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Vermicomposting of Solid Waste Generated from Leather Industries Using Epigeic Earthworm *Eisenia foetida*

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Abstract Animal fleshing (ANFL) generated as solid waste from tannery industries was vermicomposted using the epigeic earthworm *Eisenia foetida.* The mixing ratio of ANFL with cow dung and agricultural residues as feed mixtures was maintained to be 3:1:1 respectively during the vermicomposting experiments for 50 days. Vermicomposting resulted in the reduction of pH 6.74 and C:N ratio 15.5 compared to the control sample. A notable increase in earthworm biomass was also observed in the vermin bioreactor. The germination index of 84% for tomato seedlings (*Lycopersicon esculentum* cv. PKM1) was observed for the vermicomposted soil. Scanning electron microscope and Fourier transform infrared spectroscopy were recorded to identify the changes in surface morphology and functional groups in the control and vermicomposted samples. The results obtained from the present study indicated that the earthworm *E. foetida* was able to convert ANFL into nutrient-enriched products.

Keywords *Eisenia foetida* · Animal fleshing · Vermicomposting · C:N ratio · *Lycopersicon esculentum* cv. PKM1

Introduction

Animal fleshing (ANFL) is one of the major solid wastes that are generated during leather processing while scrapping out the limed hides either by hand or by machine. It is one of the low molecular weight-containing proteins that is not properly utilized or underutilized for constructive applications. There are about 3,000 tanneries in India generating 89,900 tons of ANFL as waste per year [1]. In India, Tamilnadu and Uttarpradesh are the states that have major clusters of tanneries. These fleshings are proteinaceous in nature comprising of cutaneous muscle layers and subcutaneous adhering tissues that are not required in the subsequent operations of leather manufacture. The availability of limed

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ANFL is 35% on the wet weight of the raw hides with 80% moisture [2]. The ANFL emanating from tannery industries can be subjected for biomethanation, glue manufacture, enzyme, protein, and animal feed production [3]. ANFL is a rich source for the production of organic manure that has a very high utility in the agricultural sector. Although biomethanation is a good process for energy production, it however has its own drawback of ecological significance for its incomplete biogas recovery, relatively high water consumption that is required to dilute the wastes (about 1 m^3 tap water per ton solid waste), and high financial cost in the overall process starting from collecting the wastes, biogas production, and until the disposal of end products [4]. The search for low-input basis, eco-friendly technologies for solid waste management is still under way [5].

In this regard, vermicomposting is a viable, simple, and economical process by which organic solid wastes can be efficiently managed by converting it into organic manure/soil conditioners popular with farmers [6]. Earthworms can be used for solid waste management, organic matter stabilization, soil detoxification, and vermicompost production [7]. Considerable work has been carried out on the vermicomposting of organic and industrial wastes such as guar gum industrial waste [5], aquaculture effluent solids [8], solid textile mill sludge [9], municipal solid waste [10], petrochemical sludge [11], sewage sludge [12], food waste and paper waste [13, 14], etc. Different varieties of earthworms such as *Eisenia foetida*, *Eisenia anderi*, *Lumbricus terrestris*, *Perionyx excavates*, and *Eudrilus eugeniae* have been used for the vermicomposting of these wastes. During vermicomposting, earthworms eat, grind, and digest organic wastes with the help of aerobic and some anaerobic microflora, converting them into a much finer, humified, and microbially active material. The generated product is stable and homogeneous, having desirable aesthetics such as reduced levels of contaminants [15].

The converted product can be used as a fertilizer or as a source of nitrogen for microbial populations which can be beneficial to plant growth [16]. However, recent studies show that vermicomposting of animal fleshing is scarce. Hence, the focal theme of the present investigation was to study the impact of *E. foetida* earthworm for the vermicomposting of ANFL into nutrient-rich vermin compost at low detention period. For this purpose, tannery animal fleshing was vermicomposted along with cow dung and agricultural residues.

Materials and Methods

Raw Material, Feed Mixtures, and Earthworms

Limed ANFL from goat skins collected from the commercial tannery processing industry was used as raw material. This was washed in copious water to remove adhered dirt and surface-deposited lime. The washed ANFL was treated with 10 ml l^{-1} hydrochloric acid solution for about 4–6 h to remove sulfide and calcium salts [17], followed by chopping into small pieces. These pieces were again washed thoroughly in tap water and finally stored at 4°C until the start of the experiments. Cow dung (CD) and agriculture residues were used as feed mixtures. The main characteristics of CD were pH (1:10 ratio *w*/*v* based on distilled water), 7.89; total Kjeldhal nitrogen (TKN), 0.65%; total organic carbon (TOC), 40.3%; total phosphorus (TP), 0.59%; and C:N ratio, 62. Dried agricultural residues were collected and shredded to 2–5-mm sizes before use. The main characteristics of agricultural residues were TKN, 0.9%; TOC, 50.1%; and C: N ratio, 55.66. All experiments were conducted in triplicate.

