A comparative study for pathogen removal using different filter media during vermifiltration

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ABSTRACT

The present study focuses on the effect of filter media (riverbed gravel, mud balls, coal and glass balls) for the removal of pathogens during vermifiltration of domestic wastewater. This novel study was conducted for the first time on different vermifilters (VFs) with different media, and pathogen removal was extensively investigated for a period of 3 months. Results indicated that vermifilter with riverbed material (VFR) showed maximum biochemical oxygen demand and chemical oxygen demand removal of 76 and 67%, respectively and vermifilter with mud balls (VFM) showed maximum log removal of total coliforms (2.8), fecal coliforms (2.7), fecal streptococci (2.2), *Salmonella* (2.1) and *Escherichia coli* (2.1). The area-based bacterial removal rate constant (k) was found in the range of 2.96–6.68 m/d, which is very high in comparison to the reported values. The population of total heterotrophic bacteria and total fungi was found to be 2-log (99%) higher in VFR and VFM, as compared to other media. The growth rate of *Eisenia fetida* was higher (42% increase in biomass) in glass balls vermifilter (VFG), and also the abrasions on the body wall of earthworms in VFG showed fewer injuries. Overall, the results of the study described the importance and role of each filter medium.

Key words | *Eisenia fetida*, filter media, pathogens, vermifiltration, wastewater treatment

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INTRODUCTION

Wastewater treatment by earthworms, known as vermifiltration, is proven to be an economically and environmentally preferred technology as it is a rapid, nearly odorless process, producing stable, disinfected, detoxified and highly nutritive effluent (Li *et al.* 2009). The application of vermifiltration for domestic and industrial wastewater treatment has turned out to be a competent technology compared with the conventional biofilters (Sinha *et al.* 2007, 2008; Ghatnekar *et al.* 2010). It is an organic decomposition process requiring a filter bed of suitable materials (bedding) to allow earthworms to interact with microorganisms, symbiotically and synergistically (Zhao *et al.* 2010, 2014; Liu *et al.* 2012; Xing *et al.* 2012).

Various researchers have been investigating the potential of vermitechnology for decentralized wastewater treatment. Several studies have been conducted on vermifiltration technology, which shows high efficacy for sewage treatment (Sinha *et al.* 2008; Liu *et al.* 2013; Kumar *et al.* 2014) and sludge reduction and stabilization (Xing *et al.* 2011; Liu *et al.* 2012; Yang *et al.* 2013). It has also been reported that the vermifiltration process has comparable efficiency to the activated sludge process (Li *et al.* 2009). Investigators have also reported the effect of earthworm loads (stocking density) on organic matter and nutrient removal efficiencies (Wang *et al.* 2013) and the effect of different hydraulic loading rates (HLRs) on vermifiltration (Kumar *et al.* 2014). However, the vermifilter medium is also an important design parameter to maximize the treatment efficacy of the system. Xing *et al.* (2011) performed a comparative study of synchronous treatment of sewage and sludge by vermifiltration and reported ceramsite as a better filter medium than quartz sand for vermifiltration (Xing *et al.* 2011).

Human pathogens are a typical component of domestic sewage and their control is one of the fundamental reasons for wastewater treatment. In the vermifiltration process, removal of pathogens is a complex phenomenon

doi: 10.2166/wst.2014.318

and is largely ignored. Pathogen removal in vermifilters (VFs) is closely associated with the natural die-off, predation or physico-chemical properties of the filter material, because pathogens are mainly sorbed by filter media or die off by the microflora associated with the filter bed where earthworms thrive (Kadam *et al.* 2008). Researchers have reported area-based bacterial removal rate constants for the vertical flow wetland to be 3.2 m/d for total coliforms (TC), 3.3 m/d for fecal coliforms (FC) and 2.1 m/d for fecal streptococci (FS) (Arias *et al.* 2003), 0.2–0.5 d^{-1} for constructed wetland (Hench *et al.* 2003), 0.03–0.05 d^{-1} for waste stabilization ponds (Garcia *et al.* 2008) and 0.1–0.2 d^{-1} for algal and macrophytic systems (Garcia *et al.* 2008). However, the bacterial removal rate constant in vermifiltration is yet to be determined or evaluated. Although sewage treatment by vermifiltration and its influencing factors and pollutant removal mechanism have been studied, the role of filter media in removal of pathogens in a VF is lacking. Therefore, the selection of efficient and enduring filter media to remove pathogens is the research objective of the study.

