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Influence of lignocellulolytic fungus and earthworms on microbial activity during bioconversion of Lignocellulosic organic biomass coir waste

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Abstract
The monitoring of various enzyme activities during microbial inoculated composting and vermicomposting process provides helpful information on the dynamics of significant microbial activity is beneficial for understanding the conversion taking place. In this experiment, four different fungal species and two types earthworms were inoculated in different treatments of coir waste (CW) mixed with sugarcane bagasse (SBG) to study their effect on enzymatic activities, of the final product. The results suggested that uninoculated treatments did not show any significant effect on the activities of enzymes than inoculated treatments and vermicomposting. Inoculation of fungi significantly ($p<0.05$) increased the amylase, cellulase, protease, invertase and xylanase activities. The Inoculation of *consortium*, *Aspergillus niger*, *Trichoderma viride* and earthworms in initial CW with SBG registered the highest enzyme activity in the final product. The highest value of enzyme activity was recorded in MCT3, MCT4, MCT6, VCT7 and VCT8 treatments.

Keywords: enzyme activities, coir waste, sugarcane bagasse, fungal inoculation, vermicomposting

Introduction
Composting and vermicomposting a process involving a complex ecosystem with many interacting factors, in which organic wastes are stabilized or transformed into a humic rich product by the action of microorganisms and their enzymes activities under controlled conditions, the enzymes released by the microorganisms during composting also play a key role in the biological and biochemical transformations of the matrix (Albrecht *et al.*, 2008) [1]. Although the inoculation of specific microorganisms, which are the essential factors for the successful operation of composting. In order to effectively, control the composting process it's necessary to understand the microbial community structure and its change, particularly its special role in decomposition of organic matters (Beffa *et al.*, 1996) [2]. Microbial composting and vermicomposting helps in managing large quantities of organic wastes in a sustainable manner. It is one of the technologies of integrated waste management strategies, used for the recycling of organic materials into a useful product (Manivannan, 2004; Giglotti *et al.*, 2005) [4, 3].

Coir waste, a lignocellulosic organic biomass produced during removal of coir fiber from coconut husk, accumulates as a waste material near coir processing factories causing soil and water pollution and disposal problems. It is estimated that the coir-processing factories in India produce roughly 0.5 million tones of coir pith waste every year that accumulates in the surrounding area and creates an environmental problems (Thomas *et al.* 2013) [5]. The composting and vermicomposting process is the most appropriate method for waste recycling, which besides having the advantage of reducing volume and pathogenic micro-organisms, also enables the obtainment of a final product with stabilized fertilizing characteristics that should be consciously exploited for agricultural production (Prabhakaran and Manivannan, 2014) [6]. Hence, to improve the biological degradation of coir waste, selection of effective microorganisms and earthworms enhancing the activities of lignin degrading enzymes is essential. According to the previous studies, the lignocellulolytic fungi were the most efficient microorganisms for biomass deconstruction and delignification (Dinis *et al.*, 2009) [7]. Therefore, there is an urgent need to recycle the coir waste without environmental impact. The purpose of this study was to study the effect of different lignocellulolytic microbial inoculation and two exotic earthworms on the important enzymatic activities during the bioconversion of two different organic wastes (coir waste mixed with sugarcane bagasse).

Materials and Methods
Collection of organic waste
Coir waste (CW) 15 days old were collected from coir industry in Kerala. Sugar cane bagasse (SBG) was obtained from E.I.D parry sugar mill located at Nelikkuppam, Tamilnadu, India. One month old SBG was sundried separately for 15 days to remove the odour and noxious gases. The bagasse was chopped into small pieces using chopping machine.

