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Short Communication

Influence of microbial diversity and plant growth hormones in compost and vermicompost from fermented tannery waste

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HIGHLIGHTS

- Vermicomposting proved to be a more effective method for fermented tannery waste.
- Maximum microbial population, phytohormones were recorded in the vermicompost products.
- These study results provided the scope of greater value added product from tannery waste.

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ABSTRACT

This study focuses on the effect of the epigeic earthworm *Eudrilus eugeniae* (with and without addition) to transform solid state fermented (SSF) and submerged (SmF) state fermented TFL mixed with cow dung and leaf litter into value added products in compost and vermicompost bioreactors respectively. The significant role of microbes was identified during compost and vermicompost process. In addition, three important phytohormones (Indole 3-acetic acid, Gibberellic acid, Kinetin) were also detected in the compost and vermicompost products. The results revealed that the maximum amount of plant hormones were available in the vermicompost products which may be due to the joint action of earthworm and microorganisms. The overall results confirmed that the vermicomposting process produced a greater value added product.

1. Introduction

Management of solid waste has become one of the biggest problems all over the world. The rapid increase in production and consumption, industrialization and population growth contribute to the increase in the amount of solid waste being produced every year (Khajuria et al., 2010). Tannery fleshing, the major solid waste generated in the pre-tanning operations, is about 50–60% of total waste discharged in the leather industry causing world wide disposal problem (Ravindran et al., 2014). In this regard, composting and vermicomposting are two of the best known processes for the biological stabilization of a great variety of organic wastes. Several researchers have studied the separation, combination or comparison of composting and vermicomposting process on different waste such as swine manure (Selvam et al., 2012), effluent treatment plant sludge of bakery industry (Yadav et al., 2015), distillery sludge (Singh et al., 2014) and vegetable waste (Huang et al., 2013). Nagavallemma et al. (2004) reported that the earthworm intestine contains a wide range of microorganisms, enzymes and hormones which aid in rapid decomposition of half-digested material transforming it into vermicompost in a short time (4–8 weeks). It has many advantages over traditional thermophilic composting. Some more interesting activities by earthworms are the secretion of plant hormones gibberellins, auxins and cytokinins in the vermicomposting process with the help of microbes (Sinha et al., 2011) state of synergistic interactions. In our previous studies (Ravindran et al., 2015, reported the *Eudrilus eugeniae* earthworm gut enzymes and microbial patterns, and selected plant growth hormones in vermicompost manure alone. Our current study focuses on the influence of microbial diversity and major plant growth hormones, such as (Indole 3-acetic acid [IAA], *Gibberellic acid* [GA3], Kinetin) on the compost and vermicompost

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product from submerged (SmF) and solid state fermented (SSF) tannery waste.

2. Materials and methods

2.1. Characteristics of fermented TFL, cow dung, leaf litter and earthworms

Fermented tannery fleshing (TFL) processes were well explained for both solid state fermentation process (SSF) and submerged fermentation process (SmF) in our previous publications (Ravindran et al., 2014). Fresh cow dung (CD) was obtained from a local cow-shed and consisted of a mixture of feces and urine without any bedding material. Partially decomposed leaf litter was collected from the institutional garden area and shredded to the size range of 2–5 mm before use. Selected chemical parameters of the initial raw materials were analyzed according to standard methods (APHA, 2005) in Table S1. Healthy unclitellated hatchling epigeic earthworm species *Eudrilus eugeniae* were used in these experiments.

2.2. Experimental design

Six different treatments with three different waste mixture compositions were prepared (cow dung plus leaf litter and with or without hydrolysed TFL). Composting and vermicomposting processes were conducted in circular plastic containers with a working capacity of 1 kg (Ravindran et al., 2014). One kg of feed mixtures in the ratio of 1:1 (cow dung:leaf litter) with 1 L of hydrolyzed TFL extract (SmF or SSF) was added to the respective treatments, except the control. Fifty healthy *E. eugeniae* earthworms were introduced into the three vermicompost treatment containers (control, SSF, SmF) and another set of three treatment mixtures (control, SSF, SmF) was established without earthworms (compost) to compare the results. The treatment reactor moisture content (%) was maintained at 70 ± 10. All the containers were kept in the dark at a laboratory temperature of 25 ± 3 °C. A sample (25 g) of each treatment was collected from all the reactors on day 0, 7, 14, 21 and 25 for different analyses. C:N ratios (initial day and final day), microbial(day 0, 7, 14, 21 and 25) and plant growth hormone (final day) contents were analysed in all treatment reactors.

