors for using full-scale household sand filters in this study.

Though duplicate filters helped improve statistical power, the variability observed between duplicate filters of the same set of conditions resulted in measurable standard errors and standard deviation. Variability in the performance of duplicate filters, coupled with non-

Normality of the LRV data lowers statistical power, making it more difficult to accurately and precisely estimate how each set of conditions impacted chitosan dose and filter performance. Prior research evaluating BSFs with replicate columns of the same conditions have also experienced some lack of reproducibility for experimental conditions and LRV results (Elliott et al., 2011, 2015). Different rates of maturation or ripening of the filter, including chitosan accumulation, increased biological activity in the sand column of the filter, and weekly scouring procedures are potentially responsible for variability in LRVs within each column and between duplicate columns. Additionally, regrowth in stored samples and analytical instrument imprecision may account for the variability observed in turbidity measurements. Without further investigation of these parameters, it is impossible to determine to what extent experimental design limitations and unavoidable errors in sand filtration system design and operation contribute to observed performance variability.

A primary target for this research was to evaluate if improved microbial reductions could be achieved using chitosan coagulation-flocculation as a pretreatment process before slow sand filtration. Performance was evaluated according to the WHO HWTS performance levels for bacteria and viruses. These targets are based on the acceptable risk, or tolerable disease burden, as health-based targets presented in DALYs. Based on these targets, the use of chitosan coagulation-flocculation followed by intermittently-operated sand filtration significantly increases the low average 0.42-$\log_{10}$ bacteria and 0.30- to 0.36-$\log_{10}$ virus reductions by Accusand filtration alone by an additional 0.57- to 4.33-$\log_{10}$ and 1.14- to 4.08-$\log_{10}$ for bacteria and viruses, respectively. Low average 0.15- to 0.17-$\log_{10}$ *E. coli* bacteria reductions and 0.23- to 0.35-$\log_{10}$ MS2 coliphage reductions by silica sand alone are improved by an additional 0.87- to 3.80-$\log_{10}$ and 0.76- to 3.72-$\log_{10}$, respectively. Although the 30 mg/L chitosan dose did not

consistently meet the WHO 2-star, protective performance level, this dose did reduce bacteria and virus reductions by greater than 1.5-$\log_{10}$, which still provides considerable morbidity risk reduction. The 10 mg/L doses of chitosan consistently met the WHO 2-star, protective level for bacteria, >2-$\log_{10}$, and viruses, >3-$\log_{10}$, for both sand types. This provides a substantial reduction in morbidity risks compared to the performance of the ISSFs dosed with water not pre-treated by chitosan coagulation. These results support the use of chitosan coagulation-flocculation as a pretreatment step to improve ISSF performance in terms of microbial reductions and reduced health risks.

Another main target of this research was to evaluate chitosans compared to other existing and widely used inorganic coagulants. Chitosan was identified as a potential alternative to them, especially in household settings where coagulant dose and pH are not easily optimized. Attractive properties of chitosan for water treatment are its non-toxicity, biodegradability, availability and sustainability (Renault, Sancey, Badot, & Crini, 2009). The results from this study, as well as those documenting chitosan with water treatment in the literature, demonstrate that chitosan pretreatment works over a range of doses and natural surface waters and does not significantly alter pH after treatment. Unlike inorganic coagulants, there are not substantial health risks associated with over- or under-dosing water to be treated. At optimal doses, chitosan coagulation followed by ISSF improves natural surface water qualities by reducing turbidity to below the WHO GDWQ target of 1 NTU, making it safer for household consumption. Pretreating water with chitosan also enhances bacteria and virus reductions for ISSFs to meet the WHO 2-star protective targets, thereby reducing morbidity risks from drinking water. This dual barrier system produces safer drinking water compared to conventional household sand filters. Chitosan is a more appropriate POU coagulant for household use than inorganic coagulants

because of its versatility, non-toxicity and effective microbial reduction performance over a range of doses.

5.2 Performance Compared to Prior Studies of ISSFs and Polymer Coagulation of Water

Information on turbidity and microbial reductions achieved when water is treated with other natural coagulant polymers is limited. At optimal dose and grain size conditions, chitosan coagulation coupled with ISSF achieved greater turbidity and microbial reductions than those reported for *M. oleifera* and RSF, although there were clear design and operation differences between the two studies that likely contribute to these differences (Babu & Chaudhuri, 2005). In a study evaluating coagulation with *Opuntia cochenillifera* followed by ISSF, reported average turbidity and *E. coli* removal was 77% and 2.86-$\log_{10}$, respectively (Freitas & Sabogal-Paz, 2019). Based on the results of this study, chitosan appears to enhance turbidity and microbial removal to a greater extent than observed with *O. cochenillifera*; however, differences in experimental design and filter operation between the two studies make this comparison weak. Nevertheless, the proposed dual-treatment barrier of chitosan coagulation and ISSF evaluated in this study clearly demonstrates improved reduction performance for bacteria, viruses and turbidity.

In terms of chitosan efficacy specifically, the turbidity and microbial reductions observed in this study resemble those reported in the literature. According to results reported in Abebe et al. (2016), chitosan coagulation was less effective at enhancing reduction performance for ISSFs than ceramic filters. This may be due to smaller pore sizes and different flow rates between these filter technologies. Chitosan pretreatment at optimal doses with ISSFs compared to continuously-operated RSFs achieved slightly lower LRVs for *E. coli* but greatly exceeded those reported for viruses. Again, these results are not directly comparable due to different study

parameters and conditions; however, these results suggest that chitosan may also be effective with continuously-operated SSFs at the household level.

The bench-scale columns dosed with untreated water achieved microbial reductions somewhat lower than those reported in studies evaluating BSFs in optimal conditions. This is likely explained by the differences in filter design and operating parameters between this study and what is recommended by CAWST. Physical straining through the schmutzdecke and biological activity during idle times are primary mechanisms for microbial reduction in BSFs, but the operating conditions in this study did not promote these mechanisms. Chitosan as a pretreatment significantly improved ISSF performance in terms of turbidity and microbial removal. This dual treatment barrier produced better performance improvements compared to modifications of zero valent iron coatings; however, the use of iron-oxide amended sand columns reported higher LRVs for viruses over long-term use. These comparisons are tentative due to substantial differences in both filter properties and experimental design.

5.3 Possible Mechanisms of Microbial Reduction by Chitosans & ISSFs

The specific mechanisms by which microorganisms and turbidity were removed via chitosan coagulation-flocculation and slow sand filtration were not directly investigated in this research. General principles and information from the literature may provide some insight into plausible mechanistic considerations, but these explanations and interpretations are speculative and require further testing. The mechanisms by which chitosan acts as a coagulant are documented in the literature, but the interactions between the formed chitosan-colloid floc and the sand media in ISSFs are not well-characterized. The two primary coagulation processes associated with chitosan are charge neutralization and interparticle bridging (Kumar et al., 2004; Rinaudo, 2006; Soros, 2015; Soros et al., 2019). Negatively charged particles in water, including

microorganisms, clay and other inorganic and organic material, adsorb to the cationic sites on the chitosan polymer chain. These attraction forces between the polymer and particles promote coagulation-flocculation. The resulting floc, if neutralized and dense, settles out of solution via sedimentation. The supernatant water, with remaining suspended floc, is dosed into ISSFs.