Microbial inoculants
The pure cultures of the microbial inoculants, viz., *Trichoderma viridae* (lignolytic fungi), *Aspergillus niger*, *Bacillus polymyxa* (free-living nitrogen fixing bacteria) and *Phanerochaete chrysosporium* (lignolytic fungi, basidiomycetes) were used for this study. All the microbial

inoculants were maintained on potato dextrose agar slants at 4°C and sub cultured regularly at monthly intervals. Equal quantities of each fungal inoculums were mixed together to make a consortium. Before inoculated, the complex microorganisms were cultivated by malt extract agar and the lignocellulolytic microorganisms were cultivated by potato dextrose agar. During cultivating process, the microbial colonies were counted using a standard dilution plating procedure until to reach the desired concentration for composting inoculation (Gaind *et al*, 2006) [8].

Selection of Exotic earthworms

Exotic earthworms *Eudrilus eugeniae* and *Eisenia fetida* were obtained from the stock culture maintained by the author in the laboratory, Department of Zoology, Annamalai University, Annamalainagar, India. The worms were stocked in cement tank and cow dung was used as substrate to maintain the adult earthworms. Moisture content of 65-75% was continuously maintained by sprinkling of water. This stock cultures for both species were covered with moist jute to prevent water (moisture) loss and maintained at room temperature 27±3°C inside the laboratory. The worms were adapted to laboratory conditions before inoculating into treatments.

Experimental setup

The experiments were performed in eight treatments with six replicates using cement tanks (50cm×180cm×30cm) with a hole at the bottom. Each composting and vermicomposting tank (treatment) contains 10 kg of CW mixed with SSBG in 1:1 ratio (w/w dry weight basis). The composting and vermicomposting treatments of CW and SBG were arranged in the following combinations: CT1: CW + SBG (control - without inoculums and earthworms); MCT2: CW + SBG + *Aspergillus flavus*; MCT3: CW + SBG + *Aspergillus niger*; MCT4: CW + SBG + *Trichoderma viride*; MCT5: CW + SBG + *Phanerochaete chrysosporium*; MCT6: CW + SBG + Consortium; VCT7: CW + SBG + *E. eugeniae* and VCT8: CW + SBG + S*E. fetida.* Pure cultures of *A. niger, B.* polymyxa, *P. chrysosporium*, *T. viride* and consortium of fungal species were inoculated (50 ml/kg substrate having 10^6 cells per ml) in to the respective treatments. At the initial stage (0 day) and 15 days of composting, the fungal inoculums suspension was sprayed on the raw material (Sarker *et al.*, 2013) [9]. All the composting material was turned after inoculation to spread the microbe's consortium. The experimental tanks were kept in the lab under room temperature and were covered with mosquito net to prevent any intrusion of pests.

The earthworms (*E. eugeniae* and *E. fetida)* were weighed without voiding their gut content. Afterward all earthworms of *E. eugeniae* and *E.fetida*, cocoons and feed materials returned to the respective treatment container. After the completion of pre-inoculation period of 15days, the clitellated *E. eugeniae* and *E. fetida* were weighed and inoculated in to respective each treatment (T7 and T8) at the rate of 15g/kg of waste (Manivannan *et al*, 2004) [4]. All the experimental tanks were covered with nylon mesh and maintained at the laboratory room temperature 27 ± 3°C and the moisture content in each treatment was adjusted to about 60-70% at the beginning of composting and vermicomposting and then periodically water was added during the turning of composting and vermicomposting process. All treatments were manually turned for twice a week to revolve the treatment and provide aeration.

Analysis of microbial enzyme activities

Amylase, cellulose, invertase and xylanase, activities were measured by estimation of the reducing sugars calorimetrically by DNS method. Protease was assayed by estimation of tyrosine released with Lowry's method. The values of DA were expressed as mg TPF g^{-1} h^{-1}. Enzyme assays were done as per Alef and Nannipieri (1995) [11].

Statistical analysis

Data were reported as the means of three replicates and were analyzed using a one-way ANOVA design with the software SPSS 16.0. Duncan multiple range test was used to separate the means.

