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Potential Synchronous Detoxification and Biological Treatment of Raw Sewage in Small Scale Vermifiltration Using an Epigeic Earthworm, *Eisenia fetida*

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Abstract This study assesses the potential synchronous treatment and detoxification of raw sewage during small-scale vermifiltration. A pilot small-scale bioreactor was packed with the epigeic red worm, *Eisenia fetida,* in a static black-soil medium. It was operated at a hydraulic loading rate of 0.193 $m^3/m^2/h$ and a hydraulic retention time of 0.481 hr. Vermifiltration resulted in significant reduction in toxicity using the *Daphnia* 48hr-LC_{50} acute-toxicity assay. Average removal efficiencies for BOD_5 and TSS of 90.6% and 98.4% respectively were recorded after vermifiltration. The vermifilter was more efficient than the negative control in pollutant removal. There was no statistically significant difference between the negative control and the vermifilter in the reduction of total coliforms and faecal coliforms. Principal Component Analysis (PCA) indicated that COD, turbidity, TDS, TSS, and BOD_5 have a close association and major influence on reducing acute toxicity. The high reduction in toxicity, BOD_5 and TSS attributable to vermifiltration of raw sewage indicate the success and potential of vermifiltration in clarification, detoxification and biological treatment of raw sewage.

Keywords Raw sewage, Vermifiltration, Acute-Toxicity, *Eisenia fetida*

1. Introduction

Vermifiltration uses the ecology and natural cycles of earthworms in a fixed filter medium within a bioreactor to remove pollutants from wastewater (Li *et al.,* 2008). It has been described as a sustainable, odourless and non-labour intensive method of wastewater treatment (Sinha and Bharambe, 2007, Manyuchi *et al.,* 2013). The technology requires that wastewater be fed over filter substrates such as cow dung, sawdust, horse manure or soil which have been inoculated with earthworms. Substrate-specific surface area, aeration, hydraulic conductivity and nutrient exchange are increased by the burrowing action of earthworms (Zhao *et al.,* 2012). Earthworms' bodies act as biofilters which have been found to biodegrade or bio-accumulate organic wastes, heavy metals, and solid waste. Other studies have shown that they remove the 5-day BOD (BOD_5) by over 90%, chemical oxygen demand (COD) by 80-90%, total dissolved solids (TDS) by 90-92%, and the total suspended solids (TSS) by 90-95% from wastewater (Sinha *et al.,* 2008). A removal rate of 85% COD, 74% for N and 81% P from domestic wastewater using vermifiltration technology has also been reported by Zhao *et al.,* (2012).

It has been shown that vermifiltration also offers a decomposition component through a transfer of mass from biofilms to the earthworms (Xing *et al.,* 2010) in addition to the filtration and adsorption properties of the sand and gravel matrices. By granulating the clay particles, earthworms increase their hydraulic conductivity and natural aeration. They also grind the silt and sand particles, increasing the total specific surface area, which enhances the ability to adsorb the organic and inorganic pollutants from the wastewater (Sinha *et al*., 2008).

In the present study the epigeic red worm *Eisenia fetida* was chosen for packing the bioreactor because it has been widely used in vermifiltration (Taylor *et al.,* 2003; Wang *et al.,* 2010; Wang *et al.,* 2011) and it had the highest net reproductive rate (N_{rr}) from baseline studies on earthworm culture. Vermifiltration has been documented to be successful in achieving primary and secondary treatment standards (Bajsa *et. al.,* 2003; Hait and Tare, 2011). A combination of measuring physicochemical parameters and bio-toxicity assays is gaining acceptance widely in wastewater quality testing. Toxicity assays give an indication of the complete or synergistic response to a cocktail of toxic pollutants present in the wastewater. The water flea *Daphnia magna* and *Daphnia pulex* are the most

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commonly applied zooplankton in wastewater toxicology testing. The present study assesses potential reduction in acute-toxicity during clarification and biological treatment of raw sewage by small-scale vermifiltration using *Eisenia fetida*.