The aim of the present study was to compare the effect of different filter media (riverbed gravel, mud balls, glass balls and coal) for removal of pathogens during vermifiltration. The study was carried out to compare chemical oxygen demand (COD), biochemical oxygen demand (BOD), TC, FC, FS, *Salmonella* and *Escherichia coli* removal efficiencies along with bacterial removal rate constants from synthetic domestic wastewater in different VFs. To the best of the authors' knowledge, this is the first report on this type of study.

MATERIALS AND METHODS

Experimental design

The study was carried out in the Department of Civil Engineering at the Indian Institute of Technology, Roorkee (IITR), India during the months of April–June, 2013 when the ambient temperature was 20–30 °C, which is the optimum temperature range for the growth of earthworm species (Tripathi & Bhardwaj 2004). Figure 1 shows the schematic diagram of the laboratory-scale VF. Different VFs made up of polypropylene material were set up with a cross-sectional area of 250 mm × 200 mm and a depth of 250 mm. The top layer or active layer (50 mm thick) consisted of thick matured vermicompost and the second layer (100 mm thick) consisted of different filter media: riverbed gravel (size: 6–8 mm) (VFR), mud balls (size: 5 mm) (VFM), glass balls (size: 10 mm) (VFG) and coal (size: 2–4 mm) as VFC. Third and fourth layers were 50 mm thick and comprised gravel of size 1–2 mm and 10–12.5 mm, respectively. Figure 2 shows the images of different filter media. The wastewater was applied from the top of the reactors using a peristaltic pump by perforated pipes, uniformly. Each of the VFs was inoculated with 100 earthworms (*Eisenia fetida)* to achieve a stocking density of 10,000 worms/m^3 of VF bed (Wang *et al.* 2013; Kumar *et al.* 2014) and was allowed to acclimatize for about 1 week before the study. VFs were allowed to run continuously for over a period of 70 days at HLR of 1.0 $m^3/(m^2 \cdot d)$ (50 litres per day) during all experiments (Li *et al.* 2009). Based on the flow rate and reactor configuration, the hydraulic retention time was found to

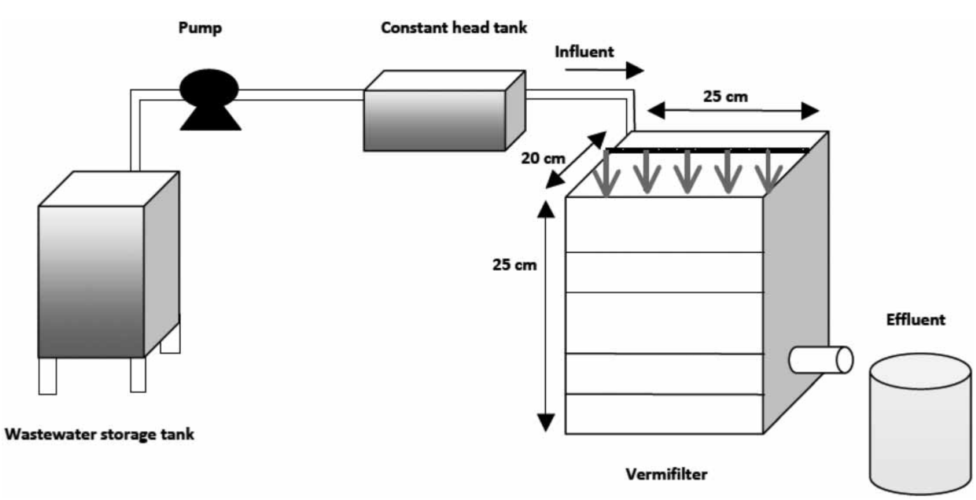

Figure 1 | Schematic diagram of a laboratory-scale VF.