Results and Discussion

In the present observation, the cellulase activity increased gradually with the decomposition process (Table 1). The highest cellulase activity was detected in *T. viride* inoculated treatment followed by combinations of fungal consortia inoculated treatment and *A. niger* inoculated treatment and vermicomposting (MCT3, MCT4, MCT6, VCT7 and VCT8). The results revealed that cellulase activity of compost obtained from different fungal inoculated treatments significantly differ than compost obtained from un-inoculated treatment. The increases in cellulase activity was due to the key enzyme β-Glucosidase, involved in the degradation of polysaccharides during composting, in which polysaccharides were degraded by hydrolyzing the reducing terminals of the β-D-glucose chains (Castaldi *et al.*, 2008) [12]. Raut *et al.* (2008) [13] also reported that fungi are primarily involved in the decomposition of cellulose, hemicellulose and lignin present in the organic matter. At the start of composting and vermicomposting, the xylanase activity was the highest in *T. viride* inoculated treatment followed by combinations of fungal consortia inoculated treatment and *A. flavus* inoculated treatment and vermicomposting. In the present study, the highest xylanase activity was obtained in *A. niger* inoculated treatment (T_2) followed by combinations of fungal consortia inoculated treatment and *T. viride* inoculated treatment was not significant (Table 2). The results of the present investigation showed a maximum activity of xylanase showed an increase during this period. Hemicellulose is the main substrate for the secretion of xylanase, therefore in the present study increased activity of xylanase was consistent with the degradation of hemicellulose as reported by Paola *et al.* (2008). Enhancement of xylanase may be due to high content of degradable organic compounds in the initial mixture (bagasse) might stimulate microbial growth and enzyme synthesis.

Proteases, considered as appropriate indicators of organic matter decomposition are important enzymes during the composting process. At the beginning of composting, the protease activity was the highest in *A. niger* inoculated treatment followed by combinations of fungal consortia inoculated treatment and vermicomposting than other

treatment (MCT3, MCT4, MCT6, VCT7 and VCT8). However, in the present study protease activity slightly decreased until the end of the composting and vermicomposting process (90 days). Proteases, considered as appropriate indicators of organic matter decomposition are important enzymes during the composting process (Lazcano *et al.,* 2008) [14]. Protease participates in transforming amino acids, proteins and other nitrogenous organic compounds of the compost matrix and its activity is related to microbial metabolic activity (Vargas-Garcia *et al.,* 2010) [15]. Hence, it was concluded CW contained a small amount of proteins, and these were degraded quickly during the composting process. Similarly, at the start of composting and vermicomposting, the amylase and invertase activity was the highest in *T. viride* inoculated treatment followed by combinations of fungal consortia inoculated treatment and *A. niger* inoculated treatment and vermicomposting (MCT3, MCT4, MCT6, VCT7 and VCT8). Moreover, in the present study amylase and invertase activity slightly decreased until the end of the composting and vermicomposting process (Table 4 and 5). The results further indicated that the activity of amylase and invertase was high in the fungal inoculated treatment may be due to the effective composting and presence of bulking agent bagasse. Further, the production of amylase depends on microbial biomass, which implies that when this biomass is degraded amylase activity decreases (Ayuso *et al.,* 1996) [16]. The results of this study indicated that the activity of amylase was high in the treatments due to the presence of bulking material sugar industrial waste bagasse.

Table 1: Changes in the cellulase activity during fungal inoculated composting and vermicomposting of CW with SBG in different treatments

