THESIS

INVESTIGATION OF PATHOGEN DISINFECTION AND REGROWTH FOR A LOW COST GRAYWATER REUSE TREATMENT SYSTEM FOR TOILET FLUSHING

Submitted by

Kristen Wiles

Department of Civil and Environmental Engineering

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Master's Committee:

Advisor: Susan De Long
Co-Advisor: Sybil Sharvelle

Lawrence Goodridge

ABSTRACT

INVESTIGATION OF PATHOGEN DISINFECTION AND REGROWTH FOR A LOW COST GRAYWATER REUSE TREATMENT SYSTEM FOR TOILET FLUSHING

Population growth in arid regions is causing water supplies to become increasingly stressed. Water conservation measures such as low-flow fixtures provide some relief, but water savings are limited and relatively small. Graywater reuse is gaining attention as a way to ease the water stress. Graywater is ideal for reuse because it is constantly available, generated on site and requires less treatment than wastewater. Reusing graywater for toilet flushing could reduce total household potable water demands by ~25%. To promote widespread adoption and therefore maximize water savings, graywater treatment technologies must be effective, low-cost, and simple to operate without compromising public health. A treatment system comprised only of filtration and disinfection could meet these constraints; however, because such a system involves minimal organics removal, research is needed to develop a treatment system that effectively inactivates pathogens and prevents regrowth. To develop a treatment system, three filter types (coarse, sand and cartridge) were tested in combination with three disinfectants (chlorine, ultraviolet radiation, and ozone). Raw graywater from the showers and hand basins of 14 student dorms was filtered and then spiked with *Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa* or the bacteriophage MS2 (virus surrogate). The log-reduction was quantified for each filter and disinfectant combination. Chlorine provided consistent log reductions of all bacteria and viruses. Chlorination post-sand filtration resulted in log-reductions of 6.5, >7.8 and >7.4 for *E. coli, S. enterica,* and *P. aeruginosa*, respectively. UV radiation post-sand filtration

provided 5.5, >8.3 and >7.1 log-reductions of *E. coli*, *S. enterica* and *P. aeruginosa*, respectively. No significant bacterial disinfection was achieved with ozone post-sand filtration. However, ozone did achieve a log-reduction of 3.7 for MS2. Chlorine post-sand filtration and UV achieved log-reductions of 3.8 and 2.7 for MS2. Disinfection results were found to be generally similar for the coarse, cartridge, and sand filters. Chlorination post-coarse filtration achieved log reductions of >7.1 and >8.0 for *E. coli* and *S. enterica.* Chlorination post-cartridge filtration provided log reductions of only 5.2 and >7.8 for *E. coli* and *S. enterica.* UV achieved log reductions between 5.5 and 5.7 for *E. coli* with all filters, and between >7.4 and >8.3 for *S. enterica.* These batch studies supported the selection of chlorination and a coarse filtration for a demonstration graywater treatment system currently installed in one of the campus residence halls at Colorado State University. Additionally, regrowth studies were conducted on graywater disinfected with chlorine. In these tests, *E. coli* and total coliforms were monitored for up to seven days. Studies indicate that regrowth of total coliforms and *E. coli* can be prevented for at least two days with adequate chlorine residual (>2.5 mg/L) and a TOC less than approximately 50 mg/L. Spiked regrowth studies support the results of initial regrowth studies. Graywater spiked with *E. coli*, *P. aeruginosa*, and *S. enterica* was disinfected with chlorine and a residual of 2.75 mg/L total chlorine prevented regrowth of all organisms for four days. Lastly, the demonstration unit was monitored and maintained over the course of the school year. Maintenance activities and observations were recorded for the development of a standard operating procedure (SOP). The SOP allows maintenance and testing to be completed by a non-professional, which was one of the criteria of the demonstration unit.

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1.0 INTRODUCTION

1.1 Background and Motivation

Water supplies in arid regions are becoming stressed as populations continue to grow. The traditional solution for stressed water supplies is to build new infrastructure, but this method is reaching its "economic, ecological and social limits" (Cooley et al. 2010). The Colorado River, for example, is a large source of water for seven states (including Arizona, California, Colorado, New Mexico, Nevada, Utah and Wyoming) and is currently over-allocated (U.S. Bureau of Reclamation 2012). The states that share water from the Colorado River include some of the "fastest growing urban and industrial areas" (U.S. Bureau of Reclamation 2012). According to the Bureau of Reclamation, recent scientific studies on climate variability and the Colorado River are predicting a decrease in water yield from the river, which will worsen the supply and demand imbalance that the Colorado River Basin is currently facing (U.S. Bureau of Reclamation 2012). This issue is not limited to the Colorado River however. Many states in the arid western United States are facing future water supply insecurity due to variable climate and growing populations.

Water conservation techniques such as low-flow water fixtures and xeriscaping, are currently being implemented by municipalities, but the water savings from these techniques are limited. Additionally, large water projects are being proposed and built in an attempt to increase future water supply security. The city of Aurora, Colorado recently completed the Prairie Water Projects, which reuses reclaimed water (Aurora Water 2010). The project treats 50 million gallons per day (gpd) and cost almost $650 million (Aurora Water 2010). The city of Highlands Ranch, Colorado, has taken a slightly different approach to securing water for future needs, through aquifer storage and recovery (Centennial Water and Sanitation District 2012). A more

cost effective solution to large water projects could be through wide-spread adoption of graywater reuse for toilet flushing.

Graywater reuse for toilet flushing can reduce the potable water demand, as well as reduce the wastewater produced in a household. Graywater is suitable for reuse because it is consistently produced on site and contains relatively little organics and pathogens compared to other household water sources such as wastewater from kitchen sinks and toilet wastewater. In order to promote widespread adoption of graywater reuse systems, the treatment process must be easy to maintain by a non-professional, inexpensive to build and maintain, consume minimal amounts of energy and most importantly, protect public health by inactivating pathogens. Although some studies have quantified select pathogens in graywater from various sources, more information about the microbiological quality of graywater is needed, specifically, information about pathogens and human viruses.

Currently, complicated graywater treatment schemes including biological treatment or membrane filtration techniques have been studied for the reuse of graywater. Biological treatment processes and membrane filtration techniques have been shown to treat graywater to near potable water quality, but both treatment methods are expensive and would require a trained operator. A more cost-effective approach would be to use a combination of coarse filtration and disinfection. However, in order to implement these low-cost treatment systems, additional study on their ability to remove pathogens and prevent regrowth must be done in order to insure that public health is protected.

1.2 Project Objectives

The objective of this research project was to determine the best combination of filtration and disinfection for treating graywater for reuse for toilet flushing. Three disinfectants were

tested in combination with three filters to determine the most efficacious process with respect to inactivation of fecal indicator bacteria (FIB), viruses, and bacterial pathogens as well as with respect to preventing regrowth. In addition, the ease of use and long-term performance of the graywater treatment process selected was evaluated using a demonstration graywater treatment unit installed in Aspen Hall. Maintenance procedures and observations were documented in order to provide a standard operating procedure (SOP) that can be used by non-professional operators. A sub-objective of this work, in collaboration with the Environmental Protection Agency (EPA), was to determine the microbiological quality of graywater, particularly with respect to pathogens and human viruses.

1.3 Thesis Overview

Chapter 3 provides a review of current literature associated with graywater characteristics, regulations associated with reusing graywater, graywater treatment technologies and disinfection technologies. Analysis of inactivation of FIB, pathogens, and viruses for filtration and disinfection combinations, as well as the results of regrowth studies is presented in chapter 4. Chapter 4 is prepared in the form of a manuscript for publication. Chapter 5 includes information about the demonstration graywater treatment system currently in use at Aspen Hall on campus at Colorado State University. Information about system design, system operation and experiences and knowledge gained through operation can be found in chapter 5. The attached appendices provide the standard operating procedure for the demonstration graywater treatment system. Information about the role this project played in collaboration with the EPA to investigate pathogens and viruses in graywater is documented in the appendices.

2.0 BACKGROUND AND LITERATURE REVIEW

Water supply concerns, aging water and wastewater infrastructure and changing and variable climate have led to concern about the way water is currently managed (Mehan 2010). Additionally, growing populations in arid regions have begun to put a strain on water resources. In order to ensure clean drinking water in the future, it may be necessary to change the way water is used and managed. Figure 2.1 shows that populations in the western United States are expected to grow in the next 15 years, particularly in states such as Arizona, Nevada and Texas.

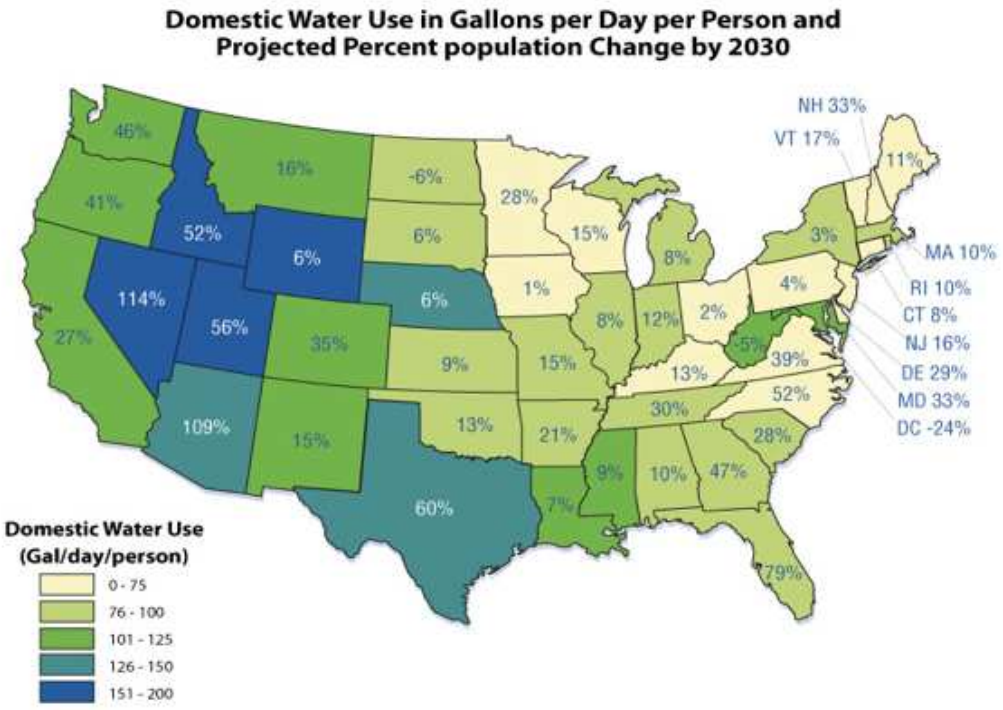

Figure 2.1 Domestic Water Use in Gallons per Day per Person and Projected Percent Population Change by 2030. (WaterSense, 2012). http://www.epa.gov/WaterSense/our_water/tomorrow_beyond.html

Water use is higher in the west, due to a greater need for outdoor irrigation in arid regions. A growing population and a relatively high demand for water have already led to a need

for increasing water efficiency. Water saving measures such as low-flow fixtures, xeriscaping, and occasionally water restrictions (for domestic irrigation) are already being promoted, and a decrease in water use has been seen as a result (Rockaway et al. 2011). However, these water saving techniques have a limited potential for decreasing water use (Rockaway et al. 2011). Graywater reuse for toilet flushing could be a solution for easing the strain on water resources without developing new water supplies, which are costly and unsustainable. This chapter provides background on the characteristics of graywater, in addition to a brief summary of graywater reuse regulations. Several techniques for the treatment of graywater and specifically, the ability of each technique to inactivate bacterial pathogens and viruses will also be discussed. Finally, an overview of disinfection methods is included.

2.1 General Characteristics of Graywater

Graywater is defined as all wastewater collected within a home except blackwater from toilets (Christova-Boal, Eden, and Mcfarlane 1996). However, wastewater from kitchen sinks and laundry water are often excluded in graywater used for reuse because the organic content from these sources is high. Therefore, graywater used for reuse, which will be referred to simply as graywater throughout this document, will only include wastewater originating from baths, showers and bathroom sinks unless specifically stated otherwise (e.g., in the discussion of previous studies that included kitchen sink water). Figure 2.2 shows the amount of water typically used in a household by water use. Graywater composes ~25% of household indoor water use. Water used for toilet flushing also accounts for ~25% of household indoor water use, indicating that sufficient graywater is typically available for reuse for toilet flushing.

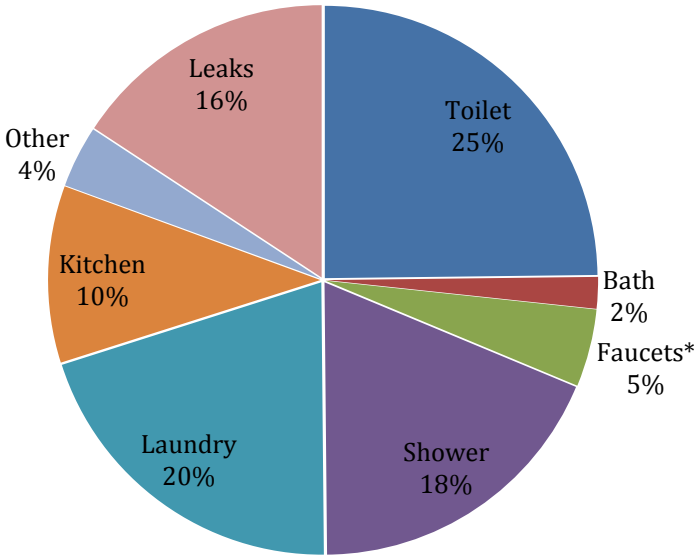

Figure 2.2 Typical Residential Indoor Water Use
Adapted from Rockaway et al., 2001
*Faucets interpolated assuming 1/3 of faucets used for bathroom washbasins and 2/3 of faucet water used for kitchen (Bergdolt et al., 2011)

The characteristics of graywater are highly variable, but may be related to graywater source, personal hygiene habits and season. Table 2.1 provides a summary of typical graywater characteristics in comparison with municipal wastewater. A few generalizations about the characteristics of graywater can be made based on source. Graywater collected from kitchen and laundry facilities has a higher organic content when compared to graywater from bathroom showers and sinks (Li, Wichmann, and Otterpohl 2009). Graywater collected from bathroom showers and sinks is referred to as light graywater (Friedler et al. 2011). The high organic content in kitchen graywater is due to the disposal of food waste. Because of the high organic content of kitchen and laundry graywater, it requires more extensive treatment and is generally considered less desirable for reuse. Light graywater, by contrast, is normally low in organic content, making it ideal for reuse. However, it should be noted that light graywater is usually higher in fecal coliforms than laundry water (Li, Wichmann, and Otterpohl 2009).

Table 2.1 Typical Characteristics of Graywater by Source

Parameter	Units	Shower/Bath[a]	Laundry[a]	Kitchen[a]	Domestic Wastewater[b]
pH		6.4-8.1	8.1-10	6.3-7.4	-
Suspended Solids	mg/L	40-120	68-250	4-185	100-360
Turbidity	NTU	28-240	14-296	-	-
BOD_5[c]	mg/L	76-200	48-380	536-1460	100-400
Total Nitrogen	mg/L-N	5.0-17	6.0-21	0.37-74	16-75
Ammonia	mg/L	<0.1-15	0.7-11.3	0.2-23	8.0-35
Total Phosphorous	mg/L	0.11-2	0.2-57	0.1-74	4.0-15
Total Coliforms	cfu/100 mL	$70\text{-}2.4\text{x}10^7$	$85 - 3.3\text{x}10^5$	-	-
Fecal Coliforms	cfu/100 mL	$1.0\text{-}3.3\text{x}10^3$	$35 - 1.09\text{x}10^3$	-	-

[a]Compiled from Eriksson et al., 2002
[b]Reynolds and Richards, 1996
[c]5-day Biochemical Oxygen Demand

Although toilet waste is not included in graywater, small amounts of fecal contamination may occur in graywater. One survey of the characteristics of graywater found that graywater contains fecal coliforms up to 3×10^3 colony-forming units (cfu)/100 mL and total coliforms up to 2.4×10^7 cfu/100 mL (Eriksson et al. 2002). Additionally, the levels of contamination have been correlated to the age of the residents. For example, families with small children and households consisting of older couples produce graywater with higher concentrations of fecal coliforms than households with young couples (Rose et al. 1991). Rose et al. (Rose et al. 1991) reported that families with children had total and fecal coliform counts averaging 3.2×10^5 and 1.5×10^3 cfu/100 mL, respectively, and families without children had low total and fecal coliform counts with both types of coliforms averaging between 6 and 80 cfu/100 mL. Additionally, fall and winter seasons are often associated with a greater risk for illnesses such as influenza or the common cold, which are caused by viruses. During these seasons of increased illnesses, the microbiological contamination levels in graywater may be different than during

times with fewer illnesses, however, there are no studies to date that have examined levels of bacteria and viruses in graywater as a function of season.

2.2 Indicator Organisms and Pathogens in Graywater

Water quality regulations are often based on indicator organisms. Indicator organisms such as total coliforms and *E. coli* are commonly used for monitoring the water quality of reclaimed water before and after disinfection (Coronel-Olivares et al. 2011). Several studies on graywater reuse have used these indicator organisms to evaluate microbiological content of influent graywater and the efficiency of graywater treatment processes (Birks et al. 2004; Rose et al. 1991; Friedler and Gilboa 2010; Friedler et al. 2011; G. P. Winward, Avery, Stephenson, et al. 2008; O'Toole et al. 2012). Typical influent ranges of total coliforms and fecal coliforms can be seen in Table 2.1.

However, public health risk is driven by the presence of human pathogens rather than indicator organisms. Graywater quality is a function of human behavior and human health, and therefore, graywater can and typically does contain human pathogens. According to Friedler et al. (2011), graywater may contain bacteria including skin pathogens (e.g. *Pseudomonas aeruginosa),* respiratory pathogens (e.g. *Legionella pneumophila*) and enteric pathogens (e.g. *Escherichia coli*). The protozoa *Cryptosporidium* and *Giardia,* and the bacteria *L. pneumophila* and Fecal enterococci were all found in at least 2 of 3 graywater samples (graywater from handbasins only) at the Millenium Dome in London (Birks et al. 2004). In a study conducted by Burrows et al. (1991), *Staphylococcus aureus* was found in the shower graywater of a U.S. military camp in concentrations ranging from 1.0 to $5x10^5$ cfu/mL. However, *P. aeruginosa* and the fungus *Candida albicans* were not detected in that study (Burrows et al. 1991). In a separate study, *Salmonella* spp., *Campylobacter* spp., *Giardia* and *Cryptosporidium* were not detected in

graywater samples from showers, baths and laundry machines (Christova-Boal, Eden, and Mcfarlane 1996). Although graywater may contain pathogens, disinfection practices can be sufficient to produce graywater of suitable quality for reuse in toilets. However, studies directly measuring pathogen inactivation, as opposed to inactivation of indicator organisms, as a function of graywater treatment technologies are lacking.

