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ORIGINAL PAPER

Is filter packing important in a small-scale vermifiltration process of urban wastewater?

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Abstract Nowadays, natural resources are under increasing stress which fosters wastewater reuse planning and emphasizes on the decentralized wastewater treatment. Vermifiltration has been described as a viable alternative to treat domestic and urban wastewater, but few studies have focused on the impact of different filter packings on vermifiltration performance. This study evaluates the effect of vermicompost and sawdust in a single-stage vermifilter (VF) for urban wastewater treatment. After an acclimation period of 45 days, urban wastewater from a combined sewage collection system was applied continuously for 24 h. Earthworm stock density was of 20 g L^{-1}, HRT of 6 h, HLR of 0.89 $m^3 m^{-2} day^{-1}$ and OLR of 7.38 g BOD_5 day^{-1}. System performance was assessed by the removal efficiencies of BOD_5, COD, TSS, NH_4^+, TN and TP, and fecal coliforms and helminth eggs elimination. Vermicompost (VE) and sawdust (SE) were tested, using an earthworm abundance of 20 g L^{-1}. Treatment efficiencies were 91.3% for BOD_5, 87.6% for COD, 98.4% for TSS and 76.5% for NH_4^+ in VE, and 90.5% for BOD_5, 79.7% for COD, 98.4% for TSS and 63.4% for NH_4^+ in SE. Earthworms contributed to reduce NH_4^+ and TN removal and to increase NO_3^- concentration. No treatment was able to eliminate fecal coliforms down to guidelines values for wastewater irrigation as helminth eggs were completely eliminated. Single-stage vermifiltration system using both filter packings is inconsistent and cannot meet EU guideline values for discharge in sensitive water bodies and WHO guidelines for irrigation with treated wastewater.

Keywords Wastewater · Vermifiltration · Vermicompost · Sawdust · *Eisenia fetida*

Introduction

Due to increasing world population, the natural resources are under increasing stress which fosters wastewater reuse planning and emphasizes on the decentralized wastewater treatment, especially in rural areas where high wastewater collection and treatment costs do not justify the installation of conventional wastewater treatment plants (WWTP) (Prasad and Kumar 2012). Decentralized wastewater treatment systems involve the collection, treatment, disposal and reuse of wastewater from households, clusters of homes and isolated communities, at or near the point of generation (Li et al. 2009). Commonly, wastewater treatments must be able to reduce organic matter and nutrients concentration, but also promote *fecal* microorganisms' elimination (George et al. 2002).

Vermifiltration has been described as a viable alternative to treated domestic wastewater in small clusters with good applicability in developing countries and in remote locations. Is a bio-oxidative process in which earthworms interact intensively with microorganisms within the decomposer community, increasing the stabilization of organic matter and greatly modifying its physical and biochemical properties (Liu et al. 2012), combining filtration processes with vermicomposting techniques. Applications include small pilot-scale tests, households and

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small WWTP, opening new opportunities for treating domestic, urban wastewater and industrial wastewater due to the low cost and sustainable nature (Sinha et al. 2008).

Earthworm species and filter media types are crucial influencing factors for the removal efficiency of vermifiltration because they are considered as the main biological components of the process and can change directly or indirectly the main removal processes of contaminants over time (Sinha 2010).

The design parameters of vermifilters (VFs) include stocking density of earthworms (Sinha et al. 2008), filter media composition (Cardoso-Vigueros et al. 2013), hydraulic loading rate (HLR) (Kumar et al. 2015) and hydraulic retention time (HRT) (Arora et al. 2014a, 2016). Studies have been made with earthworm densities of 10 g L^{-1} (Arora et al. 2014b), 30 g L^{-1} (Arora et al. 2016) and intermediate values of 22.0 to 24.5 g L^{-1} (Tomar and Suthar 2011) (Table 1). Typical HRT varies between 6 and 9 h and HLR between 2.0 and 3.0 m^3 m^{-2} day (Xing et al. 2005).

Vermifilter packing material is an important design parameter for maximizing the treatment efficiency Arora et al. (2014b). Filter medium materials should facilitate natural aeration (Cardoso-Vigueros et al. 2013) and also serve as a dwelling habitat for earthworms to thrive and perform their function proficiently. Common filter packing materials include vermicompost (Arora et al. 2014b, 2016), wood chips, bark, peat, straw (Li et al. 2008) and sawdust (Lourenço and Nunes 2017) for organic packing, and gravel, quartz sand (Lourenço and Nunes 2017), river bed gravel, mud balls, glass balls (Kumar et al. 2015), ceramsite (Xing et al. 2010) and coal for inert packing (Wang et al. 2010). Filter packings specific surface area and porosity of filter packing materials have also been reported to impact treatment performance (Toffey 2008). Besides, specific surface area and porosity of filter packing can affect the treatment performance of VF (Kumar et al. 2015).

In recent years, several studies regarding the removal of organic matter, nutrients and pathogens from domestic and urban wastewater using vermifiltration have been published. However, few have focused on the impact of different filter packings on vermifiltration performance.

The present study focuses on the evaluation of the performance of vermifiltration for the treatment of urban wastewater, studying sawdust and vermicompost as filter packing materials, considering a practical case study.

Materials and methods

Raw wastewater

The wastewater used in the study came from the urban WWTP of Messines, Algarve, with a served population of 6000 inhabitants which receives wastewater from a combined sewage collection system designed to transport both rain water and sewage together. All samples were collected

Table 1 Reported wastewater type, origin and operational parameters

Parameter	Sinha et al. (2008)	Cardoso-Vigueros et al. (2013)	Arora et al. (2014b)	Arora et al. (2016)	Kumar et al. (2015)	Tomar and Suthar (2011)
Wastewater type	Municipal wastewater	Domestic wastewater	Synthetic wastewater	Synthetic wastewater	Synthetic wastewater	Urban wastewater
Wastewater origin	WWTP	Toilets	Locally produced	Locally produced	Locally produced	Wastewater stream
Filter packing material	Garden soil, gravel	Domestic organic wastes, vermicompost, volcanic stones, gravel	Vermicompost, sand and gravel	Vermicompost, riverbed gravel, gravel	Vermicompost, riverbed gravel	Stones, sawdust, dried leaves, soil mixed with stones and pebbles
Earthworms species	Mix of *Eisenia fetida*, *P. excavatus* and *Eudrillus euginae*	*Eisenia* spp.	*Eisenia fetida*	*Eisenia fetida*	*Eisenia fetida*	*Perionyx sansibaricus*
Stock density of earthworms (g L^{-1})	10	10	18	30	16.5	22–24.5
HRT (h)	1–2	0.18	–	6	–	–
HLR (m^3 m^{-2} day)	–	–	1.3	1.0	2.5	–

on May 13 after the preliminary wastewater treatment. Wastewater used in the study was the same wastewater used in all experiments. No rain was registered during the days before wastewater collection. Wastewater physical–chemical and microbiological characterization is shown in Table 2.