2. Materials and Methods

2.1. Experimental Design: Vermifiltration System

Six glass cuboids measuring 29 cm x 29 cm x 50 cm were set up. Individual cuboids were connected to an elevated 105 L wastewater reservoir with a submersible pump, and rotating arm sprinkler as shown in Figure 1. Each cuboid was packed with a 12 cm bottom layer of a mixture of 6 mm and 19 mm gravel, river sand (5 cm), black soil (20 cm) and 6 mm gravel (2 cm). Wire gauze was used to separate the 6mm gravel from the black soil and the river sand from the bottom gravel layer. The top black soil layer was separated from the river sand by synthetic fabric. Three of the cuboids were individually inoculated with *E. fetida* at a loading density of 12 g/L adult earthworms in black-soil media. The remaining three cuboids were used as negative controls, they were not inoculated with *E. fetida*.

2.2. Bioreactor Characterization

The efficiency of a vermifiltration bioreactor/vermifilter is dependent on the hydraulic retention time (HRT), hydraulic loading rate (HLR), earthworm loading density, earthworm species used and earthworm medium used (including its porosity, depth and length). HRT is a crucial parameter as it determines the actual time the wastewater is available to earthworms so that they can interact with the wastewater thereby detoxifying it (Malek *et al.*, 2013). The HRT and HLR were determined as shown below.

2.2.1. Flow-rate Determination

The flow-rate of sewage through the vermifilter was determined by pumping water using a submersible pump and timing the volume applied to the vermifilter bed. This flow-rate did not cause ponding in the vermifilter. The submersible pump pumped the sewage at constant pressure without cavitation, since cavitation may distort flow-rate determinations. Sewage was applied to the vermifilter unit at a flow rate of 4.5 mLS^{-1} and this was maintained for all subsequent sewage treatments.