Non-clitellated specimens of *E. foetida*, weighing 200–250 mg live weight, were randomly picked from a stock culture maintained in the vermicomposting farm of our laboratory.

Experimental Design

One-liter plastic containers (diameter 14 cm and depth 12 cm) were filled with 1,500 g feed mixture. The optimized initial feed mixture (g) ratios are: 900 g of ANFL, 300 g of CD, and 300 g of AR. Nylon screen (0.6 mm mesh) was used to prevent the earthworms escaping from the vermin bioreactor. The mixtures were turned over manually every 12 h for 15 days in order to eliminate volatile substances toxic to earthworms. After 15 days, 50 non-clitellated earthworms were introduced into a vermicompost plastic container. The moisture content was maintained at 60–80% throughout the study period by periodic sprinkling of adequate quantities of water. The containers were kept in the dark under identical ambient conditions (room temperature; 25±3°C). No additional food was added at any stage during the study period. Another set of feed mixtures without earthworms was established as control to compare the results. All experiments were carried out in triplicate.

The subsamples were taken and analyzed for pH, TKN, TOC, TP, TK, scanning electron microscope (SEM), and Fourier transform infrared (FT-IR) spectra. Samples were taken from different points of the vermicompost heap (bottom, surface, side, and center) at each stage of vermicomposting containing the ANFL during a period of 0, 7, 15, 22, 29, 36, 43, and 50 days. Earthworm biomass in the vermin bioreactor was recorded at different stages of vermicomposting.

Physico-chemical Analysis

All samples were analyzed in triplicate and the results were averaged. The results were reproducible within 3–7% error limits. The pH and electrical conductivity were determined using distilled water suspension of the ANFL mixtures in the ratio of 1:10 (*w*/*v*) that had been agitated mechanically for 30 min and filtered through Whatman No. 1 filter paper. Ash content and total solids were carried out according to methods of the American Public Health Association [18]. Cation-exchange capacities of the samples were determined by the conventional ammonium acetate (NH_4OAc) method [19]. Total organic carbon was estimated according to the Walkley and Black rapid titration method [20] while TKN in accordance to the micro-Kjeldhal method [21]. Total phosphorus was measured colorimetrically with molybdenum in sulfuric acid using Varian-Cary 100 UV–visible spectrophotometer. Total potassium was determined, after digesting the sample in diacid mixture (concentrated HNO_3:concentrated $HClO_4$, 4:1, *v*/*v*), by flame photometer (Elico, CL 22D) [22]. The germination index was determined by placing a layer of compost in a Petri dish and covering it with a filter paper. Water was subsequently added until the filter paper was completely submerged and seeds of tomato seedlings (*Lycopersicon esculentum*) were separately placed on the filter paper in the Petri plate. The percentage of germination was measured after incubating the covered Petri dishes in the dark at 28°C for 5 days [23].

Fourier Transform Infrared Spectroscopy

FT-IR spectra of control and vermicompost samples were recorded using a Perkin Elmer FTIR spectrometer in the spectral range of 4,000–400 cm^{-1}. The samples were mixed with spectroscopic grade KBr and made in the form of pellets at a pressure of about 1 MPa. The pellets were about 13 mm in diameter and 1 mm in thickness.

Scanning Electron Microscopy

The control and final dried vermicompost samples were sputtered with gold for the clear visibility of the image, followed by recording the surface morphology using JEOL JM-5600 electron microscope at ×2,400 magnification.

Results and Discussion

The chemical characteristics of ANFL were carried out on a dry weight basis and the standard deviations are based on triplicate analysis. The characteristics of ANFL are pH, 7.91±0.3; TKN, 102±5.1 mg g^{-1}; TOC, 380±20.1 mg g^{-1}; TK, 2.5±0.1 mg g^{-1}; moisture content, 75.5±3.5%; ash content, 15.2±0.3%; total solids, 25.8±2.1%; and total protein, 50±1.5%.

