

Reduction of microbial risk associated with greywater by disinfection processes for irrigation

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ABSTRACT

Greywater is one of the most important alternative sources for irrigation in arid and semi-arid countries. However, the health risk associated with the microbial contents of these waters limits their utilization. Many techniques have been developed and used to generate a high microbiological quality of greywater. The main problem in the treatment of greywater lies in the nature of pathogenic bacteria in terms of their ability to survive during/after the treatment process. The present review focused on the health risk associated with the presence of pathogenic bacteria in greywater and the treatment technologies used for the disinfection processes.

Key words | biological indicator, chemical and physical disinfection, microbial risk, reuse

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INTRODUCTION

Greywater is defined as untreated household wastewater generated from showers, bathtubs, hand basins, floor wastes, laundry tubs and washing machines. In some definitions, the wastewaters generated from kitchens and dishwashers, in homes and restaurants, are included within the greywater term, while others have prohibited the inclusion of kitchen wastewater with greywater due to its lower quality and classified it with the black water from toilets (WHO 2006; Schäfer *et al.* 2006; Al-Mashaqbeh *et al.* 2012). Maimon *et al.* (2014) demonstrated that when kitchen wastewater is included with greywater, higher concentrations of *Escherichia coli* are detected, while Friedler (2004) reported that kitchen wastewater does not typically have significant faecal contamination. Indeed, high counts of *E. coli* in greywater, including kitchen water, might be due to the available organic matter and nutrients necessary for bacterial regrowth (Winward *et al.* 2008c; Zapater *et al.* 2011; Maimon *et al.* 2014).

Nevertheless, the greywater generated from kitchens and restaurants might have microbial loads, which result from the washing processes for vegetables and meat. These

wastewaters are more relevant to the greywater than to the sewage due to their lower microbial loads and chemical content, such as nitrogen levels, which are 90% less than those in sewage (Jong *et al.* 2010; Oron *et al.* 2014). In general, although there is no consensus about the classification of kitchen wastewater as greywater or black water, in this review, the term greywater will include all non-toilet wastewater resulting from households and restaurants.

Greywater represents an important alternative source of water in arid and semi-arid countries, especially those that experience water scarcity (Oron *et al.* 2014; Maimon *et al.* 2014). The quality of greywater is lower than potable water, but it is of higher quality than sewage (Prathapar *et al.* 2005; Jamrah *et al.* 2006). Greywater contains different pathogens, such as bacteria, viruses and parasites. Bacteria are the most common among these pathogens and represent the biggest challenge because they have the ability to multiply without the need for an intermediate host. Bacteria have the ability to survive for a long time under stressful environmental conditions, and have the potential to regrow after the disinfection processes for greywater. Therefore,

improvement in the collection and treatment of greywater is very important for public and environmental health (Katakiza *et al.* 2014a; Bani-Melhem *et al.* 2015). Today, a wide range of greywater treatment technologies have been investigated to restore and maintain the physical, chemical and biological integrity of greywater pollution (Wurochekke *et al.* 2014c). Some of those technologies have exhibited an efficiency for the reduction of pathogenic bacteria and are being used on land for treatment processes, while other technologies are still under investigation. Moreover, the efficacy of the treatment technology to be applied for treating greywater depends on the ability of this technology to meet the requirements of the standard limits. The main criterion for the reuse of greywater in agriculture is pathogenic bacteria. In this review, pathogenic bacteria in the greywater, the treatment technologies used for disinfection processes and the potential of greywater as an alternative resource for irrigation are discussed based on microbiological concepts.

PATHOGENS IN GREYWATER

Although greywater does not contain faeces, unlike sewage, the concentrations of pathogens are not negligible. Greywater contains several opportunistic pathogens, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, in addition to enteric organisms (Ottozon & Stenstrom 2003a; Gross *et al.* 2007; Winward *et al.* 2008a). In this section, the bacterial loads of greywater are reviewed to understand the health risk associated with greywater to humans and the environment. The concentrations of pathogenic bacteria in greywater have been reported to be in the range of 5.4–8.9 log₁₀ CFU/100 mL for total coliforms (TC), 2.8–7 log₁₀ CFU/100 mL for *E. coli*, 2.4–4 log₁₀ CFU/100 mL for enterococci, 3.2–4.4 log₁₀ CFU/100 mL for *P. aeruginosa*. *S. aureus* ranged from 3.4 to 5 log₁₀ CFU/100 mL, while *Salmonella* spp. average 4 log₁₀ CFU/100 mL (Casanova *et al.* 2001; Winward *et al.* 2008c; Santasmasas *et al.* 2013; Katukiza *et al.* 2014b; Bani-Melhem *et al.* 2015).

Casanova *et al.* (2001) investigated the microbial quality of greywater from sinks, showers, washing machines and dishwashers over a seven-month period. They revealed that greywater contained a higher concentration of TC,

faecal coliforms (FC), enterococci and *P. aeruginosa* than the standard limits recommended by the WHO guidelines (WHO 1989). Ottozon & Stenstrom (2003a) studied the prevalence of faecal contamination of greywater and associated microbial risks. The study found high concentrations of faecal indicator bacteria (coliforms, *E. coli* and enterococci) in greywater. The results of quantitative microbial risk assessment (QMRA) that was performed on *Salmonella typhimurium* and *Campylobacter jejuni* indicated that the growth conditions for *Salmonella* spp. in greywater sediments increased the probability of infection up to 1,000-fold. Recently, the QMRA wiki page, which is a community portal for current quantitative information and knowledge developed for the QMRA field, was set up as a collated database/resource relating to QMRA (2015). It contains hazard infectious doses (ID), dose response, exposure assessment, risk characteristics, and risk management of pathogens. QMRA has been used by several authors for estimating the risk infection of target pathogens in drinking water (Hunter *et al.* 2003, 2009). Petterson & Stenström (2015) have used QMRA for quantifying the log₁₀ reduction across a free chlorine disinfection drinking water. The study demonstrated the importance of accounting for variable residence time in QMRA, where the log₁₀ reduction of pathogens appeared high and small parcels of water with short residence time can compromise the overall performance of the disinfection system.

Birks *et al.* (2004) compared the microbial loads of raw greywater, rainwater and groundwater. The study noted that greywater exhibited a higher faecal bacterial load than the rainwater and groundwater, which may be due to the nutrient content in greywater. *E. coli*, FC, *S. aureus* and *S. typhimurium* were detected in washing, kitchen, cooking, bathroom and shower wastewater generated from apartments located at Gyeonggi-do in Korea (Jong *et al.* 2010). The concentrations of *E. coli* (4×10^3 CFU/100 mL) were more than the standard limits recommended by the Enforcement Decree and Regulation of the Water Supply and Waterworks Installation Act in Korea. Some pathogenic bacteria survived in the greywater even after the treatment processes – *P. aeruginosa* and *S. aureus* were not affected by the greywater treatment process and remained with concentrations ranging from 1 to 3.9×10^3 and 2.4×10^4 CFU/100 mL, respectively (Maimon *et al.* 2014).

