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A comparative study on vermifiltration using epigeic earthworm *Eisenia fetida* and *Eudrilus eugeniae*

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ABSTRACT

A comparative study was performed to treat the domestic wastewater through two different epigeic earthworms (*Eisenia fetida* and *Eudrilus eugeniae*) based vermifiltration unit (VF1 and VF2). Results revealed a significant removal of biochemical oxygen demand (88%), total suspended solids (78%), and total dissolved solids (75%) in the treated wastewater from VF1; while in VF2, it was observed to be 70, 67, and 66%, respectively, at hydraulic loading rate (HLR) of 2.5 $m^3 m^{-2} d^{-1}$. Beside this, a significant reduction of total coliform (3.1 log) was observed in VF1 as compared with 0.98 log reduction in VF2. In addition to this, an increase in earthworm biomass in reactor VF1 was found to be 11.4%; while in VF2, mortality was observed, since the earthworm species *E. eugeniae* could not survive during the process. Overall, in VF1, the effluent was rich in nitrate, phosphate, and showed the potential of *E. fetida* for wastewater treatment during vermifiltration process.

Keywords: Vermifiltration; *Eisenia fetida*; *Eudrilus eugeniae*; Hydraulic loading rate; Indicator organisms

1. Introduction

A high increase in population and elated modern lifestyle is responsible for natural resources depletion, which fosters the wastewater reuse planning. Thus, attention has been paid over a suitable decentralized wastewater treatment, especially in rural areas, where the cost of wastewater treatment is too high. Decentralized wastewater treatment involves the collection, treatment, disposal, and its reuse, which originates from an individual, clusters of homes, and isolated communities or near the point of generation [1–3]. Vermifiltration has the potential in this direction, which adapts the traditional vermitechnology to a passive wastewater treatment.

In vermifilter, earthworms stimulate and accelerate the microbial activity by increasing the population of soil

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micro-organisms [4]. The resulting effluent becomes highly nutritive and can be reused for irrigation purpose. In vermifiltration, no sludge is produced so it does not have the requirement of additional expenditure on its disposal, while in conventional treatment technologies like activated sludge process, sequencing batch reactor (SBR), upflow anaerobic reactor (UASB) produce a huge amount of sludge, for which the additional treatment is needed before its disposal [5].

Pilot-scale-level studies have been carried out on vermifilter technology and it showed a perfect efficacy for wastewater treatment with high removal rate of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and suspended solid, along with the ability to remove N and P forms [3,6,7]. It has been reported that there is very little or no problem of any foul odor during the vermifiltration process [5,6]. Vermifiltration has the potential to enhance beneficial decomposer bacteria as well as it reduces the pathogen enormously [4,8].

Previous studies primarily focused on the use of vermifiltration or its combined processes in the treatment of different types of wastewater, and the related factors contributing to its efficiency in removing pollutants using earthworm species *Eisenia fetida* [4,5]. Exotic epigeic species, *E. fetida,* has been identified as potential candidate to decompose organic content of wastewater [3,9]. Thus, as per the author's knowledge, no studies have been conducted on vermifiltration process using *Eudrilus eugeniae*. *E. eugeniae* is a species of earthworm native to tropical West Africa and presently widespread in warm regions. This species, also called as the African night crawler, has a uniform purple-gray sheen and the posterior segments which are evenly tapered to a point. Morphologically, the segments of the brandling worm (*E. fetida*) are reddish orange and brown alternately, and the posterior segments are not tapering, but blunt. Furthermore, it has been established that *E. fetida* has a unique indigenous gut-associated microflora that contributes to the development of a diverse microbial community in vermifiltration systems [10,11]. It has already been reported that *E. fetida* can consume organic matter at the rate equal to their body weight every day [12]. In this context, the present work was undertaken to study the feasibility of the vermifiltration technique for the treatment of domestic wastewater using earthworm species of *E. eugeniae,* as an alternative option in the absence of *E. fetida*.