2.3. Physico-chemical, microbiological and plant growth hormones analyses

C:N ratio was calculated from the measured values of total organic carbon and total kjeldhal nitrogen were analysed following the standard methods for testing compost materials (TMECC, 2003). During the composting and vermicomposting process, the different feed mixture sub-samples were analyzed for microbiological counts (i.e. total bacteria, fungi, actinomycetes, proteolytic bacteria and cellulolytic bacteria) and fecal coliforms were determined by inoculation of tube media using the Most Probable Number (MPN) method (APHA, 2005). All samples were assayed by dilution with three replicates of each suspension. The number of colony forming units (CFU) was expressed as CFU g^{-1} (Ravindran et al., 2015). Plant growth hormones (Indole 3-acetic acid [IAA], *Gibberellic acid* [GA3], Kinetin) were analyzed in the different treatment final compost and vermicompost products, according to the method of Unyayar et al. (1996).

2.4. Statistical analysis

Data reported here are the means of three replicates ($n = 3$). All results were subjected to a one way analysis of variance (ANOVA) using PROC GLM to test for significant differences between treatments at different times with Tukey's Studentized Range (HSD) test and Duncan's Multiple Range Test. The probability levels used for statistical significance were $p < 0.05$ for all tests.

3. Results and discussion

3.1. C:N ratio and microbial variations of feed mixtures

C:N ratios also an indicator to determine the maturity of compost and vermicompost product. In our studies (Control, SmF and SSF), C/N ratio decreased at day 25 in the range of compost (21.4–12.1) and vermicompost (17.3–10.3) from the initial mixtures (53.4–57.7) (Fig. S1). These results indicated that the vermicomposted product was more stabilized and matured than the composted product. Ravindran et al. (2015) reported that this stabilization could be due to respiratory activities of earthworms and microbes at the same time that there is an addition of nitrogen to the substrate material by earthworm excretions such as mucus, enzymes and nitrogenous compounds. Microbial activities were determined during the vermicomposting and composting process and these results were correlated with the microbes and maturity of manure. The microbial population significantly increased in earthworm (+worms) treatments compared to the without earthworms (−worms) treatments (Fig. 1). Overall, no significant differences were observed in bacteria, fungi and actinomycetes after 21 days in the composting process. However, SmF (−worms) and SSF (−worms) treatment were significantly higher than Control (−worms) at the end of the process (Fig. 1b, d and f). The highest microbial count was found at 25 days of composting process, with bacteria 91×10^5, fungi 21×10^5 and actinomycetes 66×10^5 in SSF (−worms) followed by SmF (−worms) with bacteria 85×10^5, fungi 19×10^5 and actinomycetes 65×10^5, and in Control (−worms) with bacteria 78×10^5, fungi 16×10^5 and actinomycetes 51×10^5. In this process, the microbial count slowly increased up to the end of the process on day 25. These results show that the available substrates were not completely utilized by microbes in the without worms treatments. The highest microbial count was found at 21 days of vermicomposting, with bacteria 132×10^5, fungi 28×10^5 and actinomycetes 71×10^5 in SSF (+worms) followed by SmF (+worms) with bacteria 116×10^5, fungi 26×10^5 and actinomycetes 67×10^5, and in Control (+worms) with bacteria 98×10^5, fungi 19×10^5 and actinomycetes 59×10^5 (Fig. 1a, c and e). This increase in the microbial population in earthworm treated feed mixtures may be due to a suitable conditions for the growth of microbes in the earthworm digestive tract and by the ingestion of nutrient rich organic wastes which provide energy and also act as a substrate for the growth of microorganisms. Parthasarathi and Ranganathan (2000) reported the increase of microbial populations in *E. eugeniae* processed vermicompost compared to composted manure. After 21 days of vermicomposting process, the microbial population decreased, which may be attributed to the limitation of nutrients from the feed mixtures and its revealed the complete utilization of substrates by earthworms.

The presence of fecal coliforms indicates the presence of pathogens. Fecal coliform in the range from 8×10^5 to 10×10^5 were identified in all treatments at the initial stage. In the treatment of earthworms processed mixtures (SSF, SmF and Control), the fecal coliforms were not detected at 21 days of vermicomposting (Fig. 2a). At the same time the fecal coliforms were completely reduced only at the end of 25 days in the composting process (−worms) (Fig. 2b). Monroy et al. (2009) suggested that coliforms were eliminated when they enter the food chain of the earthworms in the vermicomposting process, and the decreases in coliform