Short Communication

Ash depth filter sanitation eliminates all bacteria and makes source-separated urine waste sterile

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ABSTRACT

Remote houses cannot use sewerage systems and so they must make their own arrangements for waste disposal. A solution is the use of ash depth filters which simultaneously trap nitrogen and phosphorus from human waste streams and all bacteria during long periods (750 ml per day for more than 6 weeks) of operation by filtration under gravity. Bacteria entering the filtration system, those trapped by the filter and the living material which eventually emerges if the system is operated till it eventually fails, are characterized. Reduction of bacterial numbers is achieved by two mechanisms: physical filtration and chemical sanitation.

Key words | ash, bacteria, depth filtration, sanitation, source-separated urine

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HIGHLIGHTS

- Ash depth filters elute soluble material and then their flow properties stabilize for very long flow runs.
- Normal microflora are trapped on the surface of ash depth filters as an organic mat.
- *Pseudomonas* sp. proliferate and the composition of bacteria in the organic mat changes radically.
- Only limited numbers and types of bacteria are seen in the eluate, after long flow runs. Aseptic technique was not practiced so these might be environmental bacteria.
- Ash depth filters are remarkably odor free.

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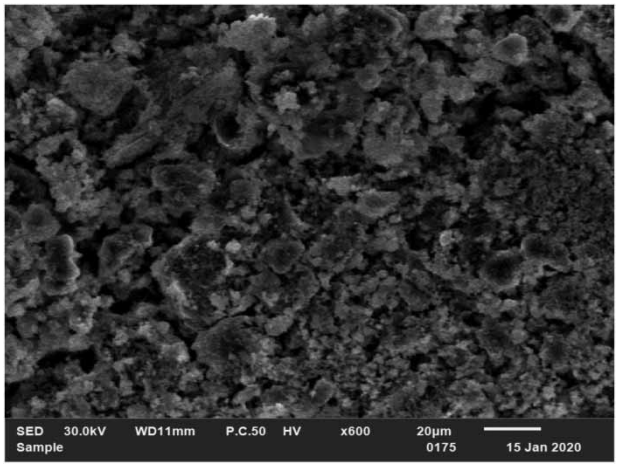
GRAPHICAL ABSTRACT



INTRODUCTION

Amelioration of human waste in remote households is needed to prevent water pollution but the infrastructure to solve waste disposal problems is prohibitively expensive. Many sanitation and industrial systems, which require filtration use thin membranes which are convenient disposable items but expensive, foul quickly and are only cleaned with difficulty (Aketagawa & Yasunaga 2020), making the alternative option of depth filters attractive. Depth filters are thick porous devices which remove particulate matter from the liquid during filtration (Hamoda *et al.* 2004). Those particles are distributed and immobilized throughout the depth filter Y dimension, rather than trapped as a crowded surface layer on a membrane filter (Ncube *et al.* 2018). This reduces blockage because suspensions can flow around previously immobilized particulate material and be trapped deep in a 3D matrix, rather than building up in a narrow surface layer which might block a 2D filter. Several inexpensive materials are commonly used for depth filtration, for example, silica, sand or diatomaceous earth (see Supplement 1) though even these can be price limiting for processes that might benefit from filtration of large volumes of suspensions, such as human waste. Efficient cheap depth filters can be composed of kitchen oak ash and these depth filters deplete human waste streams of fertility (Witty *et al.* 2020). In addition, the bacterial content of waste is reduced to zero after flow through the oak ash depth filter (OADF) causing sterile filtrate.

MATERIALS AND METHODS

For small-scale experiments to characterize oak ash as a depth filtration material, cylindrical 1 l PETE bottles were trimmed to remove the bottom of the bottle and the screw cap lid was perforated using a needle to create an end plate which allowed a good flow of water but no flow of ash particles. Oak ash from kitchen cooking fires was prepared by sieving to remove residual particles of charcoal and hearth debris as before (Witty 2016). The bottle was inverted then 300 g ash was added to make a dry filter bed within the trimmed container, trapped by the end plate. Deionized water in daily 300 ml aliquots was washed through the filter bed over 17 days. The volume of the daily eluate was measured then dried and the mass of the eluted salt measured using an open pan balance.

For larger-scale waste sanitation experiments, which approach filtration volumes suitable for domestic use, urine was collected as daily 750 ml aliquots and stored at 4 °C until used, never more than 16 h. This urine was transparent and showed no gross signs of bacteria. Gallon HDPE

