Substrate removal kinetics and performance assessment of a vermifilter bioreactor under organic shock load conditions

Tarun Kumar, K. S. Hari Prasad and Nitin Kumar Singh

ABSTRACT

In the present study, the effect of short-term organic shock loads (675, 799, 1,084 and 1,410 mg COD/L) on the treatment performance of a pilot-scale vermifilter (VF), employing an epigeic earthworm *Eisenia fetida* and treating synthetic domestic wastewater is investigated. The effect of organic shock loads on the performance and stability of vermifiltration reactor was evaluated to identify its feasibility in actual field conditions. Prior to the application of each organic shock load, normal loading conditions were maintained to achieve the pseudo steady state (PSS) conditions. The results showed satisfactory endurance against imposed organic shock loads with negligible reduction in chemical oxygen demand (COD) removals and it was almost similar to PSS condition with removal efficiencies of ~ 66, 71, 67 and 68%, respectively. The experimental COD data fit well to first-order kinetic model, with a regression value of 0.95. At the end of all shock loads, the nutritional analysis of vermicompost obtained from the top layer of VF, showed increased concentration of total nitrogen (~31 g/Kg) and total phosphorus (29 g/Kg). Besides, an augmented earthworm biomass, ~23.2% on weight basis and ~22% on number basis, was observed at the end of the study.

Key words | COD removal, decentralised wastewater treatment, *Eisenia fetida*, kinetics, organic shock loads, vermifiltration

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INTRODUCTION

The wastewater characteristics usually fluctuate due to an inappropriate contribution of runoff and rainfall during treatment, variation in population and operational problems (Orhon *et al.* 1999; Hammer & Hammer 2008). The variation in wastewater flow rate and characteristics has become common due to the modern trend of human life and activities. Such variability becomes a factor of concern where stringent discharge requirements need to be confronted. The change in wastewater characteristics generally referred to as hydraulic and/or shock loads, may be of qualitative and/or quantitative nature (i.e. change in composition, flow rate and concentration, etc.). That is why it is important to determine the stability of a treatment system against shock loads conditions, i.e. how a perturbation may destabilize or affect the treatment efficiency of a system (Xing *et al.* 1997; El Farhan & Shieh 1999; Leitao *et al.* 2006). These shock loads can manifest themselves in two ways: either as transient (for a few hours) or long term (for a few days or a week). To investigate whether wastewater treatment plants can cope with shock loads or not, stability assessment can be done under short-term hydraulic and organic shock loads (Tandukar *et al.* 2006; Gopala Krishna *et al.* 2008; Seetha *et al.* 2010).

Over the years, a number of researchers have looked upon the effects of organic shock loads in various wastewater treatment systems. Recently, ecological wastewater treatment processes have been designed as a decentralized wastewater management option (Sharma *et al.* 2014). One of the bottlenecks of these ecological systems, such as vermifilter (VF), is the lack of knowledge about the performance under severe environmental and operational variations (Mahmood *et al.* 2013). Vermifiltration has been studied extensively due to its effectiveness for removing pollutants in wastewater and its positive effects on the environment. Sinha *et al.* (2008) also reported that VF employing earthworms increases the effectiveness of

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overall treatment process, whereas the system without earthworms showed poor performance. Moreover, several proposed solutions for the treatment of diffused sources of domestic wastewater have been applied to onsite treatment in spacious rural areas, including constructed wetlands, soil infiltration trenches, and vegetation-based wastewater treatment (Cuyk *et al.* 2001; Ham *et al.* 2007; Sinha *et al.* 2008; Kaoru *et al.* 2010; Sharma & Kazmi 2014a, 2014b; Singh *et al.* 2015a). Among these technologies, vermifiltration, a process in which earthworms and microorganism simultaneously work together to treat the wastewater, is the most promising and economical method. It also separates wastewater solids by allowing it to be gravity-fed over the filtration material. Literature review revealed that *Eisenia fetida* has a unique indigenous gut-associated microflora that contributes to the development of a diverse microbial community in vermifiltration systems. In addition, *E. fetida* can ingest organic matter at a rate equal to their body weight every day (Sinha *et al.* 2008; Kumar *et al.* 2014, 2015a, 2015b). The additional features of vermifiltration system include negligible clogging and head loss. When earthworms continuously move into the VF it results in channeling of the system through their burrowing action, thus increases the hydraulic conductivity (Sinha *et al.* 2008). Despite the wide application of vermifiltration, to date, no study has been reported regarding performance of VF with organic shock load impositions.