Changes in Physico-Chemical and Earthworm Biomass of the Feed Mixtures

The vermicomposting significantly modified the physical and chemical properties of ANFL feed mixtures as shown in Table 1. The vermicompost had been processed more or less into a homogeneous mixture with a dark color than the initial feed mixtures at the end of 50 days of earthworm activity. This may be attributed to the physical and chemical breakdown during the process of ingestion and digestion of ANFL feed mixture by the earthworms. No mortality was observed during the study period. At the end of vermicomposting period, the total weight of the earthworms increased from 12.5±0.31 to 50±2.1 g. However, the maximum weight of the earthworms reached about 68.75±3.67 g during the 29th day of the study.

The decrease in pH of the final vermicompost from 7.68 to 6.74 can be attributed to the production of carbon dioxide, mineralization of nitrogen and phosphorous into nitrates/nitrites and orthophosphates, and bioconversion of the ANFL mixtures into organic acids by microbial activity [24]. Electrical conductivity (EC) of vermicompost was higher than control feed mixtures. The highest EC of 1.3 mS cm^{-1} for vermicompost than the control sample with value 0.65 mS cm^{-1} may be attributed due to the loss of organic matter and freely available ions and minerals that are generated during ingestion and excretion by the earthworms [6]. Similar kinds of results were also reported by Kaviraj and Sharma and Wong et al. [10, 25].

Total organic carbon was lower in the vermicompost than that of the control sample. The values decreased from 55.12% to 21.1% at the end of the vermicomposting period. Similar kinds of results were also reported by Suthar [5] and Gupta and Garg [7], respectively. The ingested ANFL mixtures would be homogenized through muscular action of their foregut leading to an increase in surface area for microbial action [26]. The microorganisms by biochemical mechanism degrade and provide some extracellular enzymes for ANFL waste decomposition within the worm gut leading to the loss of TOC content. TKN in the vermicompost increased from 0.81% to 1.34% from that of the control indicating low losses of inorganic nitrogen, probably because of mineralization of organic matter. The mucus, nitrogenous excretory substances, growth-stimulating hormones, and enzymes from earthworms would be the reasons for the increase in nitrogen content of the vermicompost

Table 1 Nutrient content (%) (mean±SD) in the control and vermicompost feed mixtures during the study (n=3).

Days	pH		TOC		TKN		TP		TK	
	Control	Vermicompost	Control	Vermicompost	Control	Vermicompost	Control	Vermicompost	Control	Vermicompost
0	7.7±0.23	7.68±0.23	55.27±2.2	55.12±2.2	0.81±0.02	0.81±0.03	0.275±0.01	0.275±0.01	0.167±0.005	0.168±0.005
7	7.49±0.29	7.44±0.26	51.89±1.81	48.52±1.69	0.825±0.03	0.83±0.3	0.278±0.01	0.277±0.009	0.166±0.006	0.166±0.005
15	7.17±0.21	7.21±0.21	44.52±1.55	44.47±1.55	0.83±0.03	0.84±0.03	0.281±0.009	0.282±0.009	0.165±0.008	0.164±0.007
22	7.1±0.28	7.11±0.24	44.41±1.77	43.36±1.73	0.84±0.02	0.85±0.02	0.282±0.008	0.284±0.008	0.159±0.007	0.158±0.006
29	7.05±0.21	7.02±0.21	40.02±1.60	37.87±1.32	0.89±0.03	0.95±0.02	0.287±0.01	0.288±0.01	0.157±0.007	0.154±0.007
36	7.01±0.28	6.94±0.20	37.74±1.3	35.58±1.24	0.91±0.04	1.12±0.04	0.288±0.01	0.294±0.01	0.151±0.005	0.149±0.006
43	6.95±0.20	6.8±0.27	34.17±1.1	26.93±0.94	0.96±0.04	1.3±0.03	0.29±0.009	0.297±0.08	0.147±0.006	0.145±0.005
50	6.87±0.24	6.74±0.23	30.02±1.17	21.1±0.84	0.98±0.03	1.34±0.04	0.298±0.01	0.312±0.01	0.145±0.006	0.143±0.006

sample [22, 27]. Although earthworms have a greater impact on retaining nitrogen in nitrate form, the initial nitrogen present in the waste and the extent of decomposition will decide the amount of TKN in the vermicompost.

