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Investigation of pathogen disinfection and regrowth in a simple graywater recycling system for toilet flushing

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ABSTRACT

Graywater treatment systems must inactivate pathogens, prevent regrowth, be low cost, and be simple to operate to support their widespread adoption for alleviating water stress. A treatment system comprised only of filtration and disinfection could meet these constraints. To investigate pathogen disinfection and regrowth in such a system with minimal organic matter removal, herein three disinfectants (chlorine, ultraviolet irradiation, and ozone) were tested in combination with three filter types (coarse, sand, and cartridge) for inactivation of pathogens in graywater from the showers and hand washbasins of 14 student residences. Graywater was spiked with bacterial and viral pathogens or surrogates post-filtration. Chlorination post-filtration achieved log reductions greater than 7.1, 8.0, and 7.4 for *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa*, respectively, and 3.8 for MS2 bacteriophage. UV was similarly effective, but would not prevent regrowth without a disinfectant residual. Ozonation generally was ineffective at the doses tested, with the exception that MS2 log removal was 3.7. Pathogen regrowth could be prevented for 4 d with a chlorine residual of 2.75 mg/L even for a simulated high-contamination event (6 log each pathogen). When chlorine residual was maintained, regrowth of indicators and pathogens was prevented for the light graywater investigated.

Keywords: Graywater; Disinfection; Pathogens; Regrowth; Ozonation; Chlorination; Ultraviolet

1. Introduction

Fresh water supplies are becoming increasingly stressed as populations grow, and alternative water supplies are gaining attention as a way to accommodate population growth worldwide [1–4]. Reusing graywater is an attractive alternative because graywater represents a large, continuously available water source. Further, graywater is relatively low in organic content and pathogens, and therefore is easier to treat than municipal wastewater [5]. In a review of indoor water use in North America from 1999 through 2007 [6], generation of light graywater (defined here as water from showers, baths, and bathroom washbasins) was found to be near 61 L/d per capita, while toilet demand was near 62 L/d per capita. Therefore, light

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graywater can closely meet toilet flushing demands. However, graywater recycling has not been widely implemented, in part because of the cost and maintenance requirements of graywater treatment systems. Thus, low-cost, low-maintenance treatment systems need to be developed, and these systems must also ensure protection of human health.

Previously investigated graywater treatment systems vary from biological treatment processes to simple physical treatment coupled with disinfection. Biological processes can provide good removal of organic matter, with effluent biochemical oxygen demand (BOD) concentrations below 10 mg/L [1]. However, these processes are complex to operate and thus often require a trained technician for operation and maintenance activities, making biological treatment impractical for application at the household or small building scale. Alternatively, simple treatment comprising coarse filtration and disinfection provides little removal of organic matter, but can theoretically provide good inactivation of micro-organisms and viruses in the disinfection process [1]. Simple treatment systems are advantageous for residential graywater recycling systems because they are low cost and easy to maintain [7]. However, disinfection of graywater with a relatively high organic content (e.g. BOD >10 mg/L and/or total organic carbon (TOC) >5 mg/L) has not been well studied, and this knowledge is needed to design simple treatment systems to protect public health when graywater is recycled for toilet flushing.

Further, disinfection must inactivate pathogens. Indicator organisms such as total coliforms and *Escherichia coli* are commonly used for monitoring the microbiological quality of reclaimed water after disinfection [8], but public health risk is driven by the presence of human pathogens rather than indicator organisms. Graywater is known to contain pathogenic bacteria including *Pseudomonas aeruginosa*, *E. coli*, *Legionella pneumophila*, and *Salmonella enterica*, as well as viruses [9–11]. However, studies directly measuring inactivation of pathogens and pathogen surrogates (e.g. MS2 bacteriophage), as opposed to inactivation of indicator organisms, as a function of graywater treatment technologies are limited. In particular, pathogen inactivation via simple filtration and disinfection has not been investigated for graywater with relatively high organic content.

Pathogen inactivation theoretically can be achieved using various disinfectants such as chlorine, ultraviolet (UV) irradiation, or ozone after filtration. Chlorination, commonly used in water and wastewater disinfection, is a simple and inexpensive method for disinfecting graywater. UV irradiation is sometimes preferred over chemical disinfection because there is no need for storage and replenishment. Ozone is a powerful disinfectant reported to require shorter contact times (CT) than chlorine for disinfection of *E. coli*, *Rotavirus*, and *Giardia* cysts [12]. Several studies have examined disinfection of graywater after biological treatment or membrane filtration [9,13,14], and results indicate that chlorine, UV, and ozone can inactivate micro-organisms to below stringent regulatory standards (e.g. California Title 22 water reuse standards) in graywater with a low organic content (BOD <10 mg/L and/or TOC <5 mg/L). Thus, previous findings suggest that these disinfectants might be applied to inactivate pathogens in graywater with a higher organic content.