Kinetic studies are also essential in predicting biological reactor's performance and for improved understanding of biological treatment processes (Mohanty *et al.* 2015). In this regard, different kinetic models have been proposed for different biological treatment systems. In particular, traditional zero-order, first-order, and second-order kinetic models for substrate degradation are applied for wetlands in the USA, Australia and European countries (Mitchell & McNevin 2001; Stein *et al.* 2006).

With this context, the experimental protocol of this work was planned to assess the effect of organic shock loads on the efficiency of pilot-scale VF treating synthetic wastewater with an aspiration to contribute for a better insight into the behavior of VF to augment its potential for full-scale applicability. Bearing such consideration in mind, the main objective of the present study was to gain insight about the performance of VF, in particular, to evaluate the system behavior when exposed to varying organic shock load conditions. In addition to this, an attempt has also been made by determining chemical oxygen demand (COD) removal kinetics for the stability assessment of these systems. The results presented as a part of this study are unique as it provide the first known pilot-scale study of vermifiltration reactor treating synthetic wastewater under shock loads conditions.

MATERIALS AND METHODOLOGY

Experimental set-up and operation

The study was carried out in Environmental Engineering Laboratory, department of civil engineering at Indian Institute of Technology Roorkee, Roorkee (India). A pilot-scale reactor made up of a perspex sheet was set up as shown in Figure 1. It consisted of filter bed material, a wastewater storage tank, a wastewater distribution system and effluent collection system. It was 800 mm long and 400 mm wide with a depth of 800 mm, and 650 mm of packed bedding of riverbed materials as reported in previous studies (Kumar *et al.* 2015, 2015b). An empty space or free board of around 150 mm was kept at the top to facilitate aeration. The filter bed consisted of four layers. The description of

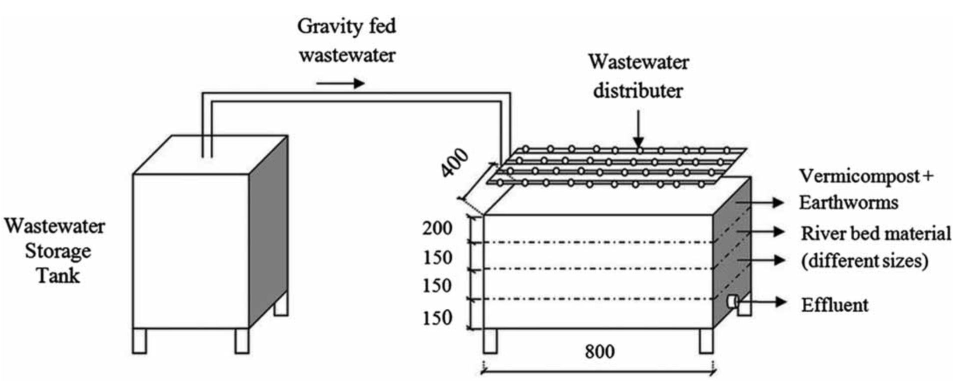

Figure 1 | Schematic view of the laboratory-scale VF (units: mm).

filter bed layers is illustrated in Table 1. The VF was inoculated with 800 numbers (weight 450 g) of earthworm species *E. fetida* based on 10,000 worms/cum stocking density. The earthworm species, *E. fetida*, was cultured as seed in the environmental engineering laboratory of IIT Roorkee before use. The juvenile earthworms were procured from Morarka Foundation, Jaipur, India, and sub-cultured according to the method as reported in previous studies (Kumar *et al.* 2015, 2015b). A centrifugal pump was installed to collect and transfer the wastewater to the overhead tank. Besides, a constant head tank was also provided which had a provision to maintain constant flow under gravity so as to reduce unnecessary energy cost. Overall, the wastewater fed from storage tank to constant head tank subsequently to the treatment unit and it passed through different layers of VF by gravity flow. Prior to analysis, a 20-day period was provided to the earthworms to acclimatize with the reactor environment. During study the atmospheric temperature varied in the range of 25–30 °C.