2.3 Water Reuse Regulations

Although graywater reuse has been investigated since the 1970's, there are no federal guidelines or regulations for the reuse of water (Pidou et al. 2007). However, 20 states allow graywater reuse of some form. These states have created individual regulations or guidelines for non-potable water reuse based on drinking water or contact water standards. Regulations put in place by the states are not consistent with one another, and often do not specify limits for the same parameters. For example, some regions regulate *E. coli*, some regulate fecal coliforms, and some regulate only total coliforms. In addition, the definition of graywater is not always consistent; states may define graywater as being inclusive or exclusive of kitchen wastewater (Glenn 2012). Table 2.2, below, provides a summary of the states that allow graywater reuse, categorized by how the graywater reuse is regulated.

Some states (e.g., Arizona) have tiered regulations based on scale and application. The amount of graywater being reused and what the graywater is being used for dictates whether or not a permit is needed, and whether or not the reused water needs to comply with certain water quality standards (Glenn 2012). For example, Arizona does not require a permit for graywater reuse systems that treat less than 400 gpd and are used for irrigation.

Table 2.2 Summary of States that Allow Graywater Reuse (adapted from Glenn, 2012)

Regulation Type	State	Water Quality Regulations?	
		Irrigation	Toilet Flushing
Tiered Regulations*	Arizona	No	NR
	California	No	Yes
	New Mexico	Yes	Yes
	Oregon	Yes	Yes
	Washington	Yes	Yes
Non-tiered Regulations, not based on scale	Florida	Yes	Yes
	Georgia	Yes	Yes
	Massachusetts	Yes	Yes
	Montana	No	No
	North Carolina	No	Yes
	South Dakota	No	No
	Texas	Yes	Yes
	Utah	Yes	Yes
	Virginia	Yes	Yes
	Wisconsin	Yes	Yes
	Wyoming	No	No
Regulations (residential subsurface only)	Hawaii	No	NR
	Idaho	No	NR
	Maine	No	NR
	Nevada	No	NR

*Regulations depend on volume of water reused.
NR denotes Not Regulated

Small scale residential systems are not regulated because the risk from "exposure to graywater is limited to homeowners" (Glenn 2012). The end use for recycled graywater also affects regulations because graywater reused for toilet flushing is perceived to have a higher risk than graywater reused for irrigation. All states using a tiered regulation scheme require permits for multi-residential or commercial treatment systems, or reuse applications that are considered high exposure (e.g. toilet flushing) (Glenn 2012).

Other states (e.g., Montana) have implemented non-tiered regulations that do not depend upon the scale of the system. Some of these states have chosen to apply existing regulations for

reclaimed water to graywater reuse applications. South Dakota, for example, allows the reuse of graywater, but does not provide water quality requirements or require permits. The remaining states, Hawaii, Idaho, Maine and Nevada have provided regulations for graywater reuse for residential subsurface irrigation applications only (Glenn 2012). Almost all states require that best management practices (BMPs) are followed. An example of some common BMPs include:

- Graywater tanks must be equipped with three-way diversion devices connected to approved sewer systems
- Graywater cannot be used to irrigate edible plants
- Graywater cannot be spray irrigated, but only applied through drip or subsurface irrigation systems (Glenn 2012)

Because it is difficult to find a standard definition for graywater, and therefore understand the associated risk with its reuse, regulations concerning graywater reuse often have limited scientific basis. While current graywater reuse regulations often serve as important guidelines, the current regulations are not based on risk assessments associated with graywater reuse.

2.4 Treatment Processes for Graywater Reuse

Many treatment technologies have been studied for the reuse of graywater, depending on the amount of water that needs to be treated, the end use of the treated graywater, and regulations in the region where graywater is being reused. In general, treatment technologies seek to remove organics (e.g., Total Organic Carbon (TOC)), pathogens, and contaminants that may affect the aesthetic quality of the graywater (e.g., suspended solids). Treatment technologies can be based on biological processes or physical/chemical processes. Biologically based treatment technologies are typically higher in cost and require a trained operator. Simple physical/chemical

treatment processes are generally composed of coarse filtration and disinfection. The corresponding types of systems are discussed in the following sections.

2.4.1 Biologically Based Treatment Technologies

Types of biological processes for the treatment of graywater include membrane bioreactors (MBRs), rotating biological contactors (RBCs), and constructed wetlands. MBRs combine bioreactors typically used in wastewater treatment with a membrane process (e.g. micro- or ultrafiltration) to achieve high quality effluent. RBC technology allows wastewater to contact disks containing biological media, which remove contaminants. Biologically based processes typically utilize several processes including filtration, settling, biological treatment and disinfection (Figure 2.3).

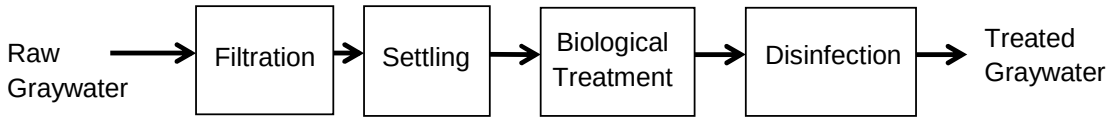

Figure 2.3: Schematic of a Typical Biological Treatment Process

The biological treatment shown in the schematic could be either an MBR or RBC. Other processes such as filtration and settling allow for the removal of large particles such as hair, while disinfection ensures that pathogens are inactivated. Biological treatment processes are used in wastewater treatment, and therefore are an obvious candidate for graywater treatment. For example, an MBR studied by Winward et al. (2008) treated graywater from baths, showers and sinks from 18 student dormitories of Cranfield University. The MBR had average removal efficiencies of 95% for biochemical oxygen demand (BOD), 45% for chemical oxygen demand (COD), >99% for total suspended solids (TSS), and 99% for turbidity (G. P. Winward, Avery,

Frazer-Williams, et al. 2008). The average influent total coliform count was 5.4 log cfu/100 mL while the effluent was 0.6 log cfu/100 mL. The study did not involve a discussion of a disinfection process and regrowth was not examined. Friedler et al. (2011) studied an RBCs that treated graywater from 14 flats and included disinfection with Ultraviolet (UV) radiation. When using UV as a disinfectant, the treatment process removed 96% of BOD, 95% of turbidity, 98% of fecal coliforms, and 96% of *P. aeruginosa*. Winward et al. (2008) studied a vertical flow reed bed (VFRB) wetland for the treatment of graywater. The VFRB had removal efficiencies of 95% of BOD, 76% of COD, 93% of TSS, and 59% of turbidity. The VFRB also had a 4.7 log reduction of total coliforms and a 2.8 log removal of *E. coli*.

Biological treatment technologies are more efficient at removing organics than simple filtration methods, thereby reducing chlorine demand and turbidity. However, organics and turbidity pose no direct human health threat and their removal is not paramount for most reuse applications. In addition, the lack of nutrients in graywater due to the exclusion of blackwater may inhibit biological processes (Jefferson et al. 2001). Therefore, the graywater sources best-suited for biological treatment are kitchen sinks and dishwashers because of the abundance of biodegradable organic substances and particulate nitrogen (Li, Wichmann, and Otterpohl 2009). However, the graywater from kitchen sinks and dishwashers is highly contaminated with thermal tolerant coliforms due to this organic matter, and therefore, is not a good candidate for water reuse (Li, Wichmann, and Otterpohl 2009).

Furthermore, although biologically based treatment processes produce a higher quality effluent than physically based processes alone, it is important to consider cost and sustainability when choosing a treatment system. Low cost systems are especially desirable in the United States where water is relatively inexpensive and reuse applications must be low in cost to be

economically feasible. Homeowners in the United States have not been polled about what payback period they would be willing to accept in order to adopt graywater reuse systems. However, in a study conducted in Guelph, Canada, homeowners wanted a payback period of ten years or less in order to strongly consider implementing a graywater reuse system (City of Guelph 2012). A survey conducted in Melbourne revealed that residents would only consider reusing graywater if the reuse system has a payback period of 2-4 years (Christova-Boal, Eden, and Mcfarlane 1996). However, graywater treatment systems that rely on biological treatment are costly to construct and maintain. These systems include many of the processes used in centralized wastewater treatment facilities, rendering them too costly and large for all but very large applications. Lastly, biological treatment requires regular monitoring, and most households do not have the knowledge or motivation to perform regular maintenance and monitoring of a biologically based treatment system. A trained water technician is needed for the upkeep of biologically based treatment technologies, adding to the cost of these systems. Thus, for the aforementioned reasons, biologically based treatment processes are not likely to be the most suitable approach to treating the graywater considered herein (e.g., graywater excluding kitchen wastewater).

2.4.2 Physical/Chemical Based Treatment Technologies

An alternative approach to biologically based treatment is to utilize physical/chemical based treatment technologies, such as membrane filtration with disinfection or coarse filtration with disinfection. Membrane filtration for the treatment of graywater is effective at removing turbidity and organics, but requires more maintenance than a coarse filtration system, as membranes are subject to fouling. Li et al. (2008) studied an ultrafiltration membrane system that

treated graywater from all household sources (including laundry machines, dishwashers and kitchen sinks in addition to baths, showers and handbasins). On average, turbidity was reduced from 140 NTU to 0.5 NTU, and TOC was reduced from 161 mg/L-C to 28.6 mg/L-C (Li et al. 2008). Despite the high removal rates, the membrane system requires maintenance that would require a knowledgeable technician. The membrane required cleaning every two weeks with an alkaline cleaning product, and the membrane filtration tank increased in suspended solids to approximately 3,000 mg/L at the end of each filtration cycle (approximately 14 days) (Li et al. 2008). Thus, membranes are a technically viable treatment option, but are cost prohibitive in most cases, limiting the widespread adoption of membrane technologies for graywater reuse (Li, Wichmann, and Otterpohl 2009).

Burrows et al. (1991) also studied a physical treatment system for the recycle of shower water for military applications. The treatment system was comprised of coagulation, flocculation, and a filter consisting of diatomaceous earth (DE) with activated carbon. The treatment system removed 53-86% of TOC, and effluent turbidity was below the limit of 5 NTUs as specified by the Department of the Army (Burrows et al. 1991). The chlorine demand was below 3 mg/L, and microbiological tests were negative for *P. aeruginosa*, *S. aureus* and *C. albicans* (Burrows et al. 1991). However, a drawback of diatomaceous earth filters is that they must be frequently backwashed and maintained much like sand filters.

Another physical-based process is coarse filtration, followed by disinfection. Although treatment technologies consisting of coarse filtration and disinfection do not remove organic content and turbidity from graywater, they are generally inexpensive, easy to maintain and capable of providing an effluent free of pathogens. For example, Brac Systems (acquired by Greyter Systems of Ontario, Canada in 2012) offers a commercial graywater recycling system

with treatment consisting of coarse filtration followed by disinfection with a chlorine puck (City of Guelph 2012). The City of Guelph, Canada conducted a pilot study in which 24 homes were equipped with the Brac treatment system and system efficacy was evaluated based on Health Canada guidelines for domestic reclaimed water use in toilets (City of Guelph 2012). The system achieved sufficient removal of turbidity to meet the Health Canada guideline maximum effluent turbidity of ≤5 NTU for only 15.3% of samples, but *E. coli* levels were below the maximum limit (200 cfu/100mL) for 90.3% of samples. It is important to note that the Brac treatment system uses chlorine pucks as a disinfectant, which can often be unreliable in achieving a consistent dose. Only 38.1% of samples from the City of Guelph pilot study had at least a minimum of 0.5 mg/L free chlorine residual (City of Guelph 2012), which can explain the presence of samples that did not meet disinfection requirements. Thus, more research is needed to develop treatment technologies consisting of coarse filtration and disinfection that result in effluent that is consistently free of microbiological contaminants.

2.5 Disinfection

Following biological treatment or physical treatment, disinfection is needed to insure pathogen inactivation. Although all disinfectants are used to inactivate pathogens and prevent microbial growth, disinfectants use different mechanisms to inactivate pathogens and the effectiveness of disinfectants may be dependent upon water quality. Disinfectants commonly used in water and wastewater treatment include chlorine, UV light, and ozone. These disinfectants are discussed further in the following sections.

2.5.1 Chlorine Disinfection

Chlorine has often been used as a graywater disinfectant due to its prevalence in water and wastewater disinfection in the United States. Chlorine is the primary chemical used for the disinfection of water because it is effective, inexpensive, and provides a measurable residual (Reynolds and Richards, 1996). Chlorine is a powerful oxidizing agent that oxidizes enzymes of microbial cells that are necessary for the cell's metabolic processes (Reynolds and Richards, 1996). A common form of chlorine used for graywater disinfection is sodium hypochlorite (NaOCl).

Chlorine disinfection of water is affected by the organic content of the water, turbidity, and biofilms formed in tanks and plumbing. LeChevallier et al. (1981) found that coliforms were more resistant to chlorine disinfection in turbid waters. Some bacteria in the turbid water were embedded into particles or surrounded by a protective material, making the bacteria less exposed to oxidation (LeChevallier, Evans, and Seidler 1981). LeChevallier et al. (1981) also showed that as TOC in a water source increases so does the chlorine demand (the amount of chlorine that reacts with substances in the water, and is thus consumed). However, Winward et al. (2008) observed that as the TOC concentration of graywater increased, survival of total coliforms did not increase for a constant chlorine residual of 1 mg/L. With TOC concentrations of 65 mg/L-C and 153 mg/L-C, the concentrations of total coliforms present after disinfection were 2.28 log cfu/100 mL and 1.86 log cfu/100 mL, respectively (G. P. Winward, Avery, Stephenson, et al. 2008). The findings of the study conducted by Winward et al. (2008) suggest that an increase in organics simply increases the chlorine demand of the water but does not necessarily result in less effective pathogen inactivation, as observed previously by LeChevallier et al. (1981). It must also be noted that chlorine consumption in a full-scale graywater reuse system will be higher

than in a bench-scale study due to consumption associated with biofilms in the plumbing (March, Gual, and Orozco 2004).

2.5.2 UV Disinfection

UV radiation as a disinfectant is sometimes preferred over chemical disinfectants (e.g. chlorine) because there is no need for chemical storage and replenishment and no harmful disinfection by-products are created. Also, UV irradiation has been shown to be more effective against viruses and pathogens than chlorine (Fenner and Komvuschara 2005). UV works as a disinfectant through photochemical damage to RNA and DNA, which renders viruses non-infective and disrupts microbial reproduction processes resulting in inactivation (Metcalf and Eddy, 2003). Disinfection by UV radiation is most commonly accomplished with lamps that operate at a wavelength of 254 nm (U.S. EPA 1999a). The UV dose is related to the intensity of the UV radiation and the exposure time to the organisms (U.S. EPA 1999a). Water quality parameters such as UV transmittance (UVT) and turbidity influence the efficacy of UV disinfection (U.S. EPA 1999a).

UV has proven effective for eliminating pathogens in drinking water applications; however, the turbidity of graywater may limit the efficacy of UV disinfection. Large particles in the graywater hinder disinfection by UV because they have the ability to shield pathogens from UV light. In a study of the effect of graywater particle size on disinfection efficacy, particles larger than 262 μm were found to be “more likely to have regions inaccessible to UV light” (G. Winward, Avery, Stephenson, et al. 2008). Several studies have also examined UV disinfection in various graywater treatment schemes. Santos et al. (2011) studied a graywater reuse system comprised of a storage tank, filtration, and disinfection by UV irradiation. The filter used in the

study was a stainless steel screen with a mesh of 0.025 mm (Santos et al. 2012). The filter effluent had a TSS of 15 mg/L and a COD of 46 mg/L, which corresponded to removals of 82% and 72%, respectively (Santos et al. 2012). The authors reported that no coliforms were detected in samples taken after disinfection; however, no influent coliform values were reported. Friedler and Gilboa (2010) studied a graywater treatment system composed of a biological treatment (RBC) followed by UV disinfection. Influent quantities of BOD and turbidity were 95 mg/L and 33 NTU, respectively (Friedler and Gilboa 2010). Average RBC effluent quantities of BOD and turbidity were 3.7 mg/L and 1.5 NTU (Friedler and Gilboa 2010). The authors observed that UV reduced fecal coliforms from 2.1×10^2 cfu/100 mL in the RBC effluent to 3.8×10^1 cfu/100 mL in the toilet bowl and *S. aureus* from 2.4×10^1 cfu/100 mL to 5.5 cfu/100 mL (Friedler and Gilboa 2010). *P. aeruginosa* and heterotrophic plate count (HPC), however, were not significantly reduced in the UV-disinfected effluent as compared to the un-disinfected effluent (Friedler and Gilboa 2010). Although some inactivation of pathogens can be achieved using UV, UV does not provide a residual disinfectant to prevent the regrowth of surviving organisms. Also, additional information is needed about the efficacy of UV disinfection in turbid water.

2.5.3 Ozone Disinfection

Ozone has been used as a disinfectant by wastewater treatment plants since the 1970s, but fewer than 10 plants in the United States currently use ozone due to issues with reliability and maintenance (Oneby et al. 2010). Ozone as a disinfectant works in the same way as chlorine: through oxidation of the cell membrane and enzymes important for the cell's metabolic processes (U.S. EPA 1999b). Ozone is an attractive choice for a disinfectant because it is considered a more effective oxidant than chlorine. Ozone is also more effective than chlorine at

removing viruses. CT values (the product of contact time and residual disinfectant) for 99% inactivation of *E. coli* are 0.034-0.05 for free chlorine, but only 0.02 for ozone (Siemens 2009). Similarly, CT values for 99% inactivation of rotavirus are 0.01-0.05 for free chlorine and 0.006-0.06 for ozone (Siemens 2009).

Ozone is not only a powerful disinfectant, but it has been shown to reduce suspended solids, turbidity, and COD in wastewaters that have undergone primary and secondary treatment (e.g., roughing filter, desanding-degreasing, sedimentation, biological treatment and secondary sedimentation) (Martinez, S.B., Perez-Parra, J., Suay 2011). A study completed by Martinez et al. (2011) concluded that primary and secondary treated wastewater from the city of Almeria, Spain, which uses ozone as a tertiary disinfectant was suitable for irrigation of food crops. The ozone treatment (a dose of 11-13 mg/L) produced a maximum reduction of COD of 88%, and a maximum removal of 75% of suspended solids (SS) (Martinez, S.B., Perez-Parra, J., Suay 2011). Influent water to the ozone treatment plant had a turbidity of less than 25 NTU (Martinez, S.B., Perez-Parra, J., Suay 2011).