An additional concern with graywater systems is regrowth of pathogens within the distribution system and at the point of use, e.g. the toilet. Households may remain empty during the workday or when residents are traveling. If graywater is not properly disinfected, regrowth of bacteria including pathogens could occur due to increased residence times as homeowners are not using water in the home during these times. Regrowth of bacteria could increase the risk from direct contact with graywater, either through splashing or aerosolizing of pathogens during toilet flushing [15]. Although inexpensive treatment methods provide little removal of organic matter, complete disinfection could prevent regrowth of organisms. Regrowth after chlorine disinfection has been studied; however, the data have been largely limited to regrowth occurring in less than 24 h [9]. A recent study reported a lack of regrowth for 15 d after filtration and disinfection; however, low organic content limited regrowth potential in that study [13]. Investigations of regrowth over longer time periods and in disinfected graywater containing organic matter, which could increase regrowth, are lacking.

Thus, the objective of this study was to investigate pathogen disinfection and regrowth in graywater treated using a simple treatment process (filtration followed by disinfection), where organic matter removal was limited. The efficacies of chlorine, UV, and ozone disinfection, in combination with several filtration methods, were evaluated for inactivation of bacterial and viral pathogens and surrogates. Regrowth was examined for up to 7-d post-chlorination at various chlorine doses to provide dosing guidance to treat graywater for recycling in toilets.

2. Materials and methods

2.1. Graywater collection and treatment system description

The graywater used throughout this study was collected from a previously described demonstration

graywater collection and treatment system installed at a student dormitory on the campus of Colorado State University (CSU) in Fort Collins, CO [16,17]. Graywater was collected from 28 students in 14 residences. The system included storage before treatment, which provided settling of solids as well as storage for equalizing diurnal flow patterns (Fig. S1). The pre-treatment storage tank was 946 L, and the average flow rate through the system was estimated to be 1,136 L/d based on regular visual observations of the tank level. Following pre-treatment storage, water passed through a filter. Each of the following filters was tested separately: a coarse 16″-long Matala medium-density filter (Matala USA, Laguna Hills, CA) (61 d of operation), a pool sand filter with a pore size of 25–50 microns (Hayward, Elizabeth, NJ) (18 d of operation), and a cartridge filter with a pore size of 100 microns containing granular activated carbon (PurFlo, Chicago, IL) (13 d of operation). The treatment system was operated with the three different filters for a total period of approximately 3 months. During this time, laboratory studies were conducted using water collected from this demonstration treatment system post-filtration (Section 2.3). For non-spiked regrowth studies (Section 2.5), graywater was disinfected with chlorine post-filtration. 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 3.785 L of water passed through the flow meter, chlorine was dosed in-line before the disinfection tank with the peristaltic pump (Fig. S1). 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 173 L, sized to provide a contact time of at least 1 h. A chlorine residual of 2 and 4 mg/L was desired in the graywater effluent, and therefore, a dose of 12 and 30 mg/L was used depending on influent graywater quality and filter performance. The graywater treatment system also included a potable make-up supply to ensure water was always available, which would be necessary when in use for toilet flushing.

2.2. Chemical and indicator organism monitoring

Standard chemical and biological parameters were measured for raw and treated graywater. TOC was measured with a Shimadzu TOC-V CSH/CSN analyzer (Shimadzu, Japan), which utilizes combustion and acidification processes. Turbidity was analyzed using a Hach 2100N nephelometric turbidimeter (Hach, Loveland, CO). Samples were analyzed for TOC as soon as possible after sample collection. Total chlorine was measured using a Hach total chlorine test kit (Method 8167) with a Hach DR2500 spectrophotometer (detection limit = 0.02 mg/L Cl_2). Ozone was measured using Hach ozone ampules (Method 8311) with a Hach DR2500 spectrophotometer (detection limit = 0.01 mg/L O_3). *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 h at 35˚C. After incubation, *E. coli* and total coliforms were enumerated following the manufacturer's instructions. The percent UV transmittance (% UVT) at 254 nm of the graywater was determined using a Thermo Scientific Genesys Spectrophotometer (Thermo Scientific, Waltham, MA).