The amount of total phosphorous in the ANFL feed mixtures increased from 0.275% to 0.312% during the course of vermicomposting period (Table 1). The total phosphorus content value was also higher than that of control feed mixtures (0.298%). The increase in TP during vermicomposting is probably due to higher phosphatase activity of earthworms contributing to mineralization and mobilization of phosphorous [28]. However, total potassium slightly decreased from 0.169% to 0.143% in the final product compared to that of the initial product. This decrease in TK can be attributed to the leaching of soluble elements by excess water that drained through mass. It was also interesting to note that the C:N ratio of vermicompost came down to 15.5 against 30.6 for the control samples. The value less than 20 for the vermicompost suggests the satisfactory degree of maturity of ANFL feed mixtures. This result also indicates that vermicompost process is better than that of control ANFL mixtures.

After the vermicomposting period (50 days), the cation exchange capacity (CEC) of 19.0 meq 100 g^{-1} was recorded. In control containers, the CEC obtained was only 17.9 meq 100 g^{-1}. This could be due to the humification process, which produces functional groups in turn increasing CEC [29]. Vermicomposting extracts had a germination index for PKM1 variety tomato seedlings (*L. esculentum*) of 84% compared to the control sample of only 74%. The results indicate that the ANFL-vermicomposted product is suitable for seed

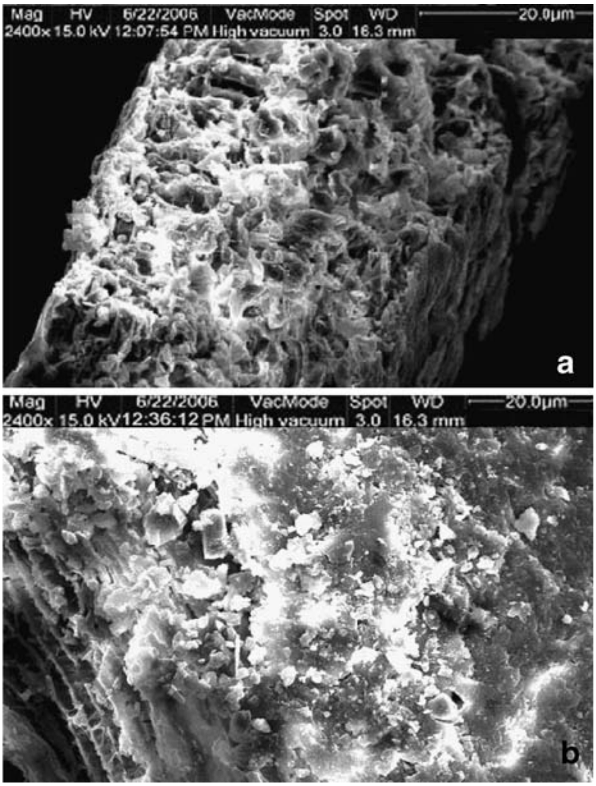

Fig. 1 **a** Scanning electron micrograph of control sample. **b** Scanning electron micrograph of vermicompost sample

germination. This germination method was however highly comparable to what Contreras-Ramos et al. [30] reported for tannery effluent composting with a germination index for cress (*Lepidium sativum*) of 48% after 90 days. Germination index values above 50% indicate that the compost was sufficiently matured.

Scanning Electron Microscopy Analysis

SEM was employed to study the morphological events of the control and final products as shown in Fig. 1a and b. In the control ANFL mixtures (Fig. 1a), the aggregates of biomass are observed to be organized into cellulose fibers and the protein matrix is firmly bounded