The hazard risks associated with the presence of pathogenic bacteria depend on the ID required to cause disease. Therefore, the bacterial contents of greywater are not necessarily an indication that the pathogen will cause infection to humans or animals. *S. aureus* and *P. aeruginosa* have the potential to cause skin infection and intestinal colonization if their concentrations exceed 10^5 and 10^6 CFU/100 mL (Maimon *et al.* 2014).

The ID for *Shigella* sp. is 10–200 cells, 10^5 cells for *E. coli*. For non-typhoidal salmonellosis, the ID is approximately 10^3 cells, while it is 10^5 cells for enteric fever by ingestion (Kothary & Babu 2001; Ryan & Ray 2004). Again, the ID of the bacteria is not the main factor that detects the pathogenicity of the bacteria. Moreover, the ability of bacteria to multiply and grow in greywater may increase their number such that it reaches or exceeds an infectious dose. The potential of bacteria to reproduce in greywater relies on the available nutrients and the environmental factors, such as pH, temperature and presence or absence of competition processes with *endogenous* microorganisms. Therefore, *Shigella* sp., which has a low ID, cannot persist for a long time in the environment. Conversely, *E. coli* and *Salmonella* spp., with a high ID, can increase in the environment due to the ability of both bacteria to grow in greywater as a secondary habitat (Gordon *et al.* 2002).

However, the health risks associated with pathogenic bacteria in greywater are not limited to their abundance and/or concentrations, but also in terms of the quality of their pathogenicity, such as antibiotic resistance. Greywater contains many antibiotic-resistant bacteria. Nuñez *et al.* (2012) examined the prevalence of antibiotic-resistant bacteria in raw greywater obtained from a channel located in the area of Ingeniero Budge, Buenos Aires Province. They showed that coliform bacteria resisted ampicillin by 34% and cephalothin by 17%. About 38% of enterococci exhibited resistance to vancomycin, while *E. coli*, *Enterococcus faecalis* and *Stenotrophomonas maltophilia* exhibited multi-resistance to antibiotics. Al-Gheethi *et al.* (2015a) studied the prevalence of antimicrobial resistance among *E. coli*, *Klebsiella pneumoniae*, *E. faecalis* and *S. aureus* in greywater generated from resultants at different locations in Penang, Malaysia. They showed that these pathogens exhibited multi-resistance to the investigated antibiotics (ampicillin, amoxicillin, ciprofloxacin, tetracycline and

erythromycin). The presence of antimicrobial resistance among the bacterial population in greywater exacerbates the distribution of antibiotic resistance among bacterial communities. This is due to the horizontal transmission of resistant plasmid genes between bacteria. Therefore, greywater is considered to be a reservoir of antibiotic resistant bacteria, which increases the potential health risk to humans.

Regarding the health risk associated with parasites, *Cryptosporidium parvum* and *Giardia lamblia*, as well as helminth ova, are the important opportunistic intestinal pathogens of humans (Rangel-Martinez *et al.* 2015). The main concerns about these pathogens lie in their ability to form cysts which can survive for a long time in the environment followed by transmission to humans via contaminated drinking water and vegetables irrigated with contaminated greywater. It has been reported previously that *C. parvum* and *G. lamblia* are more predominant in either warmer or wetter seasons. However, Rangel-Martinez *et al.* (2015) found no association between dry-cold or dry-warm periods and the presence or absence of these parasites. Further, the climate changes during different seasons might affect the number of parasites available; for example, *C. parvum* is more prevalent than *G. lamblia* (Kurniawan *et al.* 2009; Ajonina *et al.* 2012; Julio *et al.* 2012). *Cryptosporidium* spp. are capable of completing a life cycle within the same host and have the ability to cause reinfection to the host. Many reports in England, the USA and Japan have revealed the presence of correlation between the disease transmitted by polluted water and the presence of *C. parvum*, due to the potential of this parasite to resist chlorine disinfection. Cryptosporidiosis is more prevalent among immune-depressed people (Snelling *et al.* 2007).

Helminth parasites, which demonstrate a significant health risk in reused waters, include *Ascaris lumbricoides* and *Ancylostoma duodenale*. These helminths have a simple life cycle with no intermediate hosts, and are capable of causing infection via the faecal–oral route as well as having a high potential to survive and resist environmental stresses (Toze 2006). Ascariasis is the most common helminthiasis which infects children under 15 years of age (Silva *et al.* 1997; Jiménez 2003). The infection from parasites and helminth in greywater occurs after the consumption of food or water contaminated with oocysts (Carey *et al.*

2004). *C. parvum*, *G. lamblia*, *Entamoeba histolytica*, as well as *A. lumbricoides* are significantly more infectious to humans than most enteric bacterial pathogens because these pathogens have a low infective dose (<10 oocysts) (Rose & Gerba 1991; Dillingham *et al.* 2002).

Many viruses, such as rotaviruses, adenoviruses, reoviruses, caliciviruses and astroviruses have been found in wastewater. However, enteric viruses are the most common and important viruses. Enteric viruses are obligate intracellular parasites and are specialized to infect humans (Haas *et al.* 1999; Toze 2006). These viruses are more resistant to treatment processes and environment than bacteria. Therefore, the presence of these viruses in greywater represents an important risk to humans and is rarely a problem for other animals. The main concern about the presence of enteric viruses in greywater is their low dose infectivity (<10 viral particles) and their long-term survival in the environment, besides the limited extent of reduction or inactivation during treatment processes (Asano 2005). As mentioned before, the health hazards associated with greywater may turn into health risks depending on the environmental conditions and types of pathogens.

BIOLOGICAL INDICATORS OF GREYWATER

The biological indicators are microorganisms used as a model to investigate the presence and response of pathogenic bacteria to treatment processes in environmental systems. These indicators have similar characteristics to pathogenic bacteria, although the enumeration and the detection procedure is easier than for pathogens. Biological indicators include specific bacteria, fungi, viruses and parasites. The criteria for the selection of indicator bacteria include their ability to survive in the environment without an increase in their number, and a similar response to environmental factors as in the case of pathogenic bacteria. Therefore, they must be present in the contaminated environment and absent in the non-polluted environment (Cooper & Olivieri 1998; Dumontet *et al.* 1999; Lepeople *et al.* 2004; Bitton 2005; Myers *et al.* 2007).

Among several bacteria, faecal bacteria (TC, FC and enterococci) are the historical indicators. These bacteria have been used for over 100 years (Ashbolt *et al.* 2001;

US EPA 2007). The correlation between these indicators and pathogenic bacteria such as *Salmonella* spp. and *S. aureus* have been reported in the literature (Efstratiou *et al.* 1998; Efstratiou 2004). However, other reports in the literature revealed that these indicators do not correlate to the pathogens, especially in non-domestic wastewater. In some cases, the faecal indicators increased in number in the environment (Polo *et al.* 1998; Byappanahalli & Fujioka 1998). According to the US EPA, FC is used to determine whether the water meets the state water quality standards, while *E. coli* or enterococci are considered to be a health risk from recreational water. Moreover, *Enterococcus* sp. has been suggested to be useful for indicating the presence of viruses, particularly in sludge, seawater and biosolids. This is because these organisms are relatively easy to enumerate and survive longer than FC in these environments (Bitton 2005; Mote *et al.* 2012). Efstratiou *et al.* (2009) suggested that TC or FC alone might provide an adequate indicator for *Salmonella* presence in seawater. However, a statistically significant improvement demonstrated that enterococci alone might be more useful for the detection of *Salmonella* presence.