The reactor was started up in June 2013 and operated for a period of 90 days. The system was continuously fed with synthetic wastewater at hydraulic loading of 1.3 $m^3/m^2/d$. It corresponded to a hydraulic retention time (HRT) of 3 h until the system achieved steady-state condition. Pseudo steady state (PSS) condition was assumed to be achieved when the variation in effluent COD concentration was found to be insignificant, as depicted in Table 2. After this, the effect of organic shock load on the performance and stability of the system was evaluated to identify its feasibility in actual treatment conditions. For this, transient organic shock load conditions were forced upon by varying the influent concentration (COD_{Inf}) in the range 675–1,410 mg/L at constant HRT over a period of time until the reactor had reached steady state. The influent COD concentration was induced from 1.5 to 3 times of normal feed concentration as represented in Table 3. Each shock load was applied for a period of 3 h HRT and subsequent shock load was applied after attaining the PSS condition as shown in Table 3. Normal loading conditions (COD, 500–650 mg/L; HRT, 3 h) were resumed at the end of each shock load run.

Substrate loading rates: The substrate loading rates were determined by the following equation:

$$L = \frac{HLR \times C_{\mathrm{Sub}}}{(1,000\ \mathrm{mg}/L \times 1\ \mathrm{m}^3/\mathrm{kg})} \quad (1)$$

where L is the substrate loading rates ($kg/m^2/d$), HLR the hydraulic loading rate ($m^3/m^2/d$) and C_{sub} is the substrate concentration (mg COD/L).

Feed composition

The synthetic wastewater was prepared in the laboratory by dissolving molasses, urea and KH_2PO_4 to give the ratio of COD/N/P as 300/30/1 [8, 18] such that it simulates actual domestic wastewater of medium strength (Tchobanoglous *et al.* 2003). The influent had COD, 500–650 mg/L; biochemical oxygen demand (BOD), 325–415 mg/L; BOD/COD ratio, 0.65–0.69; dissolved oxygen, 2.1–3.3 mg/L; NH_4^+-N, 29–41 mg/L; NO_3-N, 0 mg/L; total phosphate (TP), 5.6–6.8 mg/L; and pH, 7.3–7.8. The large variation in influent characteristics can be attributed to composition of used molasses in synthetic wastewater preparation.

Sampling and analysis

The collected influent and effluent samples from VF were taken for the analysis of BOD, COD, total suspended solids (TSS), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3-N), pH and TP. All the parameters were analyzed according to *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.* 2005).

For the solid sample analysis, 25–100 mg of sub-sample was used for the determination of total organic carbon (TOC) by Shimadzu (TOC-VCSN) Solid Sample Module (SSM-5000A). The ash content was measured by the ignition method

Table 1 | Description of the filter bed layers for VF

Layers from top	Description of layers	Description of material in VF	Size of material	Depth (mm)
Layer 1	Active layer	Matured vermicompost	600–800 μm	200
Layer 2	Active layer	River bed material	6–8 mm	150
Layer 3	Third layer	Sand	2–4 mm	150
Layer 4	Supporting layer	Large gravel	10–12 mm	150
Empty space				150

Table 2 | Reactor performance at PSS

Parameter	Influent	VF effluent
COD (mg/L)	500–650	98–169
NH_4^+-N (mg/L)	29.9–47.1	3–8
NO_3-N (mg/L)	0	21–43
pH	7.2–7.6	7.3–7.8
COD removal efficiency (%)		63–76
Nitrification efficiency (%)		70–91

Table 3 | Influent COD and OLR at applied shock loads

S. no.	Influent COD (mg/L)	OLR (kg COD/m²/d)	COD_{max} removal (%)	Time to regain PSS condition
1	675	0.88	66	2 h 15 min
2	799	1.04	71	5 h 45 min
3	1,084	1.41	67	8 h 45 min
4	1,410	1.83	68	12 h

(550 °C for 2 h in muffle furnace) (BIS 1982). Around 5–10 mg samples were prepared for analyzing total nitrogen (TN) using CHNS analyzer. KCl (30 mL of 0.2 M KCl) extraction of 3 g of each sub-sample was used for the analysis of NH_4^+-N and NO_3-N using *Standard Methods* (Tiquia & Tam 2000; APHA *et al.* 2005). For the measurement of pH stirred 5 g of the wet sample from top of VF was taken in 50 mL distilled water and pH was measured using a pH meter with a glass electrode, previously calibrated and corrected for temperature (BIS 1982).