The processes by which bacteria and viruses are removed with ISSFs and BSFs are likely different after water has been pretreated with chitosan. Prior research has suggested the schmutzdecke plays an important role in bacterial reductions either by physical straining or reduced flow rate resulting in enhanced depth filtration (Elliott et al., 2015; Hijnen et al., 2004; Unger & Collins, 2008). Bacteria are more amenable to physical straining than viruses because they are larger. Physical straining through the schmutzdecke has little effect on virus removal, therefore other removal or inactivation mechanisms are likely responsible for virus reductions from slow sand and biosand filtration (DeLoyde, 2007; Elliott et al., 2011; Hijnen et al., 2004). Proposed mechanisms include sorption to the granular media, attachment to biofilms, predation and biological activity (Elliott et al., 2011). With the addition of a chitosan coagulation-flocculation pretreatment step, it is unclear to what extent the importance of each mechanism changes. These mechanisms were not directly studied, but speculative mechanistic considerations are proposed based on how the processes function under regular operating conditions.

Despite weekly cleaning and disruption of the schmutzdecke, high LRVs were still observed for bacteria and viruses in this study. This suggests that the development of the schmutzdecke is not necessarily important for slow sand filtration if water is pretreated with chitosan. The impact on microbial reduction performance as a result of incorporating a diffuser plate into the bench-scale column design and altering the cleaning procedure to promote

schmutzdecke growth requires further investigation. Physical straining and deep-bed filtration may be more important mechanisms for removal when operated without the biological layer and with a coagulant, which makes the particles to be removed from water larger and easier to remove.

Previous work has highlighted the importance of net electrostatic forces between viruses and granular media. Dowd et al. (1998) found that improved removal via granular filtration is achieved for viruses with higher isoelectric points. With the introduction of a cationic polymer to influent water, it is unclear if electrostatic repulsion is enhanced or negated. The extent of such effects is also influenced by the surface charge properties of the viruses themselves, which differs among them. Aforementioned differences in isoelectric points may explain why viruses are removed to a similar extent as bacterial removal.

Many of the proposed mechanisms for virus removal are also dependent on idle time within the filter. Prior studies have shown that increased idle time within the ISSF improves microbial attenuation within the filter (Elliott et al., 2011, 2008; M. W. Jenkins et al., 2011; Stauber et al., 2006). In this study, water was pretreated with chitosan and allowed to mix and flocculate for a 30-minute period before it was dosed in the filters. The daily charge volume greatly exceeded the pore volume of the filters, and effluent samples were taken after 300 mL had already passed through the media bed. This means the collected effluent spent little time within the filter column where it would be exposed to the biological processes that enhance removal. This short contact time suggests that biological mechanisms are probably not primary mechanisms for removal. Short contact times also make this dual-treatment barrier a potentially more convenient, reliable and sustainable process than traditional BSFs.

5.4 Limitations

This research demonstrates that combining chitosan coagulation-flocculation with ISSFs improves filter performance in terms of microbial and turbidity reductions; however, there were limitations to this study which could be addressed in future research.

The design of the bench-scale column filters was very basic. This was in part due to limited resources. Filters were designed with graduated cylinders and were not equipped with an upper receptacle to maintain constant head. Manual dosing introduced variability in flow rates which was dependent on how quickly the columns were refilled to maximum head. They were not designed to meet the specifications of any existing ISSF or BSF. Maximum head and sand bed depth were determined based on available supplies for filter construction. Filter operation was also not optimized based on ISSF or BSF guidelines. Optimal conditions for schmutzdecke growth were not prioritized. Absence of the diffuser plate and weekly cleaning procedures likely disrupted any biological growth on the top of the sand media bed. Idle time within the filter was also not maximized, as is suggested in BSF operation. Idle time within the filter, which allows for biological processes to occur, accounts for much of the virus attenuation typically observed in BSF use (Elliott et al., 2011). The sand types used in this study were purposely chosen based on typical grain size ranges for BSFs and RSFs; however, the target filtration rate ranges were a compromise based on column design limitations. Filtration rates were maintained within a certain range over the course of the 57-day evaluation, but that range exceeded recommended rates for BSFs and was far below those recommended for RSFs (CAWST, 2012; Crittenden et al., 2012). Maintaining filtration rate also eliminated a common variable, decline in filtration rate over time, used as a proxy for filter maturation and media aging. Because these variables were effectively removed from the experimental design in this study, the mechanisms behind

enhanced microbial and turbidity reductions with chitosan and sand filtration are likely different than those documented for SSFs and BSFs. Due to cost, time and personnel constraints, these mechanisms were not directly evaluated.

This study did not evaluate chitosan coagulation coupled with ISSF performance in terms of removal of protozoa. For the purposes of this study, it was assumed that since protozoa are larger than bacteria and the removal technology studied was filtration, bacteria can serve as a proxy for protozoa and protozoan LRVs would likely be similar to or greater than achieved bacteria LRVs.

Variability in LRVs for bacteria, viruses and turbidity may be attributable to variable water qualities, weekly scouring procedures, column design and filter maturation rates. Differences in observed LRVs between sand media types may be due to a variety of factors including flow rates, grain sizes and inorganic composition. Without further investigation, it is not possible to determine the extent to which each of these factors had an impact on LRVs. This study did coincide with the fall turnover event at University Lake, and some variability in LRVs may be associated with changes in water quality parameters over that time. The changing lake water conditions on chitosan coagulation efficacy were not directly evaluated in this research.

Unintended consequences of using chitosan with granular media filtration systems, including accelerated filter clogging, impacts on other water quality variables such as taste and odor as aesthetic concerns and impacts on microbial communities within the filter, were not systematically evaluated in this study.

There are a few notable limitations to extending these results to field use conditions. The challenge water used was of relatively good quality because it is from a protected source water of a drinking water supply. Therefore, it is unclear how chitosan pretreatment followed by sand

filtration will perform with surface waters of poor quality. Other indicator bacteria and viruses that resemble other common pathogenic microorganisms and pathogens themselves should be used to evaluate microbial reductions. The size of the columns and daily charge volume were far smaller than sand filters used in field settings, so further studies evaluating treatment effectiveness at full-size should be conducted. Finally, it is unclear how seasonal effects, including temperature and changes in source water quality, may impact chitosan-coagulation and filter performance.

5.5 Future Work

This study marks the first effort to demonstrate improved microbial reduction performance of intermittently-operated slow sand filtration systems by using chitosan coagulation-flocculation as a water pretreatment step. Due to limited resources, only basic sand filtration systems using two different sand types were evaluated. Many different aspects of conventional slow sand filtration and biosand filtration were not directly investigated in this research. Despite the limitations of this study, the results presented here suggest that many different and promising directions can be pursued with regards to combining chitosan and slow sand filter technologies.

Future work evaluating this treatment combination should be conducted with simple sand filtration systems both at bench- and full-scale with different filter design and operation parameters. The operation and maintenance of the filters could closely resemble those used in this study, i.e. intermittently-operated, short residence times for water in the filter, and absence or limited biological removal mechanisms. Studies could evaluate different operational filtration rates for sand filters receiving waters dosed with chitosans. Reduced filtration rates may further enhance microbial removal for sand filters with chitosan pretreatment, potentially reaching 3-star

performance targets outlined by the WHO. Additionally, faster filtration rates through the media bed may still provide substantial microbial and turbidity reductions at optimal chitosan doses, which could increase the quantity of water households could treat per day. Different sand media compositions within the filter columns should also be evaluated, potentially with locally sourced materials, wider ranges of grain sizes and different inorganic compositions. Adsorption of the chitosan-colloid floc particles to naturally-sourced sand media compositions may differ compared to the high-purity silica sand used in this study due to higher concentrations of iron and aluminum in natural granite or fine sand sources (Elliott et al., 2015). It would be important to validate consistent improved performance for sand filters with chitosan in low resource settings where specific, specialized sand grain sources are not readily available. Furthermore, only one sand bed depth (16 cm) was used in this investigation. Different sand media bed depths should be studied to see if microbial removal performance of chitosan-coagulation followed by sand filtration is improved to 3-star performance targets in sand columns of greater depth. Finally, the maintenance procedure for the sand beds in this study did not result in substantial declines in contaminant removal performance. Future studies could evaluate how different filter maintenance procedures improve or reduce filter performance. These should be developed in the context of real-use-conditions with the end-user in mind to ensure the additional treatment barrier is not a burden to individuals and families.