Although ozone is often considered a more effective disinfectant than chlorine, it is difficult to maintain residual ozone due to rapid decomposition, thus requiring an additional disinfectant such as chlorine (U.S. EPA 1999b). In addition, ozone is an unstable compound and must be generated on site. Ozone generators are complex, and often require a skilled technician for maintenance (U.S. EPA 1999b).

2.6 Regrowth

Storage (both pre- and post-disinfection) is an integral part of graywater reuse. Because water use follows a diurnal pattern, a large volume of graywater storage is needed for flow equalization to increase the efficiency of graywater treatment systems and facilitate process

design and control (Dixon et al. 2000). Additionally, storage pre-disinfection or post-disinfection is needed to ensure that graywater is available for use when needed and that a maximum volume of graywater can be reused (Tal, Sathasivan, and Krishna 2011). However, long-term storage of graywater can degrade water quality by leading to regrowth of microorganisms. Dixon et al. (2000) performed a study in which characteristics of untreated bath water and laundry water were recorded for up to 25 days. They found that storage for up to 24 hours could be beneficial due to settling of suspended solids and a corresponding decrease in COD; however, untreated graywater decomposes rapidly and should not be stored for more than 24 hours to prevent regrowth of organisms. Studies have found that untreated graywater stored for more than 24 hours decreased in dissolved oxygen and increased in total coliforms (Rose et al. 1991; Dixon et al. 2000).

Regrowth can also occur in post-disinfection graywater, and factors affecting regrowth include chlorine dose and contact time, residual disinfectant, suspended particle levels and organic content. For example, Huang et al. (2011) found that for a constant CT, regrowth and reactivation of antibiotic-resistant bacteria is less likely with a higher concentration chlorine dose and a shorter contact time than with a lower chlorine concentration and a longer contact time (Huang et al. 2011). It has also been suggested that suspended particles may shield bacteria attached to those particles from disinfectants, and could thus lead to higher regrowth potential for a given disinfectant dose (G. Winward, Avery, Stephenson, et al. 2008). Suspended particles, or turbidity, may also carry nutrients that support microbial regrowth after disinfection (LeChevallier, Evans, and Seidler 1981). Regrowth of pathogens may occur when a disinfectant residual is depleted. For example, Jjemba et al. (2010) studied the effluent of three wastewater treatment plants utilizing chlorine as a disinfectant. It was observed that although indicator

bacteria and pathogens were inactivated following disinfection, both indicator bacteria and pathogens (*Aeromonas* spp., *Legionella* spp., *Pseudomonas* spp., and *Mycobacterium* spp.) were present in the distribution system when the chlorine residual was depleted (Jjemba et al. 2010).

Regrowth of pathogens and indicators in graywater reuse systems on a residential or multi-residential scale has not been thoroughly investigated. March et al. (2004) studied a graywater reuse system in a hotel on Mallorca Island (Spain) with 81 rooms. The graywater consisted of water collected from bathtubs and bathroom sinks only, and was treated by filtration (nylon sock filter, 0.3 mm mesh), sedimentation and chlorination. Residual chlorine and indicator bacteria were not measured in the effluent or at the point of use, but a retention time of less than 48 hours was used for the purpose of preventing regrowth (March, Gual, and Orozco 2004). Friedler et al. (2011) studied the regrowth potential of bacteria in graywater treated by an RBC and disinfected by either chlorination or UV irradiation. Fecal coliforms, *S. aureus*, *P. aeruginosa* and HPC were monitored for up to six hours after disinfection, and none of the bacteria exhibited regrowth during this short time period (Friedler et al. 2011). A recent study by Beck et al. (2013) examined the effect of long term (> 6 hours) storage on regrowth: however, it was concluded that the low organic content of the water would not support bacterial growth even without disinfection. The graywater studied by Beck et al. (2013) had an influent turbidity ranging from 13-26 NTU, which was reduced to 1.4-6 NTU following filtration through a 10 micron filter. All samples contained less than 5 mg/L-C TOC and total nitrogen (TN) (Beck et al. 2013). The organic content and turbidity in the graywater studied by Beck et al. (2013) is low compared to typical values (Table 2.1). Therefore, there is a need to investigate long term regrowth in graywater reuse systems that do not provide significant organics removal.

3.0 INVESTIGATION OF PATHOGEN DISINFECTION AND REGROWTH IN A SIMPLE GRAYWATER REUSE TREATMENT SYSTEM FOR TOILET FLUSHING

3.1 Introduction

Fresh water supplies are becoming increasingly stressed as populations grow, and alternative water supplies are beginning to gain attention as a way to accommodate population growth worldwide (City of Guelph 2012; Nolde 1999; Pidou et al. 2007; Ward and Michelsen 2002). Thus, new ways of using and managing existing water resources will be key to accommodating population growth and to satisfying competing demands for water. Water conservation measures such as low-flow fixtures, xeriscaping and water restrictions imposed by municipal water utilities have been implemented at various levels; however, the water savings through such approaches have almost been fully realized. Reusing graywater has been gaining attention, as graywater is a large source of water that is constantly available and relatively low in organic content, and therefore easier to treat than municipal wastewater (G. P. Winward, Avery, Stephenson, et al. 2008). In a study conducted by Denver Water assessing household water use, light graywater (water from showers, baths, and bathroom washbasins) generation was reported to be approximately 15.6 gallons per capita per day (gpcd). Thus, over 5,600 gallons per person of graywater is available for reuse each year (Rockaway et al. 2011; Bergdolt, Sharvelle, and Roesner 2011). In another study, the toilet water demand was reported to be approximately 15.4 gpcd (Rockaway et al., 2011). Therefore, light graywater can meet toilet flushing demands and graywater production including laundry water well exceeds toilet demand. However, graywater reuse has not been widely implemented, in part because of the cost of graywater treatment systems. Thus, low-cost treatment systems need to be developed.

Historically, various ways of treating graywater for reuse have been investigated, from complex biological treatment processes to simple physical treatment coupled with disinfection. Biological processes provide good removal of organics, with effluent BOD quantities often below 10 mg/L (Pidou et al., 2006). However, these processes are more expensive, and a trained technician would be needed to monitor the treatment process, making biological treatment of graywater impractical for household applications. Alternatively, the simplest treatment comprising of coarse filtration and disinfection provides little removal of organics but can theoretically provide good inactivation of organisms in the disinfection process (Pidou et al., 2006). Simple treatment systems are advantageous for residential graywater reuse systems because they are low-cost and easy to maintain, and ideally would only require a manual to guide non-technical homeowners on maintenance. However, more research is needed to fully develop these technologies and ensure that simple treatment systems meet water quality goals for protection of public health. Currently, water quality goals for total and fecal coliforms range from 2.2-500 cfu/100 mL and 14-200 cfu/100 mL, respectively (Glenn 2012).

To protect public health, microorganisms in graywater must be inactivated. Indicator organisms such as total coliforms and *E. coli* are commonly used for monitoring the microbiological quality of reclaimed water after disinfection (Coronel-Olivares et al. 2011), but public health risk is driven by the presence of human pathogens rather than indicator organisms. Graywater is known to contain pathogens including *Pseudomonas aeruginosa*, *Escherichia coli*, *Legionella pneumophila*, and *Salmonella enterica* (Friedler et al. 2011; Rose et al. 1991). However, studies directly measuring pathogen inactivation, as opposed to inactivation of indicator organisms, as a function of graywater treatment technologies are lacking.

After primary treatment or filtration, disinfectants such as chlorine, UV, or ozone can be used to inactivate pathogens. Chlorine, commonly used in water and wastewater disinfection, is a simple and inexpensive method for disinfecting graywater. UV is sometimes preferred over chemical disinfectants because there is no need for storage and replenishment. Ozone is a powerful disinfectant, requiring lower CT values for disinfection of *E. coli*, *Rotavirus* and *Giardia* cysts than chlorine (Siemens 2009). Although several studies have examined disinfection of graywater after biological treatment or membrane filtration (Beck et al. 2013; Friedler et al. 2011; Kim et al. 2009), little research has been done to investigate the efficacy of a range of disinfectants on pathogens in graywater containing organics.

A large concern with graywater systems is regrowth of pathogens along the distribution system and at the point of use, the toilet. Households may remain empty during the workday or when residents are traveling. If graywater is not properly disinfected, regrowth of pathogens and bacteria could occur due to increasing residence times as homeowners are away. In addition, regrowth of bacteria could increase the risk of direct contact with graywater, either through splashing or aerosolizing of pathogens during toilet flushing (Christova-Boal et al., 1996). Inexpensive treatment methods provide little removal of organics, but complete disinfection may prevent regrowth of organisms. Though regrowth after chlorine disinfection has been studied, the data has been limited to regrowth occurring in less than 24 hours (Friedler et al. 2011). Additionally, a recent study investigated regrowth of organisms in graywater after filtration and disinfection; however, it was determined that low organic content limited the regrowth potential of the organisms (Beck et al. 2013). Relatively little has been done regarding the long-term regrowth of organisms in disinfected graywater containing organics.

This study aims to evaluate the efficacy of coarse filtration methods in combination with UV, ozone and chlorine disinfection for inactivation of pathogens and bacterial and viral indicators to produce treated graywater suitable for reuse in toilets. This study also examines the regrowth potential of pathogens in graywater treated using a simple treatment process of filtration and disinfection where little organics removal might increase the potential for regrowth of pathogens.

3.2 Materials and Methods

3.2.1 Graywater Collection and Treatment System Description

The graywater used throughout this study was collected from a graywater collection and demonstration treatment system installed at a student dormitory (Aspen Hall) on the campus of Colorado State University. Graywater was collected from 28 students in 14 dorm rooms. The average flow rate through the system was 300 gallons per day. The system consisted of storage before treatment, which provided settling of solids as well as storage for equalizing diurnal flow patterns. The pre-treatment storage tank was 250 gallons. Following pre-treatment storage, water passed through a filter. Each of the following filters was tested separately: a 16” long Matala medium density filter (Matala USA, Laguna Hills, CA) (61 days of operation), a pool sand filter with a pore size of 100 mm (Hayward, Elizabeth, NJ) (18 days of operation), and a cartridge filter with a pore size of 20-40 microns containing granular activated carbon (PurFlo, Chicago, IL) (13 days of operation). The treatment system was operated with the three different filters for a total period of ~3 months. During this time, 9 batch studies were conducted using water collected from this demonstration treatment system post-filtration (see Section 3.2.3). For non-spiked regrowth studies (see section 3.2.5), graywater was disinfected post-filtration with chlorine in-line. Chlorine was dosed by volume using a Stenner 85MP1 peristaltic pump, Stenner

PCM pump control module (Stenner, Jacksonville, FL), and Seametrics MJ 1 gallon pulse water meter (Seametrics, Kent, WA). After each gallon of water passed through the flow meter, a pre-specified chlorine concentration was dosed in-line before the disinfection tank with the peristaltic pump. Then the treated graywater entered the disinfection contact tank where it was stored prior to flowing into a toilet plumbed to the system. The disinfection contact tank was 45 gallons, sized to provide a contact time of at least 1 hour. A chlorine residual of 2-4 mg/L was desired in the graywater effluent, and therefore, a dose of approximately 20-22 mg/L was used. The graywater treatment system also had a potable make-up supply to ensure water was always available for toilet flushing.

3.2.2 Chemical and Indicator Organism Monitoring

Standard chemical and biological parameters were measured for raw and treated graywater. Total organic carbon (TOC) was measured with a Shimadzu TOC-V CSH/CSN analyzer (Shimadzu, Japan), which utilizes a combustion and acidification process. Turbidity was analyzed using a Hach 2100N nephelometric turbidimeter (Hach, Loveland, CO). Total chlorine was measured using a Hach total chlorine test kit (Method 8167) with a Hach DR2500 spectrophotometer. *E. coli* and total coliforms were enumerated using the EPA approved Colilert-24 Quanti-Tray® method (IDEXX, Westbrook, ME). Colilert-24 powder pillow indicators were added to 100-ml samples and sealed in a Quanti-Tray® and incubated for 24 hours at 35°C. After incubation, *E. coli* and total coliforms were enumerated following manufacturer's instructions. The %UVT at 254 nm of the graywater was determined using a Thermo Scientific Genesys Spectrophotometer (Thermo Scientific, Waltham, MA).

3.2.3 Laboratory Disinfection Study Set-up

Laboratory-scale disinfection studies were used to determine the potential log inactivation of pathogens using the three different filtration methods (see section 3.2.1) in conjunction with three different disinfectants. The disinfection systems were constructed using 5-gallon buckets and were plumbed for disinfection via chlorination, UV treatment or ozonation (Fig. 3.1). Graywater was collected post-filtration from the demonstration graywater treatment system and was then immediately spiked with high concentrations of pathogens or bacteriophage prior to disinfection tests. For each disinfectant tested, two-gallon aliquots of graywater were spiked with approximately 8 log/100 mL *E. coli* (American Type Culture Collection [ATCC] 25922), *S. enterica* (ATCC 14028) and *P. aeruginosa* (ATCC 27853) or MS2 bacteriophage (ATCC 15597-B1). For each filter and disinfectant combination, all bacteria into one two-gallon aliquot. *E. coli* was selected for testing in the laboratory-scale disinfection studies because it is often included in graywater reuse regulations and is a known pathogen in graywater (G. P. Winward, Avery, Frazer-Williams, et al. 2008). *S. enterica* was selected because it is an enteric pathogen and has previously been examined in graywater studies (Nolde 1999; G. P. Winward, Avery, Frazer-Williams, et al. 2008). *P. aeruginosa* was selected because it is a known biofilm former and is a skin and mucus pathogen previously found in graywater (Friedler and Gilboa 2010). MS2 bacteriophage was selected for laboratory-scale disinfection studies because it is a useful surrogate for poliovirus, which is regulated in the California Title 22 requirements for graywater reuse. MS2 is a non-enveloped virus and is more difficult to inactivate than enveloped viruses, such as influenza, making it a conservative choice for disinfection studies.

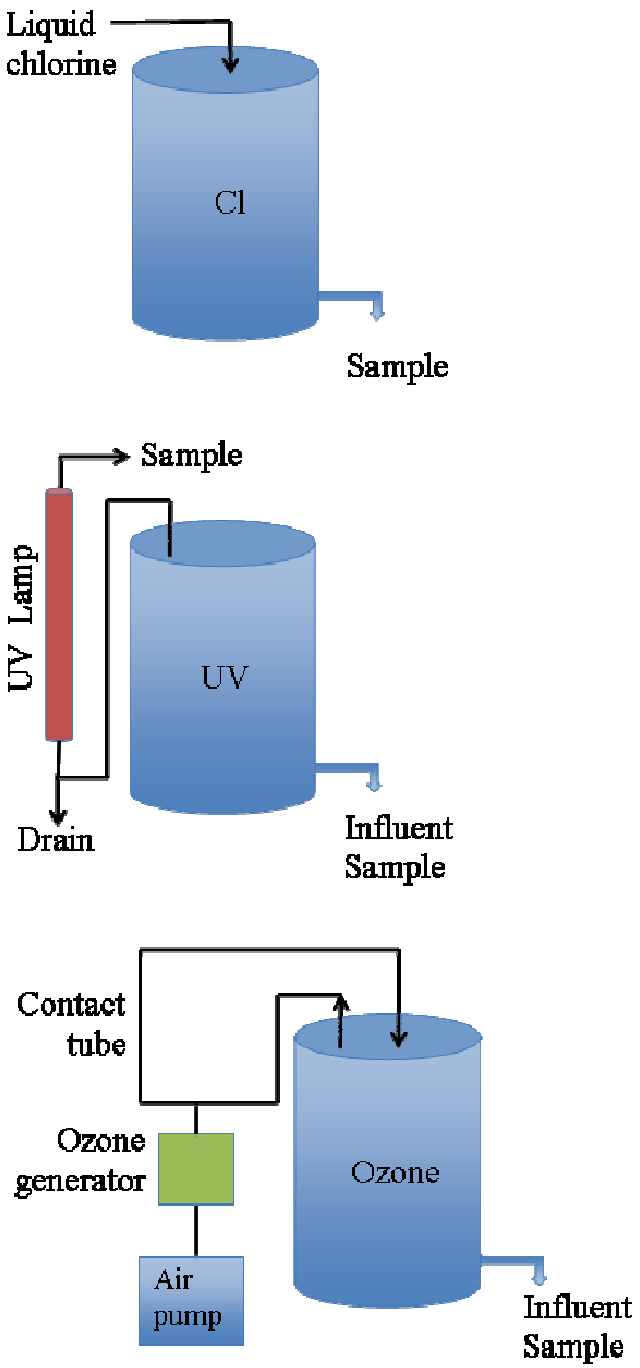

Figure 3.1 Batch Reactor Diagrams

For the chlorine batch reactor, chlorine was dosed directly into the top of the bucket using a 6% solution of NaOCl (Chlorox, Oakland, CA). Chlorine demand was estimated prior to each study. Chlorine demand was found by dosing chlorine into graywater at an amount slightly

higher than the estimated chlorine demand and then measuring chlorine consumption over time. A chlorine residual of approximately 3 mg/L was desired, so the total chlorine dose for each study was the chlorine demand plus 3 mg/L for a residual. A contact time of 60 minutes was chosen based on typical literature values ranging from 30 minutes to 90 minutes (Lechevallier, Cawthon, and Lee 1988; Burrows et al. 1991; U.S. EPA 2004). Samples were collected for pathogen and bacteriophage enumeration immediately prior to chlorination, and then post-treatment samples were collected from the sampling port (Fig. 3.1) 60 minutes after chlorine addition. It should be noted that due to high ammonia concentrations in the raw graywater, chloramine likely was formed leaving minimal free chlorine.

For the ozone batch reactor, ozone was generated in the laboratory using an aquarium air pump (Petco, San Diego, CA) and an advanced plasma gap spa ozone generator (Del Ozone, San Luis Obispo, CA). An air flow rate of approximately 1 L/min was chosen because it resulted in a maximum ozone generation rate of 1 mg/min (assuming standard temperature and pressure). Slower air flow rates generated greater percentages of ozone from air; however, the slower air flow rate provided lower ozone mass flow overall. Ozone dose was calculated using the ozone generation rate and the flow rate of graywater through the contact tube (Fig. 3.1). The graywater was re-circulated through the contact tube until the desired dose was achieved. The ozone dose used was 5 mg/L. Samples were collected for pathogen and bacteriophage enumeration immediately prior to ozone disinfection, and then post-treatment samples were collected from the sampling port (Fig. 3.1) after ozone addition.