COD removal kinetics

Among the most widely used models in the literature, three basic kinetic models named as zero-order, first-order, and second-order are frequently employed for describing kinetics of environmental systems (Singh *et al.* 2015b, 2015c). For this purpose, effluent samples from different depths of VF bed were collected and COD values were determined to assess the process behavior. These COD values were plotted as per the kinetic model equations.

RESULTS AND DISCUSSION

Performance of the pilot-scale VF against different short-term organic shock loads

The performance of the VF observed at normal operating condition (PSS at 3 h HRT) is given in Table 2. At PSS, COD removal efficiency was observed in the range of 63–76%. It may be attributed to the symbiotic activity of earthworms and aerobic microbes which accelerate and enhance the decomposition of organic matter (Loehr *et al.* 1988; Kumar *et al.* 2014) and shows the efficacy of vermifiltration process. Figure 2 shows the influent and effluent COD variation under various shock loads in VF. In all the cases, the effluent COD concentration from VF increased in duration of organic shock loads. During different shock loadings of 675, 799, 1,084 and 1,410 mg COD/L, the performance of VF in terms of COD removal efficiency was observed as 66, 71, 67, and 68% as illustrated in Table 3. Not much difference in removal efficiencies was observed under shock load conditions compared to PSS. Thus, present system can cope up with organic shock loads up to a limit as presented in this study. However, the vermifiltration system demonstrated appreciable tolerance against organic shock loads with the pollutant removal efficiency dropping down slightly. The system exhibited appreciable stability and trimness because the configuration was strong enough for minimizing the effect of organic shock load. The resilience displayed by the system could be attributed to the availability of earthworms that make it capable to overcome the effect of high concentration of organics in wastewater. Visvanathan *et al.* (2005) have also reported that *E. fetida* can consume organic matter at the rate equal to their body weight every day.

In VF, the nitrate nitrogen concentration was observed as 19–32 mg/L at PSS condition. The nitrification efficiency through vermifiltration process exhibited as 66–78%. This can be attributed to mineralization of ammonia nitrogen into nitrate form. Bajsa *et al.* (2003) have reported that earthworms secrete polysaccharides, proteins, and other nitrogenous compounds. In addition to this, the earthworms mineralize the nitrogen in the wastewater to make it available to plants as nutrients. Recently, Wang *et al.* (2011) have also investigated that oxygen is available in abundance through the burrowing action of earthworms, which 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 through biological nitrification (Kadam *et al.* 2009). During shock loading conditions, nitrate nitrogen concentrations were slightly changed and quantified as 16–26.8 mg/L.

In VF, the increased concentrations of TP were observed and quantified as 10.6–12.3 mg/L from its initial influent concentration 5.6–6.8 mg/L. This augmentation is attributed to the enzymatic and microbial action of earthworms. The

