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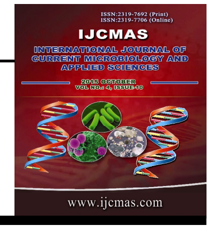

Original Research Article

Changes in Bacterial and Fungal Population of Filtermud Waste during Vermicomposting by *Eudrilus eugeniae*

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A B S T R A C T

Filter mud has significant fertilizer value but due to prohibitive cost of sludge disposal, it is dumped in open where it adversely affects the ambient environment .Conversion of industrial wastes in to beneficial vermicompost not only solves solid waste accumulation but also yield highly nutritive organic manure. Approach: The management and nutrient recovery from filtermud has been attempted by vermicomposting after mixing it with organic nutrient in appropriate quantities. The final products were nutrient rich, odour free, more mature and stabilized. The results showed that carbon content was decreased during the process and nitrogen content was enhanced. The C: N ratio decreased with time in all the feed mixtures indicating a stabilization of the waste. Bioconversion of filter mud waste using cow dung in different proportions leads to nutrients rich manure by using the earthworm *Eudrilus eugeniae.* The results suggest that the filter mud supplementary with cow dung promote the activity of earthworm and produce highly nutritive vermicompost. The chemical composition of the compost prepared by different ratios of composting using filter mud showed that the level of nitrogen, phosphorus, potassium, calcium and metals like Mg, S, Cu and Zn showed increasing trend during end of the vermicomposting. The present study recommends filtermud mixed with cow dung 1:1 ratio for vermicomposting to enhance the functioning of the earthworm and to increase fertilizer value of vermicompost.

Keywords

Cow dung, *Eudrilus eugeniae,* Vermicompost, Bioconversion and Filtermud

Introduction

Sugar industry belongs to the most important processing industries in India. According to the Indian Directorate of Economics and Statistics, India produces on average 270 million tones of sugar cane per year. During the production process considerable amounts of by products such as pressmud (p), bagasse (b), and trash (t) are produced. Pressmud generates intense heat (65°C), foul odour and takes long time for natural decomposition. However, sugar industry has considered this voluminous quantity of pressmud as a waste, and society as hazards pollutant. According to Kale (1998) an average pressmud (a byproduct of sugar processing industry containing the elements added to clarify juice and sediments, solids like bagacillo, the smaller fibers of cane sugar, and particulates and mud) production per ton of sugar is 35 kg. Pressmud has major water and air pollutant near the sugar industrial area. Filter cake or Pressmud as it is commonly known is one of the important by-products of the sugar industry. Filter mud is a soft, spongyamorphous and dark brown to brownish white material containing sugar, fibre coagulated colloids including cane wax, albuminoids, inorganic salts and soil particles. It is readily converted to a repository of macro and micro nutrients besides being a very effective soil ameliorant thorough vermicomposting (Vasanthi *et al.,* 2011).

Filter mud has significant fertilizer value as it is a rich source of organic matter, organic carbon, sugar, protein, enzymes, micronutrients (N, P and K) and macronutrients (Zn, Fe, Cu, Mn, etc.) and microbes (Sangwan *et al.,* 2008; Vasanthi *et al.,* 2014). Farmers are reluctant to apply it directly due to its bad odor, transportation cost and fear that its application may lead to crust formation, pH variation and pollution problem. Wax content of press mud (8.15%) affects the soil property by direct application (Thopate *et al.*, 1997) and its high rate of direct application (up to 100 tonnes/acre) leads to soil sickness and water pollution (Bhawalkar and Bhawalkar, 1993). Therefore, appropriate press mud management technology is desired which not only protect and conserve the environment and land resources but also to recover the nutrients present in it. The present contribution reports the results of laboratory – based experiments performed to investigate the ability of earthworms for the management of filtermud waste, after supplementary with Cow dung.

Materials and Methods

Filtermud

The filter mud was collected from the Dharani Sugar Factory near Vasudevanallur, Tirunelveli. Fresh filter mud was kept in shade for 2-3 weeks before using for the vermicomposting process. The partially degraded filter mud (FM) was then blended with Cow dung (C) in different proportions.