The design and operating parameters in this investigation were not an optimal environment for the facilitation of the biological mechanisms typically employed in SSFs and BSFs. Coagulation with chitosan may improve filter performance for traditional designs of BSFs and SSFs if use conditions are optimized. Future work should specifically study influences chitosan may have on filter maturation, schmutzdecke development, decline in filtration rates

over time and development of microbial communities within BSFs. This study did not investigate how the quality of water stored within the filter during idle times between doses changes with chitosan pretreatment. Future work should evaluate how chitosan impacts microbe attenuation during idle times. Furthermore, this study did not evaluate how chitosan may impact dissolved oxygen within the filter media bed. Future research should evaluate if chitosan accelerates or decelerates the development of anoxic conditions when used with traditional BSF setups. The results of this investigation do identify a chitosan dose that consistently achieves high microbial and turbidity reductions over time with sand filtration. Any future work with BSFs should further narrow the range of effective chitosan doses with this filtration technology. The established chitosan dose should be effective over a range of influent water qualities so the suggested use conditions are not drastically different based on geographic location or water service level. An alternative to a single optimized dose for all conditions would be a stepwise, incremental dose increase based on generic source water quality indicators such as appearance and water source characteristics. This would translate to an easily adjustable coagulant dosing system based on the needs of the individual household.

Ultimately, the optimization of chitosan coagulation with POU sand filters should be household-oriented. Any proposed use conditions, dosing mechanisms or maintenance procedures should keep the end-user in mind. Chitosan as a pretreatment mechanism prior to granular media filtration is only effective if it is used consistently. The market infrastructure for BSFs and ISSFs is already globally established. The development of a chitosan coagulation POU treatment product should be studied and optimized in a user-focused manner, based on ease-of-use, low-costs and sustainable adoption. Keeping operating parameters for BSFs and

SSFs simple, despite the additional chitosan pretreatment step, should be an important consideration when designing further investigations with this technology.

CHAPTER 6: SUMMARY AND CONCLUSIONS

This study reports the first results evaluating the effectiveness of using chitosans as a coagulation-flocculation pretreatment in natural waters to improve the removal capacity of bacteria, viruses and turbidity by intermittently-operated slow sand filtration. Extensive reductions of bacteria, viruses and turbidity were achieved by sand columns dosed with 10 mg/L and 30 mg/L chitosan-pretreated water. Sand columns dosed with water treated with 10 mg/L met the protective performance targets specified by the WHO for HWT technologies. Filter performance varied over time, possibly due to scouring procedures, variable source water quality and inconsistent flow rates.

These results were observed in simply-designed, intermittently-operated, falling-head sand filter setups. Variables such as filter media characteristics, filtration rate, microbial communities, source water quality and mechanisms for microbial reduction were not directly investigated in this study. Despite these limitations and further research questions, this study demonstrates that intermittently-operated slow sand filtration can be significantly improved using chitosan as a coagulant-flocculant pretreatment step. The chitosan coagulation-flocculation pretreatment step should be further optimized with granular filtration technologies to improve these POU systems.

APPENDIX 1: ACCUSAND U.S. SIEVE ANALYSIS PROVIDED BY UNIMIN CORPORATION

Sieve Size (mm) mm	Sieve Size, U.S. Sieves (mesh) mesh	Accusand 30/40	Accusand 40/60	Accusand 50/70
0.84	20	0	0	0
0.59	30	0.6	0	0
0.5	35	56.1	0	0
0.42	40	42.5	0	0
0.345	45	0.8	2.7	0.1
0.3	50	0.1	80.4	1
0.25	60	0	15.8	57.4
0.21	70	0	1.1	37.7
0.149	100	0	0	3.8
AFS GFN		30.1	41.7	50.7

APPENDIX 2: FULL CHARACTERIZATION OF SILICA SAND MEDIA CONDUCTED BY PENNONI ASSOCIATES INC. AND PROVIDED BY OWASA

JONES FERRY ROAD WATER TREATMENT PLANT
FILTER MEDIA REPLACEMENT AND BACKWASH IMPROVEMENTS
CARRBORO, NC

To: Kate Keenan, PE
Hazen and Sawyer
4011 WestChase Blvd, Suite 500
Raleigh, NC 27607

From: Dean Kite
Dellinger Inc.
2631 Old Charlotte Hwy.
Monroe, NC 28110

☐ Approved
☐ Approved as noted
☐ Not approved, revise and resubmit
☐ FIELD USE

We have checked the shop drawings for conformance to the contract specifications and drawings. Our approval does not relieve supplier or material manufacturer from their responsibility to comply with the contract specifications. Furthermore, our approval does not approve or disapprove the design concepts used by the Engineer.

DELLINGER, INC.
By : Dean Kite
Date : Sep 13, 2017
Contract No. CIP No. 272-40

Submittal #: 001a - SUPPLEMENT
Specification Section: 13410 – Filter Media
Items Submitted: Filter Media – Anthracite and Filter Sand (Certified Testing Reports)

Please review and return by September 15, 2017

NOTE TO ENGINEER:

1. The attached are the certified test reports for the Anthracite and Filter Sand

SAND

Pennoni
PENNONI ASSOCIATES INC.
CONSULTING ENGINEERS

Project No.: FBLP-1720

Report Date: September 13, 2017

Client: Norma Kerner
Address: F. B. Leopold - Xylem
227 S. Division St.
Zelienople, PA 16063

Project Name: Carrboro NC - #815428

Date Received: September 7, 2017

Date Sampled: September 6, 2017

Unit Specification: AWWA B100

Unit Designation and Description: Silica Sand - Lot 1R

Laboratory Number:10- 153428

Summary of Test Results

AWWA B100 - Acid and Caustic Solubilty

Acid Solubilty: 0.1% [5% max.]

Caustic Solubilty: _____

AWWA B100- Mohs Hardness

Moh's Hardness: 6.5

ASTM C1252 - Uncompacted Voids

Uncompacted Void %: _____

ASTM C127 - Specific Gravity of Coarse Aggregate

Bulk Specific Gravity (Dry): _____
Bulk Specific Gravity (SSD): _____
Apparent Specific Gravity: _____
% Absorption: _____

ASTM C128 - Specific Gravity of Fine Aggregate

Bulk Specific Gravity (Dry): 2.605
Bulk Specific Gravity (SSD): 2.620
Apparent Specific Gravity: 2.645 [~2.65]
% Absorption: 0.57

ASTM C136 - Particle Size Distribution

Sieve Size	Percent Retained	Percent Passing
#12	0.0	100.0
#14	0.0	100.0
#16	0.0	100.0
#18	1.8	98.2
#20	10.7	87.5
#25	30.6	56.9
#30	32.5	24.4
#35	13.2	11.2
#40	6.7	4.6
#45	2.7	1.9
#50	1.1	0.8

