

Comparative assessment in enzyme activities and microbial populations during normal and vermicomposting

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Abstract: Changes in extracellular enzyme activities and microbial populations were studied during the normal composting and vermicomposting of fruitpulp, vegetable waste, groundnut husk and cowdung. The microbial numbers and their extracellular enzyme profiles showed relative variation and were found increasingly more abundant in vermicompost than in normal compost leading to the conversion of agricultural waste into value added product. In vermicompost, the maximum enzyme activities (cellulase, amylase, invertase, protease and urease) were observed during 21-35 days. The cellulase, amylase and protease activities of vermicompost reached the maximum values by 28th day of 1175, 825 μg reducing sugar $\text{g}^{-1} \text{hr}^{-1}$ and 28 μmol of amino acid $\text{g}^{-1} \text{hr}^{-1}$ of vermicompost samples respectively. Similarly the invertase and urease activities reached to peak values of 876 μg reducing sugar $\text{g}^{-1} \text{hr}^{-1}$ and 197 μg $\text{NH}_4^+ \text{-N g}^{-1} \text{ha}^{-1}$ sample on 35th day respectively. Most of the enzymes showed correlation with change in number and types of different microbial groups like bacteria, fungi and actinomycetes during vermicomposting with maximum number of 126×10^6 , 28×10^4 and 93×10^5 CFU g^{-1} sample respectively. In contrast delayed greatest enzyme activities were observed on 42-49th day i.e., last days of normal composting. Earthworms stimulated biochemical activity and nutrient cycling by 40-45% contributing to the reduction of period of degradation of agricultural wastes resulting in maturation of vermicompost by 28th day.

Key words: Normal compost, Vermi compost, Cellulase, Amylase, Invertase, Protease, Urease
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Introduction

Decomposition and humification of biodegradable organic waste materials is predominantly carried out by micro organisms in the soil but the few recent studies have shown that earthworms too have roles in humification (Kadalli *et al.*, 2000; Manivnnan *et al.*, 2004; Ranganathan and Parthasarathi, 2005; Parthasarathi, 2007). Microbial composting of organic wastes through earthworm activity is called vermicomposting which does not involve a thermophilic stage. Canellas *et al.* (2002), Pizl and Novokova (1993), reported the establishment of different kinds of relationship between earthworms and microbes. They are: (1) microbes form a part of food for earthworm, (2) microbes are proliferated in the gut and vermicomposts, (3) earthworms help in the distribution of microbes and (4) together with earthworm the microbes mineralise humifies organic matter and facilitates chelation of some metal ions. The role of microbial activity in the gut and cast of earthworms and soil is very essential for the degradation of organic wastes to result in the release of nutrients to plants. In vermicomposting process during the passage through the worm's gut, organic matter undergoes physico chemical and bio-chemical changes by the combined effect of earthworms and microbial activities. Higher activities of cellulase, amylase, invertase, protease, peroxidase, urease, phosphatase and dehydrogenase in the wormcasts have been reported by Edwards and Bohlen (1996) and Sharpey and Syers (1976). Earthworms transform organic waste constituents into a more useful forms by grinding and digesting with the help of aerobic and

anaerobic microflora (Maboeta and Rensberg, 2003). The biological decomposition of organic matter is mediated by a variety of biochemical processes in which enzymes play a key role (Garcia *et al.*, 1992; Vuorinen, 1999). The major constituents like cellulose, hemicellulose, lignin, starch and different protein compounds present in waste are degraded by specific enzymes. Therefore, the quantification of enzyme activity during composting can reflect the dynamics of composting process in terms of decomposition of organic matter and nitrogen transformation. In correlation with enzyme activity the changes in microbial number and types also helpful in providing information about the maturity of the composted product (Tiquia, 2002, 2005). Characterizing and quantifying the enzymatic activity during composting can reflect the dynamics of the composting process in terms of the decomposition of organic matter and nitrogen transformations, and may provide information about the maturity of the composted product. Much of the research on vermicomposting has been focused on the changes in the chemical parameters and information available on the microbiota and enzyme activities that determine the rate of vermicomposting are very little or perhaps nil. Therefore in the present study the investigation was carried out (1) to determine and compare the extracellular enzyme activity profiles at different stages of normal composting and vermicomposting (2) to relate the activities of these enzymes to the changes in microbial population.