For the UV batch reactor, a Sterilight Copper SC1 UV lamp was used for in-line disinfection (R-can, Guelph, Canada). To determine dose, the %UVT at 254 nm of each 2-gallon water aliquot was determined as described in Section 3.2.2. %UVT typically ranged from 35-

41%. Dose was calculated using the graywater flow rate through the UV lamp and the %UVT of the graywater based on the lamp manufacturer's specifications. The dose for these studies was 28 mJ/cm^2. A dose of 28 mJ/cm^2 was used because it was the highest achievable dose that could be applied given the %UVT of the graywater used and the minimum flow rate through the UV lamp. This dose was within the range of doses tested in previous studies; Hijnen et al. (2006) reported a range of UV doses from 5-50 mJ/cm^2 for the inactivation of polivirus. Samples were collected for pathogen and bacteriophage enumeration immediately prior to UV disinfection, and then post-treatment samples were collected from the sampling port (Fig. 3.1) after passing through the UV lamp.

3.2.4 Microbiological Culturing and Analyses for Batch Studies

For each pathogenic bacterium, pure cultures were cultivated on a nutrient rich media the night before the laboratory-scale disinfection studies were conducted. A small amount of each microorganism was scraped from a pure culture stored at -80°C and placed into a test tube containing 5 mL of growth media. The cultures were then incubated aerobically at 37°C overnight. The growth media used for *E. coli*, *P. aeruginosa*, and *S. enterica* were Luria broth (LB) (BD, Franklin Lakes, NJ), tryptic soy broth (TSB) (BD, Franklin Lakes, NJ) and nutrient broth (NB) (BD, Franklin Lakes, NJ), respectively. Cell concentration for each bacterium was estimated using a standard curve relating optical density (600 nm) to the concentration of bacterial colony forming units (cfu). The volume of culture needed to produce a final concentration of log 8/100 mL in the graywater was determined based on estimated culture cfu. Prior to use for spiking the filtered graywater, each culture was centrifuged at 4,000 rpm for 5

minutes, and the supernatant was poured off. The pellet was re-suspended by vortexing in 5 mL of graywater and then used to spike the filtered graywater.

Selective plating methods were used to detect the bacterial pathogens pre- and post-disinfection treatments. *E. coli* and total coliforms were enumerated using membrane filtration and the EPA approved m-ColiBlue24® broth (Hach, Loveland, CO). 100-ml samples were filtered through a 0.45 micron glass-fiber filter and the filter was incubated on the m-ColiBlue24® broth for 24 hours at 35°C. After incubation, red and blue colonies were counted as total coliforms and blue colonies were counted as *E. coli*. Three different dilutions were plated for each sample collection event to assure readable plates. *S. enterica* were enumerated using SS agar (Sigma-Aldrich, St. Louis, MO), and *P. aeruginosa* were enumerated using commercially available mPa agar plates (Hardy Diagnostics, Santa Maria, CA). Serial dilutions were prepared for each sample, and then 50 µL or 100 µL of diluted sample were spread onto an agar plate using 6-mm sterilized glass beads (Fischer Scientific, Waltham, MA). Following incubation, plates with fewer than 300 colonies were counted.

MS2 coliphage was propagated and enumerated as described previously (Fortier and Moineau 2009; Mamane, Shemer, and Linden 2007). In brief, MS2 was propagated by incubating MS2 on a plate of *E. coli* host (ATCC 700891) overnight. Then, an MS2 plaque and agar from the plate were scraped off and placed into 10 mL of TSB supplemented with 50 µg/mL each of ampicillin and streptomycin (Thermo Fisher Scientific, Waltham, MA) and *E. coli* host. Following incubation overnight, cell debris and host were removed from MS2 in TSB by centrifugation for 10 minutes at 8,000 x g followed by filtration through a 0.45µm syringe filter. The resulting MS2 stock was then stored in a 50% glycerol solution. The titer of the MS2 stock was found to be 1.45×10^{10} pfu/mL; therefore, 2 mL of MS2 stock was spiked into graywater to

produce a concentration of log 8/100 mL. MS2 was enumerated for the laboratory-scale disinfection studies using a plaque-clearing assay, as described previously (Mamane et al., 2007). *E. coli* host was grown for 3-6 hours before each disinfection study to assure the host was in exponential growth phase for the plaque-clearing assay. Prior to disinfection studies, bottom agar was prepared using commercially available tryptic soy agar (TSA) supplemented with 50 µg/mL each of ampicillin and streptomycin (Thermo Fisher Scientific, Waltham, MA). The purpose of the antibiotics was to select for the *E. coli* host. Top agar was prepared using 6g of bacto agar added to 1L of TSB. Top agar was stored in glass vials containing 3.5 mL aliquots. For enumeration, serial dilutions of each graywater sample were prepared. Then top agar was heated until fluid, and 3.5 µL of streptomycin and ampicillin and 100 µL of *E. coli* host were added just prior to adding 100 µL of the graywater sample, as described previously in Mamane et al. (2007). Soft agar was then poured onto the TSB plates and incubated inverted for 24 hours at 35°C (Mamane, Shemer, and Linden 2007). Plaques were enumerated after the incubation period and reported as plaque-forming units (pfu)/mL.

3.2.5 Regrowth Studies

For non-spiked regrowth studies, treated graywater was allowed to sit in a 1.6-gallon toilet for seven days with the lid closed. Samples were taken each day and total chlorine, *E. coli* and total coliforms were measured. *E. coli* and total coliforms were quantified using the methods described in Section 3.2.2. An additional non-spiked regrowth study was conducted in a 5-gallon bucket. For the laboratory-scale pathogen-spiked regrowth study, graywater was collected after the pre-treatment storage tank to equalize variability in graywater quality. 1-L aliquots of raw graywater were spiked with log 6 cfu/100 mL each of *P. aeruginosa*, *E. coli* and *S. enterica*.

Each aliquot was then dosed with chlorine to attain total chlorine residuals of 1.5 mg/L and 2.75 mg/L. Chlorine doses for these residual concentrations were 45.3 mg/L and 49.5 mg/L, respectively. These chlorine doses are much higher than the chlorine dose used in the demonstration treatment unit, presumably due to the addition of such high concentrations of bacteria. Organic content of the graywater was not measured prior to spiked regrowth studies. After one hour of contact time and then daily thereafter, the chlorine residual was measured. Samples were collected for bacterial enumeration immediately prior to chlorine addition, immediately after chlorine addition, after 6 hours, and then each day for 4 days. Temperature throughout the laboratory-scale pathogen-spiked regrowth study was 27°C.

3.3 Results and Discussion

3.3.1 Raw Graywater Quality and Impact of Filtration

Average raw graywater characteristics for the demonstration graywater system during the batch studies are shown in Table 3.1. These values are relevant only to the batch disinfection studies conducted during the Spring 2012 semester. These values are typical of graywater collected from showers and sinks (Eriksson et al. 2002).

Table 3.1 Raw Graywater Characteristics

Parameter	Average	Standard Deviation
TOC (mg/L-C)[a]	44	12.2
Turbidity (NTU)[a]	32	4.2
NH_3-N (mg/L-N)[a]	8.4	2.2
Total Coliforms (log cfu/100 mL)[a]	8.4	0.6
***E. coli* (log cfu/100 mL)[a]**	4.2	2.5

[a]Hodgson, 2012

Graywater for the laboratory-scale disinfection studies was collected post-filtration, and although the filters were not expected to remove substantial levels of pathogens, it was

considered possible that filtration would change water quality parameters that would affect disinfection efficacy. The coarse and cartridge filters were found previously not to provide significant removal of organics or solids from the graywater or result in any significant change in water quality (Hodgson 2012). The coarse filter provided a 15±10% removal of TOC and a -1±7% reduction in turbidity. This was expected for the coarse filter because it only provides removal of very large solids, such as hair. The cartridge filter provided a 5±20% removal of TOC and a 5±10% reduction in turbidity. The sand filter, however, provided a statistically significant removal of TOC and turbidity, with reductions of 31±17% and 13±11%, respectively (Hodgson 2012). Although the sand filter provided slight water quality improvements, it was noted that chlorine demand after sand-filtration increased, possibly due to biological growth on the sand indicated by a decrease in TOC (Hodgson 2012).

3.3.2 Disinfection of Pathogens

The inactivation of three bacteria and one bacteriophage was quantified for each filter and disinfectant combination (Fig. 3.2). Chlorine disinfection provided consistent disinfection across all filters for all bacteria tested (Fig. 3.2). For *E. coli*, chlorination post-coarse filtration resulted in the greatest measured log reduction (7.1), and the actual achievable log reduction could be higher because this reported number was based on complete *E. coli* inactivation of the initial spike (Fig. 3.2A). Interestingly, chlorination post-coarse filtration resulted in complete disinfection of *E. coli* and *S. enterica* after only a 15-minute contact time (Appendix D).

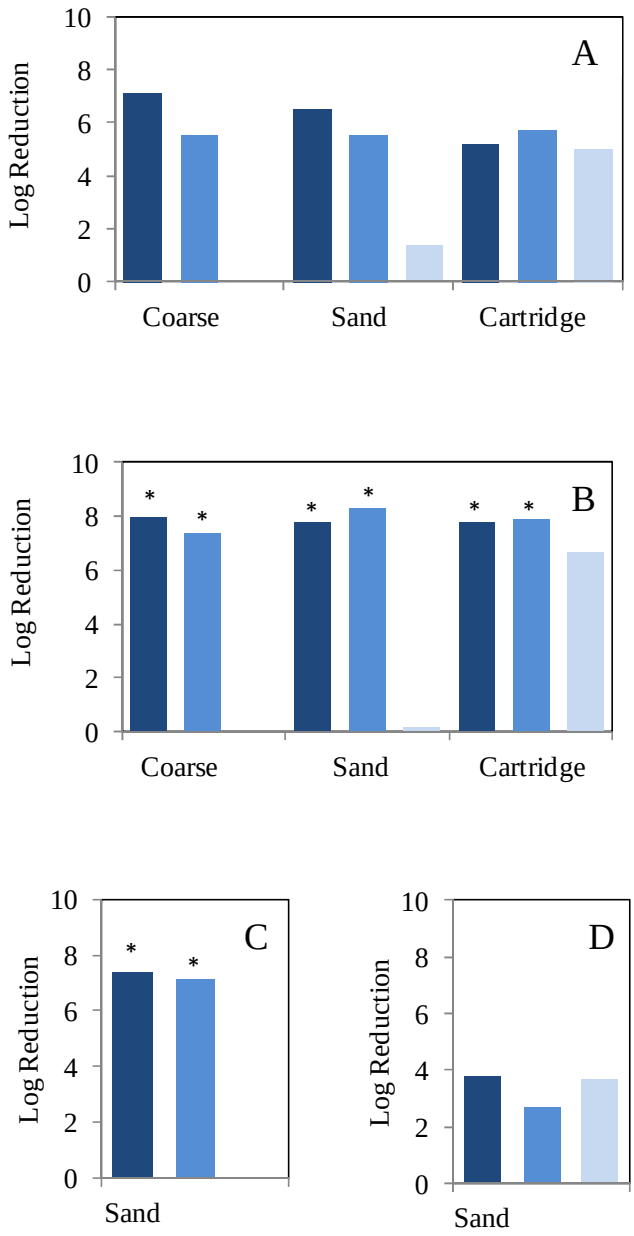

Figure 3.2 Disinfection Efficacies for Chlorine, UV and Ozone for 3 Bacteria and 1 Bacteriophage. A) *E. coli* B) *S. enterica* C) *P. aeruginosa* D) MS2 bacteriophage Chlorine results represent a contact time of 60 minutes. Log reduction of pathogens with ozone was not detected where not shown.

* indicates complete disinfection (■) chlorine, (■) UV, (■) ozone

Table 3.1 shows average water quality parameters for these tests.

For *E. coli*, the chlorination post-sand and -cartridge filtration achieved log reductions of 6.5 and 5.2, respectively (Figure 3.2A). Chlorination achieved log reductions of indigenous total coliforms of 6.4, 6.7 and 4.7 for the coarse, sand and cartridge filters, respectively (Appendix A). Chlorine also was particularly effective at disinfection of *S. enterica*. Chlorine post-coarse, -sand and -cartridge filtration provided complete disinfection of *S. enterica*, with log reductions of >8.0, >7.8 and >7.8, respectively (Figure 3.2B). Chlorine post-sand filtration achieved a >7.4 log inactivation of *P. aeruginosa*. Log reductions noted by > indicate that the log reductions achieved through disinfection were limited by the amount of pathogens spiked into the graywater. It should be noted that chlorine was present in the graywater as chloramines due to high ammonia levels in the graywater.

In comparison to other previous studies of chlorine disinfection of graywater or wastewater, the chlorination treatments studied herein demonstrated high log reductions of both indicators and pathogens. For example, the results of this study indicate that a log reduction of >7.1 could be achieved for *E. coli* with a CT of 100 mg/L-min, and a log reduction of 6.4 could be achieved for total coliforms with a CT of 297 mg/L-min. In comparison, Beck et al. (2013) reported that a log reduction of total coliforms of >3.5 could be achieved with a CT of 68 mg/L-min. Measured inactivation was limited due to the low density of total coliforms in the graywater (Beck et al. 2013). Furthermore, it is difficult to directly compare log removals for these two studies because the graywater studied by Beck et al. (2013) had an organic content that was approximately 8 times lower than the graywater studied herein; the graywater used by Beck et al. (2013) had a post-filtration (10 um) turbidity of <6 NTU and a TOC of less than 5 mg/L-C. Additionally, high log reductions of *P. aeruginosa* were achieved although past studies have demonstrated that *P. aeruginosa* can be resistant to disinfection. For example, in a study

evaluating the suitability of surrogates such as total coliforms, *E. coli*, *Enterococcus faecalis*, and *P. aeruginosa* for monitoring secondary effluent from a wastewater treatment plant, *P. aeruginosa* was found to have the lowest removal percentage, 53.57% with a chlorine dose of 30 mg/L for a 30 minute contact time (Coronel-Olivares et al. 2011). Additionally, in a study of chlorine disinfection of *P. aeruginosa* in graywater treated with a rotating biological contactor that had a relatively high organic content (average effluent COD of 40-50 mg/L), Friedler et al. (2011) only achieved an 88.5% removal efficiency of *P. aeruginosa* when the average influent concentration was 2.6 log. For the same system, Friedler et al. (2011) achieved a 99.6% removal efficiency for fecal coliforms with an average influent concentration of $1.5x10^2$ (2.2 log). Based on these results, Friedler et al. (2013) state that a treatment system producing a high quality effluent is necessary for effective disinfection; however, by contrast, the laboratory-scale disinfection study results reported herein show effective disinfection even with a relatively high organic content for all bacteria tested including *P. aeruginosa* (7.4 log).

For the bacteriophage MS2, chlorination post-sand filtration achieved a 3.8 log reduction after a 60 minute contact time. In a similar study, Beck et al. (2013) observed a 5-log inaction of MS2 with a CT above 100 mg/L-min, resulting from a contact time of 90 minutes. California Title 22 requires a 5-log poliovirus inactivation (or F-specific bacteriophage MS2 as a surrogate), which the treatment system studied herein would not likely meet. If a requirement for a 5-log reduction of viruses is widely adopted, treatment modifications, such as a longer contact time, might be able to achieve the greater required virus inactivation.

Generally, UV was nearly as effective as chlorine, even though the maximum achievable UV dose was limited slightly by the low %UVT of the graywater (between 36 and 41% UVT). UV achieved approximately a 5.5 log reduction of *E. coli* for all filters. UV was also effective at

disinfecting total coliforms with all filters, achieving log reductions ranging between 5.2 - 5.8 log (Appendix A). UV was slightly more effective at inactivating *S. enterica*, achieving log reductions of >7.4 for all filters. UV post-sand filtration provided a >7.1 log inactivation of *P. aeruginosa*.

In comparison to other previous studies of UV disinfection of graywater or wastewater, the UV treatments studied herein demonstrated log reductions of both indicators and pathogens as high as other studies despite the higher turbidity. For example, Beck et al. (2013) observed a 3.5 log inactivation of total coliforms using a UV dose of 10 mJ/cm^3 post filtration (10 um), although the reported inactivation was limited by the low density of total coliforms in the influent graywater (Beck et al. 2013). The UV treatment in the study herein achieved log reductions of total coliforms greater than 5.0 even with a turbidity over 5 times greater (Appendix A). Similarly, Friedler et al. (2011) observed a 98.2% removal efficiency of fecal coliforms (2.8 log) and a 96.4% removal efficiency of *P. aeruginosa* (2.0 log) with an average turbidity of 1.5 NTU and a UV dose of 44 mJ/cm^2. Additionally, UV disinfection of filtered-clarified treated wastewater effluent (TSS of 3 mg/L, BOD of 10 mg/L) was shown previously to achieve a 5-log reduction of *P. aeruginosa* with a UV dose of 100 mWs/cm^2 (Lorenzo Liberti et al. 2001). By contrast, in the study herein, a UV dose of 28 mJ/cm^2 achieved a >7.1 log reduction of *P. aeruginosa* post-sand filtration despite a greater turbidity. Although our study showed that UV has a disinfection rate similar to that of chlorine, additional disinfectant would be needed to provide a residual in the distribution system.

In comparison to chlorination, UV post-sand filtration achieved a lower log reduction of MS2 (2.7). Beck et al. (2013) reported a 5-log inactivation of MS2 for two of four samples following exposure to a UV dose of 100 mJ/cm^2. The UV treatment studied herein would likely

not meet California Title 22 requirements for 5-log removal of poliovirus or MS2 due to the limited UV dose. However, because an additional disinfectant is required to provide a disinfectant residual, this additional disinfectant could provide further inactivation of MS2.

Results indicate that ozone is a less effective disinfectant than both UV and chlorine in graywater with a high organic content. An ozone dose of 5 mg/L was insufficient to provide any measurable reduction of *E. coli* post-coarse filtration (Fig. 3.2A). No measureable reduction of *P. aeruginosa* with ozone occurred post-sand filtration. Ozone also achieved poor inactivation of total coliforms post-sand and -cartridge filtration, with log reductions of 0.7 and 3.5, respectively (Appendix A). By contrast, ozone disinfection post-cartridge filtration provided substantial inactivation of *E. coli* (5 log) and *S. enterica* (6.7 log). The cartridge filter provided some removal of solids and organics (TOC), which may have led to the more effective ozone disinfection (Hodgson, 2012). Overall, ozone was found to be ineffective due to size of ozone generator and high organic content in the graywater.