Indeed, the efficiency of the bacteria to be used as a biological indicator depends on the type of waste. Several studies have suggested using *S. aureus* and *P. aeruginosa* as indicators in greywater, because they have been detected even in the absence of *E. coli* (Yoshpe-Purer & Goldman 1987; Mates 1992; Tosti & Volterra 1998; Garland *et al.* 2000). Kenner & Clark (1974) reported that *P. aeruginosa* may be a better indicator than FC of pollution in potable, direct reuse, bathing and recreational water. Gross *et al.* (2007) noted that *S. aureus* and *P. aeruginosa* survived in greywater in which *E. coli* was reduced to non-detectable levels (<1 CFU/100 mL). In medical waste, *S. aureus* and *P. aeruginosa* are the biological indicators for testing the efficiency of treatment processes for those wastes (Efaq *et al.* 2015). With respect to biosolids, the US EPA (2003) demonstrated that *Salmonella* spp. are bacteria of great concern as well as good representatives of the reduction of other bacterial pathogens because they are typically present in higher densities than other bacterial pathogens and are at least as hardy.

Wheater *et al.* (1980) reported that the number of *P. aeruginosa* in freshwater streams reflected the level of domestic

pollution. Coronel-Olivares *et al.* (2011) suggested the use of *P. aeruginosa* as a new indicator for determining the quality of treated sewage effluent. This was due to its importance as an opportunistic pathogen. According to Warrington (2001), *P. aeruginosa* is the best indicator for recreational use, especially in marine water, where *E. coli* does not survive as well. Although *E. coli* is a good indicator in sewage-contaminated water supplies and chlorinated water high in organics, this bacterium is not as useful in water with high organic waste from vegetable processing. *S. aureus* has been proposed as being a useful indicator in hospital hygiene for microbiological standards (Dancer 2004). Crone & Tee (1974) found that *Staphylococcus* is more frequent than FC in swimming pools and considered staphylococci as an indicator for human contamination. Jin *et al.* (2013) used *S. aureus* as an indicator to evaluate the hydrothermal treatment process in terms of achievement of the hygienic safety of food waste.

It can be concluded that the selection of indicator bacteria depends on the type of wastewater. However, *P. aeruginosa* and *S. aureus* are the most appropriate for greywater from laundry and shower resources while *E. coli* and *E. faecalis* are the best indicators for the presence of faecal contamination.

POTENTIAL OF GREYwater FOR RECYCLING AND REUSE IN IRRIGATION

Greywater consists of all non-toilet wastewater that contains oils, fats, detergents, soaps, nutrients, salts and particles of hair, food and lint as well as different types of micro-organism. Greywater can be reused and recycled for several applications; it has been recycled as a source of biodegrading bacteria and a medium for the production of enzymes. Alrumman *et al.* (2014) used potato wastewater as a simple and cheap medium for the production of α -amylase by *Bacillus axarquiensis* compared to starch broth medium. They found that the amount of α -amylase in potato wastewater was less than in the starch medium by 13.8%. However, after the simple addition of starch, nitrogen, phosphate and calcium into the potato wastewater medium, the production of α -amylase increased four times more than production in the starch broth medium. Phong

et al. (2014) isolated 102 bacterial isolates from the wastewater of food processing plants and restaurants in Can Tho city, Vietnam. Among these bacteria, 11 bacterial isolates exhibited good lipase production. *Acinetobacteria soli* strain has been proposed for wastewater treatment because of its high ability for lipid degradation.

Moreover, in arid and semi-arid countries, greywater is used extensively for irrigation. This is due to severe water scarcity, rainfall fluctuation and the rise in water pollution (Bani-Melhem *et al.* 2015). The reuse of greywater for irrigation in those countries was intended to recover nutrients and for artificial aquifer recharge, as well as limit the resupply costs (Garland *et al.* 2000; Abu Ghunmi *et al.* 2008; Dalahmeh *et al.* 2011). Greywater is rich in nutrients that might be used for high plant growth. Alongside its benefits, greywater contents, such as soap and detergents, might have a negative effect on plants. The primary health hazard concern associated with the use of greywater is excreta-related pathogens. The presence of faecal pathogen bacteria in greywater, such as FC, *E. coli*, *S. enterica*, *S. aureus* and *P. aeruginosa*, might represent a high risk for humans and animals (Dixon *et al.* 1999; Chen *et al.* 2013; Benami *et al.* 2013; Maimon *et al.* 2014). The improper reuse of greywater for garden irrigation may pose a major hazard to the environment in the long term (Anda *et al.* 2010; Mohamed *et al.* 2012, 2013, 2014a, 2014b).

According to Al-Hamaiedeh & Bino (2010), the criteria required to reuse greywater include hygienic safety, aesthetics, environmental tolerance, and technical and economic feasibility. In this section, the potential reuse of greywater for irrigation will be discussed based on hygienic safety.

Garland *et al.* (2000) studied the effect of Igepon TC-42 (sodium N-coconut acid-N-methyl taurate, a common ingredient of soaps and detergents) on wheat and lettuce plants as well as the survival of pathogenic bacteria. They investigated three different modes: 875 mg m⁻² on growing area once a day, continuous addition of the same concentration d⁻¹ and the variable addition of 0 ± 3,000 mg m⁻² d⁻¹. They noted that the growth rate of wheat was not affected, while the lettuce yield significantly reduced with the pulse and continuous treatments. Igepon was degraded rapidly by microorganisms within 2 days, with a half-life of <1 hour. The maximum survival was recorded for *P. aeruginosa*, which persisted in the environment surrounding the

wheat for 70 days and the lettuce for 28 days. *E. coli* and *S. aureus* were reduced to below the detection limits within 35 days. According to this study, the detergent might not pose any potential growth risk to certain plants. However, the survival of some pathogenic bacteria on the irrigated plants would represent an important source and cause severe disease for plants, animals and humans.

It has been reported previously that bacteria can survive in stressful conditions by entering a viable but nonculturable (VBNC) state (Winfield & Groisman 2003). *Salmonella* spp. have high survival rates in aquatic environments (Chao *et al.* 1987), and *Salmonella* spp. spread with manure have survived for >10 months in soil (Jones 1986; Baloda *et al.* 2001). The contamination phenomenon of poultry farms has been revealed due to the ability of *Salmonella* spp. to persist for >1 year even with the use of disinfectants (Davies & Wray 1995). Jacobsen & Bech (2012) reported that *Salmonella* spp. could survive in soil for prolonged periods and could be transported to freshwater resources in high numbers.