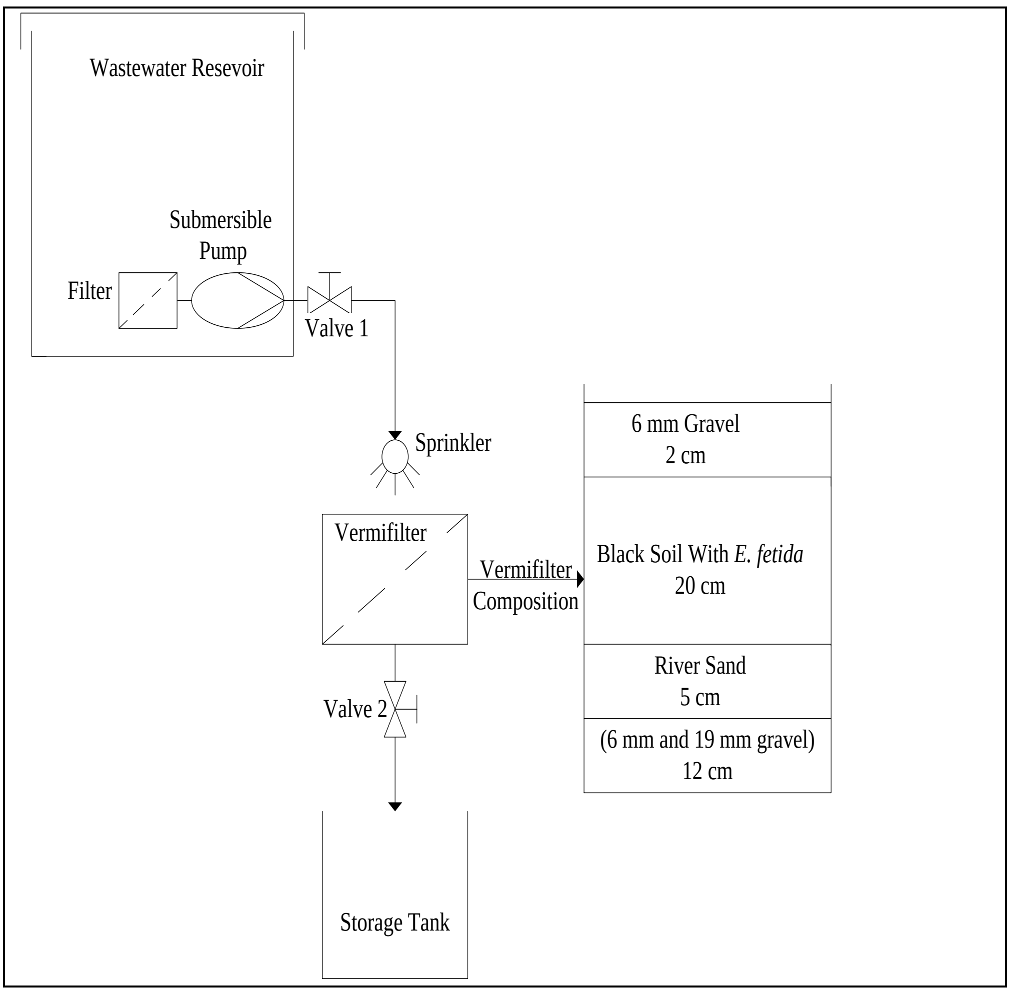

Figure 1. Schematic diagram of the vermifiltration system used in all experiments

2.2.2. Porosity Determination

The porosity of the vermifilter was determined by dividing the volume of air spaces filled by water in the vermifilter by the volume of the vermifilter and expressing it as a percentage:

Effective volume of bioreactor (44 cm x 29 cm x 29 cm)	37 004 cm^3
Volume of water added to bioreactor	12 750 /cm^3
Porosity of packed bed /%	34,5

2.2.3. Hydraulic Retention Time (HRT)

The hydraulic retention time was calculated using the formula below,

Equation 1

$$HRT = (\rho \times V_s)/ Q_{wastewater}$$

Where ρ is porosity of the entire packed bed (gravel, sand and soil), V_s is the volume of the soil profile (L) and $Q_{wastewater}$ is the flow rate of wastewater through vermifilter bed (L/hr).

$\rho = 0.345$; $V_s = 16.82$ L; $Q_{wastewater} = 12$ L/hr; HRT = 0.481 hrs

A HRT of 0.481 hr was maintained for all subsequent sewage treatments.

2.2.4. Hydraulic Loading Rate (HLR)

The HLR was calculated using the formula below,

Equation 2

$$HLR = V_{wastewater}/(A \times t)$$

Where HLR is the hydraulic loading rate (L/m^2/h), $V_{wastewater}$ is the volumetric flow rate of wastewater (m^3), A is the area of soil profile exposed (m^2) and t is the time taken by the wastewater to flow through the soil profile (h).

$V_{wastewater} = 388.8$ m^3, A = 0.0841 m^2, t = 24 hrs, HLR = 193 L/m^2/h

A HLR of 193 L/m^2/h was maintained for all subsequent sewage treatments.

2.3. Wastewater Analysis

Composite samples of raw sewage from the Chinhoyi municipality were used in this study. Earthworms were acclimatized to raw sewage for 20 days. The vermifilter was operated continuously without closing the tap at the bottom of the vermifilter. The efficiency of vermifiltration was analyzed by testing wastewater acute toxicity, physico-chemical characteristics and coliforms, before and after vermifiltration.

The BOD_5 was measured by the Dilution Method using the Acumet excel XL40 Dissolved Oxygen meter. COD was determined spectrophotometrically. The wastewater was also analyzed for TSS using a gravimetric method and TDS was analysed using the AD 8000 meter following standard procedures according to the American Public Health Authority (1995). Total coliforms, fecal coliforms and *E. coli* were measured following the Medical Laboratory Manual for Tropical Countries (Cheesebrough, 2006). The 48hr-*Daphnia pulex* acute toxicity assay was done following the ISO 6341 Method (2012). 48hr-LC_{50} of *Daphnia pulex* was determined using percentage death. Raw sewage was subjected to vermifiltration after which the above analyses were repeated to determine efficiency of the vermifilters.

2.4. Data Analysis

Wilcoxon matched pair test at 5 % level of significance was used to test for the significance of differences in water quality parameters of the raw sewage, vermifiltered sewage and sewage filtered using the control filter. The non-parametric Friedman ANOVA test (5 % level of significance) was used to test for the significance of the difference across all the treatments. These tests were done using STATISTICA 7. Principal Component Analysis (PCA) was used to investigate the main associations between the vermifiltered water quality parameters and the level of treatment of the wastewater. It was done using CANOCCO 5.

