http://dx.doi.org/10.22161/ijeab/4.2.29 ISSN: 2456-1878

Studies on Vermifiltration of Restaurant Effluent and Reuse in Benue State, North Central, Nigeria

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***Abstract*— *Vermifiltration was used for the treatment of restaurant effluents in Benue state. Four vermi-bio-filters were used; V_1 (Lumbricusterritris), V_2 (Eudriluseugeniae), V_3 (Eiseniafetida), V_4 (mixture of Lumbricusterritris, Eudriluseugeniae and Eiseniafetida) and V_5 (Control without earthworm-geofilter). The effluent from vermifilters and control were analysed for microbial load. The Earthworms' body worked as a 'biofilter' and as a bioreactor with the mechanism of 'ingestion' and biodegradation of organic wastes. Bacteria analysed in the six effluents (Staphylococcus spp, Streptococcus spp, Escherichia coli, Salmonella spp, Enterobacterspp, Proteus spp and Pseudomonas spp) and fungi (Aspergillus, Penicillium Rhizopus and Mucor. Saccharomyces cerevisae) were reduced in the range of 70.0-97.7% and 68.3-97.4% respectively. Analysis of Variance (ANOVA) to determine the differences in microbial load concentration between the influent and effluent were considered significant at 5% level ($p \leq 0.05$). The synergistic action of enzymes, microorganisms and earthworms significantly reduced the amount of microbial load compared to a geo-filter, without earthworms' presence (control). Vermifiltration is a low cost technology recommended for treatment of effluent before discharge into the environment. This is an odor-free process and no sludge formed in vermin-filtration. Vermifiltration technology can be applied as an environmentally friendly technique as the treated effluents met the set standards for irrigation purpose, parks, gardens and hydroponics.*

Keywords*— *Environment, Effluent, Microbial load, Treatment, Re-use, Vermifiltration.

I. INTRODUCTION

Wastewater treatment is the removal of contaminants from any form of wastewater and it includes physical, chemical and biological processes so that the water can be re-used (Kharwade and Khedikar, 2011). As water pollution critically affects water availability, it needs to be properly managed in order to mitigate the impacts of increasing water scarcity (UN WWAP 2017). Access to safe water is a human right (UNDP, 2006). Over (90%) of diarrhoea diseases are caused by unsafe water supply and unhygienic sanitation (WHO, 2004).Vermifiltration of wastewater using waste eater earthworms is a newly conceived novel technology with several advantages over the conventional systems. It is eco-friendly and sustainable for the treatment of wastewater, and appeared to have high treatment efficiencies without the formation of sludge (Damodhar *et al*., 2014). Earthworms feed readily upon the sludge components; rapidly convert them into vermicompost without odor, reduction in pathogens to safe levels. Treated bio-clean water can be reused for secondary purpose like floor washing, toilet-flushing, gardening, hydroponics and irrigation etc. except for direct human consumption (Aguoru*et al.,* 2015).

II. MATERIALS AND METHODS

Area of the Study

The area of study covered restaurant effluents produced within the three geopolitical zones (A, B, and C) of Benue State. Benue state coordinates are: Latitude 8^0 08' 00"N, 6^0 26' 00"S and Longitude 9^0 54' 00" E, 7^0 30' 00"W

Collection of Specimens (Earthworms) and Samples

The species of earthworms native to each of the three geopolitical zones of Benue state were collected from their habitats by Hand sorting technique as described by Glenn (2006) and identified using the method described by Ansari and Saywack (2010).Earthworms' species used were *Lumbricusterrestris* (Night Crawler), *Eudriluseugeniae* and *Eiseniafetida*. A total of fifteen restaurant water samples were collected; five samples from each zone.

Vermifilter System

The effluent was fed by gravity flow with the help of sprinkler to avoid direct hydraulic influence on the earthworms. An infusion set was used to control the volumetric flow rate of wastewater (Bhise, 2015). The body of the reactor is made of PVC drum. The depth of 40cm was divided into 4 parts in which gravel, sand and soil bed for earthworm (weight of 75g). The soil was compacted using a