Earthworm's cultures

Composting species of exotic earthworms (*Eudrilus eugeniae*) was chosen for the experiment from the vermicomposting unit of Kalapavirusam in Tenkasi, Tamilnadu (India). The stock culture of the earthworm was maintained in plastic containers using partially decomposed biowaste and cow dung as growth medium in laboratory condition for further use in the vermicomposting experiment. *E. eugeniae* a fast growing; ferocious eater of organic food does not feed much soil as substratum, the ideal ecological conditions that favours good growth.

Expertimental design

The vermibeds were prepared using Filter mud in plastic containers and watering was done regularly to moist the medium. Three vermicomposting treatments were established having 1kg of feed mixture containing CD alone and mixed with in different ratios (25, 50 and 75% filter mud) in plastic containers (Table 1). Experimental beddings were kept in triplicate for each treatment in perforated cylindrical plastic containers; Control consisted of 100% Cowdung (C) without any other ingredients. All waste mixtures were turned over manually for 15 days in order to pre-compost it so that it becomes condusive to earthworms

After 15 days of pre-composting 20 adult epigeic earthworms *E. eugeniae* species having individual live weight (350-400 mg) were inoculated into each of the reactors which were subjected for vermicomposting. All the reactors, T_0, T_{25}, T_{50} and T_{75} were operated in dark at a laboratory temperature of (26 ± 1ºC). The moisture content was maintained at 65 ± 5% by periodic sprinkling of distilled water (Vasanthi *et al.*, 2013d). During the experimental period no extra waste mixture was added at any stage in any vermicompost. All the vermicomposters were maintained in triplicate with earthworm density of twenty in each container. Control and treatments were performed in triplicate. At the end of experiment, worms, cocoons and hatchlings were removed and so produced vermicompost was air dried at room temperature and was packed in airtight plastic bottles for further physico-chemical and nutrient content analysis.

The vermicompost was harvested after appearance of black granular structure on the surface of the composting medium. Watering of the composting medium was discontinued four days before the harvesting. Vermicompost output from each treatment was calculated on dry weight basis.

Compost analysis

About 110g of homogenized wet samples (free from earthworms, hatchlings and cocoons) were taken out on the 1^{st} and 60^{th} day of composting period. Triplicate samples were collected and were stored at 4ºC for stability parameters, The pH and EC were determined using a double distilled water suspension of each waste in the ratio of 1:10 (w/v) that had been agitated mechanically for 30 min and filtered through Whatman No.1 filter paper (Trivedy and Goel, 1986). Total organic carbon (TOC) was measured using the method of Nelson and Sommers (1982). Total Nitrogen (TN) was determined after digesting the sample with concentrated H_2SO_4 and concentrated $HClO_4$ (9:1, v/v) according to Bremner and Mulvaney (1982). Total available phosphorus (TAP) was analyzed using the colorimetric method with molybdenum in sulphuric acid. Total potassium (TK) was determined after digesting the sample in concentrated HNO_3 : $HClO_4$, (4:1, v/v), by flame photometer (Bansal and Kapoor, 2000). Heavy metals' content in the vermicomposts was determined by using diacid digest of the sample. Analysis was done using atomic absorption spectrophotometer (AAS). Standard solutions were prepared by using the nitrate salts of the estimated heavy metals.

Determination of total microbial population and activity

One gram of each of the substrates vermicomposts samples on 15^{th} and 60^{th} days were collected was suspended in 1 ml sterile saline (l g NaCl in 100 ml distilled water) in a sterile test tube and was shaken thoroughly in a vortex mixer and used as inoculum for enumeration of total microbial population (fungi + bacteria) from the substrates. Using standard platinum loop, 0.01 ml of the inoculum was inoculated into nutrient agar plates for bacterial growth, Sabouraud dextrose agar plates for fungal growth and incubated at 37^{o} C for 18–24 h for bacteria, 20^{0}C and 25^{0}C for 3–5 days for fungi respectively. The different colony forming units (CFU) developing on the media were estimated according to the method of Baron *et al.*, (1994) and expressed as $CFU \times 10^5/g$ (for fungi), $CFU \times 10^6/g$ (for bacteria)