Materials and Methods

The fruitpulp, vegetable and groundnut waste was chopped to 0.5-1.0 cm bits and mixed with cowdung (FVGC) in the ratio of

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2.5 : 2.5 : 1.0 : 4.0. The FVGC mixture was incubated for precomposting for 10 days. After precomposting the mixture was mixed well and divided into two equal parts and left for maturation at room temperature under similar experimental conditions. One part was allowed for natural composting. To the other part the adult earthworms of *Eudrilus eugeniae* were added and set the experiment upto 49 days. The moisture was adjusted at 65% (W/V) by regularly sprinkling water. The composting material was turned now and then to ensure thorough mixing and uniform decomposition. Samples at weekly intervals from both natural compost and vermicompost, stored at 8°C were analysed for extracellular enzymes and microbial populations.

Cellulase activity: Four ml of carboxymethyl cellulose dissolved in 0.1 M pH 5.0, citrate buffer (1%) was added to 1.0 g sample of normal compost / vermicompost and incubated at 55°C for 60 minutes. Then the reaction mixture was centrifuged at 4000 x g for 10 minutes and passed through Whatmann No.1 filter paper. The filtrate was assayed by dinitrosalicylic acid method for amount of reducing sugar formed due to cellulolytic activity (Miller, 1959). Controls were prepared by adding carboxymethylcellulose after incubation.

Amylase activity: Four ml of starch dissolved in 0.1 M pH 4.7 acetate buffer (1%) was added to 1.0 g of normal compost/vermicompost sample and incubated at 27°C for 60 minutes. Then the reaction mixture was centrifuged at 4000 x g for 10 minutes and passed through Whatman No. 1 filter paper and the filtrate was assayed for amount of reducing sugar formed due to amylolytic activity (Miller, 1959). Controls were prepared by adding starch solution after incubation.

Invertase activity: Four ml of 0.3 M sucrose dissolved in 0.2 M pH 4.5 citrate phosphate buffer was added to 1.0 g of normal compost/vermicompost sample and incubated at 37°C for 60 minutes. Then the reaction mixture was centrifuged at 4000 x g for 10 minutes and passed through Whatman No.1 filter paper and the filtrate was assayed for amount of reducing sugar formed due to action of invertases (Miller, 1959).

Protease activity: Four ml of casein denaturalized by heating and dissolved in 0.1 M pH 8.1 tris HCl buffer (1%) was added to 1.0 g normal compost/vermicompost sample. After incubation at 51°C for 60 minutes, 4 ml of tris HCl buffer and 1 ml 17% trichloroacetic acid were added. After centrifugation at 4000 x g for 10 min, the aminoacids formed were determined by the Folin-colorimetric method (Nannipieri et al., 1980). Controls were prepared by adding buffer before incubation and casein after incubation.

Urease activity: Four ml of 0.1 M pH 7 phosphate buffer and 0.5 ml of 64 mg ml⁻¹ urea were added to 1.0 g of normal compost/vermicompost sample and incubated at 30°C for 60 min. After incubation, 10 ml of 2 M KCl was added and the mixture was kept at 4°C for 10 min to stop the enzymatic reaction. Suspensions were centrifuged for 5 minutes and the NH₄⁺ ion formed by ureases in

supernatant was determined by the Phenolphthorite method (Fawcett and Scott, 1960).

Microbial population studies: The total number of fungi, actinomycetes and bacteria present in normal compost and vermicompost samples were estimated by "Serial dilution method" (Allen, 1953). Martin rose bengal agar (RBA) for fungal culture, ken knights agar (kKA) for the culture of actinomycetes and nutrient agar (NA) for bacterial culture were used.

Statistical analysis: The significant difference ($p \leq 0.05$) between values of each sample were performed using Duncan's new multiple range (DMR) test (Duncan 1955).

Results and Discussion

Investigations revealed that normal compost and vermicompost underwent changes in microbial numbers and enzymatic activities. When the enzyme profiles were compared at different stages of composting greatest enzyme activity was observed in older composts (days 42-49) in the normal compost whereas in vermicompost the younger composts (days 21-35) showed greatest activity (Table 1). As process proceeded the cellulase and amylase activities in vermicomposting were increased by 28th day and reached the peak values of 1175 µg (Fig. 1) reducing sugar g⁻¹hr⁻¹ (Fig. 2) and 825 µg reducing sugar g⁻¹ha⁻¹ respectively and similarly the invertase and urease activities reached to peak values of 876 µg reducing sugar g⁻¹hr⁻¹ (Fig. 3) and 197 µg NH₄⁺ g⁻¹, hr⁻¹ (Fig. 4) on 35th day respectively. Enzymatic activities had decreased after 35th day as composting proceeded and this probably due to a decrease in microbial population, and available nutrients in the organic matter. Casein-hydrolysing protease activity increased sharply at the beginning of the vermicompost and reached the peak value on 28th day of 25 µmol amino acid g⁻¹hr⁻¹ followed by a sharp decline subsequently to 19 µmol of aminoacid g⁻¹hr⁻¹ (Fig. 5) which may be due to the decreasing protein content in the substrate. These results can be supported by observations of Ross and Cairns (1982). Who had reported that when earthworms were present in the ryegrass pots, the activities of invertase, amylase, urease and phosphatase further increased significantly at one or more samplings. Normal compost had shown relatively less enzyme activities till the 49th day when compared to the vermicompost. The lower enzymatic activities of normal compost might be attributed to the slow and incomplete stabilization of organic matter.