Treatments	Cellulase activity (mg reducing sugar g^{-1} $2h^{-1}$)					
	Days					
	15	30	45	60	75	90
CT1	1.24 ± 0.07^{a}	2.48 ± 0.16^{a}	2.89 ± 0.23^{a}	5.47 ± 0.30^{a}	5.25 ± 0.27^{a}	4.08 ± 0.05^{a}
MCT2	2.30 ± 0.06^{b}	4.31 ± 0.22^{b}	5.42 ± 0.27^{b}	8.59 ± 0.15^{b}	8.11 ± 0.26^{b}	7.15 ± 0.12^{b}
MCT3	3.87 ± 0.19^{cd}	5.63 ± 0.25^{c}	6.72 ± 0.21^{c}	10.87 ± 0.34^{cd}	10.36 ± 0.40^{cd}	9.18 ± 0.17^{d}
MCT4	2.33 ± 0.11^{b}	4.35 ± 0.16^{b}	5.38 ± 0.25^{b}	8.68 ± 0.28^{bc}	8.39 ± 0.22^{bc}	7.28 ± 0.15^{bc}
MCT5	3.54 ± 0.23^{c}	5.55 ± 0.29^{c}	6.99 ± 0.28^{cd}	12.30 ± 0.36^{d}	12.05 ± 0.34^{d}	10.29 ± 0.14^{e}
MCT6	3.87 ± 0.19^{cd}	5.63 ± 0.25^{c}	6.72 ± 0.21^{c}	10.87 ± 0.34^{cd}	10.36 ± 0.40^{cd}	9.18 ± 0.17^{d}
VCT7	3.80 ± 0.19^{cd}	5.63 ± 0.25^{c}	6.72 ± 0.21^{c}	10.87 ± 0.34^{cd}	10.36 ± 0.40^{cd}	9.18 ± 0.17^{d}
VCT8	3.81 ± 0.14^{cd}	5.49 ± 0.13^{cd}	6.70 ± 0.25^{c}	10.80 ± 0.34^{c}	10.35 ± 0.39^{c}	9.11 ± 0.09^{c}

All values are mean and standard deviation of six replicates; means in a column followed by the same letter(s) are not significantly different (ANOVA; Tukey's test, $p<0.05$).

Table 2: Changes in the xylanase activity during fungal inoculated composting and vermicomposting of CW with SBG in different treatments

Treatments	Xylanase activity (mg reducing sugar g^{-1} $2h^{-1}$)					
	Days					
	15	30	45	60	75	90
CT1	4.39 ± 0.47^{a}	4.63 ± 0.50^{a}	4.89 ± 0.48^{a}	5.25 ± 0.66^{a}	3.33 ± 0.51^{a}	2.02 ± 0.44^{a}
MCT2	4.42 ± 0.36^{a}	5.57 ± 0.69^{bc}	5.74 ± 0.42^{bc}	8.51 ± 0.74^{bc}	6.26 ± 0.53^{b}	5.09 ± 0.30^{b}
MCT3	5.40 ± 0.45^{b}	6.18 ± 0.72^{c}	6.36 ± 0.70^{c}	13.22 ± 0.89^{c}	9.10 ± 0.86^{c}	7.16 ± 0.55^{c}
MCT4	4.16 ± 0.51^{a}	5.42 ± 0.54^{b}	5.58 ± 0.55^{b}	8.40 ± 0.65^{b}	6.21 ± 0.40^{b}	5.27 ± 0.36^{bc}
MCT5	5.48 ± 0.62^{bc}	6.24 ± 0.71^{c}	6.69 ± 0.68^{cd}	13.15 ± 0.91^{c}	9.40 ± 0.74^{cd}	7.20 ± 0.48^{c}
MCT6	5.33 ± 0.65^{b}	6.27 ± 0.48^{c}	6.63 ± 0.61^{cd}	13.20 ± 0.85^{c}	9.37 ± 0.65^{cd}	7.66 ± 0.37^{cd}
VCT7	5.41 ± 0.45^{b}	6.15 ± 0.52^{c}	6.33 ± 0.74^{c}	13.21 ± 0.89^{c}	9.14 ± 0.86^{c}	7.12 ± 0.55^{c}
VCT8	5.42 ± 0.75^{b}	6.16 ± 0.32^{c}	6.31 ± 0.70^{c}	13.22 ± 0.79^{c}	9.10 ± 0.86^{c}	7.16 ± 0.45^{c}

All values are mean and standard deviation of six replicates; means in a column followed by the same letter(s) are not significantly different (ANOVA; Tukey's test, $p<0.05$).