3. Results and Discussion

Table 1 summarizes the effect of vermifiltration on raw sewage parameters (±Standard Deviation, SD) with Zimbabwe's Environmental Management Agency (EMA) aquatic effluent thresholds affixed for comparative purposes.

Table 1. Sewage quality parameters (±SD) relative to EMA standard guidelines for effluent. * indicates figures that were not available. RS = Raw sewage; F = Effluent from negative control; VF = Effluent from vermifiltration

Parameter	RS	F	VF	EMA
pH	8.00±1.04	8.28±0.12	8.36±0.04	6-9
TDS mg/L	656.56±47.0	691.44±56.40	674.56±26.55	≤500
EC µs/cm	1235.22±49.57	1424.000±55.14	1345.44±37.44	≤1000
TSS mg/L	1.24±0.85	0.03±0.00	0.02±0.00	≤25
BOD_5 mg/L	388.33±16.39	71.67±9.02	36.67±6.61	30
COD mg/L	412±32	300±25	247±18	≤60
Turbidity NTU	512±14	174.17±21	120±16	≤5
PV mg/L	31.38±2.1	23.62±1.4	15.75±2.3	*

Table 1 shows that vermifiltration achieved a higher removal of pollutants from sewage than the reference (control) system. TDS and EC increased after vermifiltration.

The small-scale triplicate-run earthworm-based bioreactor generally produced higher average removal efficiencies than the one without worms, achieving a 90.6% removal of BOD_5 and a 98.4% removal of TSS. This demonstrated high performance of the bioreactor. Previous vermifiltration research has also published high average removal efficiencies of more than 80-90% for BOD_5, COD, TDS and TSS (Sinha *et al.*, 2008; Manyuchi *et al.*, 2013). Wilcoxon matched pair test showed that there were no significant differences ($p<0.05$) post-vermifiltration for total coliforms, fecal coliforms and *E. coli*. Friedman ANOVA showed that there was a significant difference ($p<0.05$) in the three treatments (p =0 .00012) RS, F and VF.

In the present study vermifiltered sewage lacked odour and was clearer compared to raw sewage. The water quality parameters of vermifiltered sewage were in line with previous authors, except for the TDS. TDS remained out of the EMA range and showed an upward trend post-vermifiltration for both the bioreactor and the negative control (Table 1). Measurements for TDS represent levels of dissolved CO_3^{2-}, HCO_3^-, Cl^-, SO_4^{2-}, PO_4^{2+}, NO_3^-, Ca^{2+}, Mg^{2+}, Na^+, organic ions, and other ions. Therefore a poor TDS removal efficiency in the bioreactor suggests that the bioreactor packing material did not have sufficient cation/anion exchange capacity.

The vermifilter (VF) was more efficient in removing all the measured parameters than the negative control (F) as shown in Table 1. This is attributable to the action of the earthworms. The earthworms consume the organic matter in the sewage which subsequently reduces the BOD_5, COD, PV, TSS and turbidity. The action of the earthworm gut microbes, microbes in the sewage and their respective enzymes contribute the enzymatic aerobic and anaerobic biodegradation of complex organic matter in the sewage. An increase in HRT can increase the removal rates of all the tested parameters as a result of increased contact time for earthworms to metabolize pollutants.

Despite the high overall bioreactor performance, parameters such as BOD_5, EC, TDS, COD and turbidity remained out of the EMA range (Blue class for normal waters) post-treatment, with only pH and TSS values falling within the local recommended aquatic effluent quality thresholds under the blue class for normal waters (Table 1). However, BOD_5 reduction was statistically significant and fell close to the EMA stipulated range, which points to the potential success of vermifiltration. Turbidity of the sewage also remained high after treatment but the removal efficiency was approximately 77%. To achieve higher levels of treatment before recommendations for recycling and discharge there may be need to effect longer HRT regimes during up-scaling and implementation of vermifiltration. Biosafety issues may also remain a concern, since our study was not able to establish removal of coliforms (Table 2). Post-vermifiltration techniques would be recommended to produce an effluent that is sufficiently disinfected to be recycled for farm irrigation and watering parks and gardens.

