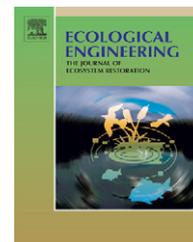


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Removal of chemical and microbiological contaminants from domestic greywater using a recycled vertical flow bioreactor (RVFB)

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ABSTRACT

Reuse of domestic greywater (GW) for non-potable purposes is emerging as an important approach to the management and conservation of water resources, particularly in rural and developing areas where small-scale decentralized treatments are suited. Recently, researchers at Ben-Gurion University (Israel) have developed a modular system – the recycled vertical flow bioreactor (RVFB) – for the removal of chemical contaminants from GW. We report on the removal of chemical contaminants, indicator organisms, and opportunistic pathogens from GW using this system.

Synthetic GW, enriched with wastes from a dining hall, was added to experimental RVFB systems. The greywater was recirculated for 2–3 days at which time half of the water was removed from each system and replaced with fresh synthetic GW. Removal of chemical and microbiological contaminants was determined by comparing influent to effluent samples obtained after 8 and 72 h treatment in the systems.

GW treatment using the RVFB system reduced effluent concentrations for NO₃-N, total ammonia nitrogen, NO₂-N, total suspended solids, boron, and anionic surfactants to below the levels considered acceptable for either recreation or irrigation. Reduction in chemical contaminants was associated with a significant increase in the number of both heterotrophic bacteria and surfactant-degrading bacteria within the RVFB.

Escherichia coli, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were present at the beginning of each treatment cycle. Treatment using the RVFB system resulted in final *E. coli* concentrations that met the USEPA water quality criteria for recreational water. Viable *S. aureus* and *P. aeruginosa* were consistently present in samples obtained at the end of the 72 h treatment period. The survival of these non-enteric opportunistic pathogens underscores the need for the consideration of additional indicator organisms to monitor and assure the safe reuse of GW.

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1. Introduction

Greywater (GW) is usually defined as all of the wastewater produced in a household except toilet wastes (blackwater:

BW). Typically, GW includes wastes from bathroom sinks, baths, and showers and may also include wastes from laundry facilities and dishwashers. Some definitions include wastes from kitchen sinks although there is no consensus on this

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(Queensland Government, 2003). GW represents the largest potential source of water savings in domestic residences, accounting for as much as 50–80% of the total water use (Christova-Boal et al., 1996; Eriksson et al., 2002; Jenssen and Vrale, 2003; Flowers, 2004). Household GW production is estimated at daily per capita volumes as high as 90–140 L (Carr et al., 2004).

Traditionally, there is no separation between GW and BW from domestic sources; both streams are mixed and treated together. GW, however, differs significantly from BW. While not of high enough quality for direct use, its better quality makes this water amenable for on-site treatment and non-potable reuse such as irrigation and toilet flushing.

Use of GW for irrigation can influence soil properties important for plant health and growth. The introduction of particulate and organic matter such as surfactants can alter soil permeability (Wallach et al., 2005; Wiel-Shafran et al., 2005). Detergents and soaps, the main sources of boron and surfactants found in domestic effluents, are more concentrated in GW because the toilet stream is excluded. While boron is an essential micronutrient for plants, excessive amounts are phytotoxic. The recommended value for irrigation water varies between 0.3 and 1.0 mgL⁻¹ for non-tolerant plants (ANZECC, 1992). In a recent study, the average concentration in GW in Israel was 0.6 mgL⁻¹ and ranged between 0.1 and 1.6 mgL⁻¹, suggesting the possibility of negative effects (Gross et al., 2005a,b). Elevated salinity due to cleaning agents may also cause environmental harm. The ratio of soluble sodium (Na) to soluble divalent cations (sodium adsorption ratio: SAR) can be used to predict the exchangeable sodium fraction of a soil and, hence, its suitability for irrigation. A SAR of 8 was suggested as the higher limit for irrigation of non-tolerant plants (ANZECC, 1992). However, long-term irrigation using water with a SAR higher than 4 can negatively alter the soil properties (i.e. a high Na concentration leads to soil dispersion). SAR of different GW sources in the Negev desert ranged between 2.8 and 6.0 and averaged 4.8 (Gross et al., 2005b). Finally, the inorganic nutrients within GW (ammonia-N, nitrate-N, nitrite-N, and phosphates) can infiltrate into the subsurface, contaminating groundwater. This is a particular problem in the case of nitrate contamination associated with “blue baby” syndrome.