2.3. Laboratory disinfection study setup

Laboratory-scale disinfection studies were used to determine the log inactivation of micro-organisms using the three different filtration methods (Section 2.1) in conjunction with three different disinfectants. Disinfection tests were conducted in a biological safety cabinet to protect researchers from pathogens. The disinfection systems were constructed using 19-L buckets and were plumbed for disinfection via chlorination, UV treatment, or ozonation (Fig. 1). The chlorination reactor was operated on a magnetic stir plate to provide mixing, and for the UV and ozonation reactors water was pumped through the systems with a Rule Model 24, 1,363 L/h bilge pump (ITT Industries, Seneca Fall, NY). 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, 7.6-L 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 were spiked into a single 7.6-L aliquot. *E. coli* was selected for testing in the laboratory-scale disinfection studies because it is often included in graywater recycling regulations and is a known pathogen in graywater [18]. *S. enterica* was selected because it is an enteric pathogen and has previously been examined in graywater studies [4,18]. *P. aeruginosa* was selected because it is a known biofilm former and is a skin and mucus pathogen previously found in graywater [19]. MS2 bacteriophage was selected for laboratory-scale disinfection studies because it is a useful surrogate for poliovirus, which

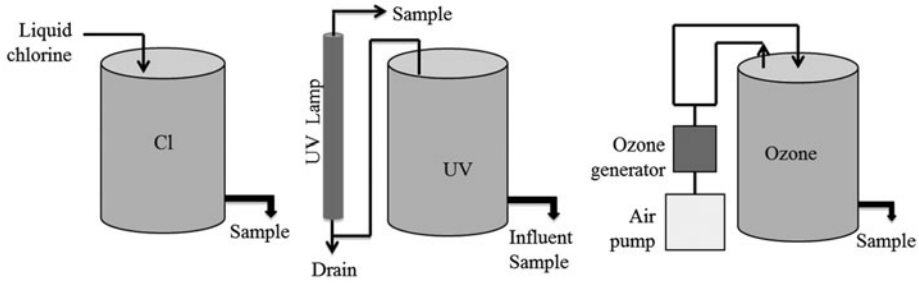

Fig. 1. Diagram of laboratory reactor setup testing filtered graywater.

is regulated in the California Title 22 requirements for graywater recycling. 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.

For the chlorine laboratory 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 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. Based on this approach, applied chlorine doses were 12 mg/L for the coarse filter, 21 mg/L for the sand filter, and 19 mg/L for the cartridge filter. CT of up to 60 min were tested [20–22], and data reported in Fig. 2 are for 60 min of contact time because this time resulted in the maximum disinfection. Chlorine residual was found to generally stabilize after approximately 20 min for graywater collected from the CSU system (data not shown); thus, for an achieved residual of 3 mg/L, a 60-min contact time corresponds to a CT of 180 mg/L-min. The actual CT for the studies depended on the achieved chlorine residual during each batch study and was 297 mg/L-min for the coarse filter, 474 mg/L-min for the sand filter, and 180 mg/L-min for the cartridge filter. Samples were collected for pathogen and bacteriophage enumeration immediately prior to chlorination, and then post-treatment samples were collected from the sampling port (Fig. 1) 60 min 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 UV laboratory reactor, a Sterilight Copper SC1 UV lamp was used for in-line disinfection (R-can, Guelph, Canada). To determine the dose, the % UVT at 254 nm of each 7.6-L graywater aliquot was determined using a Thermo Scientific Genesys Spectrophotometer (Thermo Scientific, Waltham, MA). % UVT typically ranged from 35 to 41%. Dose was calculated using the % UVT and the graywater flow rate through the UV lamp based on the lamp manufacturer's specifications. The doses for these studies were 21 mJ/cm^2 for the coarse filter, 26 mJ/cm^2 for the sand filter, and 28 mJ/cm^2 for the cartridge filter, which were the highest achievable doses that could be applied, given the measured % UVT of the graywater used and the minimum flow rate through the UV lamp (1.9 L/min). These doses were within the range of doses tested in previous studies [13,23]; Hijnen et al. reported a range of UV doses from 5 to 50 mJ/cm^2 for the inactivation of poliovirus. 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. 1) immediately after passing through the UV lamp.

For the ozone laboratory 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). This equipment was selected because it was considered practical for implementation. An air flow rate of approximately 1 L/min was chosen because it resulted in the maximum achievable ozone generation rate of 1 mg/min (assuming standard temperature and pressure). Slower air flow rates generated greater percentages of ozone from air; however, slower air flow rates provided lower ozone mass flow overall. Ozone dose was calculated using the ozone generation rate and the flow rate of graywater (7.6 L/min) through the contact tube (Fig. 1). The graywater was re-circulated through the contact tube to provide mixing, and recirculation was continued until a dose of up to 5 mg/L was achieved. There was no measurable ozone residual post-treatment for any of the tested samples. 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. 1) after