Table 2. Removal of coliforms from sewage. RS = Raw sewage; F = Effluent from negative control; VF = Effluent from vermifiltration

Parameter /100ml	RS	F	VF
Total coliforms	180^+	180^+	180^+
Fecal coliforms	180^+	180^+	180^+
E. coli	+	+	+

Table 2 indicates that the negative control and the vermifilter did not reduce the number of total coliforms and fecal coliforms in the wastewater to below 180 per 100ml.

Since *E. coli* was not removed from the wastewater (Table 2) this may be an indication that vermifiltration needs other augmentative methods that specifically remove fecal coliforms. In contrast, a study by Manyuchi *et al.*, (2013) reported a significant reduction in bacterial loads in sewage after vermifiltration. For BOD_5 loads of 200 – 400 mg/l, an HRT of 30-40 minutes is needed for their significant reduction, whilst an HRT of 1-2 hours is needed to reduce pathogens, toxic chemicals and heavy metal concentrations found in sewage (Sinha *et al.*, 2010). Zero *E. coli* per 100 ml of water is the goal for all drinking water supplies and should be the target even in emergencies (WHO, 2004). The HRT applied in this study however achieved significant reduction in BOD_5, turbidity, COD and acute toxicity as shown in Table 4. Thus post treatment of vermifiltered water to remove coliforms as opposed to increasing the HRT is advocated for. This can include treatment of vermifiltered water with chlorine dioxide.

Table 3 and 4 indicate that the 48hr-LC_{50} decreased with treatment and treated wastewater had no observable effect on the *Daphnia* at all concentrations up to 48 hours. The control (48hr-LC_{50}-81.5%) did not reduce toxicity as much as the vermifilter (48hr-LC_{50}-NOEC).

As BOD_5 decreased, electrical conductivity was increasing (Wetzel, 2001), but this inverse relationship resulted in an overall reduction of toxicity of vermifiltered sewage on the bio-indicator *Daphnia pulex* (Fig 2). The observed reduction in acute toxicity suggests metabolism and or immobilization of toxic components of the raw sewage within the bioreactor. It is reasonable to deduce that removal of organic and inorganic substances constitutes a reduction in toxicity of wastewater, further validating the utility of the *Daphnia* toxicity assays as a direct measurement of effluent toxicity (Movahedian *et al.*, 2005).

Table 3. Daphnia 48hr-LC_{50}Acute Toxicity Assay. RS = Raw sewage; F = Effluent from negative control; VF = Effluent from vermifiltration. * indicates figures that were not available

Sewage concentration %	Number of live organisms stocked	Number of dead organisms after 48 hours								
		Before treatment			After treatment without worms			After treatment with worms		
		RS	**RS**	**RS**	**F**	**F**	**F**	**VF**	**VF**	**VF**
100	10	9	10	10	6	6	6	0	0	0
50	10	7	8	8	5	4	3	0	0	0
25	10	4	5	5	0	0	0	0	0	0
10	10	0	2	2	0	0	0	0	0	0
5	10	0	0	0	0	0	0	0	0	0
0	10	0	0	0	0	0	0	0	0	0

