Original Article

Hygienic quality of artificial greywater subjected to aerobic treatment: a comparison of three filter media at increasing organic loading rates

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With a growing world population, the lack of reliable water sources is becoming an increasing problem. Reusing greywater could alleviate this problem. When reusing greywater for crop irrigation it is paramount to ensure the removal of pathogenic organisms. This study compared the pathogen removal efficiency of pine bark and activated charcoal filters with that of conventional sand filters at three organic loading rates. The removal efficiency of *Escherichia coli* O157:H7 decreased drastically when the organic loading rate increased fivefold in the charcoal and sand filters, but increased by $2 \log_{10}$ in the bark filters. The reduction in the virus model organism coliphage Φ X174 remained unchanged with increasing organic loading in the charcoal and sand filters, but increased by $2 \log_{10}$ in the bark filters. Thus, bark was demonstrated to be the most promising material for greywater treatment in terms of pathogen removal.

Keywords: aerobic treatment; column filters; greywater; hygiene; pathogen

1. Introduction

With the growing world population, the lack of clean water is becoming an increasing problem. By 2025, two-thirds of the world's population could be living under water stress and 1.8 billion people may be under extreme water stress [1]. Agriculture accounts for the largest water usage: more than 70% of the total water consumption worldwide and up to 95% in low- and mid-income countries. In fact, the yield of most crops can increase by 100–400% with irrigation [2]. The reuse of greywater for irrigation has thus become an attractive alternative [3,4]. However, irrigation with untreated greywater has been demonstrated to contribute to increased soil hydrophobicity [5,6] and levels of faecal bacteria in the soil [7]. Although greywater contains lower concentrations of pathogens than mixed wastewater, many different kinds of pathogenic organisms of faecal origin have been found in greywater [8–10]. The concentration of pathogenic microorganisms in greywater has been found to be $10\text{--}10^4$ CFU mL⁻¹ [11], although Dallas et al. [12] reported concentrations of faecal coliforms of $10^5\text{--}10^6$ CFU mL⁻¹ in greywater in Costa Rica. Studies concerning the health effects of greywater irrigation are limited, but studies investigating the health impact from irrigation of food crops with untreated wastewater have shown an increased risk of infection among farm workers and children [13]. In a recent study, Barker et al. [14] assessed the associated health risk, from an Australian perspective, of irrigation with greywater and found an increased risk

when irrigating crops intended for raw consumption (e.g. lettuce) with laundry water. Treatment methods that reduce the number of pathogenic microorganisms are thus essential if greywater is to be used for crop irrigation. Construction of greywater filters using natural, locally available materials can lower the production and maintenance costs. A number of studies evaluating the use of natural and refuse materials in greywater filters, comprising, for example, sandy loam with leaf compost or mulch, bio-char, waste paper, waste cement, waste concrete and natural clays and zeolites, have reported promising results [15–17].

Bacteriophages have been used in various studies as model organisms for viruses [18,19] and are used because they are non-pathogenic to humans, the analysis is rapid and easy and they demonstrate good survival in a laboratory environment [20]. The removal of viruses in porous media have been found to be largely controlled by electrostatic interactions between the virus particle and the media [21]. In a study concerning the attachment of enteric viruses and bacteriophages to lettuce, Vega et al. [22] found that the degree of attachment of each of the virus type studied was different. They furthermore found that the degree of attachment varied with pH. It is therefore hard to predict the effect of a filter to other viral particles than those studied and those similar in size and charge. In the present study, three filter materials (sand, pine bark and activated charcoal) were compared with respect to their ability to reduce the numbers of bacteria and bacteriophages in artificial greywater.

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The performance of the filters was investigated at three organic loading rates (OLR), to better understand filter performance capacity over a range of operating conditions in order to simulate real-life situations, as the concentration of greywater varies considerably between different countries.

2. Materials and methods

Pine bark and activated charcoal filters were compared against conventional sand filters in terms of the reduction in bacteria and viruses at three OLR. Non-verotoxin-producing *Escherichia coli* O157:H7 (EHEC) and bacteriophage Φ X174 were inoculated into synthetic greywater, in order to keep the inflow microbial concentration constant for the different OLR treatments.

2.1. Filter materials

Sand, pine bark and activated charcoal were each manually packed into duplicate acrylate plastic columns with diameter 20 cm to a depth of 60 cm. The filter materials had an effective size of 1.4 mm and an uniformity coefficient of 2.2. The specific surface area, determined according to Brunauer et al. [23], the porosity and the hydraulic conductivity of the bark, charcoal and sand used in the filters are displayed in Table 1. Characteristics of the filter materials and the column set up are described in detail in Dalahmeh et al. [24].