2. Materials and experimental methods

2.1. Experimental design

Experimental setup was placed in the solid waste laboratory of Civil Engineering Department, Indian Institute of Technology (IIT) Roorkee, India. During study, two sets of vermireactors, VF1 and VF2 were designed that inoculates the exotic epigeic earthworm species i.e. *E. fetida* and *E. eugeniae* in individual reactor, respectively. Both were taken into triplicates and consisted of plastic container having cross-sectional dimension of 250 × 200 mm and depth 300 mm. The top layer had 50 mm thick, matured vermicompost (worm bed). Initial characteristics of worm bed are depicted in Table 1. Second, third, and fourth layers were comprised of river bed material having size of 6 to 8 mm (100 mm thick), 1 to 2 mm (50 mm thick), and 10 to 12.5 mm (50 mm thick), respectively. The wastewater was applied from atop the reactors with the help of peristaltic pumps. For uniform distribution of wastewater, a 0.5 inch glass pipe with 1.5 mm diameter hole was placed on the top of vermifilter bed. Fig. 1 shows the schematic view of vermifilter. Each of the vermifilter was inoculated with a number of 150 earthworms (*E. fetida* and *E. eugeniae* in each reactor) based on the stocking density of 10,000/cum of filter bed [4]. The reactors were fed daily at HLR of 2.5 $m^3 m^{-2} d^{-1}$ for 90 d.

2.2. Earthworm species used and wastewater composition

The earthworm species, *E. fetida* and *E. eugeniae,* were cultured in the environmental engineering laboratory of IIT Roorkee before use. Both the earthworm species juveniles were procured from Morarka Foundation, Jaipur, India, and cultured in pretreated cow dung. To give some pretreatment, the fresh urine-free cow dung, already procured from a local cowshed, was partially dried in shade and homogenized manually. When the cow dung got matured, both earthworm species were released in it separately in individual trenches. After attaining maturity of both earthworm species, the clitellated earthworms were randomly picked for experimentations. The species were identified at National Zoological Survey of India, Solan, India, before culturing in the field laboratory.

For the experiments, the domestic wastewater was procured from the local institutional area of IIT Roorkee campus. The influent had COD concentration ~ 415 ± 18 mg/L, BOD ~ 240 ± 13 mg/L, BOD/COD ratio ~ 0.58 ± 0.001, total dissolved solids (TDS) ~ 543 ± 27.6 mg/L, total suspended solids (TSS) ~ 240 ± 36.3 mg/L, total organic carbon (TOC) ~ 210 ± 18 mg/L, ammonia nitrogen (NH_4^+–N) ~ 30.5 ± 10 mg/L, nitrate nitrogen (NO_3-N) ~ 4.1 ± 2.3 mg/L, total nitrogen ~ 45 mg/L, total phosphate (TP) ~ 8.1 ± 3.3 mg/L, pH of 7.1 ± 0.08, total coliform (TC) 500×10^7 MPN/100 mL, fecal coliform (FC) 110×10^6 MPN/100 mL, and fecal streptococci (FS) 150×10^6 MPN/100 mL.

Table 1
Characteristics of vermicompost from VF1 at hydraulic loading rate 2.5 $m^3 m^{-2} d^{-1}$

Parameters	Initial* characteristics of vermifilter bed	Final* characteristics of vermifilter bed
pH	7.2 ± 0.1	7.4 ± 0.26
C/*N* ratio	11.32 ± 0.15	11.08 ± 1.78
TOC (g kg^{-1})	292.1 ± 3.3	284.1 ± 2.7
Total phosphate (g kg^{-1})	25.8 ± 0.1	25.64 ± 1.12
Total nitrogen (g kg^{-1})	26.2 ± 0.65	26.2 ± 5.6
Ash content (%)	50.9 ± 1.85	51.01 ± 1.56

*Mean concentration ± standard deviation of the physicochemical parameters.