Abundant enzyme activities in vermicompost than in normal compost lead to the decomposition process by the presence of earthworms and aerobic heterotrophic microbial population. Especially during the middle stage of vermicomposting, in correlation with the introduced enzyme activity the microbial numbers reached the maximum of 126 x 10⁶, 28 x 10⁴ and 93 x 10⁵ CFU of bacteria, fungi and actinomycetes g⁻¹ samples respectively (Table 2, Fig. 6). Availability of half digested nutrient rich organic wastes by earthworm activity contributed for the proliferation of aerobic decomposing heterotrophic microbes. These results are in conformity with the

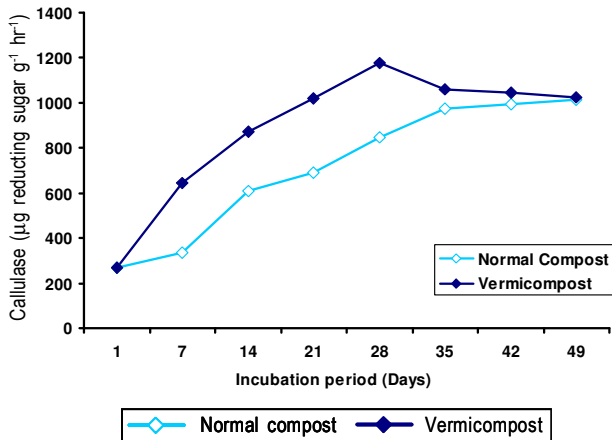


Fig. 1: Changes in cellulase activities of normal compost and vermicompost at weekly intervals $\mu\text{g reducing sugar g}^{-1} \text{hr}^{-1}$ compost

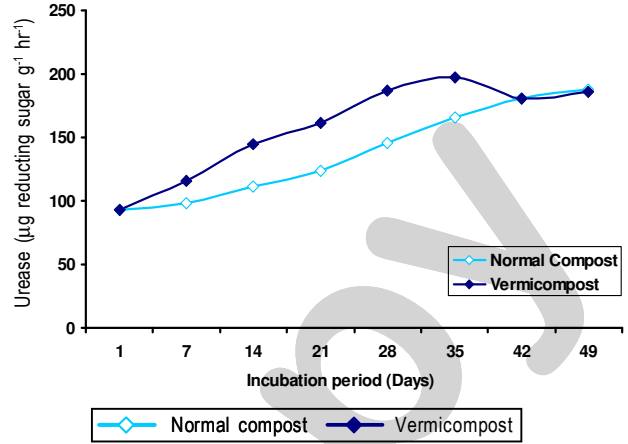


Fig. 4: Changes in urease activities of normal compost and vermicompost at weekly intervals $\mu\text{g NH}_4^+ - \text{N g}^{-1} \text{hr}^{-1}$ compost

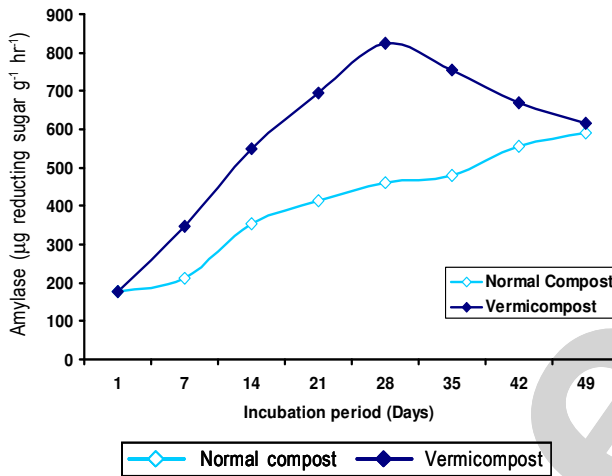


Fig. 2: Changes in amylase activities of normal compost and vermicompost at weekly intervals $\mu\text{g reducing sugar g}^{-1} \text{hr}^{-1}$ compost