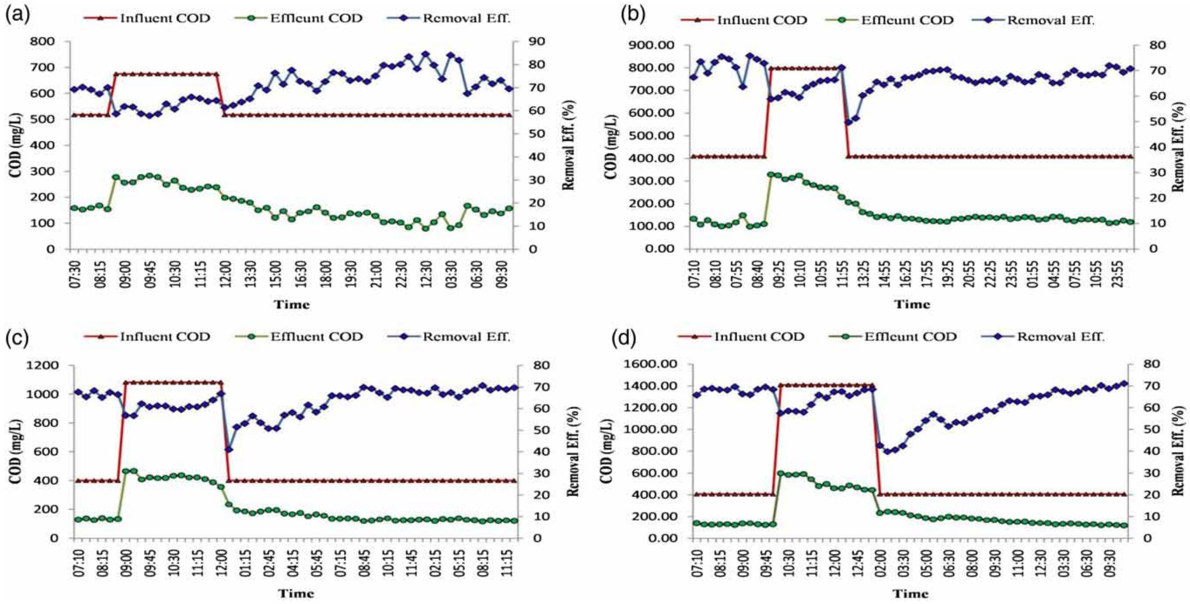

Figure 2 | Variation of COD in effluent of VF at different organic shock loads (a) 675, (b) 799, (c) 1,084 and (d) 1,410 mg COD/L.

Table 4 | Effluent COD at different depth

Time (min)	Depth (mm)	Effluent COD at different depth (C_0)
0	200	500
32	150	340
78	150	199
137	150	175
180	150	95

activities of earthworm and associated microbes in VF bed promote rapid phosphorous mineralization in the system causes increased concentration of phosphate in the effluent (Kumar *et al.* 2014). It is evident from the previous study conducted by Lee (1992) that when organic matter passes through the gut of earthworm, some amount of phosphorus gets converted into phosphate making it more bioavailable to the plants. The researchers have also demonstrated that this bioavailable form of phosphate is contributed by enzymatic activity of phosphatases in the gut of earthworms and further release of phosphate might be attributed to the P-solubilizing microorganism present in worm cast (Tchobanoglous *et al.* 2003; Kumar *et al.* 2015, 2015b). The similar results have been evident in our previous studies (Kumar *et al.* 2015, 2015b). During shock load conditions, phosphate mineralization identified slightly lesser and TP concentration remained in the range of 8–10.4 mg/L.

In VF, the higher removal of TSS concentration, around 82%, was observed. In effluent, the TSS concentration was observed as 42 mg/L from its initial influent TSS concentration of 243 mg/L. This could be attributed to the ingestion of organic and inorganic solid particles in wastewater through earthworms which excrete them as finer particles. These finer particles are further trapped in the voids of VF and causes high removal efficiency of TSS from wastewater (Sinha *et al.* 2008; Kumar *et al.* 2014).

Kinetic study of COD removal in VF

Kinetic study for removal of COD in VF was performed at fixed influent concentration, i.e. 500 mg/L and at fixed HRT of 3 h. For this purpose, effluent samples from VF were collected at fixed time intervals (at different depth of VF) during HRT and analyzed for COD determination. Data obtained from experimental evaluation, as illustrated in Table 4, were fitted in various linear equations of reaction rates. Different plots of kinetic studies for COD removal are shown in Figure 3. From the results, it could be interpreted that the biological degradation of organics follows first-order kinetics (Figure 3) with respect to initial COD concentration. The rate constant (k) for different orders of the COD removal was calculated from the slopes of the linear plots and presented in Figure 3. The R^2, which is an index of the goodness-of-fit, was found highest for the first-order model with a value of 0.951. From the correlation coefficient, it is assumed that the COD removal follows first-order kinetics better compared to others under the