ASTM C40 - Organic Impurities in Fine Aggregate

Color Plate No.: -

Fineness Modulus: N/A

ASTM C123 - Lightweight Pieces in Aggregate

Lightweight Pieces (% by mass) _____

ASTM C117 - Material Finer than the No. 200 Sieve by Washing

% Passing: _____

ASTM C142 - Clay Lumps & Friable Particles

Percent Clay Lumps & Friable Particles: _____

ASTM C29 - Bulk Density and Voids (Porosity)

Bulk Density. (pcf): _____

Void Content (%): _____

Effective Size (mm) 0.50 [0.45-0.55]
Uniformity Coefficient 1.40 [1.40 max.]
D10 (mm) 0.50
D60 (mm) 0.71
D90 (mm) 0.90

ASTM D2974 - Loss On Ignition

Loss on Ignition (%): 0.05

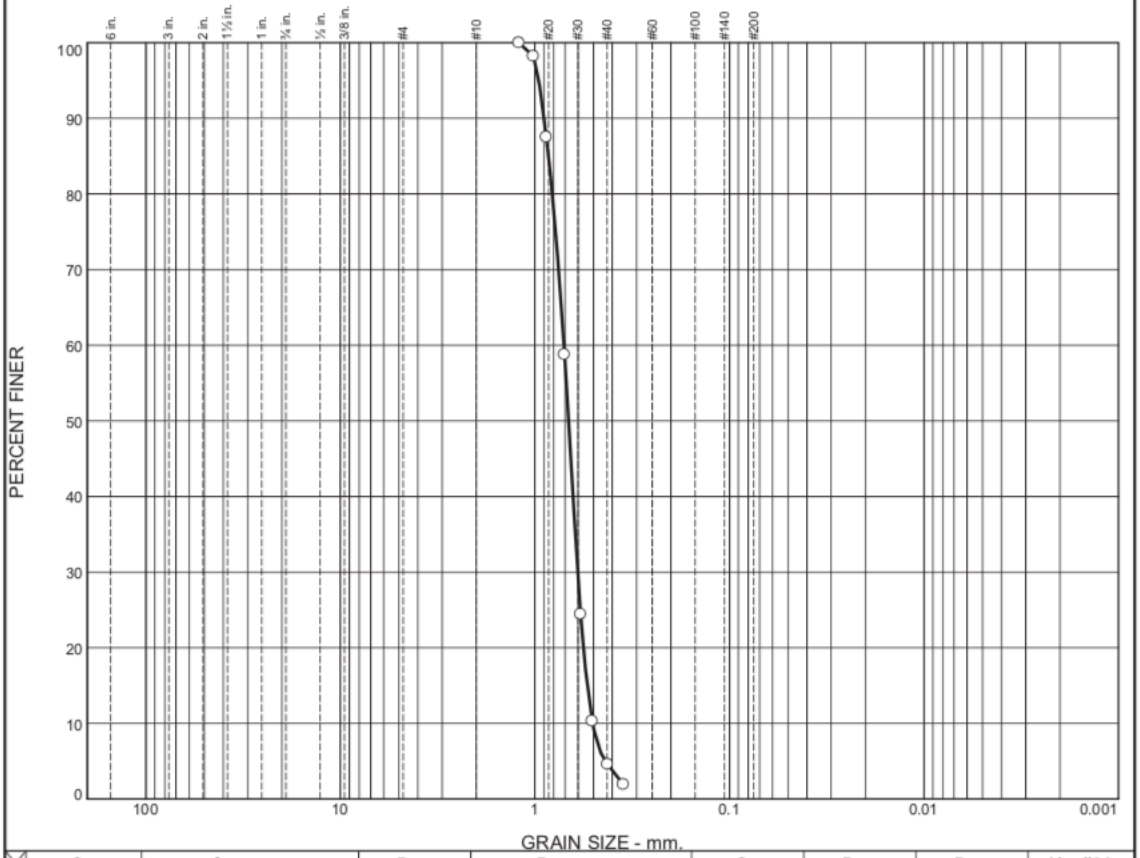
Remarks: The above sample was tested in accordance with the highlighted methods. This sample meets the requirements of the provided specification.

Chas M. Snyder, PE
Laboratory Manager

2041 Avenue C • Suite 100 • Bethlehem, PA 18017 • Tel: 610•231•0600 • Fax: 610•231•2033
www.pennoni.com

AAP AASHTO R18

Particle Size Distribution Report



	C_u	C_u spec.	D_{10}	D_{10} spec.	C_u	D_{10}	D_{60}	% - #30
○	1.40	0-1.4	0.5047	0.45-0.55	In	In	0.7090	29.2

Material and Supplier

○ Silica Sand, sampled 9/06/2017, received in lab 9/07/2017; Loss on Ignition (%) = 0.05

Project No. FBLP-1720 **Client:** Loepold / Xylem

Project: Carrboro NC - #815428

○ **Source of Sample:** Southern Products **Sample Number:** Silica Sand - Lot 1R

PENNONI ASSOCIATES INC.

Remarks:

○Specific Gravity (Dry) = 2.605
Specific Gravity (SSD) = 2.620
Specific Gravity (App) = 2.645
Absorption (%) = 0.57
Acid Solubility (% Loss) = 0.1
Moh's Hardness > 6.5

Figure 153428

Tested By: J McCarthy **Checked By:** C M Snyder PE

APPENDIX 3: PARTICLE SIZE ANALYSIS OF SILICA SAND MEDIA CONDUCTED BY TRIMAT MATERIALS TESTING, INC. AND PROVIDED BY OWASA

Hazen

Job:	Filter Media Replacement and Backwash Improvements
Location:	Jones Ferry Road Water Treatment Plant, Carrboro, NC
H&S Job No.:	32258-005
Specification No.:	13410
Equipment:	Leopold Inspection Reports
Submittal No.:	**001B**
Reviewers:	**Kate Keenan**
Date:	February 16, 2018
Review Status:	For Information Only

REVIEW COMMENTS:

Checking of shop drawing is limited to general design and general arrangement only and is not intended to be a verification of compliance with all requirements. Approval shall not relieve the Contractor from the responsibility of details of design, correct dimensions for proper fitting, coordination with other performance, or any other requirement of the Contract.

HAZEN AND SAWYER

JONES FERRY ROAD WATER TREATMENT PLANT
FILTER MEDIA REPLACEMENT AND BACKWASH IMPROVEMENTS
CARRBORO, NC

☒ Approved
☐ Approved as noted
☐ Not approved, revise and resubmit
☐ FIELD USE

We have checked the shop drawings for conformance to the contract specifications and drawings. Our approval does not relieve supplier or material manufacturer from their responsibility to comply with the contract specifications. Furthermore, our approval does not approve or disapprove the design concepts used by the Engineer.