The survival of pathogenic bacteria in greywater used for irrigation depends on the type of microorganism, source of pathogens (humans, plants), irrigated plants, frequency of irrigation, characteristics of the irrigated soil, temperature, pH and competition with protozoa (Guan & Holley 2003; García *et al.* 2010). Franz *et al.* (2011) reported very important findings, in that they found that *E. coli* strains isolated from humans survived longer than those isolated from animals (on average 211 vs. 70 days). Wang *et al.* (2014) investigated the survival time of *E. coli* O157:H7 in 14 soils collected in eastern China from the warm-temperate and subtropical zone. They showed that the *E. coli* O157:H7 survival times were between 1.4 and 25.8 days. *E. coli* O157:H7 has survived in neutral or alkaline soils and warm-temperate zones for longer than in acidic soils and subtropical zones. The survival rate also depends on the soil carbon and total nitrogen, with the minimum survival rate being recorded in the presence of amorphous Al₂O₃ and the relative abundance of Chloroflexi.

The environmental risks associated with greywater reuse are represented in the adverse effects on plant growth due to microbial contamination. However, Benami *et al.* (2013) compared the presence and abundance of *E. coli*, *K. pneumoniae*, *S. enterica*, *P. aeruginosa*, *E. faecalis*

and *Shigella* spp. in soil irrigated with treated greywater and that irrigated with freshwater (control). The study was conducted for 6 months and revealed that the abundance of these bacteria in the greywater and freshwater irrigated soils did not differ. Based on these results, they suggested that greywater irrigation has no effect on the diversity and abundance of the tested pathogens and indicators in the soil. In addition, Winward *et al.* (2008c) stated that no incidences of illness have been linked to greywater reuse and the health risks might appear to be low compared to sewage. It depends on the quality of the greywater, disinfection practices and management practices. The occurrence of faecal indicator bacteria in greywater, as reported by Casanova *et al.* (2001), Ottoson & Stenstrom (2003a) and Friedler (2004), has demonstrated the potential presence of faecally transmitted pathogens. Moreover, the WHO (2006) has reported that the disease outbreaks caused by *Vibrio cholerae*, *Salmonella* spp. and *Shigella* sp. are associated with the reuse of wastewater, excreta or greywater for irrigation of vegetables. Therefore, pathogenic bacteria associated with greywater still present a health risk even in the absence of reports in the literature; this point should be taken into consideration. The potential of pathogenic bacteria in greywater to cause human disease is discussed below.

The absence of evidence for the diseases caused by pathogenic bacteria, which have been transferred by vegetables irrigated with contaminated greywater, is due to the absence of accurate techniques that could detect the bacterial infection source. In the infections caused by parasites, it is easy to detect the source of the infection because parasites have a clear life cycle (reservoir, intermediate and vector host). In the bacterial infection as a typhoid disease, it is difficult to detect how *S. typhi* is transferred to humans – whether it is from drinking contaminated water or from contaminated food – because *Salmonella* spp. are able to grow and multiply in the environment (Lopez-Torres *et al.* 1987; Jimenez *et al.* 1989; Brettar & Hofle 1992; Winfield & Eduardo 2003). In addition, *Salmonella* spp. do not have a specific reservoir and are capable of colonizing a wide variety of microorganisms (Foltz 1969). This feature supports the *Salmonella* life cycle, as *Salmonella* spp. have the ability to pass through different hosts into the environment and back into a new host (Thomason

et al. 1977). Some authors reported that bacteriophages have the ability to identify and distinguish different strains of bacterial species isolated from different origins (disease, food, water, environment) or geographical locations (Schofield et al. 2012). Wangkahad et al. (2015) have used bacteriophages to detect the source of pathogen contamination. The study investigated the association between bacteriophages of *Aeromonas caviae*, *Enterobacter* sp. and *K. pneumoniae* in water and the contamination sources (human vs. animals) in Thailand. The study found that bacteriophages were detected in polluted samples from human faecal sources but not in non-polluted samples. The presence of *A. caviae* was associated with human faecal sources, whereas *Enterobacter* sp. and *K. pneumoniae* were prevalent in human and animal faecal sources.

In general, the concerns in terms of health risk related to the reuse of greywater in irrigation lie in the potential of these pathogens for regrowth or persistence and transfer into the food chain (Rose et al. 1991). For instance,

the ability of *P. aeruginosa* to regrow in greywater makes it an opportunistic pathogen of concern for greywater reuse (Winward et al. 2008a). Hence, the strategy of greywater reuse for irrigation purposes is subject to strict regulation in developed countries (Table 1). In contrast, although developing countries also have strict regulations for the reuse of greywater in agriculture, these countries lack the developed techniques and the power to enforce such regulations. Therefore, surface irrigation, which is not permissible in developed countries, is the most common use in developing countries.

In comparison, black water with high concentrations of pathogenic bacteria would appear to have a higher risk to humans than greywater. It has been demonstrated that the irrigation of fresh vegetables by black water represents the main cause of diarrhoeal disease due to the high microbial load in the vegetables even after the washing process (Ronner & Wong 1993). The movement of pathogenic bacteria through the soil and the contamination of

Table 1 | Regulations for irrigation process by greywater

Country	Regulations for irrigation process by greywater
USA	(i) No surface irrigation is allowed (ii) No surface application of greywater is allowed for irrigation of food plants, except for citrus and nut trees (iii) Greywater should be free from hazardous chemicals such as from washing greasy items, cleaning car parts, oily rags, or home occupational activities (iv) The direct discharge of greywater into the main sewage system is not allowed (v) Greywater applied by surface irrigation should not contain water from washing diapers or similarly soiled or infectious garments unless it is disinfected prior to irrigation
Australia	(i) Sub-soil irrigation and sub-surface irrigation are used in irrigation by primary treated greywater (ii) Surface spray irrigation, sub-strata drip irrigation, and sub-surface drip irrigation are used in irrigation by secondary treated greywater
Canada	(i) No contact between the greywater and the people around will be allowed (ii) It should be a simple system that requires minimum maintenance
WHO	Class (A) Irrigation of crops likely to be eaten uncooked, sports fields, public parks Class (B) Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees
Middle East countries	In Tunisia, Morocco and United Arab Emirates (UAE), greywater and sewage effluents have been used for irrigation of gardens in urban centres and tourist facilities. Abu Dhabi has a clear reuse strategy for treated wastewater. The high quality effluent is reused only for irrigation of green spaces in the city Egypt has adopted the regulation of wastewater reuse based on irrigation techniques, requirements for health protection. According to these regulations, the reuse of wastewater is not allowed for any edible crops or export crops. However, both crops are irrigated by wastewater regardless of the treatment level due to the absence of the advanced technologies required to produce high quality wastewater In Saudi Arabia the regulations for reuse of different wastewaters in agriculture were released in 2000. The treated wastewater for landscape irrigation and agriculture purposes shall be of tertiary quality Some countries, such as Yemen and Syria, have strict regulations for reuse of greywater but none of these are in force due to the absence of facilities

Sources: WHO (1989); Health (2000); OASIS (2002); Canadian Standards Association (2010); ACWUA (2010); Domenech & Sauri (2010); Yu et al. (2013); Oron et al. (2014).

groundwater, drinking water, runoff and erosion containing pathogens and contaminating surface water increases the possibility of human infection (Sahlstrom *et al.* 2004).