2.2. Feed characteristics and loading regimes

The bark, charcoal and sand filters were fed with artificial greywater. Artificial greywater have been demonstrated to correlate well with real greywater in greywater reuse studies [25]. The artificial greywater in this study was intended to represent greywater effluents found in low- and mid-income countries [11] and was prepared by dissolving 125 g standard nutrient broth, 16 g YES detergent, 16 g washing powder, 16 g shampoo, 10 g maize oil and 4% primary sedimentation effluent from the Kungsängen municipal sewage treatment plant (Uppsala, Sweden), in 100, 50 and 25 L of tap water to yield greywater with BOD₅ 452 ± 23, 875 ± 226 and 2400 ± 465 mg L⁻¹, respectively. The greywater was pumped intermittently onto the filters three times a day at 70%, 10% and 20% of the total daily flow (1 L filter⁻¹ day⁻¹). The hydraulic loads pumped into the filters were controlled by a computer, using the software

Labview 2009 (National Instrument Sweden AB, Stockholm, Sweden). The greywater temperature was adjusted to 25 °C in a heating tank before loading the greywater onto the filters; the ambient air temperature was 27 ± 4 °C. Over the 84 days experiment, OLRs were varied periodically every 3 weeks. A 3-week loading period had been shown in a previous study to ensure that the filters were in steady state as regards organic matter reduction [24]. The hydraulic loading rate was kept constant at 32 L m⁻² d⁻¹ and was based on US EPA guidelines [26] for sand filters with characteristics similar to those of the sand filter used in this study. In the first run the OLR was kept at 14 g BOD₅ m⁻² d⁻¹ (OLR 14), in the second at 28 g BOD₅ m⁻² d⁻¹ (OLR 28) and in the third at 76 g BOD₅ m⁻² d⁻¹ (OLR 76). In a final run carried out following the three runs with increasing OLR, the same HLR and OLR as in the first run (HLR 32 L m⁻² d⁻¹, OLR 13 g BOD₅ m⁻² d⁻¹) were used. This run (OLR 13) was conducted in order to test the reproducibility of the results obtained (i.e. whether the filters had changed during the study).

2.3. Inoculants

Non-verotoxin-producing *E. coli* O157:H7 (CCUG 44857) growing on horse blood agar plates was obtained from the National Veterinary Institute (SVA). One well-isolated colony was inoculated in unselective microbial medium (NB, Oxoid AB, Sweden) and incubated at 37 °C for 17 ± 24 h. The inoculum was used immediately after incubation. Propagation of phage Φ X174 was performed in unselective microbial medium (NB, Oxoid AB, Sweden) using the host strain *E. coli* (ATCC 13706). The phage was collected by centrifuging the solution at 2000g for 10 min, followed by sterile filtration. The phage solution was kept at 4–6 °C until use.

2.4. Microbiological sampling and analysis

Influent samples were collected from the heating tank. Filter effluents were collected in disinfected buckets and poured into sterile 25 ml tubes. The samples were analysed immediately upon collection. A 1 mL volume was extracted and further diluted in phosphate-buffered saline solution at pH 7 before enumeration of EHEC and coliphage Φ X174.

EHEC was incubated at 37 °C for 24 h on MacConkey Sorbitol agar (CT-SMAC). The plates were counted with a detection limit of 10 CFU mL⁻¹.

For analysis of Φ X174, the host was cultured in unselective microbial medium (NB, Oxoid AB, Sweden) at 37 °C for 4–12 h. Sample (1 mL) of suitable dilution was mixed with 2 mL soft agar and 1 mL host solution and poured onto blood agar base (BAB) plates (Oxoid). The plates were incubated at 37 °C for 7 ± 2 h and counted with a detection limit of 1 PFU mL⁻¹.

Table 1. Properties of the bark, charcoal and sand filters.

	Bark	Charcoal	Sand
Specific surface area (m ² g ⁻¹)	0.73	>1000	0.14
Porosity (%)	73	85	34
Hydraulic conductivity (cm h ⁻¹)	330	500	360

2.5. Statistical analysis

Analysis of covariance (ANCOVA) with 95% confidence interval was used to establish whether a statistically significant difference occurred between regression lines. All graphical plots, models and model analysis were conducted in R software [27].

3. Result and discussion

The inflow concentrations of EHEC and Φ X174 remained fairly constant between the runs with different OLR (Table 2).