DELLINGER, INC.
By : Dean Kite
Date : Feb 8, 2018
Contract No. CIP No. 272-40

To: Kate Keenan, PE
Hazen and Sawyer
4011 WestChase Blvd, Suite 500
Raleigh, NC 27607

From: Dean Kite
Dellinger Inc.
2631 Old Charlotte Hwy.
Monroe, NC 28110

Submittal #: 001b - SUPPLEMENT

Specification Section: 13410 – Filter Media
Items Submitted: Filter Media – Anthracite and Filter Sand (3rd Party Testing)
Leopold Filter Inspection Reports (Basins 1 thru 5)

Please review and return by February 23, 2018

NOTE TO ENGINEER:

1. Additional reports will be submitted for the 3rd party testing of the anthracite and filter sand as well as the Leopold Inspection Reports as they come available

October 6, 2017

Mr. Bryan Wheeler
EW2 Environmental

Re.: Jones Ferry Sand and Anthracite Lab Testing
Trimat Project # 17-1039-14

Mr. Wheeler,

As per your request, Trimat has completed the laboratory testing on the three buckets of material delivered by you to our lab on October 4th, 2017. Two buckets of silica sand and one bucket of anthracite was received from EW2 Environmental. The sand and anthracite buckets were from Filter number 5 of the Jones Ferry water treatment plant location. The materials were tested for their suitability for use as a granular filter material. The following standard tests were done on the sand and anthracite material as per the AWWA standard for granular filler material (AWWA B100-01) –

1. Acid Solubility Test for Sand and Anthracite
2. Specific Gravity Test for Sand and Anthracite (ASTM C128)
3. Sieve Analysis Test for Sand and Anthracite (ASTM C136)
4. Mohs Hardness Test for Anthracite

The test procedures in Section 5.3 of the AWWA B100-01 standard were followed for the Acid Solubility Test and Mohs Hardness Test. The Specific Gravity Test and Sieve Analysis Test were done as per their respective standards, ASTM C128 and ASTM C136. For Sieve analysis tests, the sieves were chosen as per the guidelines in section 5.3.4.3 of the AWWA B100-01 standard. Table 1 shows a matrix of the Tests performed.

Table 1. Matrix of the Tests Performed

Material	Tests	No. of Samples
Anthracite - Bag 3	Acid Solubility	2
	Specific Gravity	1
	Sieve Analysis	1
	Mohs Hardness Test	15
Sand - Bag 2	Acid Solubility	2
	Specific Gravity	1
	Sieve Analysis	1
Sand - Bag 10	Acid Solubility	2
	Specific Gravity	1
	Sieve Analysis	1

TRIMAT Materials Testing, Inc. 5900 Triangle Drive, Raleigh, NC 27617 • tel: 919.532.2211 • fax: 919.532.2212

The results of the testing are provided in Table 2. Individual test results for the gradation and specific gravity tests are attached. As observed in the results, the anthracite and sand from both bags passed the specified limits for Acid Solubility, Apparent Specific Gravity, Effective Size, Uniformity Coefficient, and Mohs Hardness.

Table 2. Results of the Testing on the Granular Filter Material

Filter Material		Anthracite - Bag 3	Sand - Bag 2	Sand - Bag 10
Acid Solubility (Average)	Result (%)	1.5	0.4	0.3
	Limit (%)	≤5	≤5	≤5
Acid Solubility Sample Variation	Result (%)	0.00	0.30	0.10
	Limit (%)	≤2.6	≤2.6	≤2.9
Apparent Specific Gravity	Result	1.681	2.547	2.535
	Limit	≥1.4	≥2.5	≥2.5
Effective Size	Result (mm)	1.20	0.61	0.60
	Limit (mm)	0.6 - 1.6 mm	0.35 - 0.65 mm	0.35 - 0.65 mm
Uniformity Coefficient	Result	1.4	1.3	1.4
	Limit	≤1.7	≤1.7	≤1.7
Mohs Hardness(average)	Result	2.9	N/A	N/A
	Limit	≥2.8	N/A	N/A

Note – 1. The limit for acid solubility sample variation was calculated as the 2% of the average of the averaged total sample weight as per AWWA B100-01 section 5.3.1.2.

Note – 2. Mohs Hardness test is only required for Anthracite.

Thank you for the opportunity to provide you with lab testing services and if you have any questions regarding the attached information, please feel free to contact us.

Thank you.

Sincerely,

Trimat Materials Testing, Inc.

K. Abhilash

Abhilash Kusam, Phd
Materials Engineer

10/17/17

Christopher Bacchi, PE
Vice President

Attachments

Trimat Materials Testing, Inc.

Particle Size Analysis of Soils

AASHTO T11

Project #: 17-1039-14 Report Date: 10/17/2017

Project Name: EW2 Environmental Sand Testing Test Date(s): 10/12/2017

Client Name: EW2 Received Date: 10/4/2017

Client Address:

Sample #: 5903 Sampled By: client Depth (ft):

Location:

Sample Description:

References: AASHTO Standards:

M92 Wire-Cloth Sieves for Testing Purposes
M231 Weighing Devices Used in the Testing of Materials
T2 Sampling of Aggregates
T27 Sieve Analysis of Fine and Course Aggregates
T248 Reducing Samples of Aggregate to Testing Size

	Particle Size Analysis / Without Hydrometer Analysis			Moisture Content	Natural
	Tare Number	I		Tare #	
A	Tare Weight	138.5	A	Tare Weight	
B	Total Sample Dry Wt. + Tare Wt.	257.1	B	Wet Weight + Tare Wt.	
C	Total Sample Dry Weight (B-A)	118.6	C	Dry Weight + Tare Wt.	
D	After Wash Weight + Tare	256.5	D	Water Wt. (B-C)	
E	Total Sample Wt. After #200 Wash	118.0	E	Dry Wt.(C-A)	
	Percent Passing #200 (1-E/C)x100	0.5%		Moisture Content (100 x D/E) (%)	

Sieve Size (mm)	Sieve Size	Retained Weight	Percent Retained	**Percent Passing Total Sample**
2.36	#8	0.0	0%	100%
2.00	#10	0.0	0%	100%
1.18	#16	0.1	0%	100%
1.000	#18	5.1	4%	96%
0.85	#20	41.9	35%	65%
0.60	#30	109.0	91.9%	8%
0.300	#50	117.9	99.4%	**0.6%**

Notes:

Reviewed by:

Technician: Brett S Junker 50740

Printed Name *Certificate #* *Signature*

TMT206 Jul-16

Trimat Materials Testing, Inc.

Particle Size Analysis of Soils

AASHTO T11

Project #: 17-1039-14 Report Date: 10/17/2017

Project Name: EW2 Environmental Sand Testing Test Date(s): 10/12/2017

Client Name: EW2 Received Date: 10/4/2017

Client Address:

Sample #: 5905 Sampled By: client Depth (ft):

Location:

Sample Description:

References: AASHTO Standards:

M92 Wire-Cloth Sieves for Testing Purposes
M231 Weighing Devices Used in the Testing of Materials
T2 Sampling of Aggregates
T27 Sieve Analysis of Fine and Course Aggregates
T248 Reducing Samples of Aggregate to Testing Size

	Particle Size Analysis / Without Hydrometer Analysis			Moisture Content	Natural
	Tare Number	J		Tare #	
A	Tare Weight	136.6	A	Tare Weight	
B	Total Sample Dry Wt. + Tare Wt.	241.1	B	Wet Weight + Tare Wt.	
C	Total Sample Dry Weight (B-A)	104.5	C	Dry Weight + Tare Wt.	
D	After Wash Weight + Tare	240.9	D	Water Wt. (B-C)	
E	Total Sample Wt. After #200 Wash	104.3	E	Dry Wt.(C-A)	
	Percent Passing #200 (1-E/C)x100	0.2%		Moisture Content (100 x D/E) (%)	

Sieve Size (mm)	Sieve Size	Retained Weight	Percent Retained	**Percent Passing Total Sample**
2.36	#8	0.0	0%	100%
2.00	#10	0.0	0%	100%
1.18	#16	0.2	0%	100%
1.000	#18	10.1	10%	90%
0.85	#20	40.3	39%	61%
0.60	#30	95.8	91.7%	8%
0.300	#50	104.2	99.7%	**0.3%**

Notes:

Reviewed by:

Technician: Brett S Junker 50740

Printed Name *Certificate #* *Signature*

Trimat Materials Testing, Inc.