In comparison to other studies, the ozone treatment herein provided little inactivation of pathogens, likely due to the high organic content of the graywater. In treated wastewater with low organic content, (TDOC 7 mg/L), a 98% removal of *P. aeruginosa* was achieved with an ozone dose of 15 ppm when the pre-disinfection concentration of *P. aeruginosa* was 8-28 cfu/100 mL (1.4 log) (L. Liberti, Notarnicola, and Lopez 1999). By contrast, no measureable reduction of *P. aeruginosa* was achieved in the study herein. For the study herein, the organic content of the graywater was 6 times larger than the water studied by Liberti et al. (1999), and an ozone dose 1/3 of that in the study conducted by Liberti et al. (1999) was used. In addition, Beck et al. (2013) found that low concentrations of total coliforms (90-440 cfu/100 mL) could be disinfected to California Title 22 standards (2.2 cfu/100 mL) at a CT of 0.4 mg/L-min (Beck et

al. 2013). The successful inactivation of bacteria using ozone in waters with low organic content indicates that the relatively high organic content of the water in this study inhibited effective ozone disinfection.

Although MS2 inactivation was only tested post-sand filtration, it is likely that the efficacy of each disinfectant on the inactivation of MS2 would be similar for the coarse filter and cartridge filter because the quality of the filtered graywater did not vary significantly between filters. Ozone disinfection post sand-filtration achieved a 3.7 log reduction of MS2, which was comparable to chlorination. This result indicates that ozone may be as effective as chlorine and UV for disinfecting viruses in graywater with a relatively high organic content. Like the UV treatment, the ozone treatment was insufficient to meet California Title 22 requirements for virus removal and treatment modifications would likely be needed to achieve the required virus inactivation.

Based on the results of the laboratory-scale studies and economic feasibility analysis conducted as part of a separate study (Hodgson, 2012), chlorination was investigated further to determine how effective it is with respect to preventing regrowth.

3.3.3 Regrowth

Regrowth of total coliforms was observed for graywater collected on some of the days tests were conducted (Figures 3.3B, C and D), probably due to the higher concentrations of organics in the filtered graywater on those days. For example, the raw graywater TOC for Fig. 3.3D was 85 mg/L-C, which is much higher than the average TOC of approximately 51 mg/L-C (+/-14.5 mg/L-C) observed for the period during which the regrowth tests were conducted (Spring, 2012 to Spring 2013). This TOC was the highest TOC observed over that period, and the second highest TOC measured was 68.2 mg/L-C out of 29 samples.

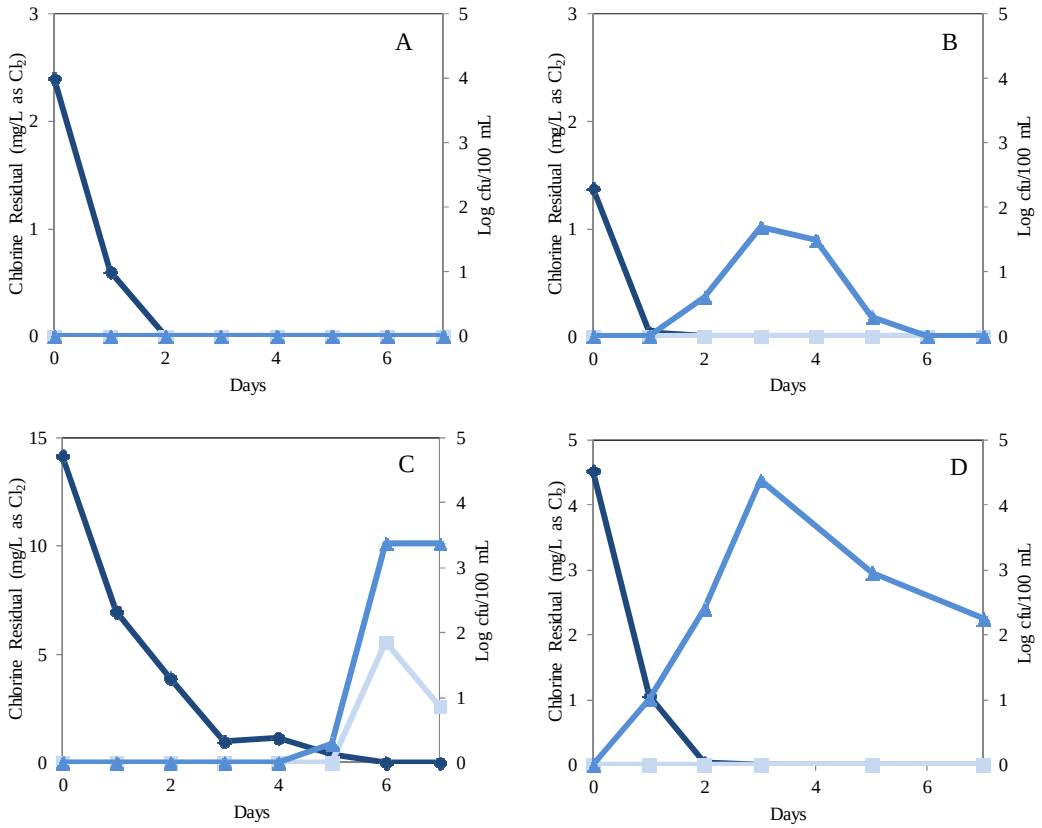

Figure 3.3 Regrowth of Total Coliforms and *E. coli* Over 7 Days. Graphs A-D represent separate collection events. Table 3.2 includes the TOC and turbidity of the raw graywater for each regrowth study. (◆) chlorine residual, (▲) total coliforms, (■) *E. coli*
The raw TOC of the graywater for Figures A-D are 27.5*, 45.0, 49.2 and 85.3 mg/L-C, respectively. *The TOC for the regrowth study in graph A was measured on graywater collected 1 day prior to the beginning of the study.

TOC values for graywater originating from bathroom sources has been reported higher than 100 mg/L-C (Surendran and Wheatley 1998), although other sources report an average of 40 mg/L-C or less (Eriksson et al. 2002). Figure 3.4 shows the results of the regrowth study conducted in a 5-gallon bucket. The influent TOC for this study was 27.5 mg/L-C and the results are consistent with the regrowth study conducted in a toilet with the same TOC (Fig 3.3A). However, these five studies show that while there is a potential for regrowth of total coliforms when the organics content of the graywater is high, regrowth of bacteria can be prevented with a chlorine residual of >2.4 mg/L for lower TOC levels (Fig. 3.3A).

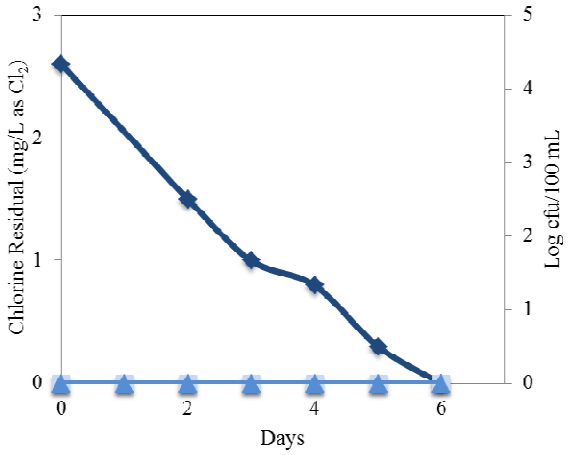

Figure 3.4 Regrowth Study (◆) chlorine residual, (▲) total coliforms, (■) *E. coli*
This regrowth test is shown separately because it was performed with approximately 3 gallons of graywater in a 5-gallon bucket. TOC of the graywater used in this experiment was 27.5 mg/L-C.

Table 3.2 Influent Graywater Quality for Regrowth Studies

Graph	TOC (mg/L-C)	Turbidity (NTU)
A[a]	27.5	28.4
B	45.0	25.8
C	49.2	30.7
D	85.3	36.8

[a]Water quality test date was 1 day prior to beginning of regrowth

Findings of this study are consistent with previously reported studies. March et al. (2004) observed that regrowth of HPC, fecal coliforms, *P. aeruginosa*, and *S. aureus* did not occur with a chlorine residual above 0.5 mg/L for up to 6 hours in treated graywater with an average TOC of 39.9 mg/L-C, although regrowth was not examined for longer periods of time in that study. In another study of chlorination following graywater treatment by an RBC, no regrowth for HPC, fecal coliforms, *P. aeruginosa*, and *S. aureus* were observed for 6 hours following chlorination (Friedler et al. 2011). Beck et al. (2013) reported that a chlorine CT of 288 mg/L-min was sufficient to prevent regrowth of *E. coli* and total coliforms for up to 15 days (Beck et al. 2013); however, total coliforms were found to be non-detect after 15 days in a non-disinfected control

sample suggesting that the low TOC graywater tested did not contain enough nutrients to support bacterial regrowth (Beck et al. 2013). Thus, the findings reported herein expand upon previous studies by demonstrating that regrowth can be prevented in treated graywater with a high organic content (TOC > 27.4 mg/L-C) over extended periods of time. This study indicates that despite the lack of removal of organics achieved by a simple treatment system consisting of only filtration and chlorination, disinfection in conjunction with a sufficient chlorine residual can prevent regrowth of indicator organisms in stored graywater for at least 2 days (Fig. 3.3).

3.3.4 Spiked Regrowth

Because pathogens are the actual risk drivers in graywater reuse, this study examined how the regrowth of pathogens compared to the regrowth of indicator organisms. The results indicate that a chlorine residual concentration of 2.75 mg/L (Figure 3.5A) prevented regrowth of all pathogens tested for at least 4 days even though pathogens were all spiked at an extremely high concentration (log 6/100 mL). Thus, this result also indicates that a residual of 2.75 mg/L can prevent regrowth even during a high-contamination event (e.g., when residents of a building are experiencing a high level of illness). By contrast, it was found that a chlorine residual of 1.5 mg/L (Figure 3.5B) was not sufficient to prevent the regrowth of total coliforms, *S. enterica* or *E. coli*.

The results of the spiked regrowth studies are consistent with the unspiked regrowth studies. A chlorine residual of 1.5 mg/L was not sufficient to completely prevent regrowth of bacteria for both the spiked and unspiked regrowth studies. A chlorine residual of 2.5 mg/L or higher, however, was sufficient in both the spiked regrowth and unspiked regrowth to prevent regrowth for at least four days as long as the TOC was relatively low (e.g., 27.5 mg/L-C).

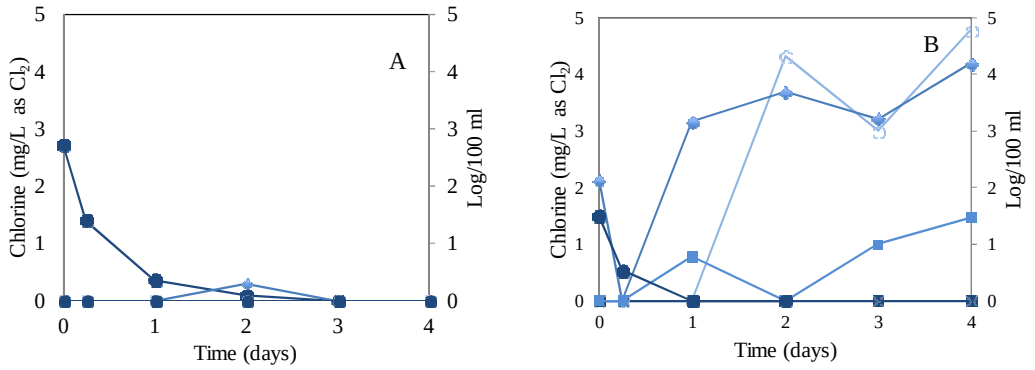

Figure 3.5 Regrowth of Spiked Pathogens in Graywater with Two Different Chlorine Residual Concentrations, 2.75 mg/L (A) and 1.5 mg/L (B) (•) chlorine residual, (■) *E. coli*, (o) *S. enterica*, (♦) total coliforms, (x) *P. aeruginosa.* Influent TOC was not measured for these tests. For graywater originating from the same source (i.e. same group of students), the range of TOC was 45.0 to 85.3 mg/L-C. The average TOC was 61.6 mg/L-C with a standard deviation of 8.3 mg/L-C.

Although the TOC of the graywater used for the spiked regrowth tests was not measured, the range of TOC found for the graywater originating from the same source as the graywater used for the spiked regrowth test was 45.0 to 85.3 mg/L-C, with an average of 61.6 mg/L-C and a standard deviation of 8.3 mg/L-C. The chlorine demand of the sample in Figure 3.5A was 46.8 mg/L and the chlorine demand of the sample in Figure 3.5B was 43.8 mg/L. The typical chlorine demand for the graywater collected in the demonstration system was approximately 17 mg/L during the Fall 2012 and the Spring 2013 semesters. The chlorine residual in the demonstration system on the day of sampling was 1.6 mg/L. The average chlorine residual for the week of sampling was 2.4 mg/L. Generally, it was found that when indicator regrowth was prevented, pathogen regrowth was also prevented for the specific species tested. Additionally, it should be noted that the pathogen-spiked regrowth studies were conducted at a relatively high temperature (27°C), and given that bacterial growth rates generally increase with temperature, the results of this study represent a conservative measure of pathogen regrowth.

Because many pathogens are not considered in water quality regulations and it is not currently possible to monitor all known pathogens, indicators that accurately represent the behavior of pathogens in graywater are needed to predict levels of pathogens in graywater. Interestingly, *S. enterica* and total coliforms exhibited a similar regrowth pattern, indicating that total coliforms may be a good indicator for *S. enterica*. These findings are consistent with previous studies comparing indicator organisms as surrogates for pathogens. *Salmonella* spp. have previously been shown to be significantly correlated to fecal coliforms in stream samples (Krometis et al. 2010). Regrowth of *E. coli* and *P. aeruginosa* was low compared to regrowth of *S. enterica* and total coliforms under the low chlorine residual of 1.5 mg/L. Because *E. coli* is a coliform bacteria, it would be expected to exhibit a similar regrowth pattern as total coliforms. However, it is possible that the laboratory strain *E. coli* used in these studies may be less resistant to disinfection and therefore less able to regrow than wildtype strains. Studies examining the regrowth of *P. aeruginosa* in reclaimed water systems have not revealed a systematic regrowth pattern. Jjemba et al. (2010) reported that 60% of reclaimed water samples were positive for *P. aeruginos*a with high levels of assimilable organic carbon (AOC), but Wang et al., (2012) found less than 10% of reclaimed water samples contained *P. aeruginosa*. Both studies also examined *Mycobacterium* spp. and *Legionella* spp., finding both bacteria more prevalent than *P. aeruginosa* in reclaimed water systems (Jjemba et al. 2010; Wang et al. 2012). Additionally, the results of this study are limited to the bacteria tested, and future work should be conducted to determine the regrowth potential of other bacteria in graywater, specifically gram-positive bacteria which may be more resistant to disinfection.

3.4 Conclusions

Graywater reuse for toilet flushing is gaining attention as a way to ease the water stress created by growing populations in arid regions. The relatively high cost and high maintenance requirements of biological treatment systems may inhibit widespread adoption and limit the potential water savings of graywater. Simple treatment systems, however, are low cost and can be maintained without a trained operator. In order for simple graywater reuse systems to be used to meet water demand for toilet flushing, public health must be protected. The results of this study indicate that a simple treatment system consisting only of filtration and disinfection can be effective at inactivating indicators and pathogens in graywater and preventing regrowth. Overall, chlorine could provide disinfection of bacteria and MS2 bacteriophage, prevent the regrowth of bacteria for at least 2 days, and provide a disinfectant residual that can be monitored in the system effluent. Although UV has a removal rate similar to that of chlorine, additional disinfectant would be needed to provide a residual. Ozone was ineffective due to the size of the ozone generator and organic content in the graywater.

In addition, residents using graywater could allow treated graywater to stay in their toilets without flushing for at least 2 days if sufficient chlorine residual is present. Based on these results, an operational recommendation is that residents switch toilets to potable water before longer absences. Finally, our results indicate that a residual chlorine level of at least 2.75 mg/L can still ensure a safe effluent with no regrowth even during a high contamination event when organics are not removed from graywater.

4.0 ASPEN HALL DEMONSTRATION GRAYWATER TREATMENT SYSTEM

4.1 Introduction

Following laboratory-scale disinfection studies and filtration studies (Chapter 4; Hodgson, 2012), a modified demonstration graywater treatment system was designed for Aspen Hall. The system treats an average of 300 gallons of graywater from 28 students in 14 dorm rooms on the first floor of Aspen Hall. The goal of the demonstration unit is to prove that a low-cost, simple graywater treatment system can produce an effluent suitable for reuse in toilets while protecting public health. Through operation of the demonstration unit during the Fall 2012 and Spring 2013 semesters, long-term system performance was evaluated and the use of graywater in one toilet temporarily plumbed in the Aspen Hall graywater room was observed.

4.2 Materials and Methods

4.2.1 System Description

Criteria for the design of this treatment system included a low operating cost, easy to maintain, and most importantly, capability to provide an effluent safe for toilet flushing (Hodgson, 2012). Through experiments and experience with filtration and disinfection alternatives (Chapter 4; Hodgson, 2012), coarse filtration and chlorine disinfection were selected for the treatment system. The coarse Matala medium density filter, the cartridge filter (100 um) with granular activated carbon (GAC) and the sand filter (20-40 um) did not have a significant effect on chlorine consumption (Hodgson, 2012). The sand filter did provide better removal of organics than the coarse and cartridge filters; however, the sand filter required more maintenance, and could have adverse effects on chlorine demand due to biological growth in the

filter (Hodgson, 2012). Therefore, the coarse Matala filter was selected for implementation in the current treatment system due to its ease of use and low cost (Hodgson, 2012).

Chlorine disinfection was selected over UV and ozone disinfection for the current graywater treatment system. Chlorine generally provided the greatest inactivation of *E. coli*, *S. enterica*, *P. aeruginosa*, and MS2. Chlorine is also a low cost disinfectant that provides a disinfectant residual that is easy to monitor in the distribution system or toilet to prevent regrowth. Figure 4.1 is a picture of the current demonstration graywater system. Figure 4.2 is a picture of the graywater treatment process.

Graywater is collected in the storage tank where compositing of the graywater as well as settling of larger solids occurs. The tank is sized such that storage is limited to 24 hours to prevent growth of organisms and deterioration of graywater quality. From the storage tank, "graywater gravity flows through the coarse filter and is dosed in-line with sodium hypochlorite before entering the disinfection contact tank" (Hodgson 2012). The treatment system also includes a potable water make up supply if graywater is depleted, and vents and overflow lines per plumbing code (IPC Appendix C). The graywater system was not hooked up to student toilets during the period of study so treated graywater was released on a flush timer. To simulate a flushing event, an electronic valve opens and the pressure booster pump (Grundfos, Olathe, KS) pulls water from the disinfection tank. An ultrasonic float switch (Flowline, Los Alamitos, CA) controls the electronic valve that releases graywater from the disinfection tank and allow water from the storage tank in to the disinfection tank.