Statistical analysis: Paired sample t-test was used to analyze the differences in vermicompost production in different treatments. The same test was used to compare the mean values of different chemical parameters of the compost (control) and vermicompost

Results and Discussion

Physico-chemical changes in filtermud waste mixture during vermicomposting

The earthworm processed waste mixture was more stabilized, odor-free, dark brown and nutrient rich material. During the process physio-chemical properties of waste mixtures is changed drastically and end material is rich in soil nutrients. Hence it is essential to specify various physico-chemical characteristics, such as pH, electrical conductivity, total organic carbon, total nitrogen, total available phosphorus, total potassium, metal content etc., to quantify the dynamics of vermicomposting process. Physico-chemical characteristics of the initial feed mixtures and vermicompost are given in table 2.

pH

pH is an important parameter in the vermicompost for promoting plant growth Goh and Haynes (1977). In the present study (Table 2), pH in the control and filtermud mixed with Cow dung in different ratio showed an alkaline trend above neutral (pH-7) at 1st day of vermicompost. After 60th days a shift from the initial alkaline status to neutral condition was observed. In all the reactors (T_0-T_{75}) the pH was in the range, 7.13–8.32. In vermicompost waste mixtures pH shifted towards to downward scale and this could be due to the production of CO_2, ammonia, NO_3^- and other intermediate organic acids during waste decomposition processes (Vasanthi *et al.,* 2013c)

Electrical Conductivity (EC)

Electrical conductivity in the reactors T_0-T_{75} varied in relation to vermicomposting period and supplement addition (Table 2). The prolongation of vermicomposting from 1 day to 60 days elevated the EC rate significantly in all the reactors. The increase in EC could be to reduction of organic matter and release of different mineral salts: phosphate, ammonium, potassium as, reported by earlier workers (Nithya *et al.*, 2015).

Total Nitrogen (TN)

The total nitrogen content after 1st day and 60th days of *E. eugeniae* action in the different vermibed was estimated. The results were compared with total nitrogen content in pre composted filter mud. In the filter mud the nitrogen content was 1.56±0.01 on 1st day and 3.69±0.02 on the 60th day in control. After supplementing the filter mud with cow dung the total nitrogen content increased in all the treatments on 1st day except T_{25} treatment compared with control. But on the 60th day in all the treatments (T_0 -T_{75}) the total nitrogen level increased significantly. More than 2 fold increase in nitrogen content was observed in all treatments. The N enrichment process during vermicomposting depends upon the microbial populations and proportion of industrial wastes which contains microbial growth retarding. Vasanthi *et al.* (2013a).

Total Phosphorus (TP)

Total phosphorus in vermicompost contributes the presence of phosphate, an important macronutrient. Phosphate content in the vermicompost must be in an optimal level to promote the vermicompost as a plant nutrient. In the present study total phosphorus was in the range 0.63- 0.96 in all the reactors at 1st day. But in the 60th day vermibeds the amount of TP increased in T_{50} and T_{75} (Table 2). In T_{25} the total phosphorus content was less on 1st and 60th day when compared with other treatments. The increase in TP content for control was in the range of (2.32±0.04). Hait and Tare (2011) had postulated that the increase in TP content during vermicomposting was through mineralization, release and mobilization of available phosphorus content from organic waste performed partly by earthworm gut phosphates and further release of phosphorus might be due to phosphate solubilizing microorganisms present in worm cast Esaivani *et al.* (2015)

Total Potassium (TK)

Potassium is one of the major nutrients essential for plant growth. The optimum presence of TK in a vermicompost elevates its nutritional value for application to crops. The total potassium content in all the vermireactors tested in the present study was in the range 0.36±0.033 - 0.57±0.00 at 1st day. But on the 60th day of vermicomposting the total potassium content level increased, The high concentration of TK in the above mentioned vermibeds (T_{50}, T_{75} and T_{25}) must be due to higher mineralization rate as a result of enhanced microbial and enzyme activities in the guts of the earthworms as reported earlier by Parthasarathi and Ranganathan, (2000).