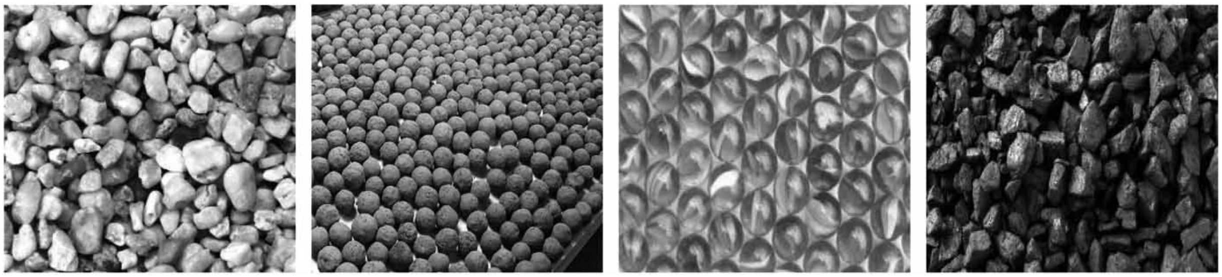

Figure 2 | Images of riverbed gravel, mud balls, glass balls and coal as filter media.

be 6 h. The performance of the system is measured in terms of BOD, COD, indicator organisms TC, FC, FS and pathogen, *Salmonella* and *E. coli*, removal efficiencies.

Wastewater composition

Synthetic sewage (Seetha *et al*. 2010; Arora *et al*. 2014a) seeded with 1% actual sewage was used to initiate the growth of coliforms and microbial population. Synthetic wastewater was used, since actual sewage causes greater fluctuations in the physico-chemical characteristics of influent, which was not desirable for the experiments. Hence to standardize the process, all other conditions were kept constant. The influent had concentrations of COD 377 ± 29 mg/L, BOD 273 ± 22 mg/L, BOD/COD ratio 0.72 ± 0.01, log values of TC $\log_{10}$ 6.7 ± 0.5 most probable number (MPN)/100 mL, FC $\log_{10}$ 5.5 ± 0.6 MPN/100 mL, FS $\log_{10}$ 5.3 ± 0.6 MPN/100 mL, *Salmonella* $\log_{10}$ 6.2 ± 0.4 MPN/100 mL and *E. coli* $\log_{10}$ 5.7 ± 0.6 MPN/100 mL, respectively.

Analytical procedure

pH and temperature were measured using a pH meter and digital thermometer. Dissolved oxygen (DO) was measured by a Hach multi-parameter kit (USA). BOD was measured by the azide modification method and COD was measured through the closed reflux spectroscopic method (DR 5000), as per standard protocols (APHA 2005). TC, FC, FS, *Salmonella* and *E. coli* were estimated by the multiple tube fermentation method (MPN method). The population of total heterotrophic bacteria and total fungi were determined by the standard plate count (SPC) technique (APHA 2005) using specific media supplied from Hi-media Laboratory, Pvt. Ltd. Earthworm number, biomass and morphology (surface structure) were also recorded on the initial and final day of experiments.

Removal kinetics

The average value of indicator organisms in influent and effluent permits the calculation of area-based first-order bacterial decay constants (k, m/d) using the following formula (Kadlec & Wallace 2009; Arora *et al*. 2014a):

$$\frac{C_o}{C_i} = \exp(-k/q) \quad (1)$$

where C_o represents indicator organisms in the effluent (MPN/100 mL), C_i represents indicator organisms in the influent (MPN/100 mL) and q represents HLR ($m^3/(m^2 \cdot d)$).

Isolating k in Equation (1) yields

$$k = q \times \ln\frac{C_i}{C_o} \quad (2)$$

Data collection and statistical analysis

The final removal efficiency for BOD and COD was calculated as percentage removal, according to Equation (3):

$$\text{Percentage reduction } (\%R) = \frac{(C_i - C_o)}{C_i} \times 100 \quad (3)$$

where, C_i and C_o are the influent and effluent concentrations, respectively, in mg/L.