http://dx.doi.org/10.22161/ijeab/4.2.29 ISSN: 2456-1878

piston. The garden soil and compositing material (sawdust and cow dung) were mixed at a volume ratio of 3:1 (Lakshmi *et al*., 2014). Pieces of baked clay pot were used to sandwich PVC drum and vermifilter to regulate temperature of the system. The system has provisions to collect the filtered water at the bottom which opens out through a pipe fitted with tap. The topmost layer of about 10 cm consists of soil bed in which the earthworms were added. The worms were given one week settling time to acclimatize in the vermifilter. A plastic mesh covered the top to prevent escape of worms and from predators. A layer of plastic mesh was placed below the layer of soil bed to allow only water to trickle down while holding the earthworms in the soil bed (Bhise, 2015). There are five vermilfter units labeled V1, V2, and V3 containing 1 kg of *Lumbricusterrestris*, *Eudriluseugeniae* and *Eiseniafetida* respectively. The fourth (V4) was a mixture of *Lumbricusterrestris*, *Eudriluseugeniae* and *Eiseniafetida*while the control was (V5) without earthworms.

Dissection of Earthworms

The peripheral surfaces of earthworms were disinfected with sodium hypochlorite (25 ppm) for 10 min before dissection. The gut contents of each segment were squeezed into sterile test tubes using a sterile scalpel, forceps, and sterile knife under aseptic conditionRavindran*et al*., (2014). The collected gut content (l g) was diluted in saline solution (NaCl, 0.9% (w/v)) and microbial diversities were determined (Prakash and Karmegam, 2010). The serial dilutions were made up to 10^{-6} dilution and an aliquot of 0.1 mL was spread onto plates of the agar media. The plates were then incubated at 30^{o}C for 24 h for bacteria and 28^{o}C for 5 days for fungi. The average per dilution will be determined and multiplied by the reciprocal of the dilution ratio and expressed as colony-forming units per milliliter (CFU/ml) of the sample (Amadi and Ayogu, 2005).Identification of bacteria will be based on Bergey's Manual of Determinative Bacteriology (Krieg and Holt, 1984).

Data analysis

Percentages were used to determine the reduction in microbial load after vermifiltration. One-way Analysis of variance (ANOVA) was carried out with a software statistical package (SPSS version 21) to test the existence of statistical significant differences and significant differences were detected the Duncan's New Range Multiple Test (DNRMT) to be significant at ($p<0.05$). The mean values compared with National Environmental Standards and Regulations Enforcement Agency (NESREA) standards for surface water (Aguoru*et al*., 2015).

III. RESULTS AND DISCUSSION

Reduction of Bacteria in Restaurant Effluent

Table 1 shows the percentage reduction of restaurant wastewater for 360 days. The mean bacteria count in Restaurant effluent in this study ranged from 3.66 x 10^{-4}CFU/ml to 5.04 x 10^{-4}CFU/ml representing 70.5 % in effluent treated by *Eudriluseugenia* (V_2) within (60) days to 93.6% in effluent treated by treatment mixture of *Lumbricusterritris*, *Eudriluseugeniae*and *Eiseniafetida*) within 180-240 dayswas lower than the findings of 5.5 x 10^{-7} to 7.4 x 10^{-7}CFU/ml before treatment by Ogidi and Oyetayo, (2012). Percentage reduction in Control (V_5) ranged from 50.9 % -55.8%. Figure 1 shows the highest result of bacteria reduction in restaurant wastewater after vermifiltartion between 121–180 days. Analysis of Variance (ANOVA) shows significance difference F (calculated) =3.36<F (tabulated) 231.6 at $P < 0.05$. The bacteria isolates from restaurant include *Escherichia coli*; *Pseudomonas* spp.; *Staphylococcus* spp., *Shigelladysenteriae*, *Proteus vulgaris*, *Klebsiella*spp and *Salmonella typhi*. These pathogens can cause human health hazards. The high occurrence of *Staphylococcus aureus*may be due to shedding of resident *Staphylococcus aureus*in human skin which may contaminate food and water during handling, processing and washing of hands after eating.

The high amount of bacteria in the restaurant effluents may be as a result of drained wastewater having contact with the soil already contaminated with decomposed waste foods dumped nearby which may be source of nutrient for the organisms along the drainage. Since most bacteria live under starvation conditions or water stress in the soil, they have adapted to quickly reproduce when water, food, and the environmental conditions are abundant. These organisms are versatile in utilizing the limited nutrient and have the ability to adapt to the toxic condition of soap and detergent contained in restaurant wastewater. Bacteria populations can easily double in 30 minutes (Nester *et al*., 2007).Foul odor during processing was trmoved (Hughes *et al*. 2011; Sinha*et al*. 2008). They create aerobic conditions in the waste materials by their burrowing actions, inhibiting the action of anaerobic microorganisms which release foul-smelling hydrogen sulfide and mercaptans (Sinha*et al*., 2009).