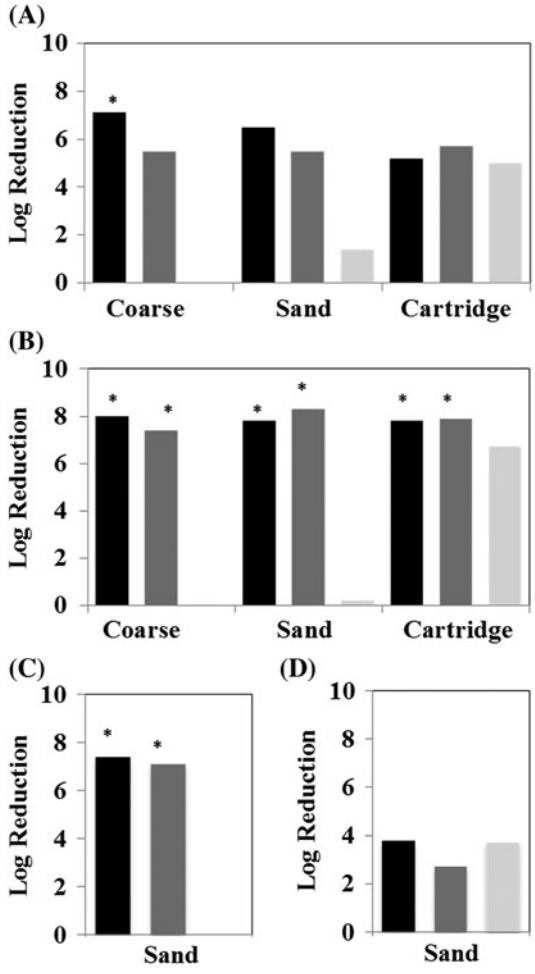

Fig. 2. Disinfection efficacies for *E. coli* (A), *S. enterica* (B), *P. aeruginosa* (C), and MS2 bacteriophage (D) with chlorine (black), UV (dark gray), and ozone (light gray). Chlorine results are for a contact time of 60 min. Chlorine applied doses were 12 mg/L for the coarse filter, 21 mg/L for the sand filter, and 19 mg/L for the cartridge filter. *indicates micro-organism levels were below the detection limit. UV achieved doses were 21 mJ/cm^2 for the coarse filter, 26 mJ/cm^2 for the sand filter, and 28 mJ/cm^2 for the cartridge filter. The applied ozone dose was 5 mg/L for all filters. For ozone, log reduction of pathogens was not detected where not shown. Table 1 shows average water quality parameters for these tests.

ozone addition. Samples were collected for a range of doses; data reported in Fig. 2 are for an applied ozone dose of 5 mg/L because this dose resulted in the maximum disinfection.

2.4. Microbiological culturing and analyses for laboratory studies

Standard safety procedures were followed to protect researchers from pathogenic organisms. For each bacterium spiked, pure cultures were grown overnight from freezer stocks in nutrient-rich media at 37˚C. The growth media used for *E. coli*, *P. aeruginosa*, and *S. enterica* were Luria-Bertani broth, tryptic soy broth, and nutrient broth (BD, Franklin Lakes, NJ), respectively. Cell concentrations were estimated using standard curves relating optical density (600 nm) to the concentration of bacterial colony-forming units (cfu) and were used to determine the volume of culture needed to produce a final concentration of 8 log/100 mL in the graywater. To remove media prior to use for spiking, each culture was centrifuged at 4,000 rpm for 5 min, and the supernatant was poured off. The pellet was re-suspended in 5 mL of graywater by vortexing and then used for spiking.

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 h at 35˚C. Selective plating methods were used to detect the bacterial pathogens pre- and post-disinfection treatments. *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). 50 µL (*P. aeruginosa*) or 100 µL (*S. enterica*) of neat or diluted sample were spread onto an agar plate using 6-mm sterilized glass beads (Thermo Fisher Scientific, Waltham, MA). Following incubation, plates with fewer than 300 colonies were counted. The limits of detection were 20 cfu/ml for *P. aeruginosa* and 10 cfu/ml for *S. enterica.* Sample filtration was avoided for pathogen enumeration to simplify analyses such that disinfection of several organisms could be tested simultaneously for multiple disinfectant doses and because the focus of these analyses was to compute log reductions. Three serial dilutions were plated for each sample collection event to obtain readable plates; however, in some cases plates did not meet accuracy criteria and so these data were not reported (*P. aeruginosa* concentrations for the sand and cartridge filters).

MS2 coliphage was propagated as described previously [24,25]. In brief, MS2 was propagated by incubating with the *E. coli* host (ATCC 700891) overnight. Then, cell debris and host were removed by centrifugation for 10 min at 8,000× g followed by filtration through a 0.45-µm syringe filter. The resulting MS2 stock was 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 8 log/100 mL. MS2 was enumerated using a plaque-clearing assay as described previously [24].