Specific Gravity and Absorption of Fine Aggregate

AASHTO T 84

Project #:	17-1039-14	Report Date:	10/17/2017
Project Name:	EW2 Lab Testing	Test Date(s):	10/13/2017
Client Name:	EW2	Received Date:	10/4/2017
Client Address:			

References:

AASHTO T85:	Specific Gravity and Absorption of Coarse Aggregate
AASHTO T19:	Bulk Density "Unit Weight" and Voids in Aggregate
AASHTO T255:	Total Evaporable Moisture Content of Aggregate by Drying
AASHTO T133:	Density of Hydraulic Cement
AASHTO T248:	Reducing Samples of Aggregate to Testing Size
AASHTO T2:	Sampling of Aggregates

Lab #: 5903 Material: Sand Bag 2 Sample Date:

Source: Sample #: 2 Sampled By: client

Flask #:		A		
Flask Wt.:	A	10	g	
Flask & SSD Wt.:	B	514.7	g	
SSD Wt.:	B - A = C	504.7	(0.1g)	
Flask, SSD & Water Wt.:	D	989.6	g	
Flask & Water Wt.:	E	683.5	g	
Wet Wt.:	D - E = F	306.1	(0.1g)	
Aggregate & Pan Wt.:	G	504.0	g	
Pan Tared Wt.:	H	0	g	
Oven Dried Wt.:	G - H = I	504.0	(0.1g)	

Bulk Specific Gravity:
I / (C - F) 2.538 (0.001)

Bulk Specific Gravity (SSD):
C / (C - F) 2.541 (0.001)

Apparent Specific Gravity:
I / (I - F) 2.547 (0.001)

Percent Absorption:
((C - I) / I) x 100 0.14% (0.1%)

C - F = 198.6 I - F = 197.9 C - I = 0.7

Remarks:

Technician: Brett S Junker (*Printed Name*) 50740 (*Certification #*) (*Signature*)

Trimat Materials Testing, Inc.

Specific Gravity and Absorption of Fine Aggregate

AASHTO T 84

Project #:	17-1039-14	Report Date:	10/17/2017
Project Name:	EW2 Lab Testing	Test Date(s):	10/13/2017
Client Name:	EW2	Received Date:	10/4/2017
Client Address:			

References:

AASHTO T85:	Specific Gravity and Absorption of Coarse Aggregate
AASHTO T19:	Bulk Density "Unit Weight" and Voids in Aggregate
AASHTO T255:	Total Evaporable Moisture Content of Aggregate by Drying
AASHTO T133:	Density of Hydraulic Cement
AASHTO T248:	Reducing Samples of Aggregate to Testing Size
AASHTO T2:	Sampling of Aggregates

Lab #: 5905 Material: Sand Bag 10 Sample Date:

Source: Sample #: 10 Sampled By: client

Flask #:		A		**Bulk Specific Gravity:**	
				I / (C - F)	2.523 (0.001)
Flask Wt.:	A	10	g		
Flask & SSD Wt.:	B	514.5	g	**Bulk Specific Gravity (SSD):**	
SSD Wt.:	B - A = C	504.5	(0.1g)	C / (C - F)	2.528 (0.001)
Flask, SSD & Water Wt.:	D	988	g	**Apparent Specific Gravity:**	
Flask & Water Wt.:	E	683.1	g	I / (I - F)	2.535 (0.001)
Wet Wt.:	D - E = F	304.9	(0.1g)		
				Percent Absorption:	
Aggregate & Pan Wt.:	G	503.5	g	((C - I) / I) x 100	0.20% (0.1%)
Pan Tared Wt.:	H	0	g		
Oven Dried Wt.:	G - H = I	503.5	(0.1g)		

C - F = 199.6 I - F = 198.6 C - I = 1

Remarks:

Technician: Brett S Junker (Printed Name) 50740 (Certification #) (Signature)

APPENDIX 4: CERTIFICATE OF ANALYSIS FOR FOOD GRADE CHITOSAN ACETATE PROVIDED BY SARCHEM LABORATORIES

Sarchem Laboratories, Inc

5012 INDUSTRIAL ROAD
FARMINGDALE, NJ 07727
P: 732 938 2777
F: 732 938 3777
INFO@SARCHEMLABS.COM
WWW.SARCHEMLABS.COM

CERTIFICATE OF ANALYSIS

Chemical Name	Chitosan Acetate			**PRODUCT #:**	S1247
CAS No:	9012-76-4	**Manufacture Date:**	Dec. 28, 2017	**Report Date:** Dec. 29, 2017	**Exp. Date:** Dec. 28, 2019
Batch #:	**SL-3308**				

	Items	Specifications	Results
Product Properties	Deacetylation	≥85%	90.3%
	Appearance	White or light yellow powder	Complies
	Odor and taste	Characteristic	Complies
	pH	3-6	4.2
	Viscosity cps	10-100	17
	Insolubles%	≤1	0.3
	Density g/ml	0.28	0.302
	Ash%	≤1	0.72
	Moisture%	≤10	8.7
	Mesh Size mesh	80 (95% pass)	Complies
	Heavy Metals ppm	≤10	Complies
	As ppm	≤0.5	Complies
	Total plate count cfu/g	≤100	Complies
	Yeast & Mold cfu/g	≤10	Complies
	E.Coli cfu/g	Negative	Negative
	Salmonella cfu/g	Negative	Negative

Approved By:	S. Kumar	02.02.18

Any claims, adjustments or returns must be made within 30 days.

APPENDIX 5: *E. COLI* KO11 LRVS FOR ALL FILTERS OVER 57-DAY OPERATING TIME

E. coli KO11 LRVs																		
Duplicate Filter #	Sand Type	Dose (mg/L)	Filter Operating Time (indicates specific days of sampling over the 57-day experiment)															
			1	6	9	13	15	20	23	27	29	34	36	41	43	50	52	55	57
1	Accusand	0	0.09	0.68	0.00	0.16	0.07	0.22	0.33	0.27	0.21	0.31	0.46	0.72	0.61	0.62	0.46	1.11	0.82
2	Accusand	0	0.22	0.41	0.06	0.06	0.19	0.24	0.30	0.13	0.30	0.22	0.40	0.53	0.60	0.98	0.46	1.07	0.91
1	Accusand	3	0.30	0.36	0.57	0.68	0.85	0.96	0.64	0.16	0.50	0.28	0.28	1.24	0.99	1.78	2.54	2.39	2.70
2	Accusand	3	0.29	0.60	0.49	0.77	0.87	0.98	0.67	0.27	0.37	0.26	0.15	1.13	0.98	1.68	2.34	2.41	2.55
1	Accusand	10	5.06	5.49	4.72	4.99	5.37	5.31	5.60	3.06	3.31	3.05	3.05	5.14	5.58	4.81	4.98	5.83	4.86
2	Accusand	10	4.95	4.82	4.19	4.75	5.37	5.46	5.46	3.26	3.48	2.60	2.99	5.58	5.88	5.22	6.03	5.39	5.27
1	Accusand	30	1.20	0.31	0.33	1.10	0.53	1.27	2.64	4.91	5.21	5.70	6.03	6.43	6.52	5.80	6.39	5.89	4.84
2	Accusand	30	1.19	0.30	0.38	1.66	0.50	4.66	4.98	4.51	5.87	6.18	6.03	6.43	6.22	5.15	5.69	6.49	4.98
1	silica	0	0.06	0.13	-0.03	-0.02	0.09	0.12	0.18	0.23	0.06	0.40	0.12	0.23	0.35	-0.15	0.30	0.41	0.15
2	silica	0	0.13	0.13	-0.02	-0.14	0.16	-0.02	0.17	0.15	0.02	0.19	0.13	0.34	0.41	0.13	0.62	0.43	0.11
1	silica	3	0.37	0.53	0.19	0.51	0.80	0.73	0.41	0.13	0.23	0.40	0.21	0.99	0.56	3.88	3.32	2.31	2.62
2	silica	3	0.28	0.32	0.27	0.55	0.82	0.99	0.29	0.18	0.16	0.27	0.10	0.69	0.55	3.96	3.20	2.59	2.56
1	silica	10	4.14	4.49	3.95	3.93	5.22	4.65	4.68	2.89	3.81	2.92	3.07	5.14	5.18	2.89	3.71	3.94	3.02
2	silica	10	3.90	4.06	4.03	3.72	4.74	4.36	4.71	2.60	3.15	2.92	3.07	5.18	5.07	3.12	3.70	3.65	3.04
1	silica	30	0.54	0.46	0.31	0.43	0.68	1.24	0.72	0.93	1.06	1.00	1.85	3.09	3.75	2.28	3.28	3.28	2.68
2	silica	30	0.35	0.27	0.44	1.82	1.22	3.32	3.10	2.88	4.10	1.28	3.01	1.15	2.23	3.02	4.79	3.51	2.91