In addition to the possible introduction of chemical contaminants into the environment, one of the largest obstacles to the reuse of GW is concern about the possible presence of pathogenic microorganisms. Activities such as hand washing and washing of diapers, as well as preparation of uncooked vegetables and meat, may introduce bacteria into GW (Burrows et al., 1991; Albrechtsen, 2002). Observations regarding bacteria in GW vary greatly between different studies. While some of these differences can be attributed to different detection methods, a major source is the variability found in the composition of GW which reflects the number and age of residents, frequency of bathing and laundering, and, in cases where kitchen waste is included, the eating habits of those living in the household (Gross et al., 2005a).

The focus of most microbiological analysis of GW has been on fecal pollution and enteric pathogens. Reports of total coliforms in GW range from as low as 10¹ to 10⁷ CFU (100 mL)⁻¹ (Rose et al., 1991; Casanova et al., 2001; Siegrist et al., 1976;

Burrows et al., 1991). In addition to total coliform organisms, enteric pathogens such as *Salmonella*, *Shigella*, and Poliovirus Type 1 have all been reported to be present in GW and concerns about the potential for re-growth or persistence of pathogenic microorganisms have been raised (Rose et al., 1991). Environmental strains of *Legionella* were observed in three raw GW samples analyzed for pathogens, as were *Cryptosporidium*, *Giardia*, and fecal enterococci (Birks et al., 2004). Since wash water is a major component of GW, several opportunistic pathogens associated with human skin – *Staphylococcus aureus* and *Pseudomonas aeruginosa* – may be present in addition to enteric organisms.

Recently, researchers at Ben Gurion University have developed a new GW treatment system, the recycling vertical flow constructed wetland (RVFCW) (Gross et al., 2007). This system combines working principles of trickling filter and vertical flow constructed wetland (CW) into modules tailored to treat different types of GW. RVFCW units have been installed at several households and pilot studies indicate they are capable of treating GW to standards compatible with irrigation and similar uses (Gross et al., 2007). We report here on the reduction in specific chemical and microbial loads during the treatment of GW using a modification of this system, the recycled vertical flow bioreactor (RVFB).

2. Materials and methods

2.1. Experimental GW treatment systems

The experimental RVFB system is illustrated in Fig. 1. Each system was constructed using two plastic containers (20 cm × 35 cm × 50 cm), with an upper “treatment container” placed over a lower “reservoir container.” Holes were punctured at even intervals in the bottom of the treatment container allowing water from this container to freely drain into the lower reservoir container. Within the treatment container, a 2 cm thick layer of pebbles (crushed limestone and dolomite: average diameter 2.5 cm) was placed over the drain holes, followed by a middle layer consisting of 12 cm of plastic filter media (Aridal Bioballs, Cfar Hasidim, Israel, www.aridalplast.com) with a high surface area (800 m²/m³) and large void volume. Each treatment container was topped with a 4 cm thick layer of peat (commercial peat from the Hula Valley, Israel). Synthetic GW was evenly poured onto the

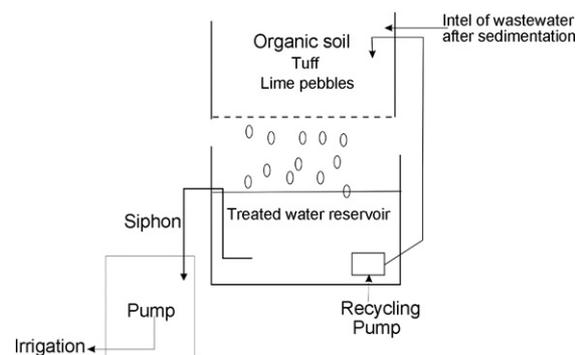


Fig. 1 – Schematic of the recycled vertical flow bioreactor (RVFB).