Total Organic Carbon (TOC)

Total organic Carbon in 1st day and 60th day's vermicompost was estimated in filter mud as well as filter mud and organic nutrient supplemented vermibeds. Total organic content in 60th day vermicompost was reduced when compared to 1st day old vermicompost. The TOC content in 60th day old vermicompost was in the range 42.18±0.35 – 52.61 ±0.15 where as it was in the range 53.54 ± 0.02 – 61.38 ±0.35 in 1st days old vermibeds. In all the treatment TOC decreased. The loss of carbon as CO2 due to microbial respiration and assimilation of simple carbohydrates leads to TOC reductions from waste mixtures. Moreover, carbo-hydrates and other polysaccharides which are considered major source of carbon are digested rapidly by earthworms and some fraction of digested substances is then assimilated into worm biomass (Vasanthi *et al.*, 2013a).

Table.1 The composition of cowdung and filter mud in different treatments

Treatment No	Description	CD(kg)	Filtermud(kg)
T_0	CD(100%)*	1	0
T_{25}	CD(75%) + Filter mud (25%)	750	250
T_{50}	CD(50%) + Filter mud (50%)	500	500
T_{75}	CD(25%) + Filter mud (75%)	250	750

*The figures in parenthesis indicates the percent content in the initial substrate material

Table.2 Variation in chemical constituents during vermicomposting of filter mud with organic nutrient supplement (g/kg)

Treatments	Days	pH	EC S/cm	TN g/kg	TP g/kg	TKg/kg	Total Organic carbon	Total Calcium	Total Magnesium	Total Sulphur	Total Copper	Total Zinc	Total Iron (Fe)	C/N ratio
T_0 control	1st day	7.83±0.03	1.07±0.00	1.56±0.02	0.66±0.08	0.53±0.033	57.68±0.88	1.58±0.0	0.69±0.00	0.62±0.00	144.8±1.74	1.08±0.00	598.6±10.5	36.00±0.05
	60th day	7.47±0.00	1.68±0.04	3.69±0.02	2.32±0.04	0.99±0.00*	52.61±0.15	3.31±0.01	1.51±0.00	1.68±0.00	390.66±14.7	1.30±0.01	719.21±5.75	14.57±0.11
T_{25}	1st day	7.23±.02	1.03±0.01	1.62±0.08	0.63±0.0	0.36±0.03	57.66±0.33	1.53±0.003	0.62±0.00	0.84±0.00	157.6±2.51	1.67±0.00	659.24±3.22	32.43±0.33
	60th day	7.13±0.00	1.94±0.00	3.07±0.00	2.26±0.0*	1.18±0.00	45.7±0.01*	3.23±0.0*	1..23±0.01*	1.34±0.0*	661±14.7	3.2±0.011*	1464.3±13.0	16.04±0.01*
T_{50}	1st day	8.02±0.03	1.28±0.00	2.02±0.08	0.96±0.03	0.57±0.00	61.38±0.35	1.61±0.00*	0.93±0.00	0.75±0.00	153.1±1.74	1.72±0.05	823.20±19.4	38.56±0.17
	60th day	7.61±0.00	1.95±0.07	4.07±0.00	3.14±0.2*	1.40±0.3*	42.18±0.35*	3.95±0.01	3.03±0.00*	2.71±0.03*	958±2.00	3.87±0.08*	2077±2.64	15.42±0.04*
T_{75}	1st day	8.32±0.03	1.22±0.00	1.84±0.05	0.86±0.03	0.53±0.03	53.54±0.02	1.58±0.0	0.81±0.01	0.71±0.02	167.13±3.15*	1.35±0.005	697.6±6.80	37.53±0.18
	60th day	7.35±0.00	1.87±0.00	3.96±0.00	2.37±0.01	1.12±0.01	43..88±0.62	3.87 ±0.0	2.81±0.00	2.11±0.00	785±7.00	2.70±0.05	1481.6±1.52	14.50±0.11