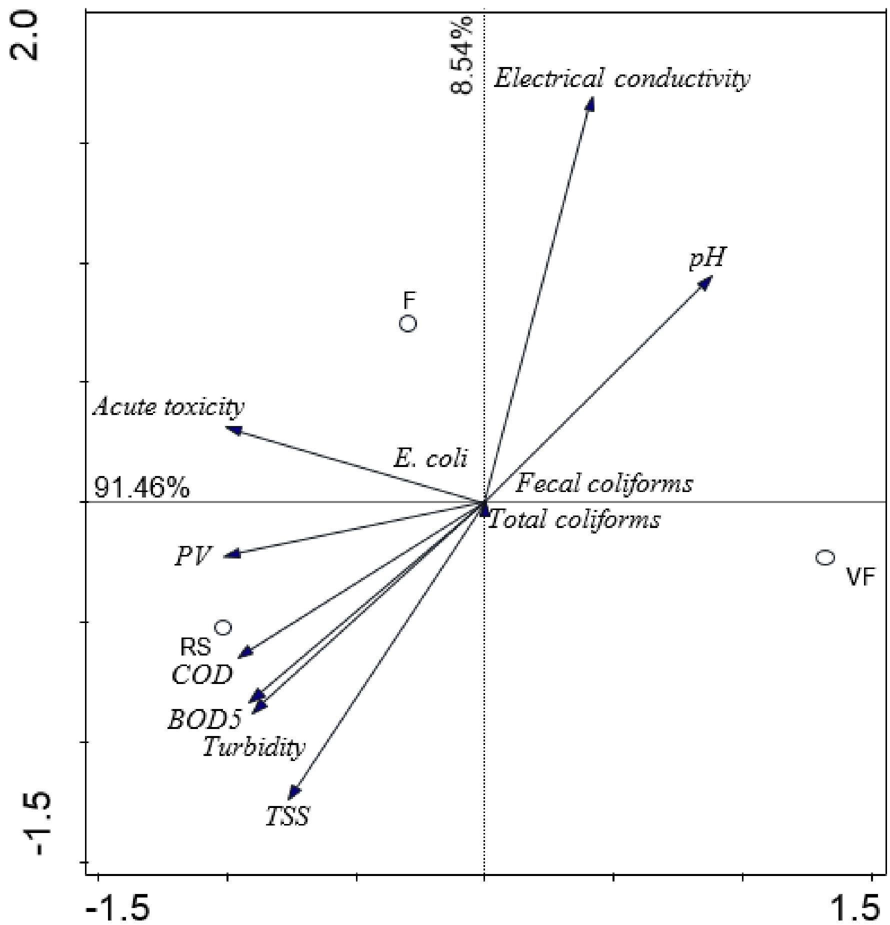

Figure 2. Principal Component Analysis showing the association among raw sewage, filtered (control) and vermifiltered sewage parameters. RS = Raw sewage; F = Effluent from reference filtration; VF = Effluent from vermifiltration

Table 4. 48hr-LC_{50} values. RS = Raw sewage; F = Effluent from negative control; VF = Effluent from vermifiltration

Treatment	**48hr-LC_{50}**
RS	39.5 %
VF	NOEC
F	81.5 %

PCA separated all tested treatments along the first axis (Figure 2). The first axis of the PCA accounted for 91.46% of the total variation observed. All treatments of the experiment were also distributed along the first axis of the PCA. RS was found to be distinctively and strongly associated with BOD_5, COD, 48 hr-acute toxicity, turbidity and TSS. These parameters were highest in raw sewage.

Separation along the second axis, accounted for 8.54% of the total variation observed. The variation resulted from electrical conductivity and pH. These two parameters were associated with vemifiltered sewage (VF) and sewage that had passed through the control (F) whereby F was found to be positively associated with the second axis and VF was found to be negatively associated with the second axis.

Notably, total coliforms, fecal coliforms and *E. coli* were found to be not associated with the tested parameters in this study.

BOD_5, turbidity, TSS, PV and COD have a synergistic effect on acute toxicity. Their reduction during treatment consequently resulted in the reduction of toxicity. This further strengthens the need to incorporate toxicity assays in determining efficiency of a water treatment process and in monitoring studies. This is because toxicity assays show the actual effect of the effluent aquatic organisms where it will be discharged.

In conclusion the high removal of BOD_5, TSS and toxicity suggests that vermifiltration is efficient in primary treatment of sewage but requires further optimization, such as higher HLR, and recirculation, to achieve efficient biological nutrient removal. The ability to reduce toxicity suggests that the bioreactor contains consortia of microflora capable of biotransformation and or biodegradation of complex toxic organic molecules which would otherwise increase effluent toxicity.

The limitation of this study is that the bioreactor did not significantly reduce the number of coliforms. This calls for post treatment of the treated effluent to remove the coliforms. However we do not encourage the extension of the HRT to remove the coliforms since it can be considered time consuming against the quick methods that can be used to remove coliforms such as chlorination. This is so because other physicochemical parameters were significantly altered using the HRT of 28.86 minutes.

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