Table 1 – Greywater stock solutions

| Greywater stock (final volume 10 L) | Laundry soap (g) | Shampoo (mL) | Cooking oil (mL) | Kitchen effluent (mL) | Other |
|-------------------------------------|------------------|--------------|------------------|-----------------------|-----------------------|
| Laundry | 1.28 | 4 | 0.05 | 10 | |
| Bath | 0.32 | 16 | 0.05 | 2 | |
| Kitchen | 0.32 | 4 | 0.2 | 10 | Ground egg and tomato |

Synthetic greywater for addition to the treatment systems was prepared by mixing the appropriate volumes of the stock solutions. Fresh stock solutions were prepared on the day they were used. Stocks were added to the RVFB as described in Section 2.

surface of the peat. It percolated through the treatment layers and was collected in the reservoir container. From the reservoir container, the GW was recirculated back to the upper layer at a rate of 60 L h^{-1} and evenly distributed via perforated tubing that was placed in a loop over the peat layer. Drainage in the system was sufficient that there was no surface ponding of water using this recirculation rate. Samples of the effluent were obtained via a three-way valve installed in the recirculation tube.

2.2. Synthetic greywater

Synthetic GW was made by combining three synthetic “waste stream” (laundry:kitchen:bath) stock solutions (Table 1). A total of 20 L (1/2 the reservoir volume) of synthetic GW was added per treatment cycle. The GW was supplemented with raw kitchen effluent from a large dining hall to provide an inocula of *Escherichia coli* and other bacteria. The volume of the raw kitchen effluent was small enough (0.1–0.2%) to not appreciably affect the chemical composition of the synthetic GW.

A total of six RVFB systems were used in this research. Initially, the systems were charged with a total volume of 40 L of synthetic GW. Each system subsequently was recharged with fresh synthetic GW at intervals of 2–3 days. This was done by removing 20 L from the lower reservoir of the treatment system (approximately 50% of the total liquid volume) and adding a total of 20 L of the GW stock solutions to the top of the treatment system. The stock solutions were added in three batches over 8 h to simulate the variable flow and composition of GW. The laundry stock was added in the morning, kitchen waste stock solution at midday, and bath waste stock in the evening.

While all of the systems were operated and recharged on the same schedule throughout the study (10 weeks), only three systems were sampled during each treatment period. Samples of GW were collected at the beginning and end of each treatment cycle. The sample representative of the beginning of the cycle (initial) was collected from the recycle loop of the system 30 min after the final GW solution (bath waste stock) had been added. Thus, a time lag was built into the initial sample since it was collected 30 min after the bath water stock, corresponding to 4 h after the kitchen stock and 8 h after the addition of the laundry stock. The final samples were collected 2–3 days later when 20 L of treated GW were drained from the reservoir and before any new stock solutions were added. Samples for chemical analysis were collected at the end of each treatment cycle only. In addition, samples of raw synthetic GW were obtained immediately after the stock solutions were made.

2.3. Chemical analysis

Samples were filtered for total suspended solids (TSS) by the gravimetric method and a sub-sample from the filtrate and the raw GW were frozen for subsequent chemical analysis. Each raw sample was analyzed for chemical oxygen demand (COD) by the potassium dichromate oxidation method and anionic surfactants by the methylene blue absorbing substances (MBAS) method (APHA, 1998). Boron was determined by the azomethine-H colorimetric method (Gupta and Stewart, 1975). The filtrates were analyzed for total ammonia nitrogen (TAN) by the nesslerization method and $\text{NO}_2\text{-N}$ by the sulfanilamide method (APHA, 1998). Nitrate-N was analyzed by the sodium salicylate method (Yang et al., 1998). The analysis of total phosphorus was conducted by the persulfate simultaneous digestion followed by the vanadomolybdate method (Gross and Boyd, 1998).