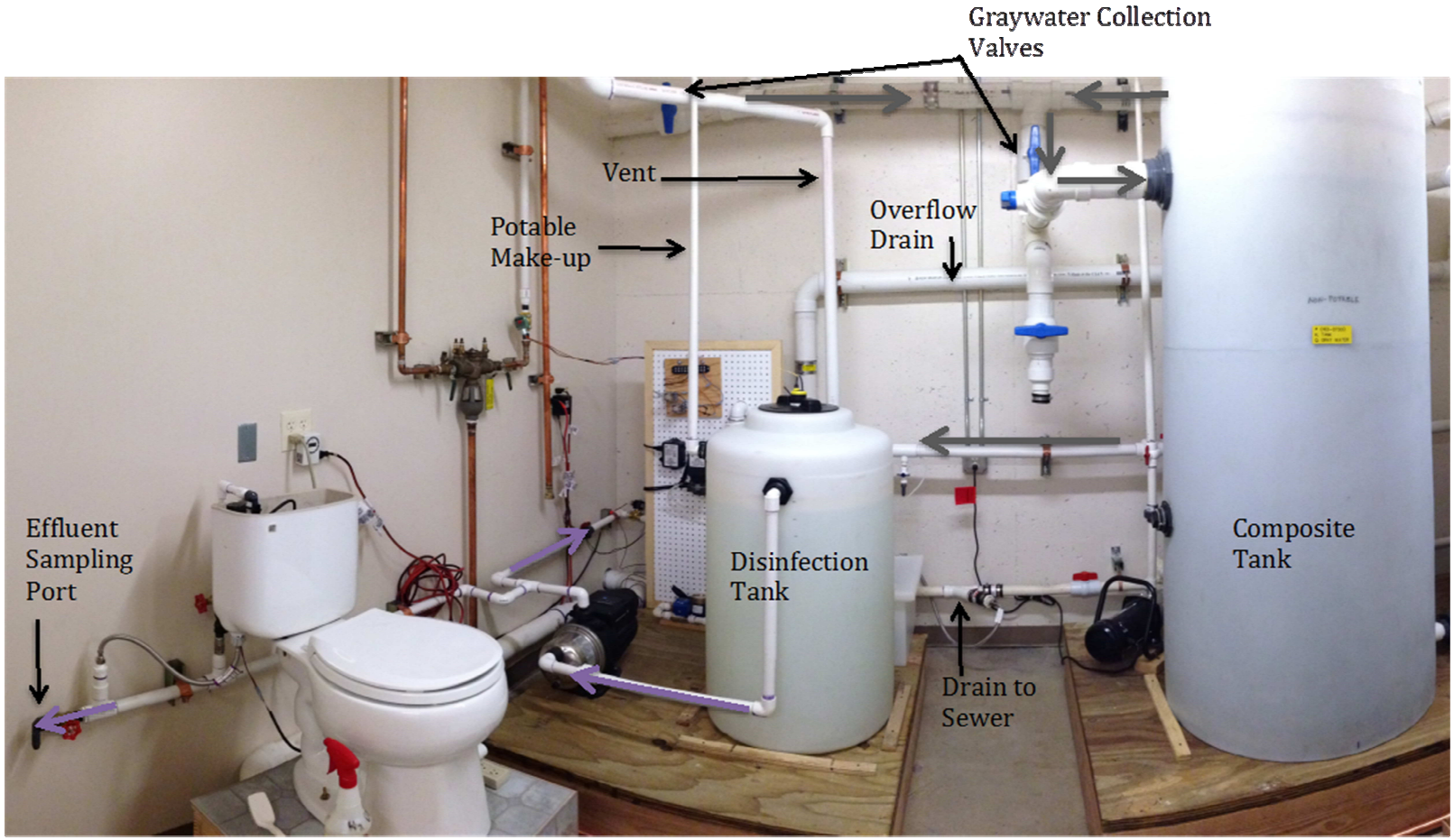

Figure 4.1 Schematic of Demonstration Graywater Treatment System
Gray arrows indicate raw graywater, purple arrows indicate treated graywater.

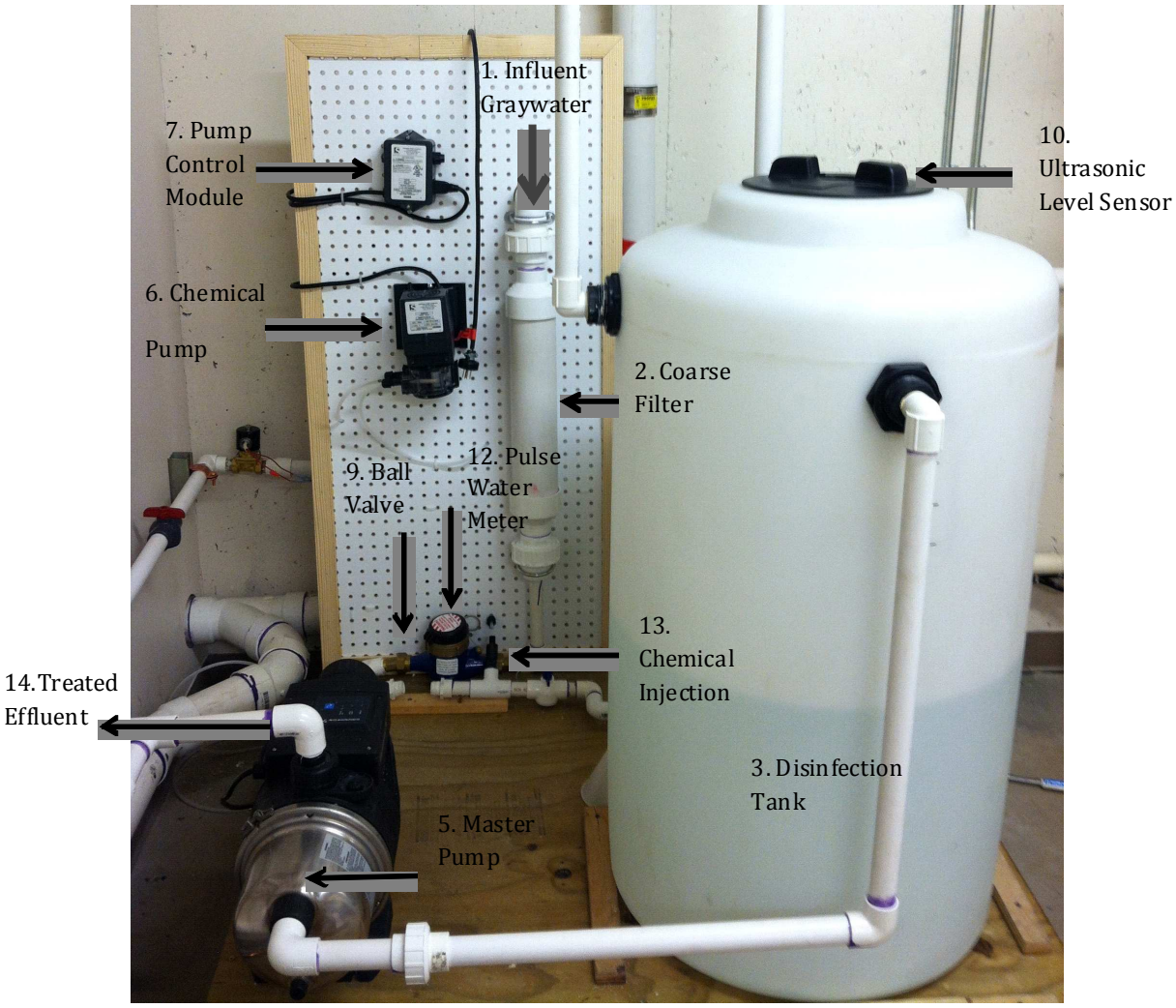

Figure 4.2 Schematic of the Graywater Treatment Process

When the ultrasonic float switch senses a low water level (Level 2; Fig 4.3) in the disinfection tank, an electronic valve opens, allowing graywater to enter the disinfection tank. At a lower water level (Level 3; Fig. 4.3), an electronic valve is opened to allow potable water to enter the tank. Water level 4 is the level at which graywater is pulled out of the disinfection tank. To document maintenance and testing procedures, an SOP was written for the graywater treatment system. The SOP contains information about system start-up, shut-down and regular maintenance. The SOP for the current graywater treatment system can be found in Appendix B.

Details about system components and operation are present in the SOP, which can be found in Appendix B.

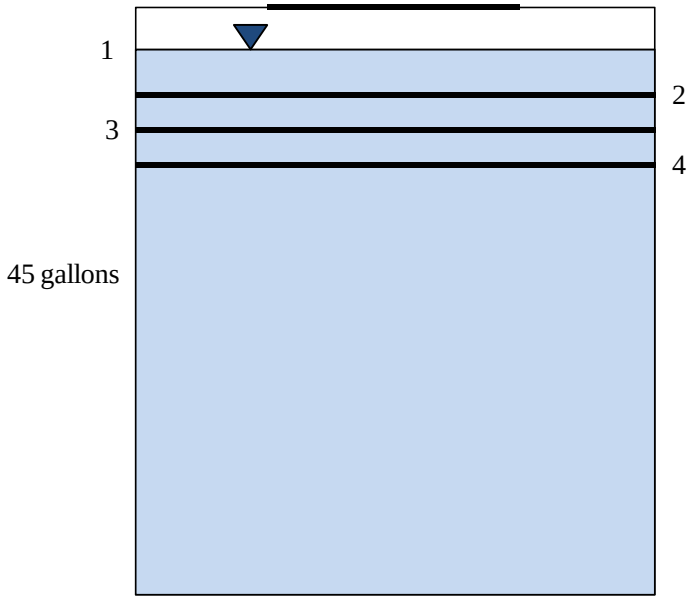

Figure 4.3 Ultrasonic Water Level Sensor Design (adapted from Hodgson, 2012) Volume between each level is approximately 3.7 gallons of graywater.

Additionally, a demonstration toilet was plumbed to use the treated graywater from the demonstration unit. The toilet flushed 5 times per day to simulate actual use of a toilet. The toilet was cleaned once per week using the same cleaning product used for cleaning student dorms, which are also cleaned once per week. Toilet components were monitored for unusual wear on components such as the toilet flapper.

4.2.2 Chemical and Biological System Performance Monitoring

Water quality parameters including TOC, total nitrogen (TN), ammonia, turbidity, total chlorine, *E. coli* and total coliforms were monitored. Influent graywater samples were taken before treatment but after the storage tank (where some settling occurs). Effluent samples were taken

after a combination of filtration and chlorination. 1-L effluent samples were collected from a sampling port that leads out of the disinfection tank (Fig. 4.1), where treated graywater was stored. Samples were also collected from the toilet plumbed to use graywater. Samples were taken from the toilet bowl with a sterile pipette. Additionally, three samples from a toilet containing potable water were analyzed for chlorine, total coliforms and *E. coli*. It should be noted that the demonstration toilet containing graywater and the potable toilet used for sampling were cleaned at the same frequency and with the same cleaning product provided by CSU Housing Facilities. Water quality parameters were measured generally as described in section 3.2.2; however, details are repeated here for convenience. TOC and TN were measured with a Shimadzu TOC-V CSH/CSN (Shimadzu, Japan), which utilizes combustion and acidification process. Ammonia was quantified using an ion-selective ammonia electrode (Fisher Scientific, Waltham, MA). Turbidity was analyzed using a Hach 2100N nephelometric turbidimeter. Total chlorine and was measured using a Hach total chlorine test kit with a Hach DR2500 spectrophotometer. *E. coli* and total coliforms were quantified using Colilert-24 Quantitray method approved by the EPA (IDEXX, Westbrook, Maine). Colilert-24 powder pillows were added to 100 mL samples of graywater, the sample was sealed in a Quantitray and incubated at 35°C for 24 hours before quantification.

4.2.3 Threshold Chlorine Residual for Preventing Pathogen/Indicator Growth

Chlorine residual was to be used as a surrogate for predicting whether or not bacterial regrowth was occurring because monitoring microbial concentrations in graywater often involves overnight culturing. Chlorine was dosed into 300-mL aliquots of graywater into 500-mL glass flasks to obtain chlorine residuals between 0.5 mg/L and 2.0 mg/L. After a contact time of 1

hour, chlorine residual was measured and 3 samples were chosen for microbiological monitoring. The three samples chosen had chlorine residuals of 0.77 mg/L, 0.93 mg/L and 1.67 mg/L. Total coliforms and *E. coli* were monitored at 0 hours (after 1 hour contact time), 6 hours, 24 hours and 72 hours.

4.2.4 Interaction of Dye with Disinfected Graywater

Where graywater reuse is allowed, regulations may require that graywater be dyed blue in order to visually communicate to toilet users that the water in the toilet is non-potable (Bergdolt, Sharvelle, and Roesner 2011). To insure that the chlorine residual would not interfere with the dye, and vice versa, a simple dye experiment was conducted to test if the dye would increase the chlorine demand of the graywater.

Graywater from Aspen Hall was disinfected with chlorine and the residual total chlorine was measured after ~1 hour of contact time. Brac Blue Dye (Brac Systems, Ontario) was added after disinfection at concentrations of 0.5, 2, 3, 5, 10 μL dye/mL graywater. Basic food coloring was also tested at concentrations of 1, 5, 1, 15, 20 μL dye/mL graywater. Chlorine residual was measured for the Brac Blue sample dosed at 3 μL dye/mL graywater after addition of dye, at 1 day, 3 days and 14 days. Because Brac Blue provided a more economic dye alternative than food coloring, only samples dyed with Brac Blue were measured for chlorine residual.

4.3 Results and Discussion

4.3.1 Demonstration Unit Effluent Water Quality

Figure 4.4 shows the average influent and effluent water quality parameters during 12 weeks of sampling over the Fall 2012 and Spring 2013 semesters. For each parameter, a Student's t-test was performed to determine if the influent and effluent were significantly

different. Filtration and disinfection did not achieve statistically significant removal of organics (TOC) or nitrogen compounds (TN and ammonia) ($p>0.1$). There was a statistically significant increase in turbidity after filtration and disinfection ($p<0.05$).

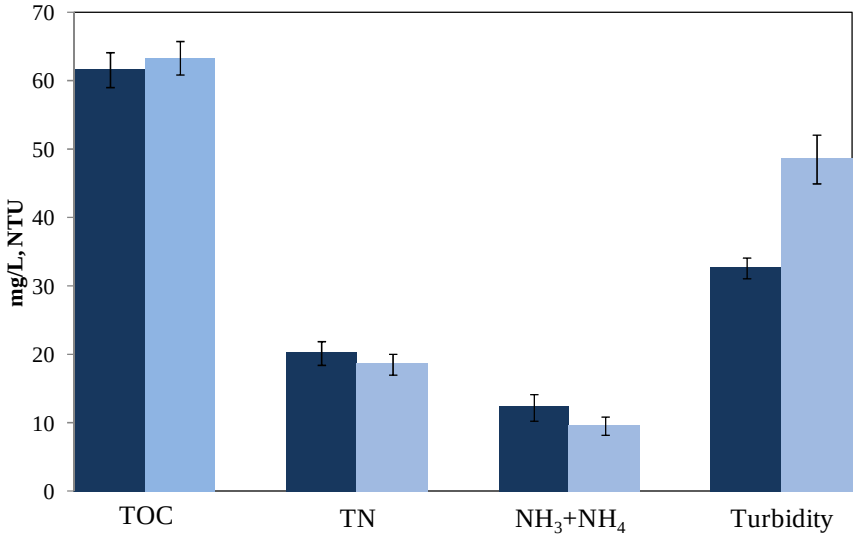

Figure 4.4 Average Graywater Quality (■) Influent (■) Influent

Table 4.1 shows the average water quality parameters of graywater in the demonstration toilet during the Fall 2012 and Spring 2013 semesters. The water quality in the toilet was not found to be significantly different from the effluent water quality. Note that the graywater quality for the Fall 2012 and Spring 2013 semesters is different than the graywater quality in the Spring 2012 semester.

Table 4.1 Graywater Quality in Demonstration Toilet

Parameter	Average	Standard Deviation
TOC (mg/L - C)	59.0	8.8
Turbidity (NTU)	42.6	10.1
TN (mg/L – N)	15.6	6.4
NH_3+NH^4	8.8	3.9

Differences in graywater quality could be due to differing personal hygiene habits and personal care products used by the students who inhabited the dormitories in the 2011-2012

school year versus 2012-2013 school year. Influent total coliforms were greater than log 7.4 cfu/100 mL for all samples. Effluent total coliforms were not detected in 5 out of 7 samples, and the greatest number of total coliforms measured was 1.6 cfu/100 mL. Similarly, total coliforms were not detected in 5 out of 7 samples from the graywater demonstration toilet. The maximum number of total coliforms detected in the toilet was 39.3 cfu/100 mL. No *E. coli* were detected in any of the effluent or graywater demonstration toilet samples. Influent *E. coli* ranged from 0 to 1,179.5 cfu/100 mL, with a median of 41 cfu/100 mL. Of the three samples collected from a potable water toilet, one of the three samples was positive for total coliforms and *E. coli*, with counts of 31.3 cfu/100 mL and 3.1 cfu/100 mL, respectively.

4.3.2 Operational Experiences

During the Fall 2012, unstable chlorine residual was observed (Fig 4.5). Abrupt losses of chlorine residual were causing growth of bacteria in the disinfection tank (Fig. 4.5). In an effort to resolve the unstable chlorine residual, the potable water make-up line was turned off because it was believed that the potable water, which had no measurable residual chlorine, might be "diluting out" the chlorine residual. In addition, black particles that were large in size were observed in the graywater samples taken after the storage tank and before treatment. The pre-treatment storage tank, which had a black biofilm near the bottom of the tank due to settling of solids during storage, had not been cleaned since installation in 2010. Therefore, the pre-treatment storage tank was cleaned during Fall Break (Nov. 19-23, 2012) by power washing and flushing with potable water. Prior to cleaning the pre-treatment storage tank, the average chlorine residual was 1.6 mg/L. After cleaning, a stable chlorine residual was observed for the remaining

3 weeks of the fall semester and again in the spring semester (Fig. 4.5). The average chlorine residual after cleaning was >3 mg/L.