Reactor structure

Reactor modules were constructed in PVC containers with a total volume of 25 L (Fig. 1) closely following the treatment scheme used in previous studies (Taylor et al. 2003). Experiments were made using vermicompost produced from municipal organic solid waste as the filtering material provided by a specialized company (FUTURAMB®). Vermicompost occupied the top 16.0 cm, underneath which was installed an inert filter constituted of 7.0 cm of gravel and 6.0 cm of quartz sand. Percolating water was collected in an equalizer located below the filtering materials. Experiments were made using vermicompost produced from municipal organic solid waste as the packing material, and sawdust produced in a local woodshop which was easily available and could be utilized without any prior treatment. Reactors were covered with a lid, leaving sufficient room and opening as to allow natural aeration. An irrigation system was attached on top of the vermifilter made from 0.5-cm-diameter regular network of HDPE flexible plastic pipes. Pipes were perforated with 0.2-cm-diameter holes separated by 2.0 cm, for wastewater irrigation, and were kept 3 cm above the filter surface to ensure optimal wastewater distribution, the creation of drop overflow, and thereby increase aerobic conditions. Gravel was separated from the equalizer by a stainless steel mesh (diameter = 0.4 cm). Quartz sand was separated from gravel and from vermicompost or sawdust by a stainless steel mesh (diameter = 80 μm). Physical–chemical characterization of vermicompost and sawdust is shown in Table 2. Parameters were determined by a commercial laboratory. The effluent from each VF was collected in the equalizer from where samples were taken. From here, recirculation was made with the help of a pump (Q_r) and mixed with raw wastewater as (Q_w) to be feed to the top of the filters ($Q_w + Q_r$).

Table 2 Characterization of the vermicompost and sawdust filter media (mean concentration ± standard deviation)

Parameter	Vermicompost	Sawdust
Bulk density (kg m^{-3})	600 ± 0.00	238.66 ± 0.00
Porosity (%)	73.7 ± 0.30	84.0 ± 0.00
Particle size (mm)	<0.1–3.0	<0.1–6.0
pH (H_2O)	6.82 ± 0.01	5.38 ± 0.06
EC (μS cm^{-1})	2530 ± 2.00	99.0 ± 1.20
Organic matter (%)	56.48 ± 0.01	77.4 ± 0.05
TOC (%)	32.76 ± 0.04	45.0 ± 0.05
TN (%)	3.64 ± 0.02	0.50 ± 0.05
C/N ratio	9.0 ± 0.03	90.0 ± 0.00
TP (mg kg^{-1})	3769 ± 0.4	<0.05
TK (mg kg^{-1})	7150 ± 0.08	0.11 ± 0.01

Process acclimation

Moisture content was held constant after placing the reactors to field capacity following procedures used by the company that provided the earthworms and the filter packing for acclimation of the earthworms. For this purpose, filters were flushed with recirculating water for 30 days. After this time, VF was flushed and recirculated permanently for 45 days with wastewater collected from urban WWTP of Messines, Algarve, to allow the growth of heterotrophic microorganisms in the filter packing. Each filter was fed, by pumping raw wastewater from a PVC container. The flow was also adjusted to permit the optimum moisture conditions for the survival of the earthworms.

Experimental design and operational conditions

After filter acclimation, four treatments were tested for packing material, identified as filter using vermicompost without earthworms (V), filter using vermicompost with the addition of earthworms (VE), filter using sawdust without earthworms (S), and filter using sawdust with the addiction of earthworms (SE). Influent wastewater flow, Q_w, and recycling flow, Q_r, were adjusted to obtain a constant Q_{mix} equal to 0.04 m^3 day^{-1}, as this was the optimal flow for maintaining the ideal moisture of the filter. Hydraulic retention time (HRT) was fixed at 6 h following previous experiments not shown here. Experiments were made for a period of 24 h with permanent continuous wastewater recirculation. Samples for chemical analysis were taken at the onset of the experiment from the influent wastewater and at the end of the treatment period from the treated effluent. Organic loading rate (OLR) was measured as g BOD_5 day^{-1}. Recirculation ratio was related to Q_r and Q_w and was fixed at 0.72. These parameters were determined using the following equations.