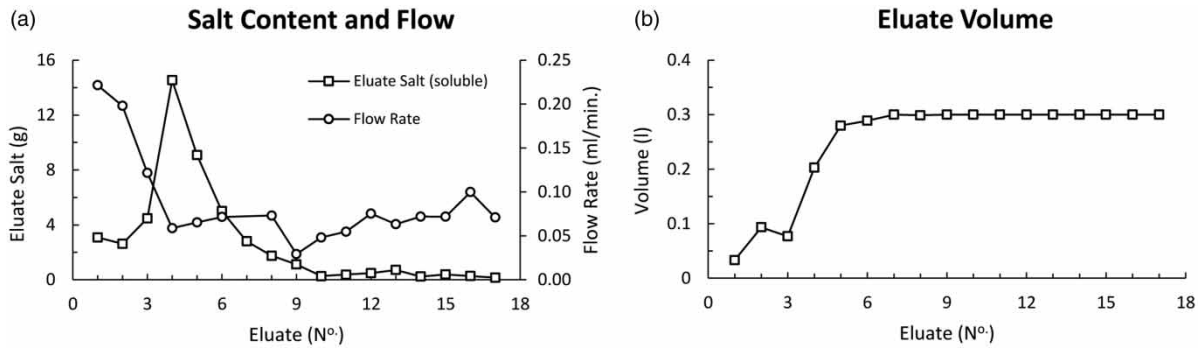


Figure 1 | (a); OADF eluate aliquots were dried at 50 °C to obtain their salts, which appeared as a peak at 1.2 l then declining to near zero. (b); Filter input volume was always 300 ml and the system took some time to become completely wet and for soluble material to be washed out. After this, input volume and output volume were always identical, as shown by the linear part of this curve.

bottles were trimmed and perforated as above and the bottle was inverted and filled with 3 l dry sieved ash (see Supplement 2). Depth filtration of the 750 ml aliquots was done at 4 °C, one aliquot per day. This process was purposefully continued until the failure point of the device (day 58) when bacterial plate counts of the eluate were marginally greater than 1 cfu/ml (at approximately 35 l) and the ash analyzed by energy-dispersive X-ray spectroscopy (see Supplement 3). Many bacteria were trapped as an organic mat on the surface of the ash bed and removed from the waste stream by filtration. This allowed for three sample types to be harvested: input urine, material from the organic mat and the pool of a few eluate-derived colonies (from plate counts).

The three sample types were analyzed for rDNA content to identify species present using DNA amplification by PCR and sequencing. This analysis was done by Scott Dowd (Shallowater, TX 79363, www.mrdnalab.com) using 16S and 18S rRNA gene primers (Caporaso *et al.* 2011) and a sequencing cycle as follows; 180 s hot start at 94 °C; 30 s at 94 °C, 40 s at 53 °C, 60 s at 72 °C for 28 cycles; final elongation for 5 min at 72 °C. DNA sequences were identified using the BLASTn program then the number of duplicates counted to show the relative abundance of single taxonomic units in the sample.

RESULTS

Flow of water showed that by aliquot 7 (2.1 l), most soluble salt had been washed out of the ash bed (Figure 1(a)) and flow characteristics had stabilized where the volume introduced into the matured system was equal to the volume of eluate (Figure 1(b)). Flow of urine aliquots showed similar desirable properties. Though suspended material accumulated as an organic mat flow continued. No bacteria were seen in the eluate, using plate counts, until late in filtration runs (day 35). Those late colonies were pooled for analysis. The number of species found in input waste was diverse (Figure 2(a), 29 species) but low in absolute number (80,000 cfu/ml, Witty *et al.* 2020) compared to the even more limited number of species seen in the ash bed eluate after device failure (Figure 2(b), 10 species) where only a small absolute number of cells were able to eventually emerge. Most species were seen in the organic mat (Figure 2(c), 46 species) as a gross organic layer. The input material harbored species expected from human urine, fecal contamination and additional environmental species, as was anticipated from the methods used, which were purposefully more similar to domestic than clinical methods. The small number of bacteria which emerged in the eluate was a subset of these species and we assume they represent the chemically resistant species. *Pseudomonas* species seen in the eluate and in the mat conspicuously increased in proportion, and this is shown as dark gray bars in Figure 2. An unexpected result was the limited number and diversity seen in the eluate (Figure 2(b)), indicating excellent sanitation of material which entered the system.

DISCUSSION

Absolute urine microbiome measurements vary artefactually depending on the method used for harvesting and the method for detecting microbes (Frimodt-Møller 2019),