2.5. Regrowth studies

Regrowth was investigated for both pathogens and indicators (i.e. total coliforms) in graywater generated in the demonstration graywater collection and treatment system (Fig. S1) with varying amounts of organic matter. For non-spiked regrowth studies, treated (filtered and chlorinated) graywater was allowed to sit in a 6-L toilet for 7 d 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 2.2. A single non-spiked regrowth study also was conducted in a 19-L bucket to confirm findings from the studies conducted with the toilet. 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 6 log 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 and 2.75 mg/L. Chlorine doses for these residual concentrations were 45.3 and 49.5 mg/L, respectively. These chlorine doses are much higher than the chlorine dose used in the demonstration treatment unit, likely because chlorine demand was increased by spiking with high quantities of pathogens as has been observed previously [13]. Organic content of the graywater was not measured directly prior to the spiked regrowth studies; however, the TOC concentration averaged 61.6 ± 8.3 mg/L in the six-month period prior to sample collection for this experiment. After one hour of chlorine 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 h, and then each day for 4 d. Temperature throughout the laboratory-scale pathogen-spiked regrowth study was 27˚C.

3. Results and discussion

3.1. Raw graywater quality and impact of filtration

Average characteristics of graywater leaving the demonstration system storage tank during the laboratory studies are shown in Table 1. These values are typical of graywater collected from showers and sinks [26].

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 could affect disinfection efficacy. The coarse and cartridge filters were found not to provide significant removal of organic matter or solids from the graywater or result in any significant change in water quality. The sand filter, however, resulted in effluent with significantly lower TOC ($p < 0.1$) and turbidity ($p < 0.05$) than measured in the influent, with average reductions of 29 ± 17% and 13 ± 11% from influent measurements, respectively. Although the sand filter appeared to provide slight water quality improvements, chlorine demand after sand filtration substantially increased with a chlorine consumption of 24.4 ± 2.8 mg/L compared to the coarse filter (13.4 ± 2.6 mg/L) and cartridge filter (17.1 ± 1.3 mg/L), possibly due to biologically mediated transformation of organic matter into a form that exerted a higher chlorine demand.

Table 1
Raw graywater characteristics

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

[a]TOC indicates TOC; NTU indicates nephelometric turbidity units.

3.2. Disinfection of pathogens

The inactivation of three bacteria and one bacteriophage was quantified for each filter and disinfectant combination (Fig. 2). Chlorination provided consistent disinfection across all filters for all bacteria tested (Fig. 2). Chlorination post-coarse filtration resulted in the greatest measured log reduction for *E. coli* (7.1; Fig. 2(A)). Further, chlorination post-coarse filtration resulted in disinfection to below detection limits for *E. coli* and *S. enterica* after only a 15-min contact time (Supplemental Table 1).

Chlorination post-sand and -cartridge filtration achieved *E. coli* log reductions of 6.5 and 5.2, respectively (Fig. 2(A)). Additionally, chlorination achieved log reductions of indigenous total coliforms of 6.4, 6.7, and 4.7 for the coarse, sand, and cartridge filters, respectively (Supplemental Table 2). Further, during stable operation of the graywater collection and treatment system with coarse filtration and chlorination, total coliforms were only detected in 2 of 6 samples (0.3 and 1.6 log cfu/100 mL), and *E. coli* was not detected in any samples (data not shown). Thus, chlorination post-coarse filtration could meet National Sanitation Foundation 350 (NSF 350) regulations (average

Table 2
Graywater quality for regrowth studies

Graph	TOC (mg/L)	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 d prior to sample collection for regrowth study.

E. coli ≤2.2/100 mL), and could likely be operated to meet the California Title 22 standard for total coliforms (7-d median ≤2.2/100 mL). Chlorine also was highly effective at disinfection of *S. enterica*. Chlorine post-coarse, -sand, and -cartridge filtration provided disinfection to below detection limits for *S. enterica*, with measured log reductions of 8.0, 7.8, and 7.8, respectively (Fig. 2(B)). Chlorine post-sand filtration achieved a 7.4-log inactivation of *P. aeruginosa* (Fig. 2(C)). Achievable log reductions could be higher for chlorination because reported values were based on inactivation of the initial spikes to below the limits of detection in numerous cases (Fig. 2). It should be noted that chlorine was present in the graywater as chloramines due to high ammonia levels in the graywater.

In comparison to 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 (applied dose of 12 mg/L). In comparison, Beck et al. [13] 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 [13]. Furthermore, directly comparing log removals for these two studies is difficult because the graywater studied by Beck et al. [13] had an organic content that was approximately eight times lower than the graywater studied herein because it was passed through a 10-μm filter prior to disinfection; the graywater used by Beck et al. [13] had a post-filtration turbidity of <6 NTU and a TOC of less than 5 mg/L. Additionally, in our study, high log reductions of *P. aeruginosa* were achieved despite past studies demonstrating 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 min contact time [8]. Additionally, in a study of chlorine disinfection of *P. aeruginosa* in graywater treated with a rotating biological contactor (RBC) that had a relatively high organic content (average effluent chemical oxygen demand (COD) of 40–50 mg/L), Friedler et al. [9] only achieved an 88.5% removal efficiency for *P. aeruginosa*, when the average influent concentration was 2.6 log. For the same system, Friedler et al. [9] achieved a 99.6% removal efficiency for fecal coliforms with an average influent concentration of 1.5×10^2 (2.2 log). Based on these results, Friedler et al. [9] stated 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).