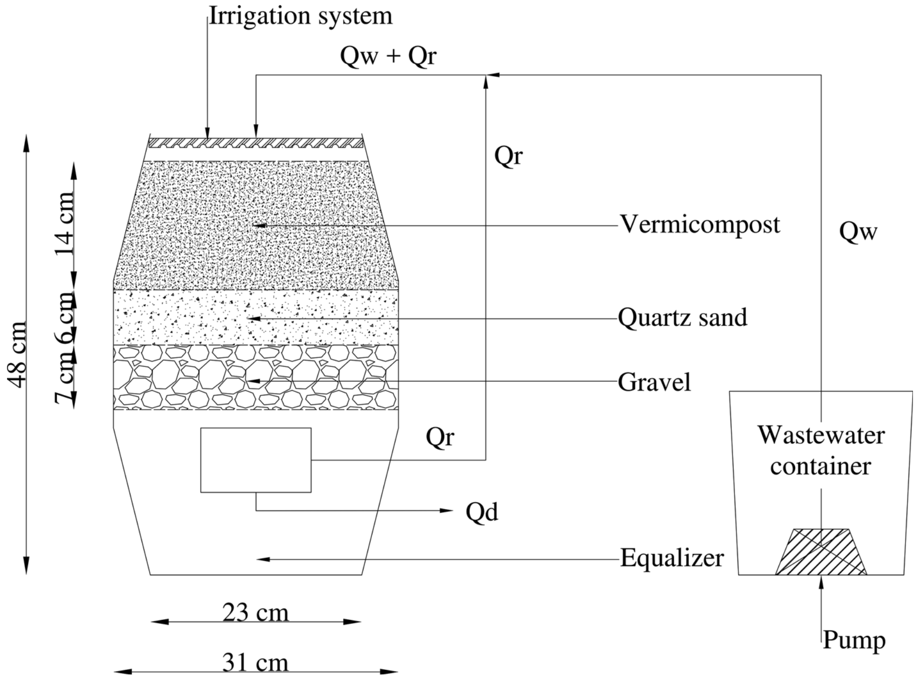
$$\mathrm{HRT} = V/Q_w \tag{1}$$

$$\mathrm{OLR} = Q_w \times \mathrm{BOD}_5 \tag{2}$$

$$\mathrm{HLR} = Q_w/A \tag{3}$$

$$\text{Recirculation ratio} = Q_r/Q_w \tag{4}$$

Fig. 1 Reactor unit design



where V (m^3) is the volume of the reactor, Q_w ($m^3\ day^{-1}$) is the influent wastewater flow rate, BOD_5 ($mg\ L^{-1}$) is the organic matter concentration in influent wastewater, HLR is the hydraulic loading rate ($m^3\ m^{-2}\ day^{-1}$), Q_{mix} ($m^3\ day^{-1}$) is the sum of Q_w and recirculating flow, Q_r (Table 3), and A (m^2) is the reactor's surface area.

Eisenia fetida (Bouché 1972) is one of the most commonly used species for soil pollution and vermifiltration research (Taylor et al. 2003). It has been shown to process organic solid wastes with high efficiency, be very proficuous, and can adapt to various environmental factors, including temperature and moisture levels (Edwards and Arancon 2004). The earthworms were provided by a company specialized in vermicomposting (FUTURAMB®) and previously installed on plastic boxes with coffee grounds at adequate moisture content for 15 days. No signs of disease and stress in the individuals were found. A stocking density of 20 g L^{-1} was used, following previous unpublished studies made at FUTURAMB®. The individuals were placed on the top of the organic filter material and were allowed to install for an acclimation period of 15 days.

During experiments, wastewater was applied continuously for 24 h. All filters were frequently monitored for foul odors, smooth percolation of wastewater through the vermicompost, and clogging. General earthworm behavior, including agility, movement, stress and health conditions, was also monitored. After this period, 200 cm^3 of treated wastewater samples was collected from the equalizer and kept in the cold (4 °C) until analysis.

Sampling and chemical and microbiological analysis

For each treatment, samples were obtained at the beginning and at the end of the treatment. Samples of raw wastewater were taken from the feeding tank, and samples of treated wastewater were taken from the equalizers (Fig. 1). All samples were analyzed immediately after sampling for pH, EC, five-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS), NH_4^+, NO_2^-, NO_3^-, total nitrogen (TN), PO_4^{3-}, total phosphorus (TP), fecal coliforms (FC) and helminth eggs. For the analysis, 5 L on each treatment was collected from the equalizer and three replicates were made for each parameter.

pH and EC were analyzed using a HANNA HI98129 meter with a precision and range ±0.01 and 0.00–14.00 for pH, ±2% and 0.0–3999 to µS cm^{-1} for

Table 3 Hydraulic parameters used in the experiments

Q_{mix} ($m^3\ day^{-1}$)	Q_r ($m^3\ day^{-1}$)	Q_w ($m^3\ day^{-1}$)	Q_r/Q_{mix}	HRT (h)	HLR ($m^3\ m^{-2}\ day^{-1}$)	OLR (g BOD_5 day^{-1})
0.13	0.09	0.04	0.72	6	0.89	7.38

EC and the later converted automatically by the equipment do total dissolved solids (TDS) in the range 0–2000 mg L^{-1}. This later parameter was obtained from EC by a conversion factor of 0.5. BOD_5 was analyzed using an OxiTop®-C respirometric system with incubation at constant temperature for 5 days (APHA 1998) with a precision, and range of ±1%. tCOD was analyzed with a photometer (NOVA 60, Merck) with a precision and range of ±5.0 mg L^{-1} and 25–1500 mg L^{-1} based on the permanganate method (APHA 1998). Dissolved fractions were determined after filtrating through a Whatman® 40-µm cellulose filter paper as dissolved COD (sCOD). Particulate COD fraction, pCOD, was obtained as the difference between total, tCOD, and soluble, sCOD. TSS was determined by filtrating the sample through a Whatman® 40-µm cellulose filter paper, drying to a constant weight at 105 °C, and weighting (APHA 1998). NH_4^+ was quantified by photometry using a HANNA HI733 m with a precision and range of ±1.0 mg L^{-1} and 0.0–99.9 mg L^{-1}, respectively. NO_2^- was analyzed with a HANNA HI708 photometer based on the ferrous sulfate method with a precision and amplitude range of ±3.0 mg L^{-1} and 0–150 mg L^{-1}, and NO_3^- was analyzed with a HANNA HI96786 photometer based on the cadmium reduction method with a precision and range of ±5.0 mg L^{-1} and 0–100 mg L^{-1}. TN analysis was performed through oxidative digestion of all nitrogenous compounds to nitrate based on the persulfate method using (APHA 1998). PO_4^{3-} analysis was made using a HANNA HI717 photometer based on the heteropolymolybdenum blue method with a precision and range of ±1.0 mg L^{-1} and 0–30 mg L^{-1}. TP was obtained by oxidative digestion of organic matter followed by a colorimetric reaction based on the ascorbic acid method (APHA 1998). FC were analyzed based on membrane filtration, subsequent culture on a chromogenic coliform agar medium with determination by the most probably number (MPN) per 100 mL^{-1}, and *Ascaris lumbricoides* were analyzed as the number of target organisms in the sample (Number 100 mL^{-1}) (APHA 1998). FC removal efficiency (K_{FC}) was calculated using Eq. (5) as proposed by Arora et al. (2014a), where C_i and C_f are the wastewater FC initial and final FC, respectively.

$$K_{FC} = \mathrm{Log}_{10}(C_i/C_f) \quad (5)$$

Statistical analysis

One-way analysis of variance (ANOVA), followed by Tukey's test at a significance level of $\alpha = 0.05$, was made to check for differences between treatments. *T* test was also performed to compare means. The statistical package SPSS® 17.0 was used in the analysis.