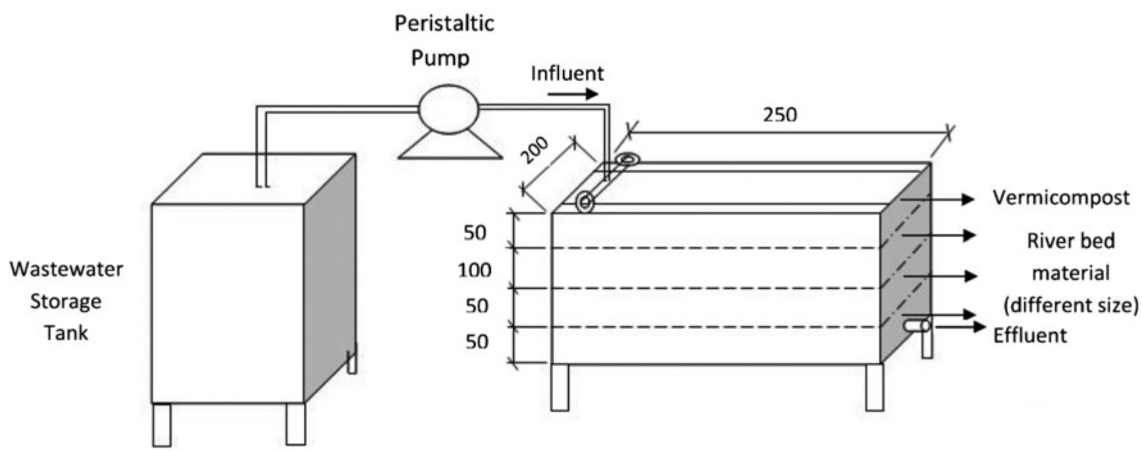

Fig. 1. Schematic diagram of the laboratory-scale vermifilter (units: mm).

2.3. Sampling and analysis

The influent and effluent samples collected from both the vermifilters, VF1 and VF2, were analyzed for BOD, COD, TOC (measured by TOC analyzer, Shimadzu, SSM-5000A), nitrogen (NH_4^+–N, NO_3–N), pH, and TP. All the parameters were analyzed according to the standard methods for the examination of water and wastewater [13].

2.4. Microbial count

2.4.1. Total bacteria

The total indigenous heterotrophic bacteria were determined by the plate pouring technique on nutrient agar (Pasteur production, Paris). The plates were incubated for three days at 30°C. All water samples were assayed by dilution with at least four replicates of each sample [13,14].

2.4.2. Indicator organisms

The TC, thermotolerant FC, and FS were monitored in all the samples using the most probable number method (MPN) by inoculation of culture tube media with Lauryl tryptose broth, EC medium, and azide dextrose broth, respectively [13].

2.5. Statistical analysis

A paired sample t-test between VF1 and VF2 was performed for each physicochemical parameter to analyze the differences. One-way analysis of variance (ANOVA) was also performed to determine the chronicle changes over the experimentations for each physicochemical parameter of wastewater using SPSS® statistical package (Window Version 14.0). All statements reported in this study are at $p < 0.05$ levels.

3. Results and discussion

3.1. Variations in physicochemical characteristics

The BOD of effluent was found significantly low in both the reactors, i.e. VF1 and VF2, but in VF1, evidenced a better removal efficiency as compared with VF2 (t-test: $p < 0.001$, Table 2). The variations of BOD concentration in VF1 and VF2 are depicted in Fig. 2.

Table 2
Characteristics of effluent collected from VF1 and VF2 at HLR 2.5 $m^3 m^{-2} d^{-1}$

Parameters	VF1*	VF2*	t-test (t- coefficient value)	Significance level
BOD (mg/l)	28 ± 7.2	72 ± 5.56	7.9582	$p < 0.001$
TOC (mg/l)	40.5 ± 14.8	88.7 ± 8.2	12.46697	$p < 0.001$
TDS (mg/l)	201 ± 18.4	321 ± 16.5	4.772431	$p < 0.001$
TSS (mg/l)	162 ± 17.4	378 ± 28	26.34297	$p < 0.001$
TP (mg/l)	34.5 ± 2.8	20 ± 1.1	−7.22276	$p<0.001$
NO_3-N (mg/l)	51.1 ± 2.6	28.4 ± 1.4	−38.9901	$p < 0.001$
NH_4^+-N (mg/l)	4.4 ± 0.5	8 ± 1.8	7.7986	$p < 0.001$
TC (MPN/100 mL)	$6.0 \times 10^{2.7} \pm 3.7 \times 10^{0.7}$	$3.1 \times 10^{5.1} \pm 1.7 \times 10^{0.4}$	7.5066	$p < 0.001$
FC (MPN/100 mL)	$5.1 \times 10^{2.5} \pm 3.7 \times 10^{0.5}$	$2.9 \times 10^{2.8} \pm 2.7 \times 10^{0.4}$	4.7704	$p < 0.05$

*Mean concentration ± standard deviation of the physicochemical parameters.