http://dx.doi.org/10.22161/ijeab/4.2.29 *ISSN: 2456-1878*

Table.1: Percentage Reduction of Bacteria in Restaurant Effluent (10^{-6} CFU/ml)

No of days	I	V_1	%	V_2	%	V_3	%	V_4	%	V_5	%	NESREA(2011) <400 CFU/ml
0-60	4.14	1.14	72.5	1.22	70.5	1.02	75.4	0.84	79.7	1.50	63.8	
61-120	3.84.	0.86	77.6	0.94	75.5	0.74	80.7	0,58	84.9	1.32	65.6	
121-180	4.60	0.98	78.7	1.06	76.9	0.78	83.0	0.64	86.1	1.68	63.5	
241-300	5.04	0.87	82.7	1.00	80.2	0.66	86.9	0.50	90.1	1.72	65.3	
181-240	3.66	0.54	85.2	0.69	81.1	0.38	89.6	0.27	92.6	1.47	59.8	
301-360	4.82	0.65	86.5	0.72	85.1	0.47	90.2	0.31	93.6	1.81	62.4	
Mean ±S.D	**4.35± 0.51**	**0.84± 0.19**		**0.94 ± 0.2**		**0.68± 0.19**		**0.52 ± 0.2**		**1.58± 0.17**		

F=3.3 D.F. = 4 ($p<0.05$)

I= influent (Before treatment)
%= percentage reduction
V1=effluent (treatment with *Lumbricusterritris*)
V2= effluent (treatment with *Eudriluseugeniae*)
V3 = effluent (treatment with *Eiseniafetida*)
V4= effluent (mixture of *Lumbricusterritris, Eudriluseugeniae*and *Eiseniafetida*)
V5= Control (without earthworm)

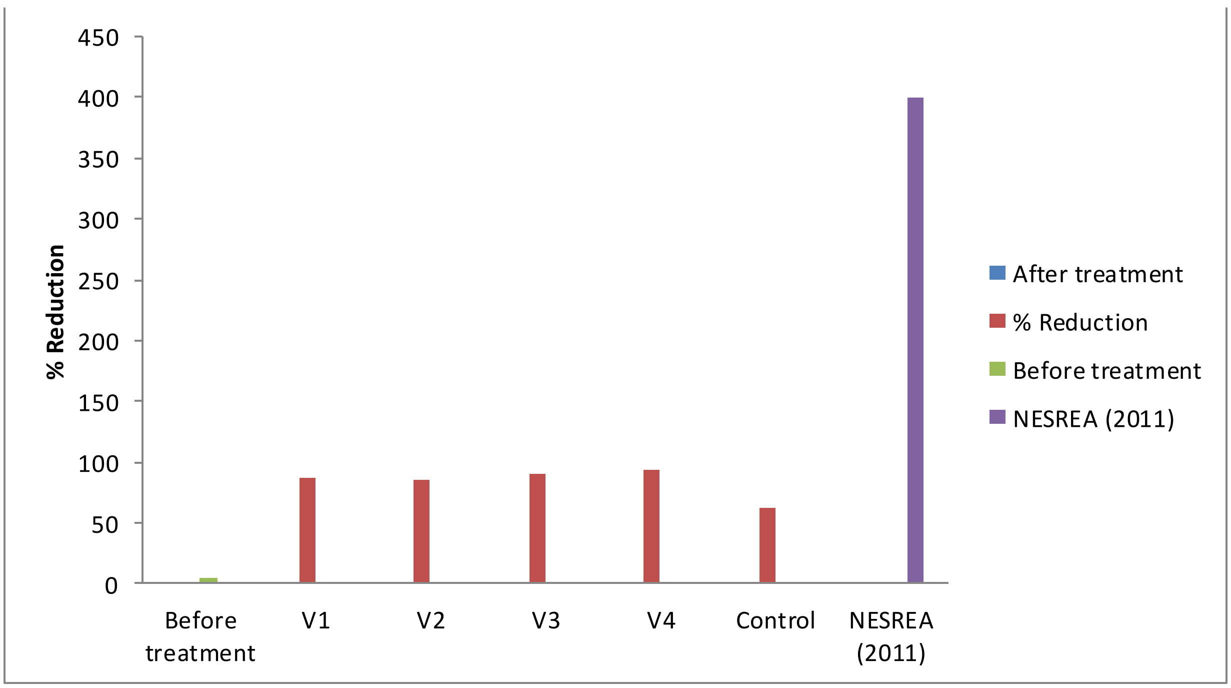

Fig.1: Vermifiltration of bacteria (10^{-6} CFU/ml) in restaurant effluent

Reduction of Fungi in Restaurant Effluent

Table 2 shows the mean Fungi count in Restaurant wastewater in this study ranged from 3.76 x 10^{-4}CFU/ml 5.22 x 10^{-4}CFU/ml shows the percentage reduction of fungi for 360 days in restaurant wastewater representing 85.8 % in effluent treated by *Eudriluseugeniae* (V_2) within (60)