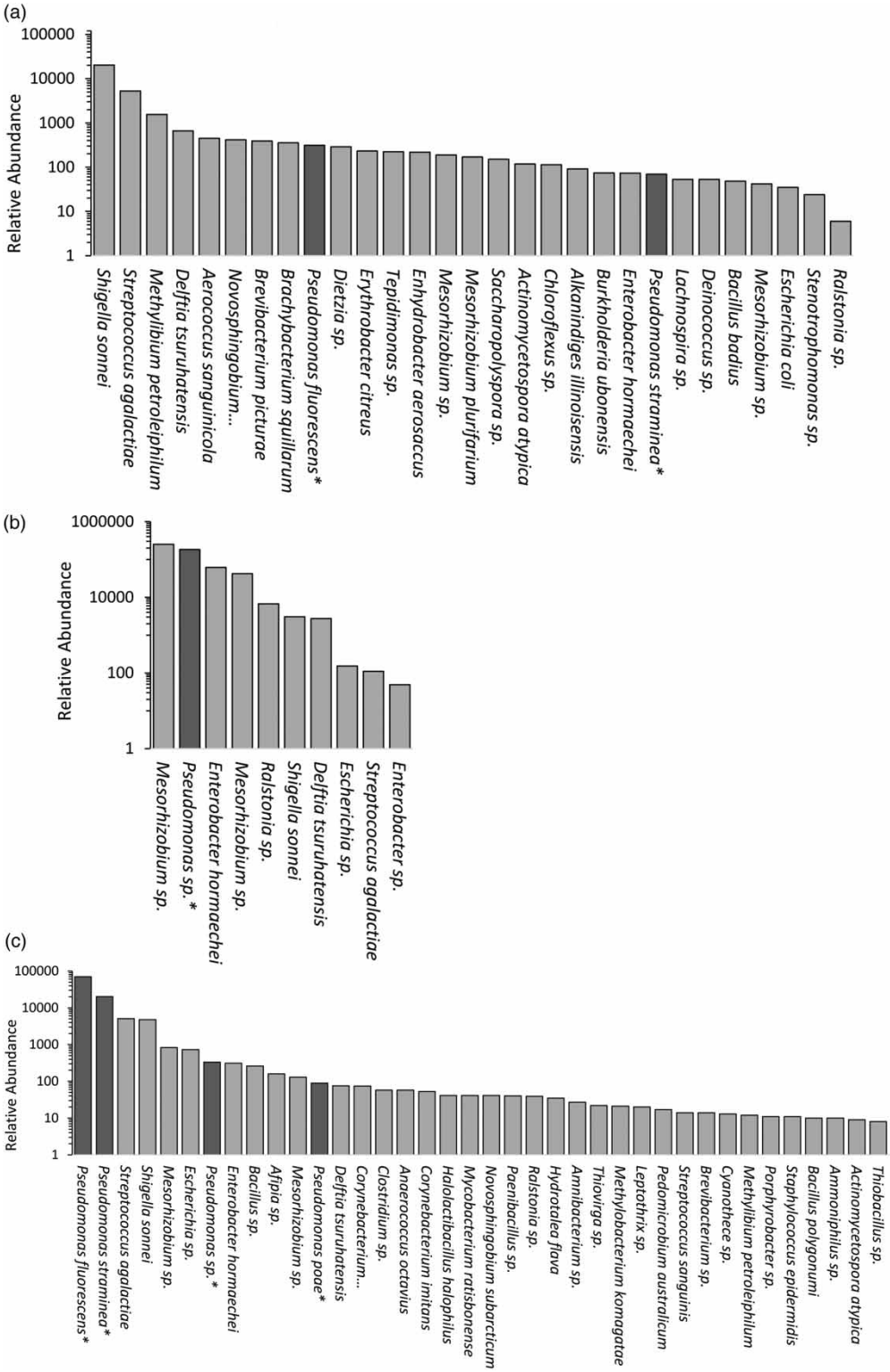


Figure 2 | Bacterial diversity (relative abundance, sequence counts) is shown, not absolute numbers. (a) Types of bacteria in normal fresh urine with 80,000 cfu/ml bacteria (Witty *et al.* 2020). (b) Very few bacteria were seen in eluate, which formed only 1220 colonies on nutrient agar, which were then pooled before analysis. (c) Types of bacteria in the abundant mat on the surface of ash, after prolonged use.

though useful information can be obtained related to diversity and relative number of species detected. For these experiments, only bacteria in input, abundant bacteria from the mat and a few bacteria from eluate were detected using 16S primers and they showed varying degrees of diversity. No significant microbes were detected using 18S primers, i.e. there was no significant presence of eukaryotes in the system.

During OADF breakdown, after long runs, *Pseudomonas* species increased noticeably in the bacterial mat and among the small number of eluate bacteria, presumably because of their resistant nature. The reason the mat had the greatest diversity of species (Figure 2(c)) probably originates in the proliferation of rare environmental bacteria as they consume their neighbors. Bacteria are completely excluded by Ash Depth Filtration for a long time and the vast majority of resistant pseudomonads are either trapped or destroyed in the waste stream, causing great reduction of microbial load and also the transformation of the population in terms of species and numbers.

In addition to the above, previous work (Witty *et al.* 2020) has shown that when waste urine aliquots are used instead of deionized water, flow also stabilizes. This was unpredictable for two reasons: particulate material in urine is a factor for decreased flow and chemical erosion of the filter bed by ammonium reaction with calcium carbonate is a factor for increased flow. It is remarkable that so few of the fecal pathogens emerged from the ash bed and, in fact, there was no gross passage of bacteria through the ash bed at all, even after more than 6 weeks of operation, suggesting ash depth filtration may also be suitable for fecal waste sanitation.

CONCLUSIONS

OADFs in one gallon containers are useful for sterilization of source-separated urine on a scale useful for domestic application. They are easy to make and operate using materials easily obtainable, i.e. HDPE containers, wood ash and a needle. It is remarkable that they run for very long periods of filtration with no loss of ash through the end plate and no significant blockage. These simple depth filters are an excellent candidate for human waste amelioration in remote habitations.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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