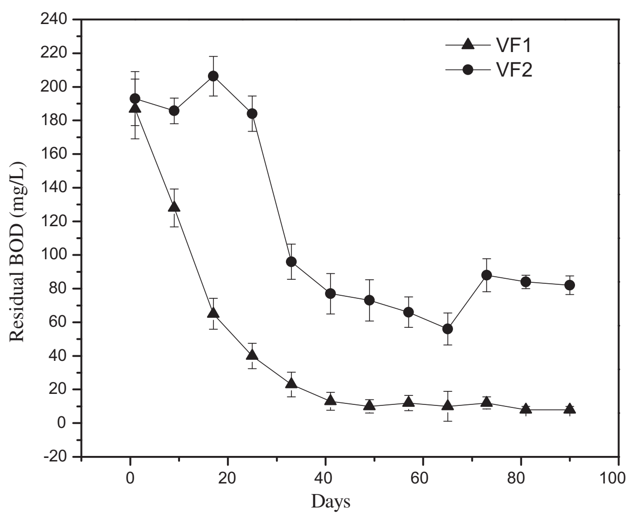

Fig. 2. BOD removal pattern in reactor VF1 and VF2.

The BOD removal in vermifilter VF1 was 88%, while in VF2 it observed to be 70%. This could be due to the symbiotic activity of earthworms and aerobic microbes that accelerate and enhance the decomposition of organic matter [15,16]. In VF2, comparatively low removal efficiency was observed as the earthworm species, *E. eugeniae*, could not thrive during the process (Table 3), which may be attributed due to high moisture content (>70%) in vermifilter, or may be due to scarcity of feed that was replaced by wastewater.

In VF1, BOD/COD ratio was observed as 0.24 ± 0.1 which is much lower as compared with VF2. This could be due to the presence of various enzymes in the gut of earthworms, which promotes the degradation of those chemicals, which may not get decomposed in geo-microbial processes. In vermifilter VF1, having earthworm species *E. eugeniae*, the BOD/COD ratio was observed to be 0.32 ± 0.06. In vermifiltration, the BOD removal efficiency was more as compared with COD in the same reactor, due to dependency of earthworms on biodegradable part of wastewater [7].

In VF1 and VF2, NH_4^+-N concentration decreased over time, from initial value of influent 30.5 ± 10 mg/L. The change in NH_4^+-N concentration is illustrated in Fig. 3(a). In VF1, more reduction was observed as compared with VF2, and it is subsequently converted into nitrate form. The nitrate-nitrogen concentration in effluent was significantly high (t-test: $p < 0.001$) as compared to VF2, due to lack of earthworm activity of *E. eugeniae*, as observed throughout the process. During the process, in reactor VF1, maximum nitrate-nitrogen concentration about 41.1 ± 2.6 mg/L was observed; while in VF2, nitrate-nitrogen concentration found to be 18.4 ± 1.4 mg/L. This may be attributed to the presence of ammonia-oxidizing bacteria that was enriched by earthworms [17]. The nitrate-nitrogen concentration profile is given in Fig. 3(b). In addition to this, the reducing concentration of ammonia nitrogen was attributed to the mineralization of ammonia nitrogen into nitrate form. It has already been reported that earthworms secrete polysaccharides, proteins, and other nitrogenous compounds, and mineralize the nitrogen available in wastewater to make it available to the plants as nutrients [18]. The final effluent in VF1 contained 4.4 ± 0.45 mg/L of ammonia nitrogen, while in VF2 it was observed as 8 ± 1.83 mg/L. Recently, Wang et al. [19] has also observed that oxygen is available in abundance through the burrowing action of earthworms that favors a microenvironment for aerobic nitrobacteria. The ammonia nitrogen is removed through rapid adsorption by media, and subsequently, it is converted from ammonia nitrogen into nitrate form in biological nitrification [20,21].