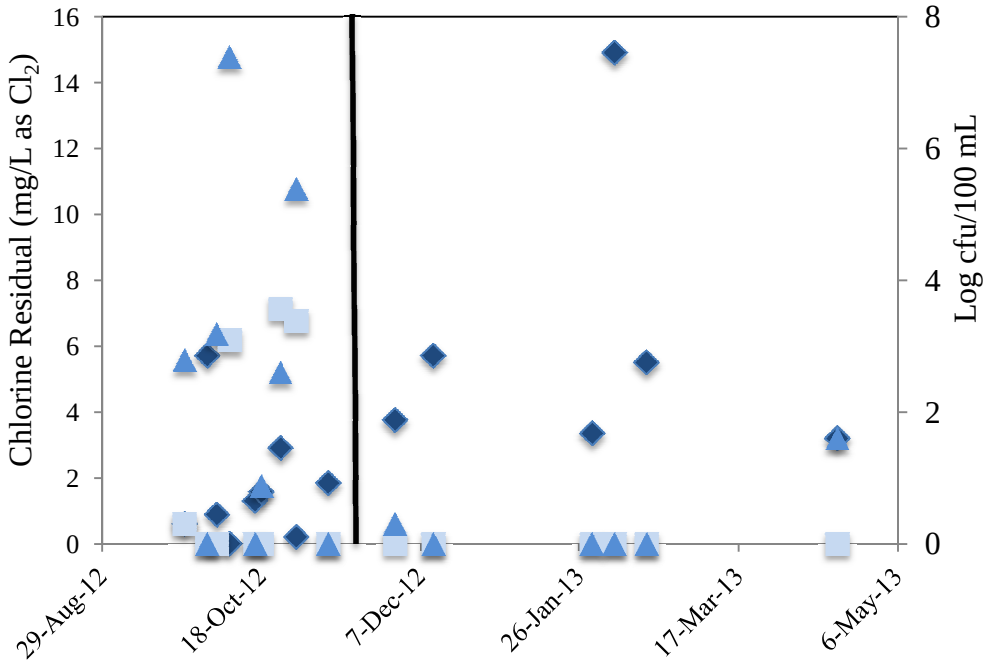

Figure 4.5 Chlorine Residual, Total Coliforms and *E. coli* During Operation; black vertical line indicates cleaning of pre-treatment storage tank
(♦) chlorine residual, (▲) total coliforms, and (■) E. coli

During the stable operation, total coliforms were observed in 2 of 6 samples (0.3 and 1.6 log cfu/100 mL), and *E. coli* was not detected in any samples. The potable water supply was turned back on in March 2013, and no change in the stability of the chlorine residual or system performance was observed. Figure 4.6 shows the inside of the storage tank before and after cleaning. A cleaning frequency of once per semester was added to the SOP (Appendix B).

Figure 4.6 Storage Tank Before (left) and After (right) Cleaning

No adverse effects on the toilet flapper and other toilet components were noticed over 8 months (September 20, 2012 through May 16, 2013) of operation with graywater (Figure 4.7). Average chlorine residual was 1.8 mg/L, ranging from 0 to 15 mg/L. Some discoloration of the toilet flapper may have occurred due to chlorination, but no shrinkage or cracking was observed. Although some blackening of the film appeared on the flapper (Figure 4.7), the flapper was still in working order. In addition, a high organic load is usually noticed at the end of the Spring semester as students leave the dorms, and it is possible that the blackening of the flapper is a result of the high organic load. A report by Kuru and Luettgen (2012) for Kohler Co. reported deteriorations of toilet flappers such as stiffening of the elastomer and geometric shrinkage and deformation on toilets using treated graywater (Kuru and Luettgen 2012).Observations from the demonstration toilet at Aspen Hall do not suggest problems as observed by Kuru & Luettgen (2012), likely a result of the use of a highly controlled dose of chlorine in the treatment system. In the study by Kuru and Luettgen (2012) some deterioration of the toilet flapper occurred in all toilets; however, the graywater effluent from the advanced oxidation (H_2O_2 and UV) treatment system caused the greatest shrinkage and deformation of the toilet flapper (Kuru and Luettgen 2012).

Figure 4.7 Toilet Flapper Monitoring; March 3, 2013 (left), approximately 5 months after operation started. Toilet flapper on May 16, 2013 (right).

Factors such as periodic cleaning of the toilet tank and chlorine residual may have an effect on the rate of deterioration of toilet components. In practice, it would be recommended that homeowners periodically check the state of their toilet flapper for failure.

4.3.3 Threshold chlorine residual for preventing pathogen/indicator growth

Although a simple graywater reuse system does not provide removal of organics, inactivation of pathogens can still be achieved through disinfection. Monitoring microbial concentrations in graywater often involves overnight culturing, which is impractical for operating a treatment system where quick changes in disinfectant dose might be necessary. Additionally, most homeowners do not have access to a laboratory and culturing supplies. To overcome these challenges, chlorine residual is proposed as a surrogate for predicting whether or not bacterial regrowth is occurring.

A minimum threshold chlorine residual is necessary to insure complete disinfection and prevent regrowth. In this bench-scale study, it was observed that a chlorine residual of 1.67 mg/L

in raw graywater (with a contact time of 1 hour) was sufficient to disinfect the graywater and prevent regrowth of total coliforms for two days (Figure 4.8). Influent TOC for the graywater used in this experiment was 85.3 mg-C/L.

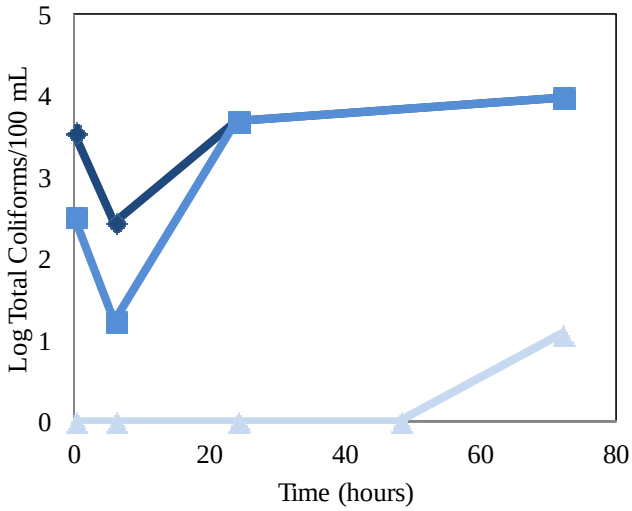

Figure 4.8 Regrowth of Total Coliforms at Three Disinfectant Residual Concentrations.
E. coli in influent graywater was measured to be 100 cfu/100 mL; however, regrowth of *E. coli* was not observed for any sample.
Chlorine residuals are (♦) 0.77 mg/L, (■) 0.93 mg/L, and (▲) 1.67 mg/L

E. coli was present in the influent water at 100 cfu/100mL but was not detected in disinfected samples. The results of this study are consistent with the regrowth studies presented in Chapter 3, where high organic content and low chlorine residual in the graywater resulted in the regrowth of bacteria after approximately two days. The results of this study are also similar to the trend observed in the full-scale system at Aspen Hall, where a chlorine residual of less than 1 mg/L tended to result in an increase of biological growth and loss of chlorine residual altogether (data not shown).

4.3.4 Interaction of Dye with Disinfected Graywater

Results of the dye test indicate that dye may have some effect on the disinfected graywater. Table 4.2 shows the residual total chlorine measurements of the Brac Blue sample (concentration 3 µL dye/mL graywater) after 1 hour, 1 day, 3 days, and 14 days. Figure 4.9 shows the Brac Blue dye samples at 1 hour and at 14 days after addition of dye.

Table 4.2 Chlorine Residual Over Time of Graywater Dyed with Brac Blue

	Chlorine Residual (mg/L)				
	Initial (no color)	1 hour	1 day	3 days	14 days
BracBlue	15.4	15.0	12.5	10.3	5.38

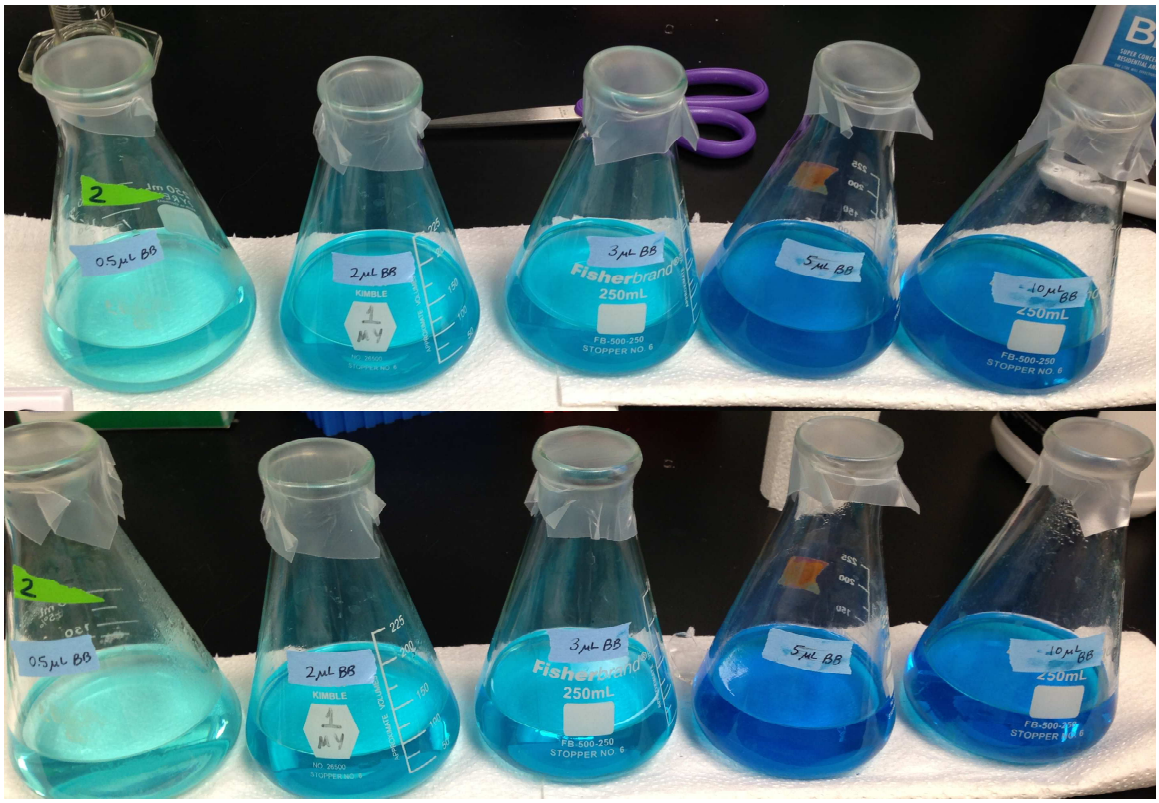

Figure 4.9 Graywater with Brac Blue Dye; 1 hour after addition of dye (top) and 14 days after addition of dye (bottom)

The photographs show that the dye is stable in disinfected graywater, even after 14 days, which is much longer than a typical residence time for treated graywater in a household. Although the dye did not react completely with the residual chlorine (Table 4.2), the chlorine residual was much higher than operating residual. Another test is recommended in which residual chlorine is closer to the operating residual to determine the effects of the dye on the disinfected graywater.

4.4 Summary

A standard operating procedure was created based on experiences with the demonstration graywater treatment system at the Aspen Residence Hall (Appendix B). Interestingly, unstable chlorine residuals in the Fall 2012 semester were likely due to settled material built up in the composite tank. Following cleaning, a stable chlorine residual was observed along with a decrease in total coliforms and *E. coli* in the treated graywater. Although solids and organics are not removed through the coarse filtration and disinfection treatment, chlorine disinfection was shown to be effective at inactivating indicator organisms. Additionally, a preliminary study suggests that Brac Blue dye does not interfere with chlorine residual, which is important to ensure protection of public health while complying with plumbing code for graywater reuse.

5.0 CONCLUSION

Water supplies in arid regions of the United States are becoming stressed as populations continue to grow, particularly in seasons of drought. Because almost all water supplies are fully developed in arid regions such as the western United States, new conservation techniques and water management practices will be needed to insure that water is available for growing populations. Low-flow fixtures have already played a role in reducing the potable water demand; however, water shortages persist due to the limited water savings of these technologies. Graywater reuse for toilet flushing is now gaining attention as a way to decrease household potable water demand by approximately 25%. If only graywater from showers, baths, and bathroom washbasins were to be reused, that provides over 10,000 gallons per person of graywater available for reuse each year (Rockaway et al. 2011; Bergdolt, Sharvelle, and Roesner 2011). Graywater from showers, baths, and bathrooms washbasins provides a constant source of water that is relatively low in organics and therefore easy to treat on-site for reuse. Additionally, unlike irrigation, the reuse of graywater for toilet flushing can be taken advantage of year-round to maximize water savings. In order for graywater reuse to be widely adopted, however, the treatment systems for graywater must be simple, inexpensive, and capable of consistently providing an effluent free of pathogens to protect public health.

The laboratory-scale disinfection studies (Chapter 3) and filtration studies (Hodgson 2012) allowed for the investigation of the optimal combination of filtration method and disinfection method. Three filtration methods (coarse, sand and cartridge) in combination with three different disinfectants (chlorine, UV and ozone) were tested. Coarse filtration was selected as the best filtration alternative due to the low cost and ease of maintenance of the filter. The cartridge filter was more costly than the coarse filter but did not provide substantial

improvements in water quality or potential disinfection efficacy (Hodgson 2012). The sand filter provided some removal of solids and organics; however, the minor water quality improvement was not considered worth the increased cost and maintenance associated with this filter (Hodgson 2012). Chlorine provided the most efficacious inactivation of both indicator organisms and pathogens. UV was only slightly less effective at inactivating pathogens; however, UV does not provide a residual disinfectant for preventing regrowth and quick monitoring of system performance. Ozone was found to be ineffective at pathogen inactivation, likely due to the limited size of the ozone generator tested and the amount of organics in the graywater.

Following the implementation of coarse filtration and chlorination in a demonstration unit in Aspen Hall, regrowth studies were conducted in a toilet plumbed with graywater to determine if public health could be protected even days after disinfection occurred. The results of the regrowth studies indicate that a chlorine residual of at least 2.75 mg/L should be maintained in the disinfection tank to insure that regrowth of pathogens does not occur. Even when the performance was challenged through spiking of pathogens into graywater at concentrations exceeding 6 log cfu/100 mL, the regrowth of pathogens *E. coli, S. enterica* and *P. aeruginosa* and indicator total coliforms was prevented for 4 days when the chlorine residual was 2.75 mg/L. Thus, given the collective results of spiked and unspiked tests, with a residual of 2.75 mg/L, it was observed that regrowth of indicators and pathogens can likely be prevented for at least 2 days with a TOC of less than 50 mg/L-C. A duration of 2 days represents a short trip where homeowners may leave their houses without flushing a toilet.

Based on the results of this study, the demonstration unit appears to be practical for use at the multi-residential scale. Although the system provides little removal of organics, disinfection can still provide complete inactivation of indicators and pathogens for the protection of public

health. Operation and maintenance of the designed treatment system is both simple and low cost. Further studies will be conducted with students using the graywater in toilets in the residence hall to determine public perception of the treated graywater. Operation and maintenance of the graywater treatment system in Aspen Hall will be turned over to certified plumbers in Spring 2014, no longer requiring engineering student and faculty expertise.

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APPENDIX A: TOTAL COLIFORM DISINFECTION RESULTS

Table A.1 Disinfection Study Results for Total Coliforms

Filter and Disinfectant		Log Reduction of Total Coliforms
Coarse Matala Filter	Chlorine	8.0*
	UV	5.5
	Ozone	-
Sand Filter	Chlorine	6.7
	UV	5.8
	Ozone	0.7
Cartridge Filter	Chlorine	4.7
	UV	5.2
	Ozone	3.5

*indicates complete disinfection

APPENDIX B: STANDARD OPERATING PROCEDURE: OPERATION AND MAINTENANCE OF A GRAYWATER REUSE FOR TOILET-FLUSHING SYSTEM AT THE ASPEN RESIDENCE HALL

A. Purpose and Applicability.

The purpose of this Standard Operating Procedure (SOP) is to provide guidance for the operation and troubleshooting of a graywater reuse for toilet flushing system at the Aspen Residence Hall. The SOP is necessary to ensure proper system operation of the collection, treatment and distribution of graywater for toilet flushing. This manual provides information on system start-up, routine maintenance and system monitoring. Figure 1 provides a schematic of the graywater treatment process. Figure 2 provides a schematic of the graywater treatment process.