The reduction in EHEC decreased with increasing OLR in the sand and charcoal filters, while the reduction increased slightly in the bark filters (Figure 1(a)). In the sand and charcoal filters, there was no increased reduction of Φ X174 with increasing OLR while the reduction not only started at a high level, but also increased with increasing organic load in the bark filters (Figure 1(b)). In the final check (run OLR 13), the reduction in EHEC was in quite good agreement with that in run OLR 14 for all three types of filters, while the reduction in Φ X174 was slightly higher in OLR 14 in the sand and charcoal filters (Figure 1(a) and (b)).

In the bark filter increased removal was observed for both EHEC and Φ X174 with increasing OLR (Figure 2). Furthermore, there was a linear correlation between the

$\log_{10}$ reduction efficiency and OLR for both organisms. The reduction in chemical oxygen demand (COD) and biological oxygen demand (BOD) was also found to correlate positively with increasing OLR (Figure 2(a)). In the charcoal and sand filters the COD reduction was also increased with increasing organic loading, from around 70% at OLR 14 g BOD₅ m⁻² d⁻¹ to over 85% at 76 g BOD₅ m⁻² d⁻¹ (Figure 2(b) and (c)). The BOD removal was high throughout the experiment in the charcoal and sand filters (97–99%), but no trend was established.

Johnson and Logan [28] showed that bacterial transport in porous media is affected by the presence of dissolved organic matter, which competes with bacteria for adsorption sites and thereby restrict bacterial adsorption. When the OLR was increased in the present experiment, the concentration of bacteria was maintained at the same level, so the same number of bacteria would have competed with increased amounts of dissolved organic matter for the available adsorption sites. As the dissolved organic matter increasingly occupied the available sites, the bacteria were flushed out of the filters in the sand and charcoal filters. For the bacteriophage Φ X174 the reduction efficiency was similar at the different OLR in charcoal and sand filters. Owing to the small size of Φ X174 (26 nm diameter according to Michen et al. [29]), the probable removal mechanism is adsorption onto the filter material. However,

Table 2. HLR, measured OLR and inflow concentrations of EHEC and Φ X174 in the artificial greywater.

	HLR [L m ⁻² d ⁻¹]	OLR [g BOD ₅ m ⁻² d ⁻¹]	EHEC [CFU mL ⁻¹]	Φ X174 [PFU mL ⁻¹]
OLR 14	32	14	1.0×10^6	5.4×10^4
OLR 28	32	28	4.3×10^6	8.1×10^4
OLR 76	32	76	1.2×10^6	2.7×10^4
OLR 13	32	13	2.2×10^6	4.6×10^4

Figure 1. Reduction in (a) EHEC and (b) Φ X174 in the different materials over the runs. The final check run (OLR 13) is represented by filled symbols.

Figure 2. Correlation between OLR and the reduction in COD, BOD, EHEC and ΦX174 in the (a) bark, (b) charcoal and (c) sand filters, with fitted regression lines displayed.

as the bacteriophage is significantly smaller in size than the bacteria, the same competitive relation with organic matter may become significant only at OLR greater than those investigated in this experiment. Furthermore, as the reduction in ΦX174 in the sand and charcoal filters was moderate throughout the experiment (~ 60 and 80% in the charcoal and sand filters, respectively), the virus affinity to the two filter materials did not appear to be great.

In the bark filter the increased removal of EHEC, ΦX174 , BOD and COD with increasing OLR could be due to increased biofilm formation. However, for charcoal and sand the COD removal was also increased, but not the EHEC and ΦX174 removal. The bark filters differed from the other two filters in a few significant ways. The total suspended solids (TSS) filtration capacity was investigated at hydraulic loading rate (HLR) $32 \text{ L m}^{-2} \text{ d}^{-1}$ and OLR $15 \text{ g BOD}_5 \text{ m}^{-2} \text{ d}^{-1}$ for 3 months in a previous study and it was demonstrated that the removal in the bark was higher

compared with the other two, 92% compared with 84% (charcoal) and 53% (sand) (unpublished data). Another factor was the minimal retention time (MRT), which increased with increasing OLR in the bark filters, from around 2.5 min in OLR 14 to almost 10 min in OLR 76 [30]. In the charcoal filters the MRT remained fairly stable at 1.5 min . The MRT in the sand filters varied between 3.4 and 4.4 min for the different OLR. Another significant difference between the filter materials was the pH: the average pH of the bark effluent at the different OLR was 6.25 ± 0.07 , in the sand 6.88 ± 0.06 and in the charcoal 7.09 ± 0.04 . According to Michen et al. [29], the isoelectric point of ΦX174 is at $\text{pH} = 6.6$ and below this pH ΦX174 has a positive surface charge. Consequently, the surface charge of ΦX174 would be positive in the bark filters and negative in the other two filters, and this, along with the difference in MRT, may be the explanation to the dissimilar behaviour in the different filter materials. Similarly, the lower pH could have affected the adsorption