In VF1, the TP concentration in the effluent significantly increased (t-test: $p < 0.001$), and it was higher than the vermifilter VF2. The TP concentration

Table 3
Growth of earthworm biomass in VF1 and VF2

	Weight of earthworms	
Earthworm species	Initial weight of earthworm biomass (g)	Final weight of earthworm biomass (g)
E. fetida	78 ± 2.64	85.66 ± 1.67
E. eugeniae	78 ± 2.64	ND

*Mean concentration ± standard deviation of the physicochemical parameters.
ND—Not detectable.

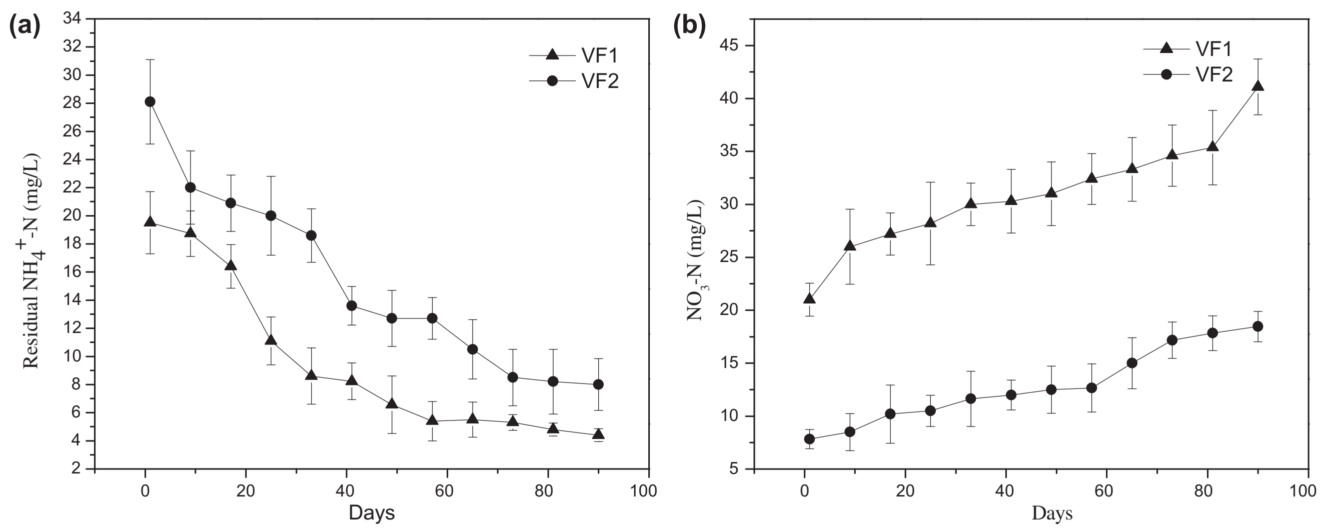

Fig. 3. Effluent concentration pattern in reactor VF1 and VF2 (a) ammonia nitrogen and (b) nitrate nitrogen.

changed in reactors, VF1 and VF2, are illustrated in Fig. 4. Maximum TP concentration in VF1 outlet was found to be 34.5 ± 3.25 mg/L. This increment can be attributed to the enzymatic and microbial action of earthworms. Activities of earthworm and associated microbes in vermi beds promote rapid phosphate mineralization in vermifiltration process that causes an increased concentration of phosphate in the effluent [22]. As phosphate is one of the key components for agriculture point of view, it showed the potential of vermifiltration for the treatment of wastewater.

TDS and TSS both reduced during vermifiltration significantly ($p < 0.05$) as shown in Fig. 5(a) and (b). Maximum TDS removal in VF1 was achieved high as 75%, wherein VF2 it could reach only up to 66%, might be due to less activity of *E. eugeniae*. Similarly, the maximum TSS removal in reactor VF1 was observed as 78%, while in VF2, the removal efficiency decreased and observed as 67% only during study period. During the process, the dissolved, suspended, organic, and inorganic solids are trapped by adsorption and stabilized through complex biodegradation processes [7]. Furthermore, the earthworms ingest solid particles of wastewater and excrete them in smaller digested particles. These finer particles, trapped in the voids of vermifilter, cause a high