STANDARD LIMITS FOR THE TREATMENT OF GREYWATER

In order to ensure the safe reuse of greywater, these wastes should meet certain limits. In some countries, there is a quality safety plan and alternative systems of treatment of greywater (Wurochekke *et al.* 2014c). Currently, many countries have regulated the microbiological standards for greywater reuse (Table 2). Moreover, most countries focus on the health or environmental risk from pathogenic bacteria, and very little is mentioned regarding the potential presence of heavy metals or disinfection by-products (DBPs) and pharmaceutically active compounds (Toze 2006). This differs from black water or biosolids where the standards focus on pathogenic bacteria as well as viruses, parasites and helminths. As well, the standards

for black water include heavy metals and toxic substances. These differences are due to the nature of black water, which is more complicated than greywater. Therefore, the discussion of standards presented here focuses only on the presence of pathogenic bacteria as a criteria for the quality of greywater. These standards range from the stringent guidelines of the US EPA and Korea, where FC should be non-detectable in water intended for urban reuse, to the less demanding guidelines of Germany and the WHO (<1,000 CFU/100 mL). In the USA, California requires that FC should not exceed 2.2 CFU/100 mL as an average over a 7-day period or 23 CFU/100 mL in 30 days. In the UK, since 1988, Defra and its predecessors have described in detail the microbiological standards of different types of wastewater. In Jordan, the standards for greywater are divided into two categories based on their utilization. In Category A, where the greywater is used for the irrigation of cooked vegetables, the FC should be <100 MPN/100 mL, while in category B, where the greywater is used for the irrigation of tree crops, the FC should be <1,000 MPN/100 mL.

Table 2 | Microbiological regulation for greywater reused for irrigation

Country/ Organization	TC	FC	<i>Pseudomonas aeruginosa</i>	References
USA (California)		2.2 MPN/100 mL (23 MPN/100 mL in 30 days)		US EPA (2004)
Australia		<30 CFU/100 mL		Health (2000)
Australia		<10 CFU/100 mL		Government of Western Australia (2005)
Germany	<10 ⁴ CFU/ 100 mL	<1,000 CFU/100 mL	<100 CFU/100 mL	Nolde (1999)
WHO		Class A (<1,000 CFU/100 mL) Class B (No standard recommended)		WHO (1989)
Mexico		≤ 2,000 CFU/100 mL		Peasey (1999)
Korea		Must not be detected		Jong <i>et al.</i> (2010)
UK	10 CFU/100 mL	–	–	BSI (2010, 2011)
Portugal	104 CFU/ 100 mL	200 CFU/100 mL	–	ANQIP (2011)
Jordan		Category A (cooked vegetables) 100 MPN/100 mL Category B (tree crops) 1,000 MPN/ 100 mL		Duqqa (2002)

DISINFECTION PROCESSES OF GREYWATER

The disinfection technologies aim to eliminate the microbial loads of greywater that might pose a potential risk for humans and plants, and thus provide safe and aesthetically acceptable greywater that is appropriate for the purpose of irrigation. These technologies include chemical (chlorination and ozonation), physical or mechanical (filtration processes) and radiation disinfection (ultraviolet (UV) irradiation). The degree of disinfection process proposed must take into account the type of reuse and the risk to the population of exposure (Matos *et al.* 2014). In this section, the disinfection techniques for greywater are

reviewed and discussed based on the efficiency in eliminating pathogenic bacteria and toxic by-products, and to reuse greywater for agricultural purposes. The advantages and disadvantages of disinfection processes for greywater are illustrated in Table 3.

Chemical disinfection

Chlorination was the most common method for disinfection in the twentieth century because it was cheap and simple (Dunnick & Melnick 1993; Winward *et al.* 2008a). The efficiency of chlorination to kill pathogenic bacteria in greywater relies on the greywater quality, such as the

Table 3 | Advantages and disadvantages of the advanced processes used for the reduction of pathogenic bacteria in the greywater

Disinfection processes	Advantages	Disadvantages
Chemical disinfection (chlorination and ozonation)	<ul style="list-style-type: none"> - Cheap and easy to use - Very powerful and fast - Effective for inactivation of pathogenic bacteria - <i>Leaves residual in treated greywater to emerge as re-contamination</i> - <i>Has flexible dosage control</i> 	<ul style="list-style-type: none"> - Generation of toxic by-products - Occurrence of resistance and regrowth among pathogenic bacteria - Decay process - Occurrence of microbial regrowth due to oxidation process of organic compounds - Levels of total dissolved solids increase in the treated greywater
Membrane filtration	<ul style="list-style-type: none"> - Efficient to remove pathogenic bacteria mechanically not chemically, which means that the bacteria on the surface of the filter are still active - No microbial resistance - No toxic by-products - No chemical additives - Eco-friendly 	<ul style="list-style-type: none"> - Insufficient to reduce pathogenic bacteria and to meet the international standards - The efficiency percentage reduces with time, number of filtration processes and with high volumes of greywater - More expensive to apply to land - Is not able to remove dissolved organic matter - Does not cause any physical or chemical damage of bacterial cells. Hence, the remaining bacteria in the disinfected greywater might grow and multiply again - High capital, operations and maintenance costs - No residual effect - The efficiency is reduced with time
Solar disinfection (SODIS)	<ul style="list-style-type: none"> - Combination of temperature and UV, which lead to destroying pathogenic bacteria effectively - Natural process - No microbial resistance - No toxic by-products - Eco-friendly - No energy is required - Short time required for disinfection process - Very cheap, no capital costs - Independent from energy sources other than sunlight 	<ul style="list-style-type: none"> - The efficiency is reduced during cloudy days - Cannot be used to treat very turbid water (>30 NTU) - Does not remove suspended particles of dissolved compounds - No residual effect

turbidity, presence of organics and particles that might protect pathogenic bacteria from the action of chlorine (Winward *et al.* 2008a). Mohamed *et al.* (2015) suggested double doses of chlorination for water with high turbidity (100 NTU). A high dose of chlorine and the removal of impurities from greywater by filtration improve the efficiency of the chlorination process (Tal *et al.* 2011). However, the free and combined chlorine residue is toxic; therefore, there is a requirement to de-chlorinate or to remove the chlorine before disposal to the environment. Recently, many developed countries such as the USA have prevented the use of chlorination due to its toxic by-products and the occurrence of resistance and regrowth among pathogenic bacteria (Morris *et al.* 1992; Nieuwen-Huijsen *et al.* 2000). The reason for the occurrence of bacterial regrowth in chlorinated greywater might be due to its highly reactive characteristics, which accelerate the chlorine decay process (Tal *et al.* 2011).