2.4. Microbiological analysis

Water samples were collected from the reservoir of each system at the beginning (initial) and end (final) of each treatment cycle and analyzed immediately upon collection. The samples were diluted in sterile diluent (peptone water) if necessary to achieve a countable concentration. Microbial populations were enumerated by viable counts using membrane filtration techniques (APHA, 1998). Two populations of microorganisms – heterotrophs and surfactant degraders – associated with the removal of chemical contaminants were monitored. Heterotrophic microorganisms, considered indicators of total aerobic microorganisms, were enumerated on Tryptic Soy Agar (BBL, Cockeysville, MD) after incubation for 2 days at 25°C . Surfactant degrading microorganisms were grown on Bushnell-Haas Agar (BBL, Cockeysville, MD) supplemented with shampoo (0.1%, v/v) as a sole source of carbon. Surfactant degraders were enumerated after 7 days growth at 25°C .

Three microorganisms with public health significance also were monitored. *E. coli*, used as an indicator of fecal pollution, was enumerated on TBX agar (Chromocult TBX: Tryptone Bile X-glucuronide Agar, <http://service.merck.de/microbiology/tedisdata/prods/4968-1.16122.0100.0500.html>). Plates were incubated at 44°C . Blue colonies, typical of *E. coli*, were counted after 24 h incubation. *S. aureus* was plated on Baird-Parker agar (<http://service.merck.de/microbiology/tedisdata/prods/4982-1.054060500.html>). Plates were incubated for 48 h at 35°C and black colonies, typical of *S. aureus*, were counted. For each set of samples analyzed, at least one colony of each different morphological type was confirmed by slide agglutination. *P.*

Table 2 – Changes in greywater chemical constituents associated with recycled vertical flow bioreactor treatment

| Chemical | Initial (\pm S.E.M.) | Final (\pm S.E.M.) | t |
|------------------------------|-------------------------|-----------------------|--------|
| Chemical oxygen demand (COD) | 339 (30.6) | 46.6 (5.5) | 15.8** |
| Anionic surfactant (MBAS) | 12.3 (1.9) | 0.2 (0.1) | 11.7** |
| Total suspended solids (TSS) | 46 (3) | 3 (0.0) | 26.5** |
| NO ₃ -N | 3.5 (0.1) | 1.8 (0.3) | 3.1* |
| Total ammonia nitrogen (TAN) | 1.2 (0.1) | 1.0 (0.06) | 1.2 |
| NO ₂ -N | 1.3 (0.5) | 0.04 (0.02) | 4.9* |
| Boron | 0.1 (0.02) | 0.15 (0.05) | 0.8 |
| Total P | 1.9 (0.2) | 0.5 (0.1) | 5.8** |

Chemical parameters are in units of mgL⁻¹. Initial and final concentrations were compared using an unpaired t-test (d.f. = 10).

** Values indicating a significant difference at $p < 0.001$.

* Values indicating a significant difference at $p < 0.05$.

aeruginosa was enumerated on *Pseudomonas* (Cimetidine) agar (<http://www.rapidmicrobiology.com/news/603h39.php?s=pseudomonas>). Plates were counted after 24 and 48 h incubation at 35 °C.

Control studies examined the persistence of *E. coli* in greywater. Synthetic greywater in sterile flasks was inoculated with *E. coli*. The static cultures were incubated under the same conditions as the RVFB systems. Samples were obtained at 24, 48, and 72 h and viable *E. coli* were enumerated using membrane filtration on TBX agar as described previously.

2.5. Data analysis

Microbiological counts were log transformed prior to statistical analysis. All statistical analysis was performed using In Stat/Prism 4.0 (GraphPad, Inc.). Comparisons between treatments were done using one-way ANOVA with *a posteriori* comparisons using Bonferroni's test or unpaired t-tests as appropriate.