http://dx.doi.org/10.22161/ijeab/4.2.29 ISSN: 2456-1878

days to 96.7% in effluent treated by treatment mixture of *Lumbricusterritris*, *Eudriluseugeniae*and *Eiseniafetida*) within 120-180 days which corroborates with the result of 4.1× 10^{-4}sfu/ml to 5.2× 10^{-4} CFU/ml before vermifiltration by Ogidi and Oyetayo (2012) Percentage reduction in Control (V_5) ranged from 68.4 % -74.8%.. Figure 2 shows the highest result of fungi reduction in restaurant wastewater after vermifiltartion between 121–180 days. ANOVA shows F (calculated) =2.72<F (tabulated) 231.6 at P < 0.05. There is significant difference between the mean values.

The fungi isolates in restaurant wastewater includes *Saccharomyces cerevisae, Mucor spp. Aspergillus fumigates* and *Penicillium spp.* The presence of *Rhizopusstolonifer*which is commonly found growing on bread and soft fruits such as bananas and grapes are capable of causing opportunistic infections of humans (zygomycosis) could be ascribed to the presence of its spores (Aslankoohi, 2013). *Aspergillusniger*is known to causes a disease called black mold on certain fruits and vegetables. Inhalation of *Aspergillus*sp. can result in Asthma with difficulty in breathing. A large Aspergilloma in the lungs can block respiratory gas exchange and cause death due to asphyxiation (Ronald, 2003). *Rhizobium* sp. on the other hand can infect leguminous plant roots, where they cause formation of tumorous growth root nodules with which they live in a mutually beneficial relationship with leguminous plant thus enhancing their growth.The microbial removal can be attributed to the presence of earthworms, which is known to reduce organic matter content of wastewater thus making the environment unsuitable for pathogens. This is because under favorable conditions, there is a symbiotic interaction of earthworms and microorganisms to enhance the decomposition of the organic matter that causes the release of coelomic fluids from their body cavity (Sinha *et al.*, 2008). This fluid has antibacterial properties, which destroy all the pathogens from the media in which it inhabits (Kumar *et al.*, 2014). Bacteria and fungi results in the restaurant effluent are within the permissible limit of 400CFU/ml set by National Environmental Standards and Regulations Enforcement Agency (NESREA, 2011).

Table.2: Percentage Reduction of Fungi Load in Restaurant Effluent

No of days	**I**	**V_1**	**%**	**V_2**	**%**	**V_3**	**%**	**V_4**	**%**	**V_5**	**%**	**NESREA (2011) <400CFU/ml**
0-60	4.43	0.36	91.9	0.32	92.8	0.26	94.1	0.22	95.0	1.40	68.4	
61-120	5.22	0.56	89.3	0.46	91.2	0.33	93.7	0.18	96.7	1.48	71.6	
121-180	4.60	0.40	91.3	0.46	90.0	0.39	91.5	0.34	92.6	1.32	71.3	
241-300	3.76	0.24	93.6	0.30	92.0	0.28	92.6	0.20	94.7	1.10	70.7	
181-240	4.20	0.54	87.1	0.61	85.5	0.44	89.5	0.32	92.3	1.06	74.8	
301-360	5.12	0.60	88.3	0.52	89.8	0.48	90.6	0.30	94.1	1.40	72.7	
	4.55± 0.3	**0.45± 0.1**		**0.44± 4.9**		**0.36 ±0.1**		**0.26 ±0.06**		**1.29 ±0.2**		

F=2.72 D.F. = 4 (p<0.05)

I= influent (Before treatment)
%= percentage reduction
V1=effluent (treatment with *Lumbricusterritris*)
V2= effluent (treatment with *Eudriluseugeniae*)
V3 = effluent (treatment with *Eiseniafetida*)
V4= effluent (mixture of *Lumbricusterritris, Eudriluseugeniae*and *Eiseniafetida*)
V5= Control (without earthworm)