Most DBP of chlorination are carcinogenic; one of them is nitrosodimethylamine, which has been reported to be a probable human carcinogen (Pehlivanoglu-Mantas *et al.* 2006). Cantor *et al.* (1987) reported the first indication of the association between bladder cancer risk and the consumption of chlorinated water. The bacterial resistance of chlorination has been reported since the 1980s. Ridgway & Olson (1982) indicated that bacteria isolated from a chlorinated system have more resistance to the combined and free forms of chlorine than those isolated from an un-chlorinated system. Recently, some authors have revealed several pathogenic bacteria that are resistant to chlorination (Dungeni *et al.* 2010; Cherchi & Gu 2011). The formation of DBPs such as total trihalomethane and haloacetic acid were also reported in the disinfection process of water by titanium dioxide photocatalysis.

The problem of bacterial resistance to chlorine has been extended to include resistance to antibiotics. The authors reported that there is a correlation between the resistance to chlorine and antibiotics, as bacterial cells exposed to chlorine may acquire resistance to antibiotics (Murray *et al.* 1984; Shi *et al.* 2013). In greywater, the ability of pathogenic bacteria to resist chlorine is due to the bacteria having been in contact with high concentrations of chlorine during the washing processes, thus, the efficiency of chlorination to eliminate these pathogens would be weak.

In order to increase the efficiency of chlorination, Santasmasas *et al.* (2013) suggested that the disinfection of greywater by chlorination might be more effective if the greywater were treated using a biological oxidation process, as this would lead to degradation of organic matter and thus reduce the toxic by-products. In their study, the authors treated greywater in four stages: a screening process, in which the debris was separated; then biological oxidation to decompose the organics; a filtration process using ultrafiltration membrane technology; finally, the greywater generated was disinfected with chlorine. They showed that chlorination reduced the TC and FC by 6 log₁₀ CFU/100 mL and that the disinfected greywater met the standard limits recommended for reuse in irrigation.

Benami *et al.* (2015) monitored the presence of FC, *E. coli*, *S. enterica*, *S. aureus* and *P. aeruginosa* during the greywater treatment and disinfection process (raw, biologically treated, and disinfection by chlorine). The evaluation study was conducted bi-monthly over the course of a year. The samples were analysed using both culture and molecular methods based on DNA-based quantitative polymerase chain reaction (qPCR). The culture-based method revealed that the pathogenic bacteria were inactivated by chlorine. However, the DNA-based analyses indicated that there was no significant difference between the pathogenic bacteria in the raw and disinfected greywater. To understand the discrepancies between the results, the authors repeated the analysis of *E. faecalis* as a model bacterium, and obtained similar results. Based on these results, the authors concluded that the inactivation efficiency cannot be estimated by DNA-based qPCR. In fact, the explanation of these results is the viable but nonculturable (VBNC) state of the inactivated bacteria. It has been reported that under unfavourable conditions, bacteria may transfer into a dormant state called VBNC, which means that there are slow metabolic processes and no cell division (Oliver 2005); in such a case, the culture-based technique is not able to detect the bacterial cells. Therefore, the molecular-based technique will be more accurate to detect the presence or absence of VBNC bacterial cells (Chen *et al.* 2012). However, some authors reported that this technique might give false positive results. For this reason, a decade ago the molecular-based technique using qPCR was developed for greater accuracy. The development included using propidium

monoazide or ethidium monoazide dyes (Fijalkowski *et al.* 2014). In addition, new technologies have been introduced to detect the viability of bacterial cells, such as the detection of metabolic activity, the positive energy status, responsiveness and the reverse transcription quantitative PCR assay (Fittipaldi *et al.* 2012; Jiang *et al.* 2013).

Helmi *et al.* (2015) have investigated techniques to detect the viability of a bacterial population in chlorinated and non-chlorinated water. The techniques used included flow cytometry (FCM), solid-phase cytometry (SPC), epifluorescence microscopy (MSP) and culture method performances. The total bacterial counts were quantified by using 4',6'-diamino-2-phenylindole (DAPI) (for MSP) and SYBR Green II (for FCM), and the viable and non-viable bacterial cells were distinguished by using SYBR Green II and propidium iodide dual staining (for FCM). In FCM and SPC, the Chemchrome V6 was used to distinguish the active bacterial cells, while 5-cyano-2,3-ditoyl tetrazolium chloride (CTC) was used in the MSP method. The study recorded significant correlation between total bacteria and active cells as determined by microscopy and FCM. In contrast, the correlation between bacterial counts and active cells was not significant, as determined using solid-phase and FCM. Moreover, the culture-based method using R2A medium showed bacterial recovery after chlorination. Therefore, FCM might be a useful and powerful technique for drinking water production monitoring.

In general, chlorination has become an unacceptable process for disinfection of water due to the toxic by-products, bacterial resistance and bacterial regrowth after the disinfection process. Therefore, the alternative technology used is ozonation. However, the responses of microorganisms to ozonation have been reported to be similar to those of chlorination (Xu *et al.* 2002; Vital *et al.* 2008, 2010).

Membrane filtration

A mechanical disinfection process by filtration would be more efficient to remove pathogenic bacteria but not for the inactivation of bacterial cells, as the cells removed by the filtration systems are still active. This technique could not increase the microbial resistance to antibiotics or produce toxic by-products. Numerous systems of membrane filtration have been developed for the removal of pathogenic

bacteria, the efficiency of which depends on the pore size of the membrane filter. However, the physiochemical characteristics of greywater significantly affect the filtration process.

Winward *et al.* (2008c) evaluated the efficiency of three configurations of constructed wetland: horizontal flow reed bed, vertical flow reed bed (VFRB) and a green roof water recycling system (GROW). A membrane bioreactor (MBR) and a membrane chemical reactor (MCR) were used for the removal of heterotrophic bacteria, TC, *E. coli*, enterococci, sulphite reducing Clostridia, *S. aureus* and *P. aeruginosa* from the greywater. The MBR was conducted in continuous flow and under aerobic conditions to provide a mixing of the biomass. The MCR consisted of an advanced oxidation process using UV light and titanium dioxide in a closed reactor, and membrane filtration was used for the disinfection process. They reported that the three constructed wetlands met the German reuse standard for TC and *P. aeruginosa* (4.0 and 2.0 log₁₀ CFU/100 mL, respectively) but not the California State standards (2.2 log₁₀ CFU/100 mL). Among the constructed wetlands, VFRB provided the best overall removal of faecal indicators, in which 44% of the treated samples met the California State standards. MBR provided the highest quality treated greywater and had the most robust treatment efficiency. This system was not affected by an increase in the influent greywater strength.

Jong *et al.* (2010) investigated the efficiency of an MBR consisting of anaerobic-anoxic-oxic reactors, and a membrane filter (pore size 0.45 µm) to remove *E. coli*, *S. aureus* and *S. typhimurium* from greywater. The maximum removal of these bacteria after the anaerobic-anoxic-oxic reactors was 20%, 5.5% and 5.6%, respectively. After the membrane filter, *E. coli* reduced by 67.5 compared to 27.7 and 20.4% for *S. aureus* and *S. typhimurium*, respectively. However, the *E. coli* count in the treated effluent was still more than the standard limit recommended for irrigation reuse (1.3×10^5 CFU/100 mL).