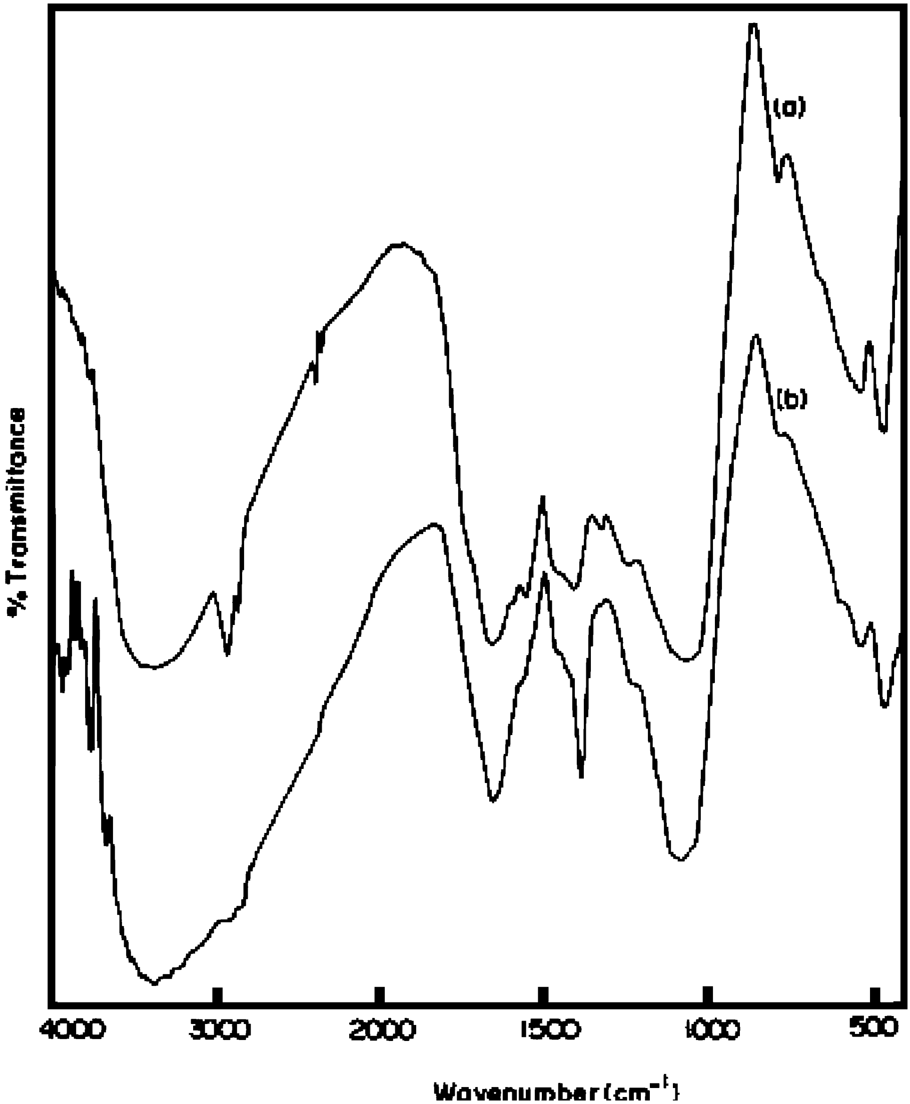

Fig. 2 FT-IR spectra of control and vermicompost samples

with lignin-containing cellulose fibers of mixtures. However, in the vermicomposted final product (Fig. 1b), the protein and lignin matrix is disaggregated by earthworms. Normally, earthworms grind the feed mixtures in the gut that contains numerous hydrolytic bacteria and helps in the progressive degradation. Moreover, the ANFL-containing mixtures in the presence of earthworm confirm more numerous surface irregularities in the final day that proves to be a good vermicompost manure.

FT-IR Analysis

FT-IR spectra of control and vermicompost samples are shown in Fig. 2. A strong hydrogen band due to -OH stretch was observed at 3,400 cm^{-1}. A band at 1,400 and 1,100 cm^{-1} appeared due to cellulose and C–O stretch in cellulose and hemicellulose, respectively. The bands at 1,440 cm^{-1} and 1,650 cm^{-1} absorbed for lignin were also observed. A band at 1,540 cm^{-1} appeared due to the presence of amides. This N–H band was characteristic of the control product. These bands appear and confirm to the partial mineralization of mixtures. But in the final vermicomposted sample, the deformation of bands at 1,100 and 2,925 cm^{-1} is in accordance for cellulose, hemicelluloses, fat, and lipid. The appearance of a sharp band due to nitrate vibration was also found [31, 32]. These results confirm the reduction of aromatic structures, polypeptides, and polysaccharides in the vermicomposting process associated to extensive organic matter mineralization and indicate the maturity and stability of the final product than the control.

Conclusions

Vermicomposting of animal fleshing obtained as waste from tannery industries was carried out using the epigeic earthworm *E. foetida.* Vermicomposting resulted in the drop of pH, increase in nitrogen content, and decrease in TOC and C:N ratio compared to the control sample. The value of C:N, 15.5, for the vermicompost suggests the satisfactory degree of maturity of ANFL feed mixtures. The evolution of carbon dioxide led to the reduction of TOC values in the final products. The increase in earthworm biomass in the vermin bioreactor, the germination index of 84% for tomato seedlings, and other physicochemical characteristics of the vermicomposted soil give the scope for producing organic manure from animal fleshing. SEM and FT-IR results also substantiate the usefulness of vermicomposting techniques. Thus, the results obtained from the present study indicate that the earthworm *E. foetida* was able to convert ANFL into nutrient-enriched products and can play a major role in solid waste management.

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