The pathogen removal efficiency for TC, FC, FS and pathogens is calculated using Equation (4):

$$\text{Log removal value } (K) = \log_{10}\frac{C_{in}}{C_{out}} \quad (4)$$

where C_{in} and C_{out} represent the influent and effluent pathogen concentration, respectively in MPN/100 mL.

Analysis of variance (ANOVA) was conducted to analyze the data collected in all experiments, using the SPSS software program, version 11.5. Mean values between different filter media were compared by using one-way ANOVA and Student's *t*-test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Physico-chemical performance

The average pH of the influent was 8.0 ± 0.3. The pH of effluent from all the VFs increased initially during the treatment, then settled in the neutral range (7.6 ± 0.2) signifying the inherent capability of earthworms to act as buffering agent and neutralize pH (Arora *et al*. 2014b). Temperature is one of the key factors that affect the efficacy of wastewater treatment by vermifiltration. Throughout the study period, the average temperature of the effluent was $27.4 \pm 1.5\,^{\circ}$C (ranged 20–28 °C). The filter media provide resistance to the adverse impact of VF under lower and higher temperature conditions (Li *et al*. 2009) and have a buffer capacity to withstand the variations of temperature (Sinha *et al*. 2008). Therefore the choice of filter media is important as it serves as a dwelling habitat for earthworms to thrive and perform their function proficiently in the optimum temperature range (Tripathi & Bhardwaj 2004). DO is an important factor that signifies the environmental conditions prevailing inside the reactor and it is one of the significant parameters in the outflow, because low oxygen water is toxic to aquatic organisms. The values of DO increased from 0.5 ± 0.5 mg/L in the influent to 5.0 ± 1.0 mg/L in the effluent. This suggests that earthworms are responsible for creating aerobic conditions inside VFs by their burrowing action (Wang *et al*. 2011). The design of the VF is such that oxygen could penetrate to the bottom, thus increasing the efficiency of treatment. Higher DO in effluent reduces the septic condition and brings a good chance of wastewater reuse for irrigation purposes (Holenda *et al*. 2008). It is to be noted that no significant difference of pH, temperature and DO values was observed statistically between the VFs with different filter media.

The variations in BOD and COD removal with different filter media are described in Figures 3(a) and (b). It can be seen that the concentrations of BOD and COD decreased remarkably during 70 days of operation. In addition, the maximum BOD removal efficiency was 75.9% and COD removal was 66.7% in VFR (Figure 4). This can be attributed to the suitable endemic habitat provided by the riverbed gravel for the joint activity of earthworms and aerobic microbes to degrade the accumulated organic matter faster and loosen the compacted substrate to improve air

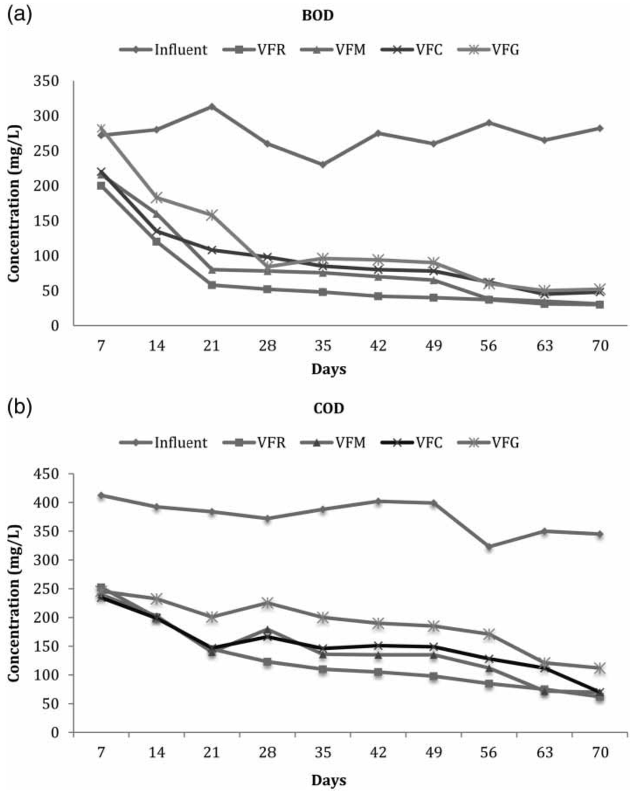

Figure 3 | Variation in (a) BOD and (b) COD with time in different VFs.