B. Definitions

Refer to Figure 1 for labeled components of the Graywater Treatment System

1) Influent Graywater – Untreated graywater from showers and sinks
2) Coarse Filter – The coarse Matala filters the graywater after the composite tank before the disinfection tank
3) Disinfection Tank – 65 gallon tank stores treated graywater for toilets
4) Chemical Tank – 7 gallon chemical tank stores 8.15% NaOCl (Clorox Bleach)
5) Master Pump – Grundfos pressure booster pump distributes treated graywater to toilets
6) Chemical Pump – Stenner fixed output peristaltic metering pump doses chemical into graywater
7) Pump Control Module (PCM) – Stenner control module meters chemical dose of peristaltic pump
8) Freshwater Solenoid Valve – Electronic solenoid valve controls influent freshwater into the disinfection tank (not pictured)
9) Graywater Ball Valve – Electronic ball valve controls influent graywater from composite tank into the disinfection tank
10) Ultrasonic Level Sensor – Flowline ultrasonic level controls the graywater ball valve and freshwater solenoid valve to refill disinfection tank when necessary
11) Composite Tank – 250 gallon tank collects, composites and settles initial graywater (not pictured)
12) Pulse Water Meter – Records the amount of water passing through the meter and works with chemical pump to dose volumetrically
13) Chemical Injection – Point at which chlorine is dosed in-line
14) Treated Effluent – Graywater that has been filtered and disinfected and is ready to be used for toilet flushing

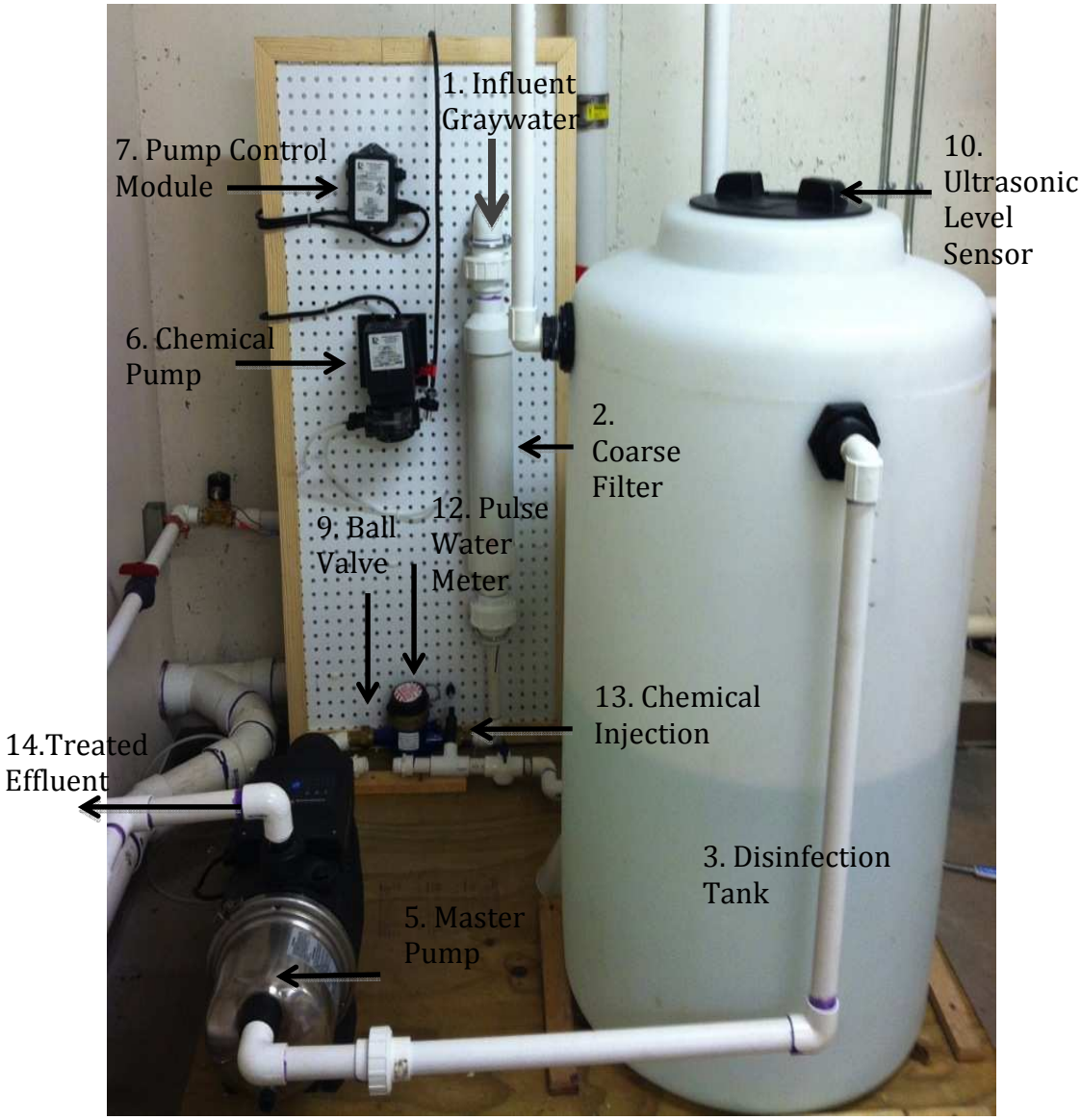

Figure B.1: Aspen Graywater Treatment System Diagram (not pictured, 4. Chemical Tank)

C. Health and Safety Warning

1) Contact with untreated graywater presents potential health risks due to possible pathogens in the water. Minimize contact with untreated graywater.
2) Sodium hypochlorite (NaOCl) poses health risks if mishandled. Follow proper storage and handling outlined on chemical label.
3) Wear gloves and safety goggles to minimize health and safety risks.

D. System Start-Up

1) Collect graywater in the composite tank. This will take approximately 24 hours. Close the valve directly ahead of the filter to prevent graywater from flowing into the disinfection tank at this time.

2) Prime master pump. If the pump has been primed recently and still has water in the pump no re-priming is necessary. It is best practice to drain pump during periods of prolonged downtime.
3) Ensure that the chemical tank is full of 8.15% NaOCl (Clorox Bleach). If a different concentration is used the chemical dose will need to be adjusted based on selected NaOCl concentration.
4) Provide power to the treatment system (Do Not Open Graywater Influent Line!). This provides power to all system components. The disinfection tank will fill with freshwater.
5) Prime the chemical pump. Unplug the chemical pump from the PCM and provide the chemical pump with power until the NaOCl has been pumped from the chemical tank into the dose-line. This should be observable through the clear pump tubing.
6) Plug the peristaltic pump back into the PCM and ensure that the dose level of the PCM is set according to the predetermined chlorine dose. Setting should be 40% on the PCM dial. Note that this may change based on graywater composition but should remain relatively constant throughout the semester. All necessary chlorine dose adjustments are controlled with the PCM.
7) Turn on the master pump. The indicator light will change from red to green and the pump will complete the priming process.
8) Open the manual valve directly ahead of the filter at this time. Once the pump turns on and distributes water to toilets, the low water level will signal the electronic valve to open and allow graywater to flow from the composite tank into the disinfection tank.
9) At this time the system is primed and ready to distribute treated graywater to the toilets. Open the distribution valve and the system is operating normally.

E. Maintenance

Table 1 provides a list of monitoring and maintenance activities, the frequency at which they should be performed, the duration of each activity and who should perform the activity.

1) Weekly monitoring
    i. It is paramount to maintain chlorine in the chemical reservoir. The chemical reservoir should be checked on a weekly basis and refilled if half empty. The reservoir must be refilled with 8.15% NaOCl (Clorox Bleach). Do not refill with a different concentration of bleach, this will require a dose a change on the PCM.
    ii. Check the residual chlorine in the disinfection tank by opening the sample port to the left of the toilet. Be sure to flush a reasonable amount (approximately 1 liter) of water to get a representative sample from the disinfection tank. A sample can also be taken by unscrewing the lid on the disinfection tank, but the lid must be replaced immediately to assure the Echopod reads the proper water level. Test the residual chlorine using the Colorimetric Chlorine Test Kit from Hach (Model #CN-66T). Chlorine

residual should be at least 1.5 mg/L. If below 1.5 mg/L, increase the dose slightly on the PCM.

2) Periodic cleaning of coarse filter
    i. The coarse filter should be removed and cleaned occasionally. This should be done every three months or as needed. Additional cleaning may be necessary when the graywater fill rate between the composite tank and disinfection tank is significantly slower then initial system start-up or if a spike in chlorine demand is observed or larger solids are seen in the disinfection tank. Cleaning of the filter is necessary when the rate of water moving through the pulse water meter (see #12, Figure 1) is noticeably slower than the initial rate. Under normal operation, a small white dial with a silver dot on the pulse water meter will spin very quickly. If the filter is clogged, this white dial will spin very slowly, indicating that very little water is passing through the filter. In this case remove the filter and backwash by rinsing the filter with freshwater into a drain. If the filter is not cleaned after backwashing dispose of the old filter and install a new one.
3) Short-term System Downtime
    i. If the student body is going to be gone for a known short period of time (<2 weeks, ex. Fall break) temporarily shut down the system. Three days before the break stop collecting graywater and empty the composite tank. Close the influent graywater valve to the disinfection tank and open the valve for bypass to sewer. The system will now operate on freshwater only and prevent prolonged storage of graywater in the toilet tanks over the break.
4) Long-term System Downtime
    i. If the student body is going to be gone for a long period of time (>2 weeks, ex. winter break) shut down the system according to the short-term procedure outlined above. Additionally, once the system is no longer in use turn off the system and empty the disinfection tank and master pump. It is a good practice to remove and clean the coarse filter at this time.
5) Cleaning of composite tank
    i. The composite tank should be cleaned before the beginning of each semester. This should be done when graywater is not being collected. Empty the composite tank, use fresh water to rinse the side walls of the tank and wash settled solids out of the tank drain. Fill the composite tank with fresh water, add 180 mL of bleach and let sit overnight. The next day, drain and rinse the composite tank.
6) Building a new filter
    i. Components needed: 2x 3” to 1.5” PVC reducing coupling, 2x 1.5” PVC threaded pipe fitting, 3” PVC (16” length), 1.5” PVC (for connecting coupling and fitting), Matala filter material.
    ii. Fill 3” PVC with Matala filter material, prime and glue reducing couplings to the end of the Matala filled PVC. Use the 1.5” PVC to prime and glue the reducing coupling to the pipe fitting. Once assembled, connect the threaded pipe fitting to the existing plumbing.

F. System Monitoring

1) Low water level in the disinfection tank
    i. If water is below the low water level then there is an issue with water supplies to the disinfection tank. Check to see if there is graywater in the composite tank, if so then there is an issue with the ultrasonic switch or graywater ball valve. A low water level also indicates an issue with the freshwater make up supply this could result from an electrical or mechanical issue in the ultrasonic switch or freshwater solenoid valve.
2) Master pump working properly
    i. A green ready light indicates the pump is on and operating under normal conditions. A red light indicates an issue has occurred and the pump shut off. This will occur if insufficient water was supplied to the disinfection tank or if the electrical connection to the pump was interrupted. If there is a red light, check if there is sufficient water in the disinfection tank and that nothing is blocking the master pump water supply or if the electrical supply was interrupted from the plug or breaker. Once the error is resolved turn the pump on and ensure that a green indicator light is achieved.
3) Empty chemical reservoir
    i. In the case that the chemical reservoir is empty or an issue occurred with the delivery of chlorine to the graywater, immediately turn off all power to the system, close the influent graywater valve from the composite tank to the disinfection tank and drain the disinfection tank. Once the disinfection tank is empty, leave the influent graywater supply valve closed and restore power to system. The system is now flushing toilets with freshwater. Refill the chemical reservoir and make sure the chemical pump is primed. Open the influent graywater valve between the composite tank and disinfection tank to restore system to normal operations.

G. Quality Control and Quality Assurance

Periodic testing of total chlorine residual and total coliform levels should be performed to ensure that the quality of treated water is sufficient for use for toilet flushing. The frequency of testing should be in accordance of requirements by the Colorado State University Health Department. All tests should be performed in accordance to Standard Methods for the Examination of Water and Wastewater.

H. System Shut Down

1) Close the valve above the composite tank and open the valve to sewer to stop collecting graywater in the composite tank.
2) Shut off the power to the potable water valve to prevent potable water from entering the disinfection tank.
3) Empty the composite tank by opening the valve at the bottom of the tank. Be sure to close the valve at the bottom of the disinfection tank to prevent any backflow from the composite tank into the disinfection tank.
4) Once the composite tank is empty, open the valves at the bottom of the disinfection tank and let the water empty to the sewer.

5) Close the valve at the bottom of the disinfection tank, but leave the valve to sewer open. This should allow water from the filter to drain to the sewer.
6) Close the valve to sewer.
7) Open the sampling port between the composite tank and the filter and use a bucket to collect any water left in the line.
8) Close the valve between the toilet and the pump and open the sampling port near the toilet and allow water to drain from the line.
9) If the graywater system is to be left empty, turn off all power to the system and stop at this step. If potable water is to be used in the system, continue to step 10.
10) Turn the power to the potable water valve back on and allow the disinfection tank to fill with potable water. At this point the system will now operate with City water. However, it is not considered potable because tanks and lines have not been disinfected and tested. Non-potable signs must remain at each toilet.

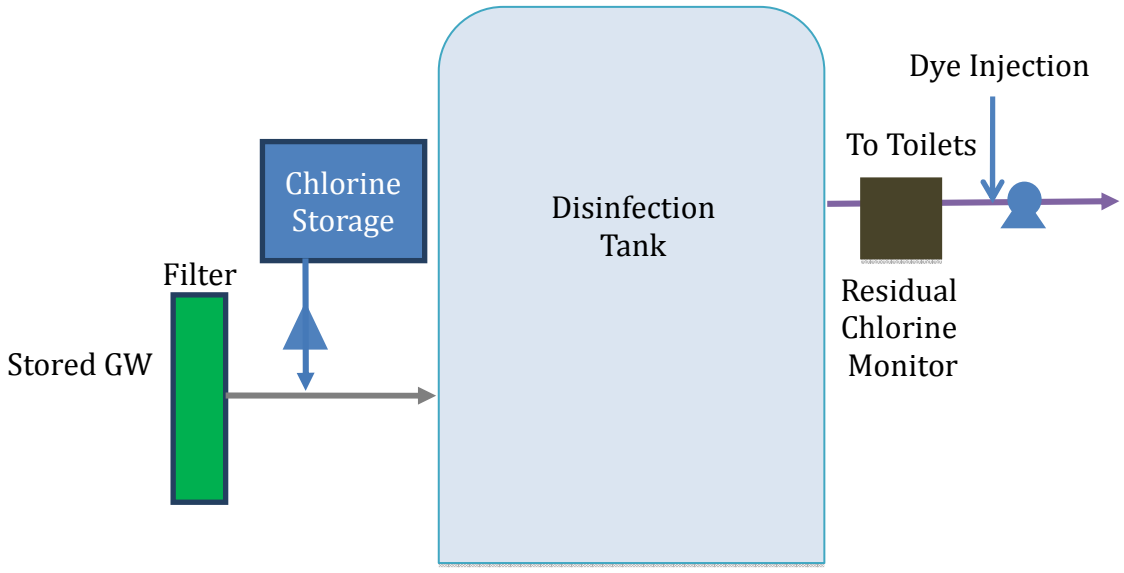

Figure B.2: Graywater Treatment Process Schematic

Table B.1: Monitoring and Maintenance Activities

Activity	**Frequency**	**Duration**	**Assigned to**
Clean composite tank	Twice per year	1-1.5 hours	Plumber
Check chlorine residual	Once per week	5 minutes	Student/Plumber
Clean coarse filter	4 times per year	20 minutes	Plumber
Fill chemical reservoir	Once per week	5 minutes	Student/Plumber
General system monitoring	Once per week	5 minutes	Student/Plumber
System start up	Twice per semester	1 hour	Plumber
System shut down	Twice per semester	1 hour	Plumber

APPENDIX C: COLLABORATION WITH EPA TO INVESTIGATE PATHOGENS AND VIRUSES IN GRAYWATER

Bacteria and Virus Sample Collection

In collaboration with the U.S. EPA to investigate pathogens and viruses present in graywater, graywater samples were collected from the treatment system in Aspen Hall and sent to the EPA to identify the bacteria and viruses present via pyrosequencing. In addition to characterizing the raw graywater, the temporal variability of bacteria species and viruses across seasons was investigated. Knowledge about bacteria species and viruses present in raw graywater will help guide further studies on which bacteria species are important to monitor. Raw graywater samples for bacterial and viral analysis were collected during March/April 2012 and May 2012. Additional samples of bacterial DNA only were collected December 2012 and April 2013. For each bacterial DNA and virus collection period, a total of 12 samples (6 bacterial DNA, 6 viruses) were collected and processed during 6 collection events in two weeks. For bacterial DNA collection, at least 50 L of raw graywater was collected during each sampling event in a large 35 gallon tank in order to obtain a representative mixed sample. 1-L samples were obtained from the mixed sample and taken to the lab for processing. For each collection event, bacterial DNA was extracted from each of the 3 100-mL aliquots of a raw graywater sample using a PowerWater DNA extraction kit (MoBio, Carlsbad, CA). DNA from the 3 extractions was pooled after elution. The elution volume for each extraction was 100 μL; thus, the total volume of each DNA sample sent to the EPA was 300 μL.

Viruses were concentrated from raw graywater following an ultrafiltration protocol provided by the EPA. In brief, 5.0 g of sodium polyphosphate was added to 50 L of raw,

unstored graywater. Using a peristaltic pump, all 50 L of raw graywater was processed through a Rexeed 25S hollow-fiber ultrafilter (Dial Medical Supply, Chester Springs, PA), and retentate was collected in a sterile 1-L bottle. After all 50 L were processed, the direction of pumping was reversed and 500 mL of an elution solution was circulated back through the hollow-fiber ultrafilter. Elution solution was made with 0.1 g sodium polyphosphate (Sigma Aldrich, St. Louis, MO), 0.1 mL Tween-80 (Sigma Aldrich, St. Louis, MO) and 0.01 mL Y-30 antifoam emulsion (Sigma Aldrich, St. Louis, MO) added to 1 L reagent grade water. Samples were shipped overnight on dry ice to the EPA (Cincinnati, OH) for processing the following day.

In addition to DNA extraction and virus concentration, an experiment was conducted to determine if the source of the bacteria found in graywater was bacteria in collection pipe biofilms or the students using the showers. Three rooms from the student dormitories plumbed to the graywater collection system were chosen for sampling. Room 1 was a model room and students had never used the shower. Rooms 2 and 3 were occupied during the semester. First, 10 L of potable water from one showerhead was collected. Then, three simulated showers were conducted. Next, one simulated shower was conducted in each of the three rooms. A collection tank was used to collect the graywater from the simulated showers before entering the compositing tank. Due to the large size of the collection tank used for compositing the simulated showers, it was unable to be autoclaved or acid-rinsed between samples (simulated showers). Instead, the collection tank was thoroughly rinsed with potable water and a small amount of chlorine between samples. To simulate a shower, the showerhead was turned on for 8 minutes, and a nickel sized amount of Tresemme Natural shampoo and two minutes of hand washing with Dove body soap were mixed with the shower water. Following the simulated shower, 2 L of water from the collection tank were taken immediately to the laboratory for DNA extraction.

DNA was extracted using a PowerWater DNA extraction kit (MoBio, Carlsbad, CA). DNA was extracted from 7 L of the potable water collected from the showerhead. For each simulated shower experiment, 1 L of graywater was used in the DNA extraction. The elution volume of the DNA sample was 100 μL.

APPENDIX D: CHLORINE DISINFECTION BATCH STUDY RESULTS

Results of the chlorine disinfection batch studies after 15 minutes of contact time. Results reported in Chapter 3 are after 60 minutes contact time.

Table D.1 Chlorine Disinfection Batch Study Data

Filter and Organism		Log Reduction
Coarse Matala Filter	*E. coli*	7.1
	P. aeruginosa	NQ
	S. enterica	8.0
	MS2	NQ
	Total Coliforms	4.1
Sand Filter	*E. coli*	5.7
	P. aeruginosa	5.7
	S. enterica	7.8
	MS2	1.7
	Total Coliforms	5.9
Cartridge Filter	*E. coli*	NQ
	P. aeruginosa	NQ
	S. enterica	0.4
	MS2	NQ
	Total Coliforms	NQ

NQ indicates not quantified

LIST OF ACRONYMS AND ABBREVIATIONS

ATCC: American Type Culture Collection
BOD: biochemical oxygen demand
cfu: colony-forming unit
COD: chemical oxygen demand
CT: chlorine contact time, the product of chlorine residual concentration and contact time
DE: diatomaceous earth
EPA: Environmental Protection Agency
FIB: fecal indicator bacteria
GAC: granular activated carbon
gpcd: gallons per capita per day
gpd: gallons per day
HPC: heterotrophic plate count
LB: Luria broth
MBR: membrane bioreactor
NB: nutrient broth
NTU: Nephelometric Turbidity Units
pfu: plaque-forming unit
RBC: rotating biological contactor
SOP: standard operating procedure
TDOC: total dissolved organic carbon
TN: total nitrogen
TOC: total organic carbon
TSB: tryptic soy broth
TSS: total suspended solids
UV: ultraviolet
UVT: ultraviolet transmittance