Katukiza *et al.* (2014a) studied the treatment of greywater in Uganda using a two-step crushed lava rock filter. The filter unit consisted of two filters in series. The filters (R1 and R2) were composed of 10 cm of crushed gravel under-drain (for the sedimentation process) and graded crushed lava rock (2.56–5 mm for the first filter and 1.18–2.56 mm for the second filter). Crushed lava rock was

chosen in the study as it has a higher specific surface area and porosity compared to sand and gravel (Kalibbala *et al.* 2012; Sekomo *et al.* 2012). The hypothesis for treatment was based on a two-step design; the primary treatment of greywater was performed by R1, while the secondary treatment was performed by R2. Katukiza and co-workers found that the sedimentation process did not reduce the bacterial loads. The TC, *E. coli* and *Salmonella* spp. counts did not differ significantly before and after the sedimentation process. The log reduction was 3.9, 3.8 and 3.2 after R1 and R2 for TC, *E. coli* and *Salmonella* spp. respectively. However, the bacterial loads of the treated effluents did not meet the WHO guidelines for greywater. The concentration of *E. coli* in the treated effluents was 3.7 log₁₀ CFU/100 mL compared to 3 log₁₀ CFU/100 mL required by the WHO; in addition, the *Salmonella* spp. was 2.73 log₁₀ CFU/100 mL. Based on these results, the designed filter system was insufficient to reduce the pathogenic bacteria in greywater and to meet the international standards for reuse for agricultural purposes.

Molaei (2014) studied four types of filtration system for the removal of *Salmonella* spp. and *E. faecalis* from artificial greywater in Sweden. These filters were biochar, bark, activated carbon and a mixture of bark and activated carbon. The filtration systems were designed in column experiments (height 65 cm, diameter 4.3 cm) for 60 days. The biochar filter was the most effective in removing *Salmonella* spp. (3 log reductions). The efficiency of the bark and filter mixture ranged from 1 to 2 log reductions. The activated carbon filters exhibited the highest efficiency for removing *Salmonella* spp. and *E. faecalis* (7 vs. 5 logs, respectively). The high removal of active carbon appeared to be efficient for the disinfection of greywater. However, this efficiency was recorded during the first week and decreased significantly after 15 days to 1 log reduction. After 30 days of the filtration process, the concentration of *Salmonella* spp. increased to >3 log₁₀ CFU/100 mL. For all the filter types investigated, the efficiency percentage reduced after 1 week of the disinfection process. In order to achieve greater removal of pathogenic bacteria, the filter should be replaced every week at least, which means that the current disinfection process would be more expensive to apply to land.

Bani-Melhem *et al.* (2015) evaluated a submerged membrane bioreactor (SMBR) system (pore size of 0.04 mm)

for removing TC and FC from greywater in Jordan. The SMBR was operated for 42 days at constant transmembrane pressure (13 kPa). They noted that 99.99% of the TC and FC concentration was removed and that the greywater met the standard limits recommended for reuse in irrigation.

According to the above literature, the disinfection of greywater using membrane filtration has some efficiency for the removal of pathogenic bacteria. However, these technologies have many disadvantages. Membrane filtration is less effective in highly polluted greywater and requires constant maintenance. This technology is not able to remove dissolved organic matter, and does not cause any physical or chemical damage to bacterial cells. Hence, the remaining bacteria in the disinfected greywater might grow and multiply again.

Solar disinfection

The disinfection process, which does not lead to complete damage of the bacterial cells to prevent regrowth after the disinfection process, would be inadequate. For instance, although the disinfection process by UV irradiation is safer in terms of toxic by-products, the ability of bacteria to resist UV disinfection and regrow after the disinfection process has been recognized (Alonso *et al.* 2004; Munir *et al.* 2010; Ting *et al.* 2011; Wang *et al.* 2012). Huang *et al.* (2013) reported that the population of antibiotic-resistant bacteria in water increases when exposed to low doses of UV light or chlorine. Therefore, the selection of the disinfection process for greywater should be in terms of being free from toxic by-products and having the ability to completely destroy bacterial cells.

The potential for bacteria to regrow depends on the inactivation mechanism caused by the disinfection process. An understanding of the factors affecting bacterial growth would help in the selection of an appropriate process that has the ability to cause complete damage to the bacterial cells. Among the many factors that affect bacterial survival and growth in the environment, some are chemical, such as pH, water activity, moisture content, nutrients and disinfectants, while others are physical, such as temperature, pressure and irradiation (Johnson *et al.* 2005; Hungaro *et al.* 2014).

Among the various factors, temperature is the most critical in terms of the survival of the bacteria (Tang 2009).

Hence, temperature is a basic consideration in the development of several disinfection technologies, such as pasteurization, which has been used for over 200 years. Most pathogenic bacteria are mesophilic, with an optimal temperature of 37 °C. Therefore, thermal treatment above the optimal temperature would lead to denaturation of the proteins, enzymes and nucleic acids of bacterial cells. However, the thermal disinfection of greywater has become increasingly unacceptable in recent years due to the high energy and increase in the Earth's temperature.

Thermal disinfection at moderate temperature (45 °C) has been reported as being effective for the reduction of *E. coli*. Wastewater treated in such a manner has met the WHO guidelines (<3 log₁₀ CFU/100 mL) after 48 hours and US EPA (<14 CFU/100 mL) after 1 week of treatment (45 °C) (Al-Gheethi *et al.* 2013a). These results indicate that a moderate temperature is effective as a disinfection process for wastewater. However, the treatment periods required to achieve the standards are lengthy. Thus, this process might be inappropriate for a high volume of greywater or even for a private house because the greywater is generated daily, whereas this process requires 1 week to meet the greywater standards for reuse. Therefore, Al-Gheethi *et al.* (2013b) used solar disinfection (SODIS) for the elimination of FC, *E. faecalis*, *Salmonella* spp. and *S. aureus* in secondary effluent and lake water. The treatment process was conducted inside transparent polyethylene terephthalate bottles for 1, 2, 3, 4, 5, 6, 7 and 8 hours. It was revealed that the temperature of the samples increased to >45 °C during the treatment process. FC, *Salmonella* spp. and *S. aureus* were eliminated from the secondary effluent after 8 hours and after 7 hours from the lake water. No regrowth of these pathogens was observed, as determined by the absence and presence technique using an enrichment medium. *E. faecalis* reduced to less than the detection limits in the lake water but was still detectable in the secondary effluent. In order to eliminate *E. faecalis*, Al-Gheethi *et al.* (2013b) stored the treated samples inside closed PPT bottles at room temperature for 2 weeks; the bacteria were eliminated during the storage period. This was because *E. faecalis* is aerobic and died off in the closed bottles due to the absence of oxygen and competition from indigenous microorganisms.