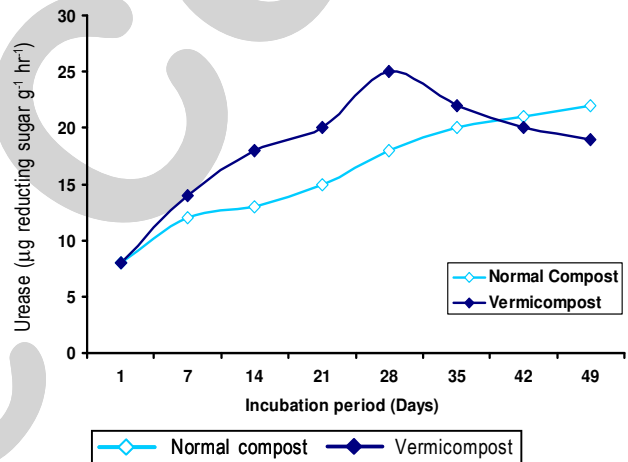


Fig. 5: Changes in protease activities of normal compost and vermicompost at weekly intervals $\mu\text{mol - amino acid g}^{-1} \text{hr}^{-1}$ compost

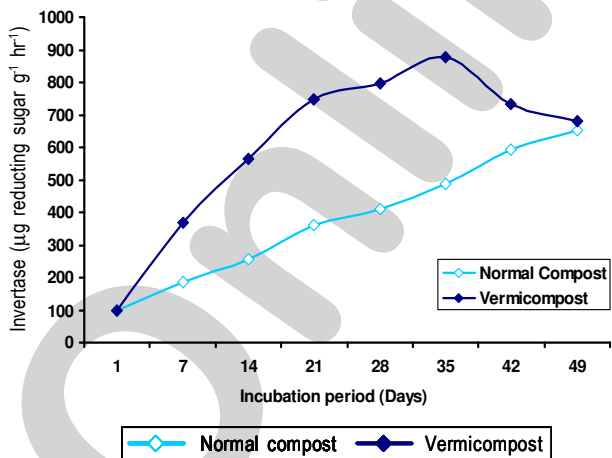


Fig. 3: Changes in invertase activities of normal compost and vermicompost at weekly intervals $\mu\text{g reducing sugar g}^{-1} \text{hr}^{-1}$ compost

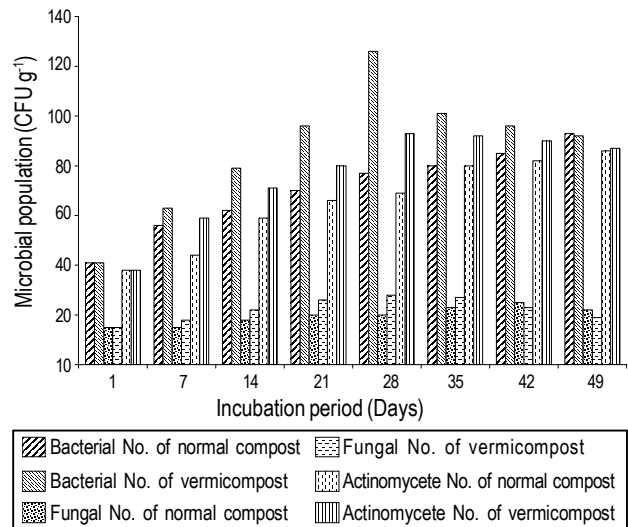


Fig. 6: Changes in different microbial populations of normal compost and vermicompost at weekly intervals

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Table - 1: Activities of different enzymes in compost and vermicompost

Sampling period (days)	Cellulase ¹		Amylase ²		Invertase ³		Urease ⁴		Protease ⁵	
	Ncom*	Vcom**	Ncom*	Vcom**	Ncom*	Vcom**	Ncom*	Vcom**	Ncom*	Vcom**
1	268 (100) ^a	268 (100) ^a	176 (100) ^a	176 (100) ^a	100 (100) ^a	100 (100) ^a	93 (100) ^a	93 (100) ^a	8 (100) ^a	8 (100) ^a
7	334 (124) ^b	646 (241) ^b	213 (121) ^b	347 (197) ^b	187 (187) ^b	367 (367) ^b	98 (105) ^b	116 (125) ^b	12 (150) ^b	14 (175) ^b
14	608 (227) ^c	872 (325) ^c	354 (201) ^c	549 (312) ^c	256 (256) ^c	566 (566) ^c	111 (119) ^c	145 (156) ^c	13 (162) ^b	18 (225) ^c
21	691 (257) ^d	1019 (380) ^d	414 (235) ^e	696 (395) ^d	363 (363) ^d	746 (746) ^d	124 (133) ^d	161 (173) ^d	15 (187) ^c	20 (250) ^c
28	849 (317) ^c	1175 (438) ^e	461 (261) ^e	825 (469) ^e	412 (412) ^e	797 (797) ^e	146 (157) ^e	187 (201) ^e	18 (225) ^d	25 (312) ^d
35	973 (362) ^f	1058 (394) ^f	479 (272) ^f	756 (429) ^f	487 (487) ^f	876 (876) ^f	166 (178) ^f	197 (212) ^f	20 (250) ^e	22 (275) ^e
42	994 (370) ^g	1044 (390) ^g	556 (318) ^g	668 (379) ^g	593 (593) ^g	733 (733) ^g	181 (194) ^g	181 (194) ^g	21 (262) ^e	20 (250) ^c
49	1012 (377) ^h	1026 (382) ^h	590 (335) ^h	615 (349) ^h	654 (654) ^h	680 (680) ^h	188 (202) ^h	186 (200) ^e	22 (275) ^e	19 (237) ^c