3. Results

3.1. Chemical contaminants

There were statistically significant reductions in the concentration of COD, anionic surfactant, TSS, NO₃-N, NO₂-N, and total P after treatment in the RVFB (Table 2; Fig. 2). There was a slight reduction in TAN and a slight increase in boron, however, neither of these changes was statistically significant. GW treatment using the RVFB systems reduced effluent concentrations of NO₃-N, total ammonia nitrogen, NO₂-N, TSS, B, and anionic surfactants (MBAS) to below the levels considered acceptable for either recreation or irrigation (Halperin and Aloni, 2003; ANZECC, 1992).

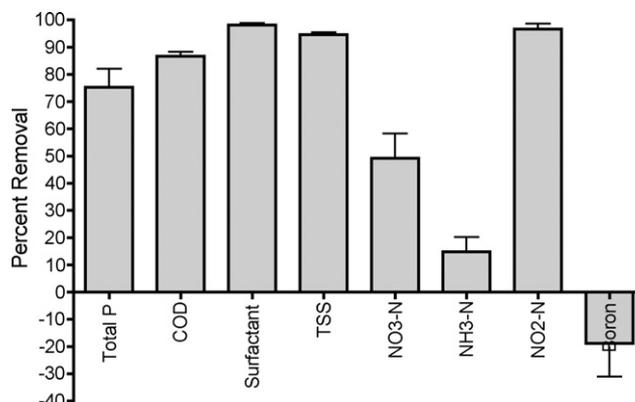


Fig. 2 – Change in chemical contaminants resulting from treatment in the RVFB. Values are mean (\pm S.E.M.) % change between final concentrations and mean initial concentration.

3.2. Microbial populations

3.2.1. Heterotrophs and surfactant degraders

Both heterotrophic and surfactant degrading microorganisms increased significantly (Table 3; Fig. 3) compared to the concentrations added with the raw GW. Total heterotrophs increased to a steady-state population in the RVFB of over 10⁶ CFU (100 mL)⁻¹, an increase of over one order of magnitude from the concentration present in the raw GW. Populations of surfactant degrading microorganisms also increased over one order of magnitude compared to the raw GW indicating that conditions within the RVFB systems were favorable for the enrichment and growth of these organisms.

Table 3 – Changes in concentration of microorganisms involved in removal of contaminants

| Organism | Raw GW (\pm S.E.M.) | Final (\pm S.E.M.) | t (d.f.) | p |
|----------------------|------------------------|-----------------------|------------|-------|
| Heterotrophs | 4.7 (0.5) | 6.6 (0.1) | 5.2** (22) | 0.001 |
| Surfactant degraders | 4.4 (0.1) | 6.7 (0.2) | 6.0** (14) | 0.001 |

Initial and final concentrations are the geometric mean of samples. Concentrations are in logCFU (100 mL)⁻¹. Means were compared using an unpaired t-test.

** Values indicating a significant difference at $p < 0.0001$.

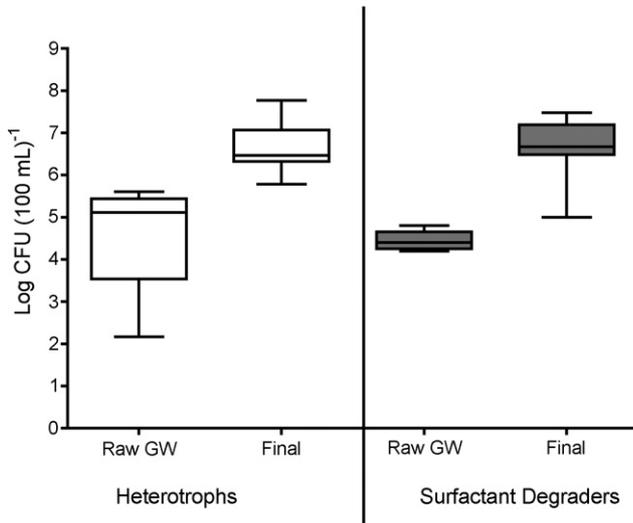


Fig. 3 – Concentration of microbial populations involved in contaminant removal.