SODIS is a technique used extensively for drinking water; this technique improves the microbiological quality

of drinking water due to a combination of the temperature and UV, which effectively destroys pathogenic bacteria (WHO 2002; Gomez-Couso *et al.* 2009). In the study conducted by Al-Gheethi *et al.* (2013b), the authors combined three factors: temperature, UV and anaerobic condition. This treatment exhibited efficiency in the reduction of faecal indicators and the elimination of pathogenic bacteria.

Pansonato *et al.* (2011) demonstrated the efficiency of SODIS in the inactivation of pathogenic bacteria in greywater. They investigated SODIS for the inactivation of *E. coli* in greywater. The samples were exposed to an average solar radiation intensity of 518 W/m². The results indicated that the SODIS system has the potential for TC and *E. coli* inactivation in pre-treated greywater by >2 log₁₀.

Currently, the US EPA (2015) uses the SODIS method as a model for the design of residential systems for the sanitation of greywater for the irrigation of lawns and gardens. This project started in August 2014. It is expected that a system will be invented that can increase the efficiency of the solar radiation and heat for the inactivation of microbes.

Other treatments and disinfection processes of greywater

Gross *et al.* (2007) studied the recycled vertical flow bio-reactor system for removing pathogenic bacteria (*E. coli*, *S. aureus* and *P. aeruginosa*) from greywater generated from the kitchen. In this system, the greywater was subject to preliminary sedimentation and then pumped into plastic containers consisting of three layers: the upper container contained organic soil with lime pebbles and heterotrophic bacteria as decomposing organisms; the lower container was used as a lower 'reservoir container'; and the middle layer consisted of plastic filter media. The greywater generated from the reservoir container was pumped again into the upper container. The treatment process was conducted for 2–3 days, and no disinfection process was performed in this treatment. Samples from the input and output points were collected and subjected to microbial analysis, which revealed that at the end of the treatment process, *E. coli* was reduced to less than the detection limits (<1 CFU/100 mL). However, the concentrations of *P. aeruginosa* did not change in the samples collected before and after the treatment process. *S. aureus* declined in the treated

effluent but still averaged 100 CFU/100 mL. In this study, although no disinfection process was implemented, *E. coli* was completely removed. However, *S. aureus* and *P. aeruginosa* survived during the treatment process. This might be because the kitchen greywater represents a secondary environment for *E. coli*, while *S. aureus* and *P. aeruginosa* are indigenous bacteria in these wastes. Thus, the survival of these bacteria was greater than for *E. coli*. In addition, the biodegradation of organic matter by decomposing bacteria during the treatment process would prevent the regrowth of *E. coli*. Friedler (2004) reported that kitchen wastewater does not contain faecal contamination. Therefore, such waste might only contain a small concentration of *E. coli*, whereas, in the presence of the organic matter and nutrients in kitchen wastewater, *E. coli* might regrow to high concentrations (Winward *et al.* 2008c; Zapater *et al.* 2011; Maimon *et al.* 2014).

The disinfection of greywater through the use of plant products that have antimicrobial properties was studied by Winward *et al.* (2008b). They assessed the antimicrobial properties of essential oils (EOs) from origanum oil (*Thymus capitatus*) and carvacrol as a disinfection process for greywater in the UK. They found that 468 mg/L of origanum oil reduced TC to less than the detectable levels after 30 min of contact time. The inactivation rate of TC increased with an increase in the contact time. No regrowth of TC was recorded in the greywater over 14 weeks, despite the organic concentrations and particulate size in greywater reducing the efficacy of the disinfection process with origanum EOs. The study opened up a new direction for the disinfection of greywater. EOs are aromatic compounds extracted from plants (Sangwan *et al.* 2001). EOs have the ability to inactivate bacterial cells by disruption of the cell membrane, causing leakage of the cell contents and eventual cell lysis (Burt 2004). As yet, toxic by-products generated from the disinfection process using EOs have not been reported. However, in this study, the authors reported that disinfection with origanum EO was limited by organics. Thus, they indicated that disinfection by origanum EO would be more effective for preventing bacterial regrowth at a low concentration if the greywater was treated by a MBR (Jefferson *et al.* 2001). Otherwise, high concentrations of origanum EO should be used. Finally, the authors concluded that the disinfection of greywater with

EOs appears to be impractical due to the land area required to produce sufficient origanum EO and the anticipated costs.

STORAGE OF GREYWATER

The storage of greywater is an important element in all greywater recycling systems. Clearly, water needs to be stored until its utilization. However, the microbial load of the stored water needs to be evaluated carefully. Rose *et al.* (1991) reported that coliform bacteria increased to 1 and 2 log₁₀ CFU/100 mL during the storage period over 48 hours. Furthermore, Dixon *et al.* (1999) indicated that TC increased by 2 log₁₀ CFU/100 mL after 24 hours of storage. In contrast, Gerba *et al.* (1995) found that TC and FC reduced by 3 logs in stored greywater. Previous studies for *Salmonella* sp. and *Campylobacter* sp. seeded into greywater indicated that no regrowth occurs (Rose *et al.* 1991; Ottoson & Stenstrom 2003b). Al-Gheethi *et al.* (2013a) noted that the concentrations of *E. coli* and *E. faecalis* reduced in the stored effluents to less than the detection limits after 2 weeks of storage at room temperature. The effluent met the standard requirements for irrigation. It has been suggested that the presence of competing microorganisms prevents the regrowth of pathogenic bacteria or induces them to enter into a viable but non-culturable state (Ottoson & Stenstrom 2003b). Moreover, Al-Gheethi *et al.* (2013a) reported that the storage system for secondary effluents should not be more than 2 weeks due to the increase in antimicrobial resistance among the surviving bacteria. They noted that after 4 weeks of storage, the concentrations of TC and *Salmonella* spp. reduced. However, the resistance to ampicillin, amoxicillin, cephalexin, cefuroxime and ciprofloxacin increased significantly among these bacteria.

The microbiological qualities of greywater during the storage period depend on the nature of these waters. The potential of pathogenic bacteria to multiply and grow in the treated water is less than that in untreated waters. Nevertheless, this potential will rely on the nature of the treatment processes and storage conditions of the treated waters. The storage conditions for the disinfected greywater affect the ability of bacteria to regrow and multiply. The bacterial cells inactivated using UV irradiation could grow again

under suitable storage conditions. Al-Gheethi *et al.* (2013b) found that *Salmonella* spp. *S. aureus* and *E. faecalis* grew again in the effluents treated by SODIS for 6 hours, when the samples were stored at 37 °C for 4 days. Generally, bacterial regrowth may occur if the disinfection process does not lead to physical damage of the bacterial cell, as in the case of ozonation and different bio-filter systems (Berney *et al.* 2006; Vital *et al.* 2010).

CONCLUSIONS

Greywater is considered to be an alternative resource for irrigation. These waters have lower microbial loads than sewage. However, there is still a potential health risk. Therefore, effective disinfection processes rely on the ability to divert microbial cells rather than inactivation only. Advanced technical analysis to evaluate the viability of microbial cells in greywater is required before the reuse of disinfected greywater for agriculture proposes.

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