$P < 0.05$ - Significant * - non – significant

Table.3 Microbial count of *E. eugeniae* worked out filter mud turn vermicomposting after supplementation with cow dung

Treatments	15th day of vermicomposting		60th day of vermicomposting	
	Bacteria×10^4/g	Fungi×10^3/g	Bacteria×10^6/g	Fungi×10^4/g
T_0	224.33±2.19	231.00±0.58	247.00±4.73	223.00±1.53
T_{25}	283.67±1.76	267.00±5.51	291.67±0.88	249.33±2.9
T_{50}	292.33±1.45	280.67±6.44	297.00±1.15	275.67±2.85
T_{75}	231.67±1.20	215.00±2.89	263.33±1.67	262.67±1.45

Calcium (Ca)

Calcium content in the vermicompost was higher than initial feed substrates. The increase in Ca level was higher in 60th day treatment than in 1st day treatment. In the 60th days vermicompost the highest Ca level 3.95±0.01 was observed in T_{50} treatment. The lowest level of Ca was noticed in filter mud vermibeds in T_{25} (3.23±0.0). When compared to control, the hike in Ca level in $T_{50} > T_{75}$ on 60th day. Suthar (2007) reported that Ca metabolism in earthworm is primarily associated with gut secreted enzymes. He had also reported that in few endogeic and anecic worms the calcium gland is considered to play an important role in calcium secretion. A similar increase in calcium was reported by some researchers who used *E. eugeniae* in vermicomposting process (Vasanthi *et al.*, 2014).

Metals (Mg, S, Cu, Zn)

The trend of percent increase in Fe of the vermicomposts was in the order of $T_{50} > T_{75} > T_{25} > T_0$. Total iron content in vermicomposted material was in the range of 719.21± 5.75 - 2077±2.64 in different vermibeds. The maximum decrease in iron was in T_{25}. The difference in the Iron content of the vermicompost obtained from different treatments was significant (P<0.05). Senthilkumari *et al.* (2013) also reported similar increase of metals in the vermicompost. In vermicomposted material, Cu content increased in the order: $T_0 > T_{25} > T_{50} > T_{75}$ in different treatments (Table 2). Similarly Zn content also increased in all treatments, it was in range of 1.30 ± 0.01 – 3.87 ± 0.08 mg $kg^{-}1$ in vermicomposts and in range of 1.08 ± 0.00 - 1.72 ± 0.00 mg $kg^{-}1$ in initial feed mixtures. These results are supported by previous studies also, which have reported higher concentration of metals in final vermicomposts as compare to initial metal levels (Gupta and Garg, 2008). Mineralization of partially digested worm faecal by detritus communities (bacteria and fungi) and their action in the foregut resulted in high level of extractable or available trace elements in vermicompost (Vasanthi *et al.*, 2013b).

Carbon and Nitrogen Ratio (C/N)

The C/N ratio is traditionally used to establish the maturity degree of compost (Bernal *et al.*, 1998). The C/N ratio in all the experimental set up including control (T_0-T_{75}) was in the range 32.43±0.33 to 38.56 ±0.17 at 1st day of vermicomposting. But prolongation of *E. eugeniae* activity on vermibeds had reduced the C/N ratio significantly (Table 1). The C/N ratio was in the range 14.50 ± 0.11 -16.04 ±0.01 after

60^{th} of day vermicomposting. The decrease in C/N ratio was high in filter mud with cow dung (T_{25}), whereas in other treatments, (T_{50} and -T_{75}), the C/N ratio on 60^{th} day was reduced when compared to T_0. The C/N ratio is a factor related to the decomposition of the waste material and, even if it is recognized as a factor related negatively with the growth of earthworms and reproduction activities. In the present study, the observed decreasing trend in C/N ratio during vermicomposting is in good agreement with other authors (Vasanthi *et al.*, 2013c)