3.2.2. Indicator organisms and opportunistic pathogens

E. coli, *S. aureus*, and *P. aeruginosa* were present in samples from all of the systems at the beginning of each treatment cycle (Table 4). Control studies (data not shown) indicated that the concentration of *E. coli* changed by less than 2% in untreated static samples of GW over 3 days. There were statistically significant ($F = 611.3$; $d.f. = 36$) changes in the concentration of *E. coli* over the treatment cycle in the RVFB. There was no significant change (Bonferroni’s multiple comparison test $t = 0.26$; $p > 0.05$) in the concentration of *E. coli* over the initial sample compared to the raw GW. Subsequently, the concentration of *E. coli* showed a statistically significant (Bonferroni’s multiple comparison test $t = 33.56$; $p < 0.001$) decline, with the final concentration of *E. coli* in the RVFB being between four and five orders of magnitude lower than the initial concentration (Table 4; Fig. 4). The concentration of *E. coli* at the end of the treatment cycle generally was below detection (< 1 CFU (100 mL⁻¹)).

There was a statistically significant ($F = 95.62$; $d.f. = 36$) decline in the concentration of *S. aureus* associated with treatment using the RVFB. As with *E. coli*, there was negligible change (Bonferroni’s multiple comparison test $t = 1.71$; $p > 0.05$) in the concentration of *S. aureus* during the initial sample compared to the raw GW. The overall decline in the concentration of *S. aureus* in the final sample, however, was significant (Bonferroni’s multiple comparison test $t = 8.70$; $p < 0.001$). The removal of *S. aureus* in the RVFB was much lower than that of *E. coli*. *S. aureus* could always be recovered from the effluent at the

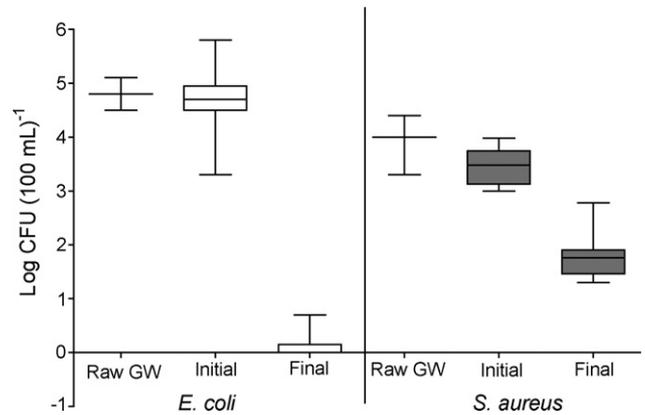


Fig. 4 – Changes in concentrations of *E. coli* and *S. aureus* during treatment of greywater using the RVFB system.

end of the treatment cycle at concentrations between 10 and 100 CFU (100 mL)⁻¹ (Table 4; Fig. 4). There was no significant change ($F = 0.49$; $d.f. = 9$) in the concentration of *P. aeruginosa* in the RVFB system (Table 4).

4. Discussion

The reuse of GW is an attractive addition to water management options. One of the major concerns limiting GW reuse is the possible presence of pathogens. Given the likely contact of individuals, including those in at-risk groups (e.g., immunosuppressed), with GW during its reuse for non-potable applications such as irrigation, the treatment of GW is necessary.

Large-scale GW treatment systems have been developed and some are currently being used by municipalities and housing units. These systems, which include ultrafiltration; direct disinfection; and constructed wetlands, are expensive and technically complex (Christova-Boal et al., 1996; Jefferson et al., 1999; Al-Jayyousi, 2002).