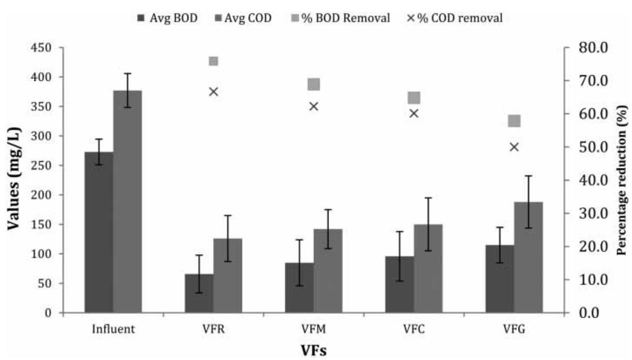

Figure 4 | BOD and COD removal efficiency in different VFs. *Note:* Left Y-axis shows the concentration (Avg ± SD) and right Y-axis shows the percentage removal (%).

permeability in the filter bed, which promotes BOD and COD removal (Sinha *et al*. 2008; Xing *et al*. 2010, 2012). In a favorable habitat this activity works better. In VFG and VFC, BOD and COD removal was not significant. This could be related to increased humidity and scouring of the VF, which are not beneficial for the performance of the vermifiltration process. BOD removal from wastewater creates an unsuitable physico-chemical environment for pathogens to thrive and thus makes septic bacteria more susceptible to die-off in the filter bed (Kadam *et al*. 2008). This might be a reason that BOD and COD removal is related to pathogen removal.

Pathogen removal performance

The log removal profiles for TC, FC, FS, *Salmonella* and *E. coli* were comparatively higher in VFR and VFM as seen in Figures 5(a)–(e). There is no significant difference in pathogen removal efficacy between VFR and VFM (t-test, $P > 0.05$) statistically. The irregular shaped, smooth surface of gravel and spherical shaped mud balls in VFR and VFM, respectively, provided a more favorable, aerobic habitat for earthworms to thrive well and interact with microorganisms, thereby causing them to release the antibacterial substance which in turn may be responsible for considerable reduction of coliforms and pathogens (Sinha *et al*. 2008; Arora *et al*. 2014b). Statistically, there is a significant difference in pathogen removal efficacy in all the different VFs as shown by single factor ANOVA ($P < 0.05$).

Based on the average indicator bacteria population in influent and effluent and HLR of the VF, the area-based