The superior log reductions observed herein are likely due to the higher applied chlorine doses, which were required because organic matter was not removed. Friedler et al. [9] used biological treatment to reduce organic content and chlorine demand, and therefore, dosed chlorine at less than 9 mg/L. Consequently, they were unable to completely inactivate *P. aeruginosa* in their system, despite its relatively low concentration (2.6 log). Disinfection efficacy has been shown to increase with initial applied chlorine dose when micro-organisms are particle-associated because higher chlorine doses lead to increased particle penetration [5,27]. However, the maximum allowable chlorine dose is limited by the fact that residual chlorine should not exceed 4 mg/L to avoid corrosion of fixture components [28]. Thus, simple graywater treatment systems that do not remove organic matter may have an unexpected benefit: chlorine disinfection efficacy is improved relative to more complex biological systems (e.g. RBCs) because applied chlorine dose can be increased. Systems with fine filters (10 μM) or membrane bioreactors capable of removing the majority of particles represent exceptions, but such systems are costly. Minimal particle association also may have contributed to higher observed log reductions for spiked bacteria; however, high log reductions of indigenous total coliforms support the former conclusion.

Chlorination also removed the bacteriophage MS2, although log reductions were lower than for bacteria. Chlorination post-sand filtration achieved a 3.8-log reduction after a 60 min contact time (CT of 474 mg/L-min). Beck et al. observed a 5-log inactivation of MS2 with a CT above 100 mg/L-min, resulting from a contact time of 90 min, although as noted previously,

organic content was low in those tests. However, 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 36–41% 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 and 5.8 log (Supplemental Table 2). 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 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. [13] observed a 3.5-log inactivation of total coliforms using a UV dose of 10 mJ/cm^3 post filtration (10 μm), although the reported inactivation was limited by the low density of total coliforms in the influent graywater [13]. The UV treatment studied herein achieved log reductions of total coliforms greater than 5.0 even with a turbidity over five times greater (Table S2). Similarly, Friedler et al. [9] 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 (total suspended solids 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 mJ/cm^2 [29]. Similary, herein a UV dose of 28 mJ/cm^2 achieved >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). Consistently, Beck et al. [13] reported a 5-log inactivation of MS2 for only two of four samples following exposure to a UV dose of 100 mJ/cm^2 in graywater with low organic matter. Thus, 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 when organic matter is high (>10 mg/L BOD and/or TOC >5 mg/L). An applied ozone dose of 5 mg/L was insufficient to provide a measurable reduction of *E. coli* post-coarse filtration (Fig. 2(A)). 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 (Supplemental Table 2). 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 organic matter (TOC), which may have led to the more effective ozone disinfection [16]. However, overall ozone was found to be ineffective due to the size of ozone generator used in this study and high organic content in the graywater.

In comparison to other studies, the ozone treatment provided little inactivation of pathogens, likely due to the high organic content of the graywater. In treated wastewater with low organic content (total dissolved organic carbon of 7 mg/L), a 98% removal of *P. aeruginosa* was achieved with an ozone dose of 15 mg/L when the pre-disinfection concentration of *P. aeruginosa* was 8–28 cfu/100 mL (0.9–1.4-log reduction) [29]. By contrast, no measureable reduction of *P. aeruginosa* was achieved in this study. However, the organic content of the water investigated by Liberti et al. [29] was sixfold lower and their ozone dose was threefold higher. In addition, Beck et al. [13] 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 with low organic content (<5 mg/L TOC). When higher concentrations of total coliforms were present (2,050–3,330 cfu/100 mL) the California Title 22 standard could not be met even for a CT of 2 mg/L-min (maximum ozone dose of 5 mg/L) [13]; however, Beck et al. still reported log reductions of nearly three. Via modeling, Beck et al. recommended an applied ozone dose of 5–7 mg/L to meet regulations for graywater with a low organic content (TOC <5 mg/L). 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.

Interestingly, results indicate that ozone may be as effective as chlorine and UV for disinfecting viruses in graywater with a relatively high organic content. Ozone disinfection post sand-filtration achieved a 3.7-log reduction of MS2 (Fig. 2(D)), which was comparable to chlorination. Although MS2 inactivation was only tested post-sand filtration, the efficacy of ozone likely would be similar for the coarse and cartridge filters because the quality of the filtered graywater did not vary substantially between filters. Thus, like UV treatment, ozone treatment was insufficient to meet California Title 22 requirements for virus removal. Moreover, poliovirus has been shown to be more resistant to ozone than MS2 [30], and thus, a 6.5-log inactivation of MS2 is required via ozonation to meet California standards [31]. Ishida et al. [31] successfully demonstrated a 6.5-log reduction of MS2 with an applied ozone dose of 5–7 mg/L for filtered graywater. However, for graywater with high organic content, treatment modifications clearly would be needed to achieve the required virus inactivation.