APPENDIX 6: MS2 COLIPHAGE LRVS FOR ALL FILTERS OVER 57-DAY OPERATING TIME

MS2 Coliphage LRVs																			
Duplicate Filter #	Sand Type	Dose (mg/L)	Filter Operating Time (indicates specific days of sampling over the 57-day experiment)																
			1	6	9	13	15	20	23	27	29	34	36	41	43	50	52	55	57
1	Accusand	0	0.27	0.09	-0.25	-0.23	0.52	0.09	0.15	0.20	0.32	-0.11	0.09	0.17	0.07	1.95	0.70	1.11	1.01
2	Accusand	0	0.64	-0.16	-0.26	-0.02	0.32	0.09	0.07	0.00	0.28	-0.20	0.11	-0.01	0.00	1.89	0.51	0.80	1.06
1	Accusand	3	0.41	0.92	0.26	1.13	1.96	1.65	0.82	0.51	0.60	0.25	0.27	1.38	1.61	2.73	3.96	3.59	3.51
2	Accusand	3	0.47	0.94	0.90	0.99	1.88	1.75	0.77	0.63	0.70	0.42	0.22	1.71	1.96	2.79	4.00	2.98	3.89
1	Accusand	10	4.07	5.98	5.33	4.54	5.17	5.18	5.56	3.43	4.21	4.10	3.78	3.26	3.64	4.33	4.03	4.92	3.94
2	Accusand	10	3.77	5.28	4.50	4.39	4.95	5.23	5.24	3.37	4.31	4.56	4.03	3.54	3.97	4.60	4.34	4.28	3.65
1	Accusand	30	2.48	1.46	1.38	3.17	2.91	3.10	4.04	4.12	4.60	4.71	4.85	5.22	5.05	4.64	4.86	5.33	4.03
2	Accusand	30	3.80	1.53	1.87	3.58	2.91	3.91	4.45	4.67	5.07	4.72	5.08	5.55	5.12	4.77	5.08	4.85	4.29
1	silica	0	-0.19	0.07	-0.55	0.07	0.94	0.62	0.27	0.35	0.67	-0.13	0.35	0.40	0.33	0.80	0.65	0.99	0.35
2	silica	0	0.35	0.56	-0.80	0.13	0.65	0.50	0.27	0.26	0.28	-0.35	0.27	0.25	0.05	0.39	0.54	0.30	0.28
1	silica	3	0.73	0.12	-0.55	1.10	1.37	1.23	0.85	0.40	0.28	0.81	0.08	1.15	0.96	2.52	2.75	2.63	2.44
2	silica	3	0.52	0.36	-0.16	1.13	1.75	1.33	1.01	0.47	0.49	0.30	0.10	1.16	1.25	2.54	2.61	2.22	2.72
1	silica	10	3.26	3.88	4.73	4.28	5.11	4.92	4.60	3.80	3.49	3.57	3.29	3.46	3.58	4.36	3.92	3.59	3.36
2	silica	10	3.60	4.08	4.55	4.07	5.04	4.60	4.35	3.42	2.93	3.18	2.99	2.43	2.61	4.93	4.24	3.57	3.25
1	silica	30	1.43	1.42	1.23	2.55	2.50	2.72	2.78	2.20	2.01	1.66	2.21	2.96	3.50	2.55	2.54	3.12	2.19
2	silica	30	2.50	1.45	1.63	2.81	2.93	4.13	4.38	3.66	3.06	2.20	2.96	2.66	3.50	3.68	3.92	4.13	3.01

APPENDIX 7: TURBIDITY LRVS FOR ALL FILTERS OVER 57-DAY OPERATING TIME

Turbidity LRVs																		
Duplicate Filter #	Sand Type	Dose (mg/L)	Filter Operating Time (indicates specific days of sampling over the 57-day experiment)															
			1	6	9	13	15	20	23	27	29	34	36	41	43	50	52	55	57
1	Accusand	0	0.18	-0.68	-0.03	-0.13	0.18	0.18	0.34	-0.24	-0.83	0.19	0.13	-0.04	-0.01	0.30	0.33	0.38	0.32
2	Accusand	0	0.28	0.34	-0.03	0.09	0.36	0.28	0.62	-0.01	-0.09	0.24	0.15	0.12	0.04	0.26	0.31	0.35	0.36
1	Accusand	3	0.62	0.16	0.49	1.02	0.65	0.62	0.52	0.34	0.13	0.32	0.23	0.89	0.81	1.25	1.33	1.07	1.15
2	Accusand	3	0.41	0.36	0.46	0.55	0.52	0.41	0.48	0.18	0.17	0.37	0.04	0.80	0.84	1.06	1.25	1.02	1.05
1	Accusand	10	0.70	0.22	0.56	0.88	0.88	0.70	0.84	1.37	1.15	1.14	1.17	1.27	1.02	1.54	1.16	1.10	1.13
2	Accusand	10	0.62	0.77	0.72	0.73	0.81	0.62	0.68	1.34	1.26	1.35	1.30	1.37	1.22	1.58	1.41	1.00	1.27
1	Accusand	30	0.62	0.25	0.06	0.47	-0.03	0.62	0.59	1.11	1.05	1.15	1.23	0.75	0.73	0.85	1.21	0.60	0.74
2	Accusand	30	0.38	0.28	0.19	0.31	0.11	0.38	0.27	1.04	1.05	1.07	1.18	1.02	0.71	1.12	1.12	1.05	0.96
1	silica	0	-0.25	-0.02	-0.10	0.06	0.16	-0.25	0.26	-0.05	-0.12	0.08	0.05	0.03	0.02	0.22	0.24	0.24	0.17
2	silica	0	-0.14	-0.19	-0.03	-0.24	0.28	-0.14	0.39	-0.18	0.00	0.10	0.01	0.02	-0.05	0.19	0.22	0.21	0.19
1	silica	3	0.46	-0.11	0.25	0.58	0.33	0.46	0.10	0.15	0.20	0.22	0.18	0.44	0.70	1.10	1.06	1.02	1.00
2	silica	3	0.60	0.03	0.24	0.53	0.41	0.60	0.19	0.17	0.20	0.24	0.21	0.75	0.72	0.90	1.06	0.83	0.93
1	silica	10	0.68	0.58	0.43	0.51	0.63	0.68	0.65	1.40	1.39	1.10	1.07	0.33	0.84	1.32	1.40	0.89	1.03
2	silica	10	0.75	0.43	0.52	0.35	0.71	0.75	0.66	1.26	1.20	1.28	1.09	1.18	0.85	1.49	1.32	1.02	1.24
1	silica	30	0.62	-0.23	-0.12	0.17	0.12	0.62	0.32	0.98	0.86	0.76	1.19	0.46	0.63	0.88	0.81	0.75	0.54
2	silica	30	0.64	-0.29	-0.38	0.09	0.39	0.64	0.51	0.97	1.16	0.78	0.94	0.93	0.91	0.92	1.05	0.71	0.52