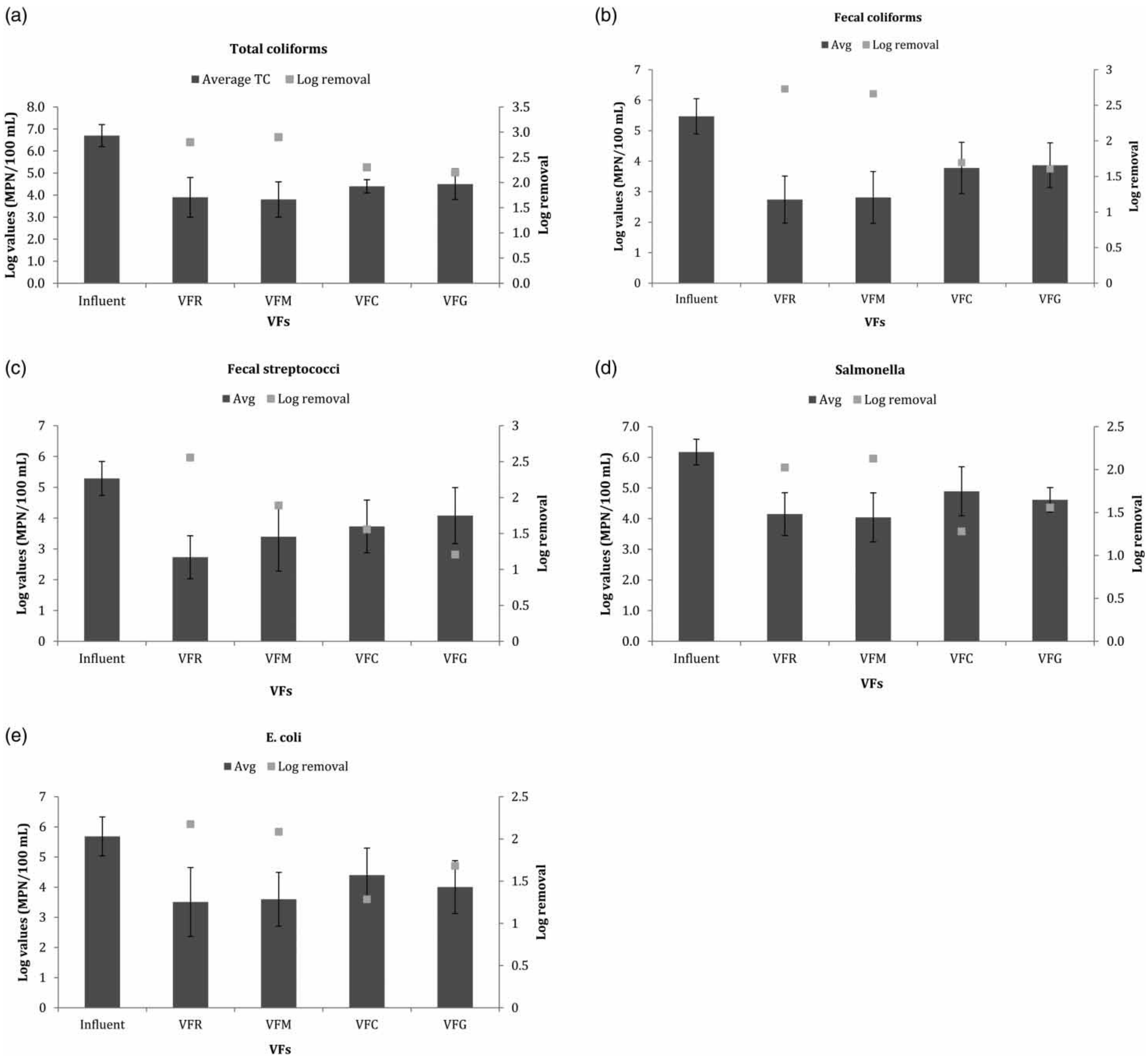

Figure 5 | Log removal profile for (a) TC, (b) FC, (c) FS, (d) *Salmonella* and (e) *E. coli* in different VFs. *Note:* Left Y-axis shows the log values (Avg ± SD), right Y-axis shows the log removal.

Table 1 | Bacterial removal rate constant k (m/d) for different VFs

	VFR	VFM	VFC	VFG
TC	6.45	6.68	5.30	5.07
FC	6.28	6.13	3.90	3.69
FS	5.89	4.35	3.59	2.78
Salmonella	4.66	4.90	2.95	3.59
E. coli	5.01	4.80	2.96	3.87

rate constants, k, for TC, FC, FS, *Salmonella* and *E. coli* (m/d) were estimated according to Equation (2) (Arora *et al.* 2014a). The calculated k for the bacteria are given in Table 1. These k values are also comparable with the reported values of bacterial die-off rate constants in a conventional treatment system. The bacterial removal rate constant of all studied bacteria was significantly different in all the VFs as shown by the single factor ANOVA ($P < 0.05$).