Based on the results of the laboratory-scale studies and an economic feasibility analysis conducted as part of a separate study [16], chlorination was investigated further to determine its effectiveness for preventing regrowth.

3.3. Regrowth

Regrowth was investigated for pathogens and indicators (i.e. total coliforms) in graywater with varying amounts of organic matter, due to collection on different days (Table 2).

Regrowth varied and was likely impacted by the concentration of organic matter present (Fig. 3). Regrowth was successfully prevented when TOC was at the lower end of the observed range (27.5 mg/L; Fig. 3(A)); this finding was confirmed via an additional regrowth study with a similar organic concentration conducted in a 19-L bucket (Fig. S2). For graywater with mid-range TOC content (45.0–49.2 mg/L), regrowth was only completely prevented when a high chlorine residual was achieved (Fig. 3(C)). For graywater with the highest TOC content (85.3 mg/L), regrowth was not prevented even with a chlorine residual above 4 mg/L. However, this organic concentration is substantially higher than the average TOC of approximately 51 mg/L (±14.5 mg/L) observed over a four-month operational period for the demonstration graywater collection and treatment system [16]. In fact, this TOC was the highest observed over that period, and the second highest TOC measured was 68.2 mg/L out of 29 samples. Similarly, for graywater originating from bathroom sources average

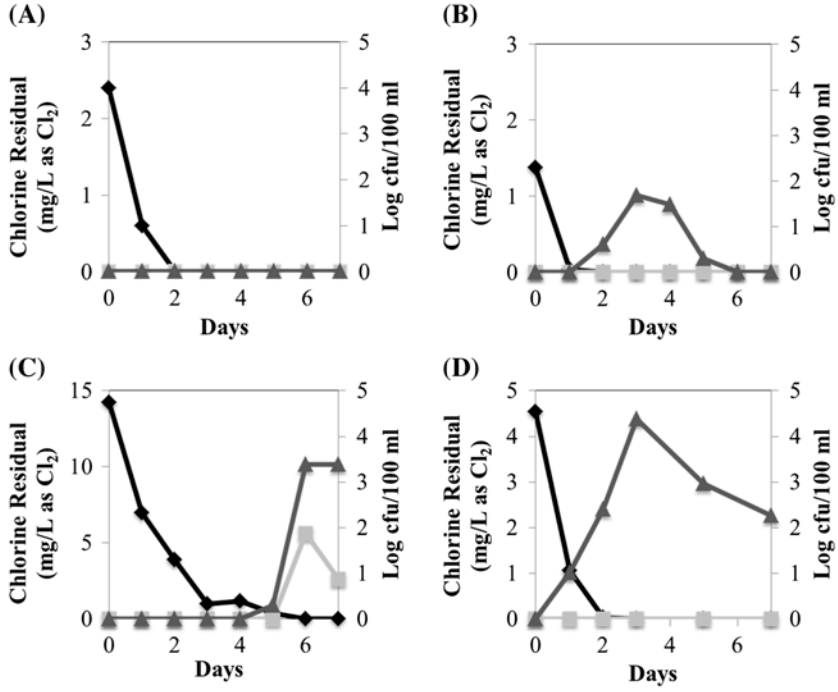

Fig. 3. Regrowth of total coliforms and *E. coli* over 7 d. A-D represent separate collection events, and Table 2 shows raw graywater TOC and turbidity for each event. (♦) chlorine residual, (▲) total coliforms, (■) *E. coli*.

TOC values have been reported to be 40 mg/L or less [26]; although higher values of ~100 mg/L have been reported [32]. While there is potential for regrowth of total coliforms when the organic matter content of the graywater is high, regrowth of bacteria can be prevented, and water quality standards (NSF 350) can be met with a chlorine residual of ≥2.4 mg/L for more typical TOC levels (Fig. 3(A)).

Findings of this study are consistent with previously reported studies. Chlorination prevented regrowth of heterotrophic plate counts, fecal coliforms, *P. aeruginosa*, and *Staphylococcus aureus* for up to 6 h with a chlorine residual above 0.5 mg/L when applied to graywater pretreated via an RBC (average BOD of 3.7 mg/L (COD of 47 mg/L)), although regrowth was not examined for longer periods of time in that study [9]. Beck et al. [13] 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 d [13]; however, total coliforms were found to be non-detect after 15 d in a non-disinfected control sample, suggesting that the low TOC graywater tested did not contain enough nutrients to support bacterial regrowth [13]. 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) over extended periods of time.