Results and discussion

Earthworms showed good survival in the filter using vermicompost (VE) and in the filter using sawdust (SE) during the whole experiment, as individuals accommodated to the experimental conditions with no evidence of decrease in population numbers. Individuals meandered throughout all the volume of the organic filter packing, while not trying to escape, meaning that wastewater was not toxic and the environment was suitable. In our study, during the first 15 h, wastewater percolated smoothly into all reactors, but some clogging was observed in the control filter using vermicompost without earthworms (V) after that period, as indicated by an abnormal accumulation of wastewater on the surface of the filter bed. No clogging was reported in the remaining filters during the whole experiment: vermicompost with earthworms (VE), control filter with sawdust without earthworms (S), and filter with sawdust and earthworms (SE).

The ratio BOD_5/COD is one important way to assess the biodegradability of wastewater, as in a raw urban wastewater the BOD_5/COD ratio varies between 0.3 and 0.8 (Tchobanoglous et al. 2003). Besides, with a BOD_5/COD ratio of 0.5, wastewater is considered to be easily treatable by biological processes (Tchobanoglous et al. 2003). Also, the common BOD_5/COD ratio in a treated wastewater varies between 0.1 and 0.3 (Henze and Comeau 2008). In a typical urban wastewater, BOD_5, COD and TSS have average concentrations of 350, 750 and 400 mg L^{-1}, respectively (Henze and Comeau 2008) (Table 4). Comparing our results with the results from the literature (Table 4), BOD_5 (210 ± 10.0 mg L^{-1}), COD (450 ± 10.0 mg L^{-1}) and TSS (158 ± 3.46 mg L^{-1}) were all lower than published ones. This could be justified by solids sedimentation in the PVC container during the study. The BOD_5/COD ratio found for the wastewater was 0.47 indicating good biodegradability (Tchobanoglous et al. 2003).

The content of the individual nutrients in wastewater should correspond to the bacteria needs, and there should be a balanced relationship between carbon, nitrogen and phosphorus, as this is crucial to the effectiveness of the biodegradation processes. The concentration of NH_4^+ (46.4 ± 0.26 mg L^{-1}) obtained was similar to values referred by Henze and Comeau (2008) of 45 mg L^{-1}, supporting the argument that it could be mainly from domestic sources as urine or cleaning agents. Nearly 75% of the TN in a typical urban wastewater is NH_4^+ and the majority (70–90%) comes from urine, while the final 20% comes from cleaning agents, disinfectants and food wastes (Hughes et al. 2008). *Fecal* coliforms concentration ($5.7 \times 10^8 \pm 3.98 \times 10^1$ MPN 100 mL^{-1}, Table 4) was relatively high if compared with the literature (George et al. 2002).

Table 4 Characterization of influent wastewater and typical values from literature data

Parameter	Value[a]	Typical values		
		Henze and Comeau (2008)	USEPA (2004)	Tchobanoglous et al. (2003)*
pH	8.48 ± 0.03	n.a.	n.a.	n.a.
BOD_5 (mg L^{-1})	210 ± 10.0	350	221	300
COD (mg L^{-1})	450 ± 10.0	750	580	650
TDS (mg L^{-1})	532 ± 5.00	n.a.	n.a.	n.a.
Turbidity (NTU)	148.3 ± 7.51	n.a.	n.a.	n.a.
TSS (mg L^{-1})	158 ± 3.46	400	243	500
NH_4^+-N (mg L^{-1})	49.4 ± 0.31	45	9	n.a.
NO_2^--N (mg L^{-1})	2.0 ± 0.75	0.2	$\Sigma < 1$	n.a.
NO_3^--N (mg L^{-1})**	0.2 ± 0.12			n.a.
TN (mg L^{-1})	68.3 ± 0.31	60	51	70
PO_4^{3-}-P (mg L^{-1})	16.3 ± 0.75	10	n.a.	n.a.
TP (mg L^{-1})	5.7 ± 0.12	15	9	15
BOD_5/tCOD	0.47 ± 0.03	0.47	0.38	0.44
COD/NH_4^+-N	9.1 ± 0.23	16.7	64.4	17.2
FC (MPN 100 mL^{-1})	$5.7 \times 10^8 \pm 3.98 \times 10^1$	1.0×10^{12}**	1.0×10^7	2.2×10^6
Helminth eggs (No. L^{-1})***	8.00 ± 6.24	13	n.a.	n.a.

n.a. Not available

* Average concentration

** Converted by mass equation

*** As total coliforms

**** As *Ascaris lumbricoides* eggs

[a] Mean concentration ± standard deviation

Treatments showed a good efficiency for removing BOD_5, COD and TSS from wastewater (Tukey's test, $p < 0.05$, Table 5). BOD_5, COD and TSS values in all treatments met the EU standards (Directive 91/271/EEC, May 21, 1991) for wastewater discharge, namely of 25 mg L^{-1}, or a minimum removal of 70–90% for BOD_5, 125 mg L^{-1} or a minimum removal of 75% for COD and 35 mg L^{-1} or a minimum removal of 90% for TSS. Removal efficiencies for BOD_5 were 91.27 ± 0.55% in VE, 96.19± 0.00% in V, 90.48% in SE and 92.06% in S as removal efficiencies for COD were 87.56 ± 0.45% in VE, 86.67 ± 0.89% in V, 79.70 ± 0.92% in SE and 77.63 ± 1.80% in S. As for TSS, removal efficiencies were 98.42 ± 0.00 ± 0.55% in VE, V, SE and S (Table 5). Dissolution of earthworm castings may have contributed to increase BOD_5 values in VE and SE (18.33 ± 1.15 mg L^{-1} and 8.00 ± 0.00 mg L^{-1}) compared to V and S (20.0 ± 1.00 mg L^{-1} and 16.67 ± 1.15 mg L^{-1}). Vermifiltration contributed to higher COD removal in treated wastewater (56.0 ± 2.0 mg L^{-1} in VE and 91.33 ± 4.2 mg L^{-1} in SE, Table 5). COD removal efficiency was lower compared to BOD_5 (91.27% in VE and 90.48% in SE for BOD_5 and 87.56% in VE and 79.70% in SE for COD, Table 5), due to the fact that earthworms are mainly responsible for the removal of biodegradable substances. In comparison, Sinha et al. (2008) reported removal of TSS in the ranges of 90–92 and 90–95%, for COD and BOD_5, respectively. Xing et al. (2010) reported that the presence of earthworms was responsible for about 57–79% reduction in TSS in wastewater, which was lower than the values obtained here. The vermifilter system with sawdust was less efficient in reducing turbidity from wastewater (2.28 NTU ± 0.08 in SE and 1.17 NTU ± 0.14 in S), and earthworms in fact contributed to increase turbidity (3.94 NTU ± 0.16 in VE and 2.28 NTU ± 0.08 in SE) comparing to the systems without earthworms (V and S) (Table 6).