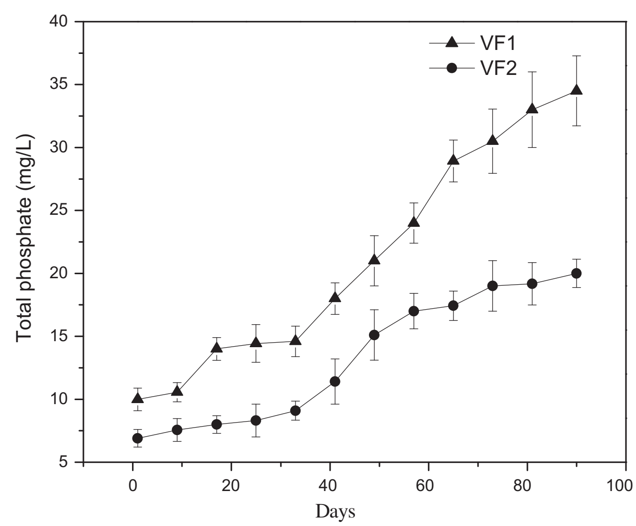

Fig. 4. Total phosphate concentration pattern in reactor VF1 and VF2.

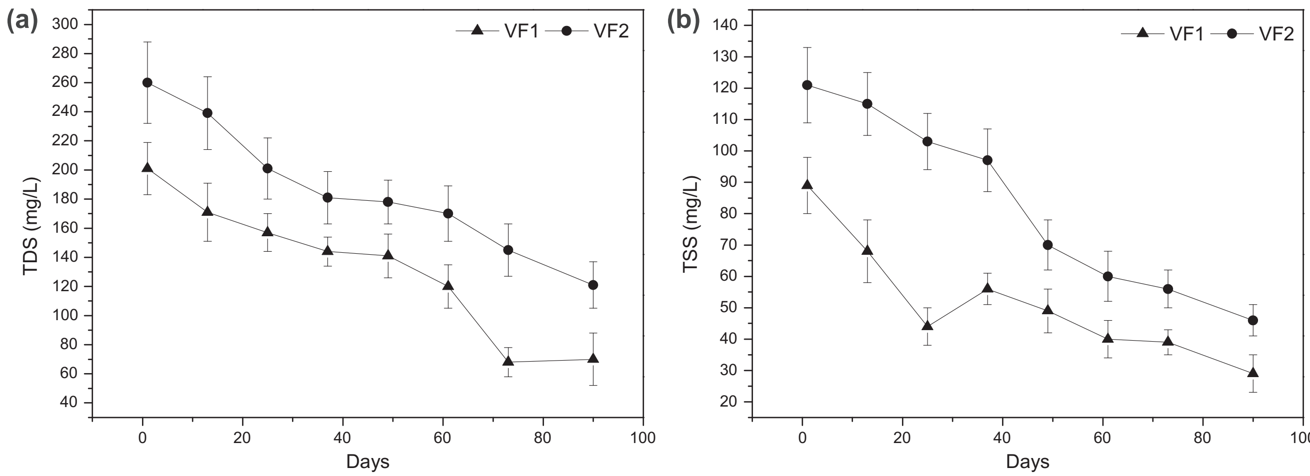

Fig. 5. (a) TDS and (b) TSS removal pattern in reactor VF1 and VF2.

removal efficiency of TSS and TDS from wastewater. This phenomenon was found dominating in VF1 reactor, due to extravagant potential of *E. fetida*, which has a unique indigenous gut-associated microflora that contributes to the development of a diverse microbial community in vermifiltration system. During the process, a tortuous behavior for TDS and TSS concentration was also observed. This unfavorable performance might be due to turbulence, which led to washout of the influent solids and settled biomass [23]. The stimulatory effect of earthworms could also be a reason behind this tortuous behavior, in which mucus and cast are produced. Mucus is a source of easily assimilable carbon for micro-organisms, while casts are often enriched with available forms of C, N, and P [24]. Thus, the leaching of vermicast gives rise to such behavior as observed in study. In addition to this, the adsorption of the impurities in the wastewater cannot get fully adsorbed on the sand and gravel particles, and it would be washed away from the reactor [25].

In VF1, TOC concentration in effluent was significantly low (t-test: $p < 0.001$) as compared with VF2. Fig. 6 shows the TOC concentration profile during the study. In VF1, the maximum TOC removal was observed as 85% at the end of the run; while inVF2, the TOC removal was observed to be 62%. This could be due to the increased carbon consumption in metabolic activity during the vermifiltration process [21].