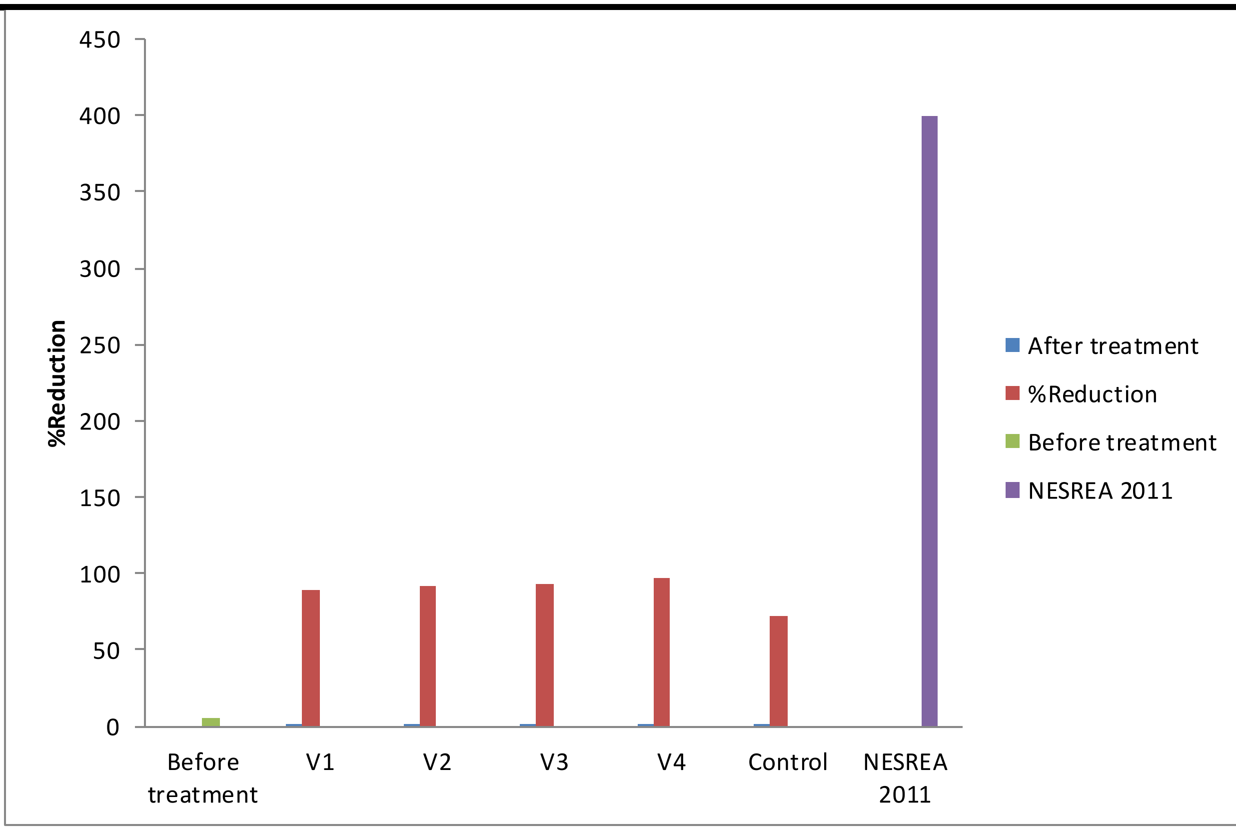

Fig.2: Vermifiltration of fungi (10^{-6} CFU/ml) load in restaurant effluent

IV. CONCLUSION

Wastewater treated with earthworms (vermifiltration) has proven that earthworms are capable of reducing microbial load in wastewater, thus reducing the high rate of pathogenic organism using ecofreindly method that is sustainable. Among the three earthworm species, *EiseniaFetida*is best suited for treatment of waste water, followed by *Lumbricusterritris* and lastly, *Eudriluseugeniae*. There is no foul odor as the earthworms arrest rotting and decay of all putrescible matters in the wastewater and the sludge. They also create aerobic conditions in the soil bed and the waste materials by their burrowing actions, inhibiting the action of anaerobic microorganisms which release foul-smelling hydrogen sulfide and mercaptans.No sludge formed in the vermin-filtration unit during the wastewater treatment. The waste water treated through this method was clear and good enough for irrigation purpose or parks and gardens.

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