of EHEC in the bark filter. Lewis et al. [31] also found bark to have excellent pathogen removal abilities, they achieved a $2.7 \log_{10}$ reduction in *Enterococcus faecalis* and a $3 \log_{10}$ removal of bacteriophage MS2 in steam-exploded bark (SEB) columns. When mixing *E. faecalis* (Gram negative) and *E. coli* (Gram positive) in a suspension with SEB they found a lower removal of *E. faecalis*, elucidating the effect the microorganisms cell properties have on the adsorption, while also suggesting that most of the *E. faecalis* removal in the SEB columns had been by non-specific entrapment. The high removal observed in the bark filters in this study could have been due to entrapment in the biofilm or small pores of the material, or due to adsorption onto the filter material. It may have been due to a combination of entrapment and adsorption, enhanced by the low pH. Another possible contribution is the release of ecotoxicological substances by the bark [32]. For a deeper understanding of the processes involved in pathogen removal in bark filters, further investigations are required.

4. Conclusion

The reduction of EHEC and Φ X174 was found to increase with increasing OLR in bark filters. In sand and charcoal filters the removal of EHEC was decreased while the removal of Φ X174 was unaffected by OLR. Bark displayed the greatest potential for pathogen removal of the potential greywater filter materials investigated here. The bark filters differed from the other two in two significant ways: the pH was lower and the MRT was greater. Two likely pathogen removal mechanisms in the bark filters are entrapment in the biofilm and small pores of the filter material or adsorption onto the filter material, or a combination of the two.

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References

- [1] FAO, *Coping with water scarcity: Challenge of the twenty-first century*, 2007, <http://www.fao.org/nr/water/docs/escarcity.pdf>.
- [2] FAO, *Water at a Glance: The relationship between water, agriculture, food security and poverty*, 2008, <http://www.fao.org/nr/water/docs/waterataglance.pdf>.
- [3] E. Madungwe and S. Sakuringwa, *Greywater reuse: A strategy for water demand management in Harare?*, Phys. Chem. Earth A/B/C 32 (2007), pp. 1231–1236.
- [4] J. Wimpenny, I. Heinz, and S. Koo-Oshima, *The wealth of waste - The economics of wastewater use in agriculture*. FAO Water Reports 35, 2010, <http://www.fao.org/docrep/012/i1629e/i1629e00.htm>.
- [5] J. Tarchitzky, O. Lerner, U. Shani, G. Arye, A. Lowengart-Aycicegi, A. Brenner, and Y. Chen, *Water distribution pattern in treated wastewater irrigated soils: hydrophobicity effect*, Eur. J. Soil Sci. 58 (2007), pp. 573–588.
- [6] S.S. Dalahmeh, L.D. Hylander, B. Vinneras, M. Pell, I. Oborn, and H. Jonsson, *Potential of organic filter materials for treating greywater to achieve irrigation quality: A review*, Water Sci. Technol. 63 (2011), pp. 1832–1840.
- [7] M.J. Travis, A. Wiel-Shafran, N. Weisbrod, E. Adar, and A. Gross, *Greywater reuse for irrigation: Effect on soil properties*, Sci. Total Environ. 408 (2010), pp. 2501–2508.
- [8] J. Ottoson and T.A. Stenström, *Faecal contamination of greywater and associated microbial risks*, Water Res. 37 (2003), pp. 645–655.
- [9] J.B. Rose, G.S. Sun, C.P. Gerba, and N.A. Sinclair, *Microbial quality and persistence of enteric pathogens in greywater from various household sources*, Water Res. 25 (1991), pp. 37–42.
- [10] G.P. Winward, L.M. Avery, R. Frazer-Williams, M. Pidou, P. Jeffrey, T. Stephenson, and B. Jefferson, *A study of the microbial quality of grey water and an evaluation of treatment technologies for reuse*, Ecol. Eng. 32 (2008), pp. 187–197.
- [11] A. Morel and S. Diener, *Greywater management in low and middle-income countries, review of different treatment systems for households or neighbourhoods*, Swiss Federal Institute of Aquatic Science and Technology (Eawag), 2006, <http://www.north-south.unibe.ch/content.php/publication/id/1733>.
- [12] S. Dallas, B. Scheffé, and G. Ho, *Reedbeds for greywater treatment - case study in Santa Elena-Monteverde, Costa Rica, Central America*, Ecol. Eng. 23 (2004), pp. 55–61.
- [13] A. Melloul, O. Amahmid, L. Hassani, and K. Bouhoum, *Health effect of human wastes use in agriculture in El Azzouzia (the wastewater spreading area of Marrakesh city, Morocco)*, Int. J. Environ. Health Res. 12 (2002), pp. 17–23.
- [14] S.F. Barker, J. O'Toole, M.I. Sinclair, K. Leder, M. Malawaraarachchi, and A.J. Hamilton, *A probabilistic model of norovirus disease burden associated with greywater irrigation of home-produced lettuce in Melbourne, Australia*, Water Res. 47 (2013), pp. 1421–1432.
- [15] S. Ahsan, S. Kaneco, K. Ohta, T. Mizuno, and K. Kani, *Use of some natural and waste materials for waste water treatment*, Water Res. 35 (2001), pp. 3738–3742.
- [16] K. Bratieres, T.D. Fletcher, A. Deletic, and Y. Zinger, *Nutrient and sediment removal by stormwater biofilters: A large-scale design optimisation study*, Water Res. 42 (2008), pp. 3930–3940.
- [17] M. Rozic, S. Cerjan-Stefanovic, S. Kurajica, V. Vancina, and E. Hodzic, *Ammoniacal nitrogen removal from water by treatment with clays and zeolites*, Water Res. 34 (2000), pp. 3675–3681.
- [18] J. Zhuang and Y. Jin, *Virus retention and transport as influenced by different forms of soil organic matter*, J. Environ. Qual. 32 (2003), pp. 816–823.
- [19] S. Van Cuyk and R.L. Siegrist, *Virus removal within a soil infiltration zone as affected by effluent composition, application rate, and soil type*, Water Res. 41 (2007), pp. 699–709.
- [20] J.W.T. Wimpenny, N. Cotton, and M. Statham, *Microbes as tracers of water movement*, Water Res. 6 (1972), pp. 731–739.
- [21] N. Tufenkji, *Modeling microbial transport in porous media: Traditional approaches and recent developments*, Adv. Water Resources 30 (2007), pp. 1455–1469.
- [22] E. Vega, J. Smith, J. Garland, A. Matos, and S.D. Pillai, *Variability of virus attachment patterns to butterhead lettuce*, J. Food Protection 68 (2005), pp. 2112–2117.
- [23] S. Brunauer, P.H. Emmett, and E. Teller, *Adsorption of Gases in Multimolecular Layers*, Amer. Chem. Soc. 60 (1938), pp. 309–319.