Earthworms significantly degrade the wastewater organics by enzymatic action in their gut, helping in the degradation of several compounds which could not be decomposed by microorganisms (Sinha 2010; Malek et al. 2012). This may explain the higher COD efficiencies obtained in VE and SE, where microbial stimulation, biodegradation and enzymatic degradation of solid wastes by earthworms work simultaneously (Sinha 2010). In fact, vermifiltration is effective due to the biological, physical and chemical reactions, including the adsorption of molecules and ions, oxidation–reduction in organic matter, and

Table 5 Parameters and efficiencies[1] for the different treatments

Parameter	Raw wastewater	Experiment							
		VE	η (%)	V	η (%)	SE	η (%)	S	η (%)
BOD_5 (mg L^{-1})	210 ± 10.0	18.33^{bc} ± 1.15	91.27^{ab} ± 0.55	8.00^{a} ± 0.00	96.19^{c} ± 0.00	20.0^{c} ± 1.00	90.48^{a} ± 0.48	16.67^{b} ± 1.15	92.06^{b} ± 0.55
COD (mg L^{-1})	450 ± 10.0	56.0^{a} ± 2.00	87.56^{b} ± 0.45	60.0^{a} ± 4.00	86.67^{b} ± 0.89	91.33^{b} ± 4.20	79.70^{a} ± 0.92	100.67^{b} ± 8.10	77.63^{a} ± 1.80
TSS (mg L^{-1})	532 ± 5.00	2.5^{a} ± 0.00	98.42^{a} ± 0.00	2.5^{a} ± 0.00	98.42^{a} ± 0.00	2.5^{a} ± 0.00	98.42^{a} ± 0.00	2.5^{a} ± 0.00	98.42^{a} ± 0.00
NH_4^+ (mg L^{-1})	49.4 ± 0.31	11.60^{c} ± 0.15	76.51^{b} ± 0.24	8.57^{b} ± 0.32	82.64^{c} ± 0.50	18.08^{d} ± 0.76	63.40^{a} ± 1.19	2.54^{a} ± 0.31	94.86^{d} ± 0.48
TN (mg L^{-1})	68.3 ± 0.31	60.0^{d} ± 3.00	12.20^{a} ± 4.40	22.0^{c} ± 0.00	67.80^{b} ± 0.00	9.3^{b} ± 0.58	86.34^{c} ± 0.84	2.0^{a} ± 0.00	97.07^{d} ± 0.00
TP (mg L^{-1})	5.7 ± 0.12	11.7^{b} ± 0.58	-105.88^{a} ± 10.18	11.7^{b} ± 0.58	-105.88^{a} ± 10.18	0.6^{a} ± 0.00	89.41^{b} ± 0.00	0.065^{a} ± 0.00	98.85^{b} ± 0.00

[1] Mean concentration ± standard deviation. Values followed by the same letter within each line are not significantly different (ANOVA; Tukey's test, $\alpha = 0.05$)

the synergetic effects of earthworms with microorganisms (Bouché and Soto 2004).

The higher removal BOD_5 and COD efficiencies in VE compared to SE may be related to the higher C/N content in sawdust compared to that of vermicompost (45.0 ± 0.05% to 32.76 ± 0.04%, Table 2) since more carbon content (as carbonaceous BOD_5) from sawdust may have been released to the wastewater. Specific surface area and porosity of filter packing are one of the factors that affect the treatment performance of biological filtration (Toffey 2008). A filter packing with low granulometry improves biomass accumulation and attains higher treatment efficiency as compared to the performance of media with low specific surface area (Taylor et al. 2003). Since vermicompost has lower granulometry compared to sawdust (Table 2), its higher specific surface may have created better conditions for microorganisms to survive and grow. This could justify the significantly higher BOD_5 removal efficiencies (Tukey's test, $p < 0.05$) in VE (91.27 ± 0.55%) and V (96.19 ± 0.00%). As organic solid particles are retained in the pores of the filter packing, high removal efficiencies for TSS are usually obtained (Sinha et al. 2008). In our experiments, there was no significant difference in TSS removal efficiency in systems with or without the presence of earthworms (Tukey's test, $p > 0.05$), indicating that the removal process is essentially physical.

In our study, compared with raw wastewater (46.4 ± 0.26 mg L^{-1}), NH_4^+ concentrations decreased in all experiments (11.60 ± 0.15 mg L^{-1} in VE, 8.57 ± 0.32 mg L^{-1} in V, 18.08 ± 0.76 mg L^{-1} in SE and 2.54 ± 0.31 mg L^{-1} in S, Table 5). Vermifiltration contributed to decrease NH_4^+ removal efficiency (Tukey's test, $p < 0.05$) (76.51 ± 0.24% at VE and 63.40 ± 1.19% at SE) as V had an efficiency of 82.64 ± 0.50% and S had an efficiency of 94.86 ± 0.48% (Table 5). NH_4^+ is generated by organic nitrogen mineralization leading to ammonia emissions being the first inorganic nitrogen form produced during biological wastewater treatment (Henze and Comeau 2008). Vermicasts increase nutrient content in soil (Edwards et al. 2011) as N cycling is directly influenced by earthworms. In their studies, Kadam et al. (2009) concluded that NH_4^+, as the dominant type of N in domestic wastewater, was removed through rapid adsorption by the filter packing and subsequently converted into NO_3^- through nitrification. The increase in NH_4^+ on VE and SE compared with V and S may be due to the ion leachate from earthworm castings during treatment. Besides, vermicompost packing may have contributed to increase NH_4^+ due to the fact the vermicompost is mainly constituted by earthworm castings and is rich in heterotrophic bacteria which increase organic nitrogen mineralization (Sinha et al. 2008). Also, the excess of ammonium