Microbial community diversity in different VFs

The SPC measures the population count of prevailing microbial diversity in different VFs. As shown in Table 2, distribution of the microbial population varies with filter media. During the experiment, it was observed that the average population of total heterotrophic bacteria and total fungi of the active layer in VFR and VFM was found to be 99% (2 log) higher than in VFC. The differences in the distribution of microbial diversity in different VFs is due to the particle size and surface structure of the media, which have a greater capacity to protect microbial biomass by providing a sorptive, high surface area environment for closer microorganism attachment (Arias *et al.* 2003). This suggested that the presence and activity of earthworms in their suitable habitat might result in higher microbial diversity. In vermifiltration, bacteria are the microorganisms that work together with earthworms and, under favorable conditions, earthworms and microorganisms act 'symbiotically and synergistically' to accelerate and enhance the decomposition of the organic matter in the waste. It is the microorganisms which break down the cellulose in the waste (Sinha *et al.* 2008). There is a unique and natural mechanism in which earthworms graze on the surplus harmful and ineffective microbes in wastewater and maintain a culture of effective biodegrader microbes to function well (Xing *et al.* 2011). Therefore in a suitable endemic environment like riverbed material, the population of bacteria will be higher and therefore the VF will work more efficiently. For any system to work efficiently, it is important that the living species that maintain the working of the system should be well adapted and adjusted to the habitat of the system, and a favorable habitat allows them to work better and more efficiently (Arora *et al.* 2014b).

Table 2 | Microbial community diversity in different VFs

Vermifilters	Total heterotrophic bacteria	Total fungus
VFR (10^5 CFU/mL)	70 ± 20	27 ± 12
VFM (10^5 CFU/mL)	35 ± 15	17 ± 10
VFC (10^3 CFU/mL)	84 ± 53	44 ± 20
VFG (10^2 CFU/mL)	60 ± 10	3.0 ± 1.0

Data are represented as Avg. ± SD.

Earthworm biology

The average initial earthworm number and biomass were 100 and 40 g, respectively in all the VFs. The percentage increase in the number and biomass of earthworms at the end of the run is given in Table 3. The maximum percentage increase in numbers of earthworms was found in VFG at 21%. Glass balls have a very smooth surface, which is an advantage for earthworms in moving around and performing their functions but the development of the microbial community on the surface was low (Table 2); so the treatment efficiency was lower than other VFs. In VFC a

Table 3 | Growth characteristics of earthworms

	No of earthworms			Biomass of earthworms (g)		
Vermifilters	Initial	Final	Percentage increase	Initial	Final	Percentage increase
VFR	100	108	7.4	40	52	23.0
VFM	100	119	15.9	40	54	22.2
VFC	100	98	−2.0	40	46	−6.5
VFG	100	127	21.2	40	71	42.2

decrease in the number of earthworms was observed. The reason for this is that coal has a very rough surface which resulted in abrasions on the outer skin lining of the earthworms, affecting their skin and health and resulting in the death of earthworms.

No clogging was observed in VFR and VFM throughout the study. There was a dynamic balance between the earthworm active mass and its number in VFR and VFM due to the favorable habitat provided for the earthworms. The study proves that gravel and mud balls media showed better treatment efficiency. Riverbed gravel is a cost-effective filter medium, which is easily available and can be utilized without any prior treatment, whereas mud balls and glass balls are costly and rarely available. Coal medium may be completely disregarded, as it showed poor results. However, further combinations of filter media into one single unit may bring more desirable results.

CONCLUSION

The present study explored the suitability of filter media with high pathogen removal efficacy as vermifilters, and naturally occurring riverbed material and mud balls were found to be better suited for the treatment. Overall, the observed trend of VFs in terms of treatment efficiency was observed to be VFR $\geq$ VFM $>$ VFG $>$ VFC. This can be attributed to the larger particle size of media, good permeability, higher porosity and smooth surface. The higher BOD, COD, and pathogen removal efficiency, higher microbial diversity in the filter bed, higher percentage increase in earthworm number and biomass, and no abrasions on the body wall of earthworms in VFR and VFM showed that riverbed and mud balls media are promising bed materials for vermifiltration. This approach can lead to deeper understanding of the role of the filter media and bedding during vermifiltration and the interrelationship between earthworms and microorganisms in their optimum filter bed.

ACKNOWLEDGEMENT

The authors are highly thankful to the Department of Science and Technology, New Delhi, Government of India, for providing financial support for the study.

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First received 10 April 2014; accepted in revised form 2 July 2014. Available online 22 July 2014