3.4. Spiked regrowth

Because pathogens, not indicator organisms, are the driver for risk in recycled graywater, this study examined how the regrowth of pathogens compared to the regrowth of indicator organisms. The results indicate that a chlorine residual concentration of 1.5 mg/L (Fig. 4(A)) was not sufficient to prevent the regrowth of total coliforms, *S. enterica* or *E. coli*. By contrast, a residual of 2.75 mg/L (Fig. 4(B)) prevented regrowth of all pathogens tested for at least 4 d even though pathogens were all spiked at a high concentration (6 log/100 mL); by contrast, in raw graywater *P. aeruginosa* has been reported at 4 log, while other pathogens (e.g. *Salmonella*) are often non-detect [18,33]. Thus, this result also indicates that a residual of 2.75 mg/L could prevent regrowth even during a high-contamination event (e.g. when residents of a building are experiencing a high level of illness). TOC of the graywater used for the spiked regrowth tests was not measured and TOC of influent graywater to the treatment system was also not measured during the month when the spiked regrowth studies took place. However, TOC concentration ranged from 45.0 to 85.3 (average 61.6 ± 8.3) mg/L in the six-month period prior to sample collection, when the measured chlorine residual of treated graywater was most often greater than 3.4 mg/L (the upper detection limit based on method). On the day that graywater was collected for the spiked regrowth experiment, the measured chlorine residual in the treated graywater was 1.6 mg/L. Since the chlorine dose was not modified prior to the regrowth experiment, a residual chlorine of 1.6 mg/L indicates that TOC of the graywater sample collected was likely within the range of observed TOC (45.0 and 85.3 mg/L), and probably on the upper end of that range. Therefore, findings are applicable for graywater with a comparable TOC content to that studied here. It should be noted, however, that the applied chlorine doses for the spiked regrowth test were relatively high (45.3 and 49.5 mg/L for A and B, respectively) because spiking increased chlorine demand as observed previously [13]. Therefore, patterns of pathogen regrowth could differ with realistic pathogen loads and chlorine doses. Generally, though, it was found that when indicator regrowth was prevented, pathogen regrowth was also prevented for the specific species tested. Additionally, the pathogen-spiked regrowth studies were conducted at a relatively high temperature (27˚C), and given that bacterial growth rates generally increase with temperature,

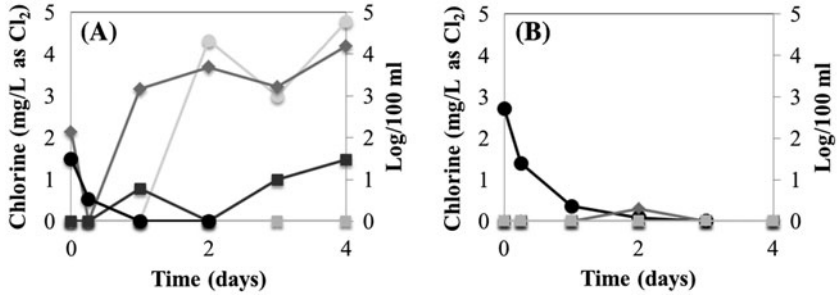

Fig. 4. Regrowth of spiked pathogens in graywater with two different chlorine residual concentrations, 1.5 mg/L (A) and 2.75 mg/L (B). (●) chlorine residual, (■) *E. coli*, (●) *S. enterica*, (♦) total coliforms, (■) *P. aeruginosa*.

the results of this study represent a conservative measure of pathogen regrowth.

The results of the spiked regrowth study 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 regrowth studies (Figs. 3(B) and 4). A chlorine residual of 2.5 mg/L or higher, however, was sufficient to prevent regrowth for at least 4 d as long as the TOC was within the range studied here (average 61.6 ± 8.3 mg/L).

Because many pathogens are not considered in water quality regulations and monitoring all known pathogens is not currently possible, indicators that accurately represent the behavior of pathogens are needed to predict pathogen levels 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 [34]. 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, 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. [35] reported that 60% of reclaimed water samples with high levels of assimilable organic carbon were positive for *P. aeruginos*a, but Wang et al. [36] 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 [35,36]. 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.

4. Conclusions

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 recycling 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. Ozonation was not effective for the size of generator tested, and UV was effective but would still require chlorination to prevent regrowth. In contrast, chlorination alone could provide disinfection of bacteria and MS2 bacteriophage, provide a disinfectant residual that can be monitored in the system effluent, and prevent the regrowth of bacteria for several days (~4 d). Temporarily switching to potable water would be recommended if systems are unused for longer absences. For typical light graywater, a total chlorine residual of 2.75 mg/L was sufficient, and when chlorine residual was maintained, regrowth of indicators and pathogens was prevented. Further, this chlorine residual prevented regrowth even during simulated high-contamination events. Thus, monitoring chlorine residual real time can be used to detect system upsets and is recommended to ensure public health is protected.

Supplementary material

The supplementary material for this paper is available online at http://dx.doi.org.10.1080/19443994.2016.1159992

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