3.2. Variations coliform removal efficiencies

The average value of TC and FC in the influent was $3.15 \times 10^{6.25} \pm 3.8 \times 10^{0.9}$ and $1.43 \times 10^{5.1} \pm 0.6 \times 10^{0.35}$ MPN per 100 mL, respectively, as illustrated in Table 2. In case of TC, the log reduction of 3.12 was observed

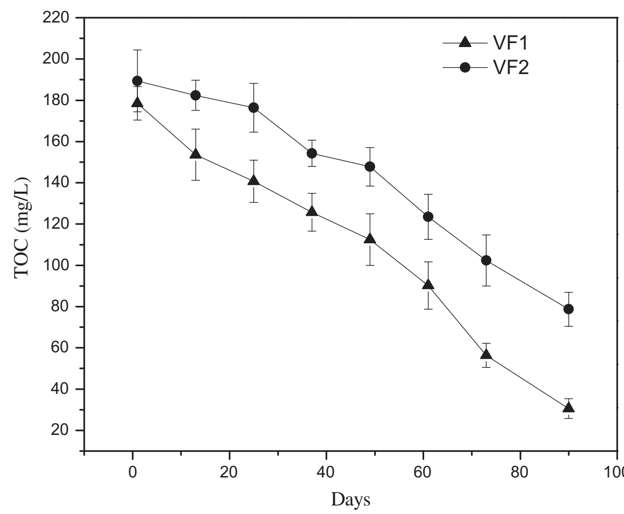

Fig. 6. TOC removal pattern in reactor VF1 and VF2.

in the effluent from VF1 as compared with 0.98 log reduction in VF2. The overall average log reduction of FC was 2.18 (99%) in VF1, and 1.02 (90%) in VF2. Another observation was that the final treated wastewater from VF1 was less biologically contaminated, which contained lower numbers of FC (3 log) than the permissible limit (i.e. 3 log or 1,000 MPN/100 mL) as reported earlier for unrestricted irrigation [26]. This indicates the considerable reduction of pathogens in vermifiltration, which renders it as a pathogen-free process. The possible reason for the pathogen reduction in VF1 can be attributed to various earthworm-mediated actions, such as intestinal enzymatic action, secretion of coelomic fluids having antibacterial properties, and selective grazing [27]. It can be inferred from this present study that vermifiltration may achieve complete sanitization under favorable environmental conditions.

3.3. Earthworm biomass

In vermifilter, VF1, the earthworm biomass was significantly increased at the end of run, as depicted in Table 3.Wherein reactor VF2, initial decline of *E. eugeniae* was observed after 3 d, when both reactors started from 15 June 2013. Furthermore, the mortality was consistently increased and at the end of the run, on 15 August 2013, no *E. eugeniae* was found alive in VF2. This might be due to high moisture content during the process, which was introduced due to the direct application of wastewater in the reactor, so the species could not acclimatize fast enough to this condition during the process [28]. *E. fetida* showed excellent growth, and the biomass found varying between 8.2 and 11.4%. The initial weight of earthworm was taken as 78 ± 2.64 gm. The health of earthworms was observed excellent in VF1, which exhibits the better performance of *E. fetida* against VF2 having species of *E. eugeniae*.

4. Conclusion

As presented by the results, the earthworm species *E. eugeniae* experienced no sustainability during vermifiltration for the treatment of wastewater. Besides this, *E. fetida* proves its utility in vermifiltration process, and the treated wastewater sustainably meets the requirement for irrigation and can be reused at source itself, thus conserving the water, and overcome the load on the exhausting conventional resources. The BOD, COD, TSS, and TDS were reduced significantly, and a considerable reduction was observed in TC, FC, and FS at HLR of 2.5 $m^3\,m^{-2}\,d^{-1}$. Beside this, in VF1, no sludge was produced and solids present in wastewater were consumed by earthworms (*E. fetida*) as they convert the waste available in wastewater into value added vermicompost, which can be used as fertilizer as having good content of nitrogen and phosphate. The effluent contained higher value of nitrate and phosphate, which is suitable for sewage farming or horticulture. A significant increase in earthworm biomass at the end of the run showed a better performance of vermifilter having species of *E. fetida*.

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