APPENDIX 8: PERCENT DECREASE IN pH FOR ALL FILTERS OVER 57-DAY OPERATING TIME

Percent Decrease in pH, influent to effluent (%)																		
Duplicate Filter #	Sand Type	Dose (mg/L)	Filter Operating Time (indicates specific days of sampling over the 57-day experiment)															
			1	6	9	13	15	20	23	27	29	34	36	41	43	50	52	55	57
1	Accusand	0	-5.85	0.14	-4.30	7.11	2.38	1.52	12.18	-5.79	6.01	1.10	-1.27	-22.06	3.15	-1.21	0.83	-0.27	2.51
2	Accusand	0	-6.26	-1.68	-5.06	1.98	1.83	-2.00	8.79	0.79	6.63	0.83	-0.28	-10.83	-1.94	-0.54	1.11	0.80	1.86
1	Accusand	3	-1.48	-6.05	-9.41	-7.41	5.33	-3.18	8.33	0.95	8.30	-1.25	-2.90	-10.22	7.03	-2.72	-3.85	-0.27	3.62
2	Accusand	3	-1.73	2.90	-8.46	-6.64	-1.17	0.14	8.46	-2.37	7.32	-1.39	0.53	-10.85	8.27	4.90	0.55	-0.41	3.62
1	Accusand	10	0.75	0.54	-4.94	1.14	-6.07	2.86	5.38	-1.68	5.94	-1.79	-1.39	-15.85	11.62	4.17	-0.69	0.00	1.72
2	Accusand	10	2.34	6.53	-4.65	2.39	-6.37	-2.00	6.03	0.82	6.32	-3.21	-1.39	-5.70	13.91	3.54	-0.41	0.41	0.67
1	Accusand	30	-0.37	-0.82	-5.71	2.56	5.04	-0.28	-1.80	-4.89	5.77	0.55	-0.98	-1.21	4.17	1.97	-0.41	1.62	1.73
2	Accusand	30	-1.51	1.21	-5.71	3.93	-5.60	2.86	5.63	-4.31	5.90	1.09	-1.84	-14.79	3.42	1.06	0.41	1.35	2.51
1	silica	0	-2.09	0.41	-5.67	-0.87	-2.65	-5.32	4.99	-3.03	5.88	2.83	5.91	-1.91	8.56	-2.32	1.92	-2.06	4.52
2	silica	0	-3.87	-9.02	-6.13	5.98	4.78	-0.14	8.30	-4.46	6.63	0.14	5.03	-10.01	7.06	-6.68	1.38	-0.13	5.74
1	silica	3	-5.94	6.00	-2.02	-0.14	8.94	3.26	12.00	-0.55	3.33	-0.83	-6.59	-7.28	7.90	4.14	-1.96	-1.80	-0.13
2	silica	3	-1.23	-0.27	-2.86	-7.26	3.35	-0.14	11.76	1.21	3.73	-2.10	-2.76	-0.79	6.39	3.75	-1.39	-0.27	0.80
1	silica	10	1.12	6.41	-1.09	0.00	-6.37	1.25	3.52	0.14	3.86	-2.35	-1.53	-30.36	8.94	-0.14	1.88	-1.80	4.25
2	silica	10	1.73	4.95	-2.20	6.21	-2.60	3.52	5.90	0.41	4.26	-2.21	-2.39	-17.47	10.42	0.67	2.01	-1.38	2.23
1	silica	30	0.98	4.54	-7.39	1.01	2.19	3.39	-2.50	-3.87	3.44	2.43	0.55	-1.76	0.27	-2.47	-0.83	-0.69	3.28
2	silica	30	-0.50	-0.96	-11.43	8.31	-1.42	2.99	-1.94	-4.60	4.36	-0.42	2.05	-22.24	-4.85	-1.63	0.27	2.27	1.20

APPENDIX 9: FILTRATION RATE FOR ALL FILTERS OVER 57-DAY OPERATING TIME

Filtration Rate (m/hr)																		
Duplicate Filter #	Sand Type	Dose (mg/L)	Filter Operating Time (indicates specific days of sampling over the 57-day experiment)															
			1	9	13	15	20	23	27	29	34	36	41	43	50	52	55	57
1	Accusand	0	0.75	0.45	0.58	0.46	0.48	0.41	0.35	0.53	0.58	0.36	0.66	0.62	0.53	0.64	0.74	0.68
2	Accusand	0	0.58	0.48	0.73	0.50	0.57	0.46	0.35	0.43	0.61	0.35	0.61	0.63	0.46	0.82	0.74	0.65
1	Accusand	3	0.66	0.29	0.54	0.35	0.42	0.47	0.47	0.50	0.42	0.62	0.46	0.57	0.68	0.89	0.59	0.82
2	Accusand	3	0.72	0.27	0.45	0.47	0.41	0.37	0.42	0.50	0.46	0.52	0.49	0.53	0.60	0.71	0.56	0.60
1	Accusand	10	0.59	0.53	0.51	0.58	0.86	0.51	0.61	0.48	0.46	0.67	0.85	0.74	0.55	1.04	0.88	0.97
2	Accusand	10	0.72	0.75	0.60	0.66	0.81	0.59	0.55	0.54	0.46	0.64	0.71	0.68	0.77	0.83	0.83	0.68
1	Accusand	30	0.37	0.39	0.44	0.39	0.60	0.60	0.32	0.40	0.45	0.41	0.52	0.64	0.57	0.65	0.63	0.84
2	Accusand	30	0.45	0.47	0.38	0.51	0.36	0.56	0.32	0.39	0.39	0.55	0.36	0.59	0.64	0.73	0.53	0.75
1	silica	0	1.77	1.88	1.35	1.73	1.77	1.48	1.15	1.57	1.71	0.82	1.77	1.03	1.49	1.30	1.57	1.55
2	silica	0	2.25	1.45	1.08	0.66	1.21	1.59	0.78	1.77	1.38	1.30	1.66	1.12	1.41	1.35	1.67	1.27
1	silica	3	2.25	0.95	0.97	0.62	0.25	0.95	0.64	0.61	0.99	0.72	0.63	0.94	1.22	1.27	1.35	1.42
2	silica	3	2.09	1.48	1.01	0.81	0.24	0.93	0.91	0.81	1.16	0.70	1.44	1.19	1.03	1.59	1.12	1.64
1	silica	10	2.01	1.67	1.36	1.11	1.05	1.55	1.44	0.70	0.89	0.80	0.93	0.93	1.10	1.24	1.42	1.25
2	silica	10	2.15	1.66	1.29	1.57	1.64	1.49	1.24	0.90	1.21	0.75	1.17	1.31	1.06	1.12	1.36	1.44
1	silica	30	1.46	1.32	1.51	1.22	1.20	1.04	0.64	0.76	1.16	0.87	0.81	0.73	1.28	1.08	0.89	1.06
2	silica	30	1.73	1.54	1.42	1.62	0.54	0.91	0.66	0.80	1.10	0.47	0.68	0.75	0.91	0.74	0.94	1.01

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