The bulk of the small systems being proposed for the treatment of GW fall into one of two categories. Many are filtration systems providing minimal treatment. Others are small-scale domestic wastewater treatment systems. As such, they are not designed to handle the differences in both flow and composition between GW and wastewater. In a recent case study, five different commercial GW treatment systems (filtration through 130 μm sieve, filtration through sand, filtration through tuff, horizontal and vertical flow wetlands) were tested in households (Gross et al., 2006). Treated waters from all of the tested systems did not meet the Israeli guidelines for

Table 4 – Changes in indicator organisms and opportunistic pathogens

| Organism | Raw GW (±S.E.M.) | Initial (±S.E.M.) | Final (±S.E.M.) |
|----------------------|------------------|-------------------|-----------------|
| <i>E. coli</i> | 4.8 (0.2) | 4.7 (0.1) | 0.1 (0.05) |
| <i>S. aureus</i> | 3.9 (0.3) | 3.5 (0.3) | 1.8 (0.4) |
| <i>P. aeruginosa</i> | 4.3 (0.5) | ND | 4.7 (0.5) |

Concentrations are the geometric mean of samples. Concentrations are in log CFU (100 mL)⁻¹.

reuse of water in habitat areas (Halperin and Aloni, 2003), suggesting that systems should be designed to specifically treat GW.

The RVFB was designed to address the limitations of existing commercial GW treatment systems. There are four modules in the RVFB. Upon discharge from the household, GW is introduced by subsurface infiltration into an organic soil module. In this module, the large negatively charged available surface area allows adsorption of contaminants. The soil module can be planted with native vegetation or, as in this study, left unplanted. The unsaturated trickling module beneath the soil module is composed of an extremely porous material chosen to maximize the available surface area and allow significant microbial growth. This module functions in a manner similar to a trickling filter. The trickling module is responsible for the (re)aeration of the material being treated. This not only may enhance nitrification of the waste, but also may prevent the development of unpleasant odors associated with anaerobic conditions and promote the removal of enteric microorganisms (e.g., *E. coli*) sensitive to high concentrations of oxygen. The lime pebble module is used for additional physical filtration and to buffer potential losses in alkalinity due to production of acidity in the nitrification process. Finally, beneath these modules, there is the reservoir/recirculation module. This functions as a flow equalizer. Because treated water is constantly recirculated to the front of the system, shocks due to sudden changes in influent composition or volume are attenuated. This is particularly important when GW from a single household is being treated since domestic activities (i.e., laundry) result in rapid, short-term changes in the waste composition.

The benefits of soil-based systems for wastewater treatment are: low construction and operational costs, easy maintenance, effective and reliable wastewater treatment, tolerance to both great and small volumes of water and varying contamination levels (Hammer and Bastian, 1989). Wetlands have been found to be effective in reducing biochemical oxygen demand, suspended solids, nitrogen, and phosphorus, as well as for decreasing the concentrations of metals, organic chemicals, and pathogens. In addition, they can be aesthetically pleasing and provide habitat for wildlife and human recreation.

The removal of TSS, $\text{NH}_3\text{-N}$, and anionic surfactants in the current study were generally in agreement with removals reported in previous work with RVFCW systems. Removals of COD and boron were less than that previously reported while removal of $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ were better (Gross et al., 2007). This is attributed to the different environmental conditions under which the systems were operated, slight changes in setup, the presence or absence of plants in the systems, and the GW sources in the different studies.

There is extensive research demonstrating the effectiveness of soil based treatment systems and constructed wetlands in the control of pathogens in domestic wastewater (Kadlec and Knight, 1996; Karathanasis et al., 2003; Hench et al., 2003). These studies have demonstrated significant reductions in indicator organisms, and thus presumably, in pathogens using a variety of constructed wetlands (Perkins and Hunter, 2000; Green et al., 1997; Steer et al., 2002). Removal efficiencies ranged from less than 50% to greater than 99%,

depending on the type of system, the operating characteristics, and the microbial load (Haberl et al., 1995; Green et al., 1997; Leonard, 2000; Karathanasis et al., 2003).