Table 6 BOD_5/COD and COD/NH_4^+-N, nutrient concentration[1], and pH, TDS and turbidity[1] for the different treatments

Parameter	Raw wastewater	Experiment			
		VE	V	SE	S
BOD_5/COD	0.47 ± 0.03	$0.33^c \pm 0.03$	$0.13^a \pm 0.01$	$0.22^b \pm 0.02$	$0.17^{ab} \pm 0.02$
COD/NH_4^+-N	9.1 ± 0.23	$4.8^a \pm 0.22$	$7.0^b \pm 0.55$	$5.1^a \pm 0.40$	$39.7^c \pm 1.21$
NO_2^- (mg L^{-1})	2.0 ± 0.75	$3.2^b \pm 0.68$	$3.5^b \pm 0.92$	$0.6^a \pm 0.60$	$0.7^a \pm 0.17$
NO_3^- (mg L^{-1})	0.2 ± 0.12	$4.9^c \pm 0.29$	$1.5^b \pm 0.21$	$0.0^a \pm 0.00$	$0.0^a \pm 0.00$
PO_4^{3-} (mg L^{-1})	16.3 ± 0.75	$10.7^c \pm 0.12$	$11.1^c \pm 0.25$	$1.3^b \pm 0.01$	$0.03^a \pm 0.06$
pH	8.48 ± 0.03	$7.85^a \pm 0.04$	$8.37^c \pm 0.02$	$8.22^b \pm 0.00$	$8.46^d \pm 0.01$
TDS	532 ± 5.00	$476^c \pm 3.51$	$418^b \pm 2.89$	$423^b \pm 1.00$	$363^a \pm 1.00$
Turbidity (NTU)	148.3 ± 7.51	$3.94^c \pm 0.16$	$4.73^d \pm 0.11$	$2.28^b \pm 0.08$	$1.17^a \pm 0.14$

[1] Mean concentration ± standard deviation. Values followed by the same letter within each line are not significantly different (ANOVA; Tukey's test, $\alpha = 0.05$)

in wastewater may contribute to earthworm' stress (Hughes et al. 2008). The former authors reported ammonium concentration of 25 mg L^{-1} in treated effluent after vermifiltration and a LC50 of 1.49 mg L^{-1} and a 0% survival rate above 2.0 mg L^{-1}. The low toxicity of ammonium in our study may be attributed to the rapid conversion of ammonium to nitrate.

All treatments contributed to decrease BOD_5/COD ratios (Tukey's test, $p < 0.05$), but in the presence of earthworms the BOD_5/COD ratios were higher (0.33 ± 0.03 and 0.22 ± 0.02 in VE and SE, and 0.13 ± 0.01 and 0.17 ± 0.02 in V and S, respectively (Table 6). This may be due to the release of dissolved organic compounds from the vermicastings. When comparing the two filter materials, no significant difference was found in BOD_5/COD ratio (Tukey's test, $p > 0.05$). Degradation of organic fractions of wastewater produces some acidic species of mineralized organic materials (CO_2, NH_4^+, NO_3^- and organic acids) which play an important role in shifting of pH of treated water. This may justify the decrease in pH in all treatments. Besides, vermifiltration contributed to decrease pH from raw wastewater (7.85 ± 0.04 at VE and 8.22 ± 0.00 at SE, Table 6). Edwards et al. (2011) and Arora et al. (2014b) have reported the influence of earthworms in making pH converge to neutrality in soil, solid organic wastes treatment and vermifiltration. Hughes et al. (2008) have also found that vermicompost as filter packing has high buffering capacity for pH.

Carbon-to-nitrogen ratio in raw wastewater plays an important role in wastewater treatment and is measured by the COD/NH_4^+-N ratio change (Cardoso-Vigueros et al. 2013). Vermifiltration had a significant influence in COD/NH_4^+-N ratio (Tukey's test, $p < 0.05$). For TN removal, rates by nitrification may be improved when carbon-to-nitrogen ratios in wastewater are in between 5:1 and 10:1 (Roy et al. 2010). The filter with vermicompost and earthworms showed the highest nitrification (Tukey's test, $p < 0.05$), as the lowest COD/NH_4^+-N, 4.8 ± 0.22 mg L^{-1}, was obtained in VE (Table 6). Nitrification coupled with denitrification seems to be the major N removal process involved in many vermifiltration systems, while insufficient available organic C (as COD) is considered to be responsible for the inhibition of denitrification (Sinha et al. 2008). NO_2^- is an intermediate product of nitrification, and its concentration in wastewater is usually negligible (Henze and Comeau 2008). Comparing vermicompost and sawdust, the first contributed do increase NO_2^- (3.2 ± 0.68 mg L^{-1} in VE and 3.5 ± 0.92 mg L^{-1} in V) and also to increase NO_2^- relatively to raw wastewater (0.2 ± 0.12 mg L^{-1}). No statistically significant difference was obtained between treatments and NO_2^- concentration (Tukey's test, $p > 0.05$, Table 6). In nitrification, the adsorbed NH_4^+ is subsequently converted to NO_3^-, carried out by autotrophic bacteria using molecular oxygen as an electron acceptor (Zhang et al. 2005). Nitrification step for NH_4^+ removal led to a substantial increase in NO_3^- in VE and V as no NO_3^- was found in SE and S (Table 6). NO_3^- concentration increased in the treatment using vermicompost with VE registering 4.9 ± 0.29 mg L^{-1} and V registering 1.5 ± 0.21 mg L^{-1} (Table 6), in comparison with raw wastewater (0.2 ± 0.12 mg L^{-1}) (Table 4). The presence of earthworms contributed to increase NO_3^- from 0.2 ± 0.12 mg L^{-1} in raw wastewater to 4.9 ± 0.29 mg L^{-1} in VE and 1.5 ± 0.21 in SE ($p < 0.05$, Table 6). Vermicompost is rich in nitrifying bacteria which help effluent mineralization (Sinha et al. 2008). This aspect is also supported by Cardoso-Vigueros et al. (2013) who found a positive correlation between earthworm density and nitrifying bacteria, helped by abundant oxygen due to the burrowing action of earthworms. At the same time, earthworms excrete polysaccharides, proteins and other nitrogenous compounds as they mineralize nitrogen in wastewater (Sinha 2010). The highest rates of mineralization occur in the vermicasts, which greatly enhances the availability of inorganic nutrients.