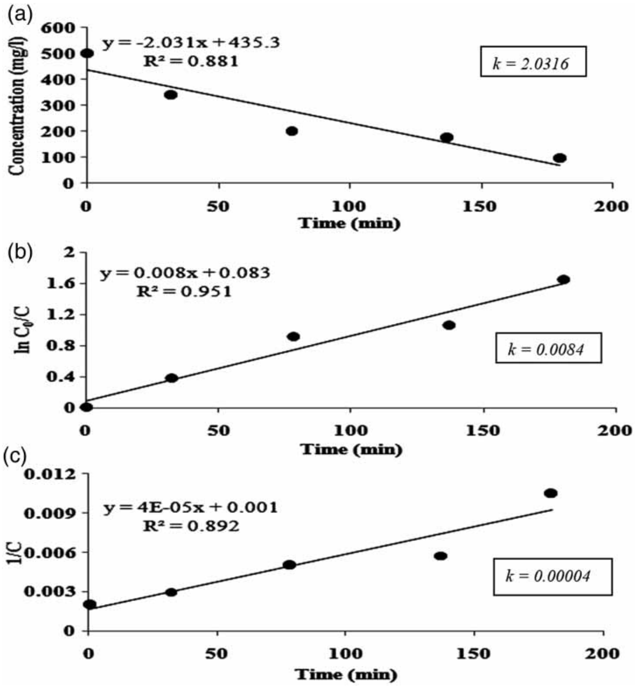

Figure 3 | Kinetics for COD removal (a) zero-order, (b) first-order, (c) second-order (influent COD = 500 mg/L; HRT = 3 h); k, rate constant; R^2, coefficient of regression; C_0 = conc. at zero time; C = conc. at time t; t = time.

Table 5 | Characteristics of vermicompost in VF

Parameter	Initial characteristics of vermicompost	Final characteristics of vermicompost
pH	7.3–7.6	7.4–7.8
C/N ratio	10.57–10.76	9.47–10.5
TOC (g/Kg)	282–296	294–305
Total nitrogen (g/Kg)	26.2–28	30–32.2
TP (g/Kg)	23.4–25	28–30
Ash content (%)	49–51	53–56

described experimental conditions (Suthar & Singh 2008; Singh *et al.* 2015b; 2015c).

Quality of vermicompost

The initial and final characteristics of vermicompost in VF have been illustrated in Table 5. In VF, the end product as vermicompost, collected from the top layer of VF, was dark brown in color and C/N ratio quantified as 9.47–10.5 which revealed stability of waste that deposited after vermifiltration process. However, the value of TOC slightly increased due to deposition of organic matter on the top surface which finally augmented the ash content and identified as 53–56% from its initial value 49–51%. The TN and TP increased in final vermicompost found on top layer and observed as 30–32.2 and 28–30 g/Kg at the end of the run due to the activity of earthworm. The value of pH in final vermicompost varied in the range of 7.4–7.8 and considered as almost neutral condition (Hughes *et al.* 2007; Sinha *et al.* 2008).

Earthworm growth and reproduction

During the vermifiltration process, the earthworm biomass plays an important role for evaluating its long-term performance. At the end of the experiment, the number of earthworms were found to be increased by about 986, while the weight of earthworms was observed as 549 g, that revealed about 23.2% increase in earthworm biomass on number basis, while on weight basis it could be 22%. This may be attributed to continuous substrate (organic matter) availability to the earthworms and better environmental conditions for their propagation inside VF. This indicates that the designed system capable to be highly resilient to the organic shock loads up to three times the average organic loading, as there was no significant change noted on the performance of the system during the applied shock loads.

CONCLUSIONS

Based on the experimental analysis, the following can be concluded:

- The performance of a pilot-scale VF, employing *E. fetida* earthworm and using synthetic wastewater as feed, was investigated under 1.5–3-fold short-term organic shock loads at optimum HRT. Four different organic shock loads were undertaken in this study and each shock load was applied for a period of 3 h HRT, after which normal loading conditions were resumed.
- The maximum effluent COD concentration were 229, 231, 357 and 451 mg/L under the shock loads of 675, 799, 1,084 and 1,410 mg COD/L, respectively. The maximum (71%) COD removal was observed at an organic loading rate of 1.04 kg/m^2/d (799 mg COD/L). The vermifiltration system regained stability quickly after application of applied perturbations of organic matter concentration. The results of the COD kinetic study

suggested compliance of the well-known first-order model with organic matter degradation in VF.

- The population of earworms was found to be increased at the end of experimental study. An enhanced concentration of TN and TP in vermicompost suggests that it can be utilized as a nutrient rich product for plants growth. The results obtained in this study indicated that VF employing *E. fetida* is capable of absorbing the organic shock loads being experienced generally by wastewater treatment plants. This study also promotes an understanding of organic matter removal in the system and experimental results can be used for estimating treatment efficiency of full-scale reactors under similar operational conditions.

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