Research on the removal of pathogens in GW is much less extensive than studies involving domestic wastewater. *E. coli* declined to non-detectable concentrations in a recirculating, hydroponic system designed for the treatment of GW during spaceflight (Morales et al., 1996; Garland et al., 2000). Gerba et al. (1995) reported a significant reduction in total and fecal coliforms in water hyacinth dominated constructed wetlands for the treatment of GW. The reduction in indicator organisms, however, was not sufficient to allow agricultural reuse of the water (USEPA, 2004). Therefore, they recommended that an additional disinfection step be incorporated into the treatment train.

Our research indicates removal of *E. coli* from GW in excess of 99.99% (4 logs) can be obtained using the RVFB. Treatment using this system consistently resulted in *E. coli* levels in the effluent that met the USEPA criteria for water reuse (USEPA, 2004). It should be noted, however, that the initial concentration of *E. coli* in the GW tested was lower than the concentration of *E. coli* in GW reported by some investigators. Thus, additional research is needed to establish the maximum removal that can be obtained using the RVFB.

There are a variety of physical, chemical, and biological mechanisms known to be involved in the removal and decay of bacteria in constructed wetlands and soil treatment systems. These may include sedimentation, mechanical filtration, adsorption, die-off, predation, antibiotic production and excretion by plants and other bacteria, and exposure to stressful abiotic conditions such as pH, temperature, and oxygen (Soto and Garcia, 1999; Green et al., 1997; Leonard, 2000; Axelrood et al., 1996; Decamp and Warren, 1998, 2000; Collings and Phillips, 2001). In all likelihood, the same general mechanisms are responsible for microbial removal in the RVFB.

The removal of microorganisms in the RVFB varied depending on the specific microorganism under investigation. While removal of *E. coli* was excellent, removal of both *S. aureus* and *P. aeruginosa* were much lower, always remaining below a 99% (2 log) removal. Thus, these organisms were frequently found in treated effluent in the absence of *E. coli*.

S. aureus has been recognized as the cause of community-associated infections. Outbreaks of *S. aureus* have been associated with a variety of contact sports (Begier et al., 2004; Lindenmayer et al., 1998; Stacey et al., 1998). In addition, swimmers and others involved in water contact sports are at increased risk of *S. aureus* infection (Cohen, 2005). *P. aeruginosa* is widely distributed in aquatic and terrestrial habitats. It can be found in the gut of many warm-blooded mammals and concentrations of *P. aeruginosa* in sewage may exceed 10^5 CFU (100 mL)⁻¹ (Howard et al., 2004). *P. aeruginosa* is associated with a variety of opportunistic infections. It causes 10–11% of all nosocomial infections and has the highest fatality rate of all hospital-acquired bacteremias (Frank et al., 2005; Navon-Venezia et al., 2005; Trautmann et al., 2005).

Several studies have reported the presence of *Pseudomonas* and/or *Staphylococcus* in water samples when *E. coli* could not be detected and have proposed the adoption of these organisms as supplemental indicators of water quality (Bahlaoui et al., 1997; Mates, 1992; Yoshpe-Purer, 1987; Tosti and Volterra,

1998; Garland et al., 2000). Our study indicates that both *S. aureus* and *P. aeruginosa* could be found in the majority of GW samples in which *E. coli* was reduced to non-detectable (<1 CFU (100 mL⁻¹)) levels and, therefore, supports the use of *S. aureus* and *P. aeruginosa* as supplemental indicators of the microbiological safety of water.

The RVFB is a useful addition to GW treatment options, particularly for small-scale decentralized use. Our research indicates that this treatment can produce treated GW in compliance with USEPA water quality criteria for reuse. The presence of non-enteric opportunistic pathogens, however, underscores the need for the consideration of additional indicator organisms to monitor and assure the safe reuse of GW.

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