Microbial Population in vermicompost

The microbial count observed in the 15^{th} and 60^{th} day of vermicomposting is given in the table 3. In the microbial count observed during 15^{th} day of vermicomposting, maximum bacterial count was noticed in sample (T_{50}). The bacterial Population was 292.33±1.45×10^4CFU/g. The observed values were (T_{25}) 283.67±1.76× 10^4CFU/g, (T_{75}) 231.67±1.20×10^4CFU/g, when compared to control (T_0) 224.33±2.19 ×10^4CFU/g. The maximum fungal count was recorded in the sample (T_{50}) 280.67±6.44×10^3CFU/g. The observed values are (T_{25}) 267.00±5.51×10^3CFU/g, (T_{75}) 215.00±2.89×10^3CFU/g when compared to control (T_0) 231.00±0.58 ×10^3CFU/g. The bacterial and fungal counts were to be increasing from 15^{th} day to 60^{th} day filter mud compost. In the present study the observation of bacterial and fungal count was found to be increased in 60^{th} day of vermicomposting, In the sample (T_{25}) 291.67±0.88 ×10^6CFU/g. The observed values are (T_{50}) 297.00±1.15×10^6CFU/g, (T_{75}) 263.33±1.67×10^6CFU/g, when compared to control (T_0) 247.00±4.73×10^6CFU/g. The maximum fungal count was observed in treatment (T_{50}) 275.67±2.85 ×10^4CFU/g and the minimum fungal count was noticed in (T_{25}) 249.33±2.9×10^4CFU/g. when compared to control (T_0) 223.00±1.5310^{4}CFU/g.

An increased number of bacteria and fungi in the treatments compared to the control were observed (Table 3). Thus *E.eugeniae* contributed to the increase of the microbes of the organic matter. Similar increases were also observed in other vermicomposts (Karmegam and Daniel, 2009; Prakash *et al.*, 2009). This observation parallels that of Parthasarathi (2007) who reported increased microbial population, microbial activity and N, P and K content in the vermicompost at 31ºC and 60–70% moisture during vermicomposting of sugar industrial wastes.

Although the earthworms enhance soil microbial activity by improving the environment favorable for microbes (Syers *et al.*, 1979) the fate of microorganisms during the casting analysis proved the removal of *Salmonella sp.*, *Shigella sp.*, and faecal coliform, in 35 days. However, the *Pseudomonas sp.*, cellulolytic *Bacillus sp.*, and heterotrophic bacterial population were increased at the end of vermicomposting period, indicating the selective nature of earthworms in the removal of microorganisms. This corroborates with the findings of the researchers proving that earthworms include microorganisms in their substrates as a food source and can digest them selectively (Edwards and Bohlen, 1996); (Bohlen and Edwards, 1995). The total CFU/g was higher in the final vermicompost and in the compost than in the initial vermibed mixtures. The study conducted by Raphael and Velmourougane (2011) clearly showed that the general and functional microbial groups in vermicompost of coffee pulp using the exotic worms (*E. eugeniae*). Similar increases in microbial population were reported in other vermicomposting systems also (Prakash and Karmegam, 2010).

Similar type of result was also obtained in our present study. The results indicated that the organic substrates used in the present study could initiate the proliferation of the microorganisms and the earthworm species used in the present study also acted as an organic nutrient for the rapid microbial colonization whereby increases the microbial activity. The statistical analysis of "t" test of microbial count of all treatments shows significance at 5% level ($p \leq 0.05$).

In conclusion, the present investigation clearly suggests that the incorporation of Cow dung with filter mud convert this huge waste into highly valuable vermicompost. Due to excellent level of nutrient release into the filter mud by the action of *E. eugeniae*, the vermicompost becomes a good plant growth promoter. The results suggest that the filter mud supplementation with cow dung 50% wastes promotes the activity of the earthworm and produce highly nutritive vermicompost. The bacterial and fungal counts were found to be maximum in T_{50} vermibed. The study recommends that filtermud mixed with cow dung1:1 ratio is suitable mix for making vermicompost rich in nutrients and microorganisms which can be used as suitable organic soil amendment.

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