The presence of earthworms contributed to decrease TN removal efficiency (Tukey's test, $p < 0.05$) (12.40 ± 4.40% at VE and 86.34 ± 0.84% at SE) as V had an efficiency of 67.80 ± 0.00% and S had an efficiency of 97.07 ± 0.00% (Table 5). Also, when comparing both filter packings, vermicompost (VE and V) contributed to reduce TN removal efficiency (Tukey's test, $p < 0.05$, Table 5).

In the present study, PO_4^{3-} concentrations decreased in all treatments relatively to raw wastewater (Tukey's test, $p < 0.05$), while no statistically significant difference was obtained for PO_4^{3-} between VE and V (Tukey's test, $p > 0.05$, Table 6). The presence of earthworms did not help to improve TP removal as no statistically significant difference was obtained between treatments and TP (Tukey's test, $p > 0.05$, Table 5). Vermicompost contributed to increase TP concentration compared to raw wastewater (11.7 ± 0.58 mg L^{-1} in VE and 11.7 ± 0.58 mg L^{-1} in V) (Table 5). On the contrary, sawdust contributed to reduce TP from raw wastewater with 0.6 ± 0.00 mg L^{-1} in SE and 0.065 ± 0.00 mg L^{-1} in S (Table 5). Due to this, TP removal efficiencies in treatments using vermicompost were negative (−105.88 ± 10.18% at VE and −105.88 ± 10.18% at V) (Table 5). TP removal in SE and S suggests that sawdust may have contributed to remove organic and PO_4^{3-} from wastewater due to absorption of inorganic constituents by different biological or non-biological components. Moreover, in the filters with sawdust it was possible to observe a statistically significant difference between SE and S (Tukey's test, $p < 0.05$, Table 6) with SE ending with higher PO_4^{3-} concentration (1.3 ± 0.01 mg L^{-1}) than S (0.03 ± 0.06 mg L^{-1}). PO_4^{3-} increase during vermifiltration is due to enzymatic and microbial activity due to the presence of earthworms (Hait and Tare 2011). Increase in TP concentration during vermifiltration has been reported by other authors (Cardoso-Vigueros et al. 2013; Arora et al. 2016; Kumar et al. 2015). The vermicastings can increase the levels of nutrients in vermifilter effluents more significantly, as indicated above, which can explain the negative removal efficiencies obtained for TP in our study.

According to EU standards, VE and V exceeded total nitrogen and total phosphorus emission limits of 15.0 and 2.0 mg L^{-1}, respectively. According to this regulation, these two parameters are especially important in sensitive water bodies and fundamental nutrients responsible for eutrophication processes. Nutrient increase is also supported by the fact that earthworms contributed to increase ion concentration in treated effluent since TDS was 476 ± 5.31 mg L^{-1} in VF and 423 ± 5.31 mg L^{-1} in SE, compared with 418 ± 2.89 mg L^{-1} in V and 363 ± 1.00 mg L^{-1} in S. This is also supported by the fact that pH followed the mineralization process and oxidation of organic compounds as expected (Table 6).

Removal of pathogens is one of the main objectives when treating wastewater for discharge in water bodies or reuse for irrigation. Fecal coliforms typical concentration in raw wastewater is usually between 10^6 and 10^8 MPN 100 mL^{-1} depending on both raw wastewater composition and treatment efficiency (George et al. 2002). *Ascaris lumbricoides* eggs are a good indicator of parasitological quality as 99.9% of removal must be achieved (WHO 2006). All *fecal* coliforms (concentration, Log_{10} FC, K_{FC} and k_{FC}) and helminth eggs parameters during the study are given in Table 7. Log_{10} FC values were all between the values reported by WHO (2006) with 4.70 ± 0.01 in VE, 4.78 ± 0.03 in V, 4.72 ± 0.02 in SE and 3.26 ± 0.24 in S. No statistically significant difference was obtained for Log_{10} FC value between VE and V treatments (Tukey's test, $p > 0.05$). In filter with sawdust, vermifiltration did not contribute to decrease Log_{10} FC value (Tukey's test, $p > 0.05$). No statistically significant difference was obtained for K_{FC} and k_{FC} values between treatments (Tukey's test, $p > 0.05$). *Ascaris lumbricoides* eggs were all removed of 100% in all experiments (Table 7). Based on fecal coliforms concentration in raw wastewater and the maximum concentration permitted by WHO (2006) of 6–7 Log_{10} units for unrestricted irrigation, it is possible to predict that the minimal K_{FC} and k_{FC} values in the final effluent obtained from vermifiltration should be 5.91 and 11.70, respectively.

Working with vermifiltration, Arora et al. (2014a, b) studied the removal of *E. coli* from urban wastewater having obtained a reduction from a mean Log_{10} value of 4.48 MPN 100 mL^{-1} to 2.80 MPN 100 mL^{-1}. Using vermicompost as filter packing, Arora et al. (2016) obtained an effluent wastewater with a mean Log_{10} value of 2.50 MPN 100 mL^{-1} starting from an influent wastewater of 5.48 MPN 100 mL^{-1}. In their studies, Kumar et al. (2015), using as filter packing, vermicompost and river bed material, vermicompost and wood coal, vermicompost and glass balls, and vermicompost and mud balls, reported a reduction in fecal coliforms of 3.4 ± 0.67, 2.9 ± 0.88, 2.6 ± 0.45 and 2.6 ± 1.05 Log_{10} MPN 100 mL^{-1}.