- [24] S. Dalahmeh, M. Pell, B. Vinnerås, L. Hylander, I. Öborn, and H. Jönsson, *Efficiency of Bark, Activated Charcoal, Foam and Sand Filters in Reducing Pollutants from Greywater*, *Water Air Soil Poll.* 223(7) (2012), pp. 3657–3671.
- [25] F. Hourlier, A. Masse, P. Jaouen, A. Lakel, C. Gerente, C. Faur, and P. Le Cloirec, *Formulation of synthetic greywater as an evaluation tool for wastewater recycling technologies*, *Environ. Technol.* 31 (2010), pp. 215–223.
- [26] EPA, *Onsite Wastewater Treatment Systems Manual*, EPA 625/R-00-008, 2002.
- [27] R Development Core Team, *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria, 2010.
- [28] W.P. Johnson and B.E. Logan, *Enhanced transport of bacteria in porous media by sediment-phase and aqueous-phase natural organic matter*, *Water Res.* 30 (1996), pp. 923–931.
- [29] B. Michen, F. Meder, A. Rust, J. Fritsch, C. Aneziris, and T. Graule, *Virus removal in ceramic depth filters based on diatomaceous earth*, *Environ. Sci. Technol.* 46 (2011), pp. 1170–1177.
- [30] S. Dalahmeh, M. Pell, L. Hylander, B. Vinnerås, C.H. Lalander, and H. Jönsson, *Simple greywater treatment - effect of loading regime on pollutant reduction in bark, charcoal and sand filters*; Under review.
- [31] G.D. Lewis, T.D. Lomax, and M. Kimberley, *Removal of virus particles, bacteria and bovine serum albumin from water by steam-exploded Pinus radiata bark*, *Water Res.* 29 (1995), pp. 1689–1693.
- [32] V. Ribe, E. Nehrenheim, M. Odlaare, and S. Waara, *Leaching of contaminants from untreated pine bark in a batch study: Chemical analysis and ecotoxicological evaluation*, *J. Hazard. Mater.* 163 (2009), pp. 1096–1100.