Figures in parentheses indicate percentages same letter are not significantly different ($p \leq 0.05$) from each other; (1) μg reducing sugar per gram; (2) μg reducing sugar per gram; (3) μg reducing sugar per gram; (4) μg NH_4^+ per gram; (5) μ moles of amino acid per gram, * Normal compost ** Vermicompost

Table - 2: Colony forming units(cfu) of different microbes during composting and vermicomposting

Sampling period (Days)	Bacteria ^a x 10 ⁶		Fungi ^b x10 ⁴		Actinomycetes ^c x10 ⁵	
	Ncom*	Vcom**	Ncom*	Vcom**	Ncom*	Vcom**
1	41 ^a	41 ^a	15 ^a	15 ^a	38 ^a	38 ^a
7	56 ^b	63 ^b	15 ^a	18 ^b	44 ^b	59 ^b
14	62 ^c	79 ^c	18 ^b	22 ^c	59 ^c	71 ^c
21	70 ^d	96 ^d	20 ^c	26 ^{de}	66 ^d	80 ^d
28	77 ^e	126 ^e	20 ^c	28 ^e	69 ^e	93 ^e
35	80 ^f	101 ^f	23 ^d	27 ^e	80 ^f	92 ^e
42	85 ^g	96 ^d	25 ^e	23 ^c	82 ^g	90 ^e
9	93 ^h	92 ^e	22 ^d	19 ^b	86 ^h	87 ^f

Values are g^{-1} compost, *Normal compost, **Vermicompost, ^aNutrient agar, ^bRose Bengal agar, ^cKenknights agar same letter are not significantly different ($p \leq 0.05$)

result of earlier works like Kale *et al.* (1988) who had reported higher counts of actinomycetes and bacteria when the *E. eugeniae* and *P. excavatus* worked organic waste mixed with soil. Jambhekar (1992) noticed a considerable increase in total viable counts of actinomycetes and bacteria in the worm treated compost than the control. Parthasarathi and Ranganathan (1998) had reported recently an increase of bacterial population in *Lampito mauritii* and *Eudrilus eugeniae* worked vermicompost. The increase of microbial population may be caused by congenial condition for the growth of microbes with in the worm 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 as reported by Tiwari *et al.* (1989).

Earthworms voraciously feed on organic wastes while utilizing only a small portion for their body metabolic activities excrete a large part of the consumed materials in a half digested form (Edward and Lofty, 1977; Kale and Bano, 1986). The half digested material decompose rapidly and is transformed into vermicompost within a short time since the intestines of earthworms harbour wide range of microorganisms, enzymes, hormones *etc.* The feeding activity of

the earthworms and their voiding of microbially and enzymatically enriched casts could have also contributed to higher enzyme activities of vermicompost.

Most important feature of vermicompost is that, during processing of the various organic wastes by earthworms, many of the nutrients are changed to available forms that are more readily taken up by plants (Chaudhuri, 2005). Earthworms fragment the substrate in the process of feeding and thereby increase the surface area for further microbial colonization. The enhanced microbial activity accelerated the decomposition process leading to humification thus oxidizing unstable organic matter to stable form. During the passage through the gut of earthworms the surviving microorganisms are voided along with cast. So vermicompost not only provides mineralogical nutrients to soil but also contributes to the biological fertility factor by adding beneficial microbes to soil. Hence, vermicompost can be considered as the best organic manure than farmyard manure. Thus vermicomposting can also be considered as a low cost technology system for the processing or treatment of wastes and utilization of earthworms may be an answer as an ecologically sound, economically viable and socially acceptable technology.

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