Guidelines for wastewater reuse in irrigation indicate a pH between 6.0 and 9.0, a BOD_5 concentration ≤10 mg L^{-1} (for food crops consumed uncooked) or ≤30 mg L^{-1} (for non-food crops and food crops consumed after processing), a TSS concentration between ≤30 mg L^{-1} (for processed food crops) and, for fecal coliforms and helminth eggs, a maximal MPN of 10^3 100 mL^{-1} (or 3.0 Log_{10}) and 1 unit L^{-1}, for

Table 7 Fecal coliforms[1] in treated wastewater

Parameter	VE	V	SE	S
FC	$5.07 \times 10^{4b} \pm 1.53 \times 10^3$	$6.03 \times 10^{4c} \pm 4.04 \times 10^3$	$5.3 \times 10^{4b} \pm 3.00 \times 10^3$	$2.0 \times 10^{3a} \pm 1.00 \times 10^3$
Log_{10} FC value	$4.70^b \pm 0.01$	$4.78^b \pm 0.03$	$4.72^b \pm 0.02$	$3.26^a \pm 0.24$
K_{FC}	$3.693^a \pm 0.01$	$3.618^a \pm 0.03$	$3.674^a \pm 0.03$	$5.139^b \pm 0.24$
k_{FC}	$7.484^a \pm 0.03$	$7.320^a \pm 0.06$	$7.445^a \pm 0.05$	$10.412^b \pm 0.49$
Helminth eggs removal efficiency η (%)	$100.00^a \pm 0.00$	$100.00^a \pm 0.00$	$100.00^a \pm 0.00$	$100.00^a \pm 0.00$

[1] Mean concentration ± standard deviation. Values followed by the same letter within each line are not significantly different (ANOVA; Tukey's test, $\alpha = 0.05$)

agricultural irrigation (USEPA 2004). For pathogens only, WHO (2006) indicates a maximum MPN of 10^3 100 mL^{-1} (for unrestricted use), a maximum MPN of 10^4 100 mL^{-1} for restricted use and ≤ 1 No. L^{-1} for helminth eggs. In all experiments, pH and TSS comply with these limits. As for BOD_5, all treatments attained the limit concentration for non-food crops and crops consumed after processing (≤ 30 mg L^{-1}), but only vermicompost without earthworms attained the limit concentration for food crops consumed uncooked (≤ 10 mg L^{-1}) (Table 6). Also, none of the experiments reduced fecal coliforms to less than 10^3 MPN 100 mL^{-1} or a Log_{10} value less than 3.0 (Table 7). All treatments removed helminth eggs with an efficiency of 100%. This could be related due to the destruction of the three layers of protective shells that constituted helminth eggs. Nevertheless, all values related to fecal coliforms and helminth eggs were in accordance with the proposed by WHO (2006) for primary and secondary wastewater treatment technologies.

The results using single-stage vermifiltration were not completely positive since the efficiencies obtained for some of the parameters were still short to attain the EU regulation for discharges in sensitive water bodies (TN and TP) and USEPA and WHO guidelines for irrigation (fecal coliforms). The removal efficiencies were nonetheless higher than those registered in similar conditions as, e.g., in Arora et al. (2016), with 85.5% for BOD_5, 77.8% for COD and 82.2% for TSS. As for NH_4^+, high removal efficiencies with VF may be only attainable with vertical stage VF since Yang et al. (2015) reported an increase in NH_4^+ removal with the depth of filter packing. Fecal coliform removal efficiencies here obtained do not meet guidelines as a maximal MPN of 10^3 100 mL^{-1} for irrigation through VF has shown to not attain such level of use.

Table 8 resumes the parameters removal efficiencies obtained in the literature. Comparing these data with the values obtained in our study, the best removal efficiency for BOD_5 (96.19% working with filter with vermicompost without earthworms) was just lower than efficiencies obtained by Sinha et al. (2008), 98%, and Cardoso-Vigueros et al. (2013) (99%). For COD, the best removal efficiency (87.56% working with filter with vermicompost and earthworms) was just lower than the obtained by Cardoso-Vigueros et al. (2013), 92%, and for TSS, in all treatments, removal efficiency (98.42%) was higher when compared with the literature. When analyzing the NH_4^+ removal, the value obtained in filter with vermicompost and earthworms, 76.51%, was lower than the efficiencies obtained by Cardoso-Vigueros et al. (2013)—98%, and Arora et al. (2016)—90%. In what concerns TP, Cardoso-

Table 8 Treatment efficiencies obtained in the literature using VF

Parameter	Sinha et al. (2008)	Cardoso-Vigueros et al. (2013)	Arora et al. (2016)	Arora et al. (2014a)	Kumar et al. (2015)
BOD_5	98%	99%	86%	76%	81%
COD	45%	92%	78%	67%	72%
TSS	90%	97%	82%	–	75%
NH_4^+	–	98%	90%	–	76%
TN	–	78%	–	–	–
TP	–	[a]	[a]	–	[a]
Fecal coliforms*	–	–	2.82	2.70	3.40

* As Log_{10} FC value

[a] Authors reported an increase in TP final concentration

Vigueros et al. (2013), Arora et al. (2016) and Kumar et al. (2015) all registered increases in TP concentration in treated effluent, which is in line with our results. The latter two authors obtained in their studies treated effluent with less than a Log_{10} of 3.0 of fecal coliforms, which clearly surpasses our results.

Conclusion

Vermicompost and sawdust showed high treatment efficiency for BOD_5, COD and TSS. Moreover, the values using single-stage vermifiltration were not completely positive given that the efficiencies obtained for TP and TN were still above EU guideline values for discharge in sensitive water bodies, and fecal coliforms were above the WHO guideline value for irrigation.

Earthworms contributed to reduce treatment efficiencies for BOD_5, NH_4^+ and TN and to increase treatment efficiency for COD. In vermicompost, earthworms contributed to increase NO_3^-. Comparing with raw wastewater, vermicompost contributed to increase TP. No treatment eliminated fecal coliforms down to guidelines values for wastewater irrigation, but helminth eggs were completely eliminated.

In order to attain EU guideline values for discharge in sensitive water bodies and WHO guideline values for irrigation, alternative treatment technologies are needed, namely sequential vermifiltration systems or vermifilters followed by wetlands, working as hybrid systems suited for small communities.

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