Table 3: Changes in the protease activity during fungal inoculated composting and vermicomposting of CW with SBG in different treatments

Treatments	Protease activity (mg tyrosine $g^{-1}2h^{-1}$)					
	Days					
	15	30	45	60	75	90
CT1	2.15 ± 0.34^{a}	2.56 ± 0.37^{a}	2.65 ± 0.39^{a}	3.18 ± 0.41^{a}	3.06 ± 0.30^{a}	2.27 ± 0.24^{a}
MCT2	3.18 ± 0.31^{c}	3.25 ± 0.32^{b}	3.24 ± 0.30^{b}	3.69 ± 0.37^{b}	3.16 ± 0.41^{ab}	2.19 ± 0.22^{a}
MCT3	2.36 ± 0.21^{ab}	3.29 ± 0.30^{b}	3.37 ± 0.37^{bc}	4.21 ± 0.56^{c}	4.14 ± 0.53^{b}	3.34 ± 0.42^{bc}
MCT4	3.14 ± 0.35^{c}	3.17 ± 0.35^{b}	3.28 ± 0.39^{b}	3.87 ± 0.35^{bc}	3.13 ± 0.43^{ab}	3.06 ± 0.35^{b}
MCT5	2.28 ± 0.22^{ab}	3.20 ± 0.37^{b}	3.46 ± 0.32^{bc}	4.38 ± 0.52^{cd}	4.22 ± 0.50^{bc}	3.29 ± 0.39^{bc}
MCT6	2.19 ± 0.27^{a}	3.26 ± 0.33^{b}	3.37 ± 0.35^{bc}	4.59 ± 0.55^{d}	4.33 ± 0.55^{bc}	4.10 ± 0.51^{c}
VCT7	2.20 ± 0.21^{ab}	3.25 ± 0.20^{b}	3.31 ± 0.57^{bc}	4.25 ± 0.56^{c}	4.10 ± 0.53^{b}	3.36 ± 0.42^{bc}
VCT8	2.19 ± 0.26^{ab}	3.22 ± 0.30^{b}	3.33 ± 0.37^{bc}	4.22 ± 0.26^{c}	4.15 ± 0.53^{b}	3.38 ± 0.45^{bc}

All values are mean and standard deviation of six replicates; means in a column followed by the same letter(s) are not significantly different (ANOVA; Tukey's test, $p<0.05$).

Table 4: Changes in the amylase activity during fungal inoculated composting and vermicomposting of CW with SBG in different treatments

Treatments	Amylase activity (mg reducing sugar $g^{-1}2h^{-1}$)					
	Days					
	15	30	45	60	75	90
CT1	1.03 ± 0.15^{a}	1.15 ± 0.10^{a}	1.36 ± 0.18^{a}	1.54 ± 0.25^{a}	1.23 ± 0.19^{a}	1.17 ± 0.24^{a}
MCT2	1.24 ±0.12^{ab}	1.08 ± 0.17^{a}	2.20 ± 0.23^{b}	2.25 ±0.25^{b}	2.19 ± 0.05^{b}	1.25 ± 0.07^{ab}
MCT3	1.29 ± 0.17^{a}	2.19 ± 0.15^{a}	2.35 ±0.25^{bc}	3.28 ± 0.33^{c}	3.17 ± 0.31^{c}	1.08 ± 0.41^{a}
MCT4	1.27 ±0.16^{ab}	1.26 ± 0.14^{a}	2.18 ± 0.10^{b}	2.29 ± 0.22^{b}	2.14 ± 0.17^{b}	1.27 ± 0.28^{ab}
MCT5	1.20 ±0.19^{ab}	1.28 ± 0.06^{a}	2.15 ±0.21^{bc}	2.08 ± 0.33^{c}	2.70 ± 0.31^{c}	1.18 ± 0.15
MCT6	1.29 ±0.15^{ab}	2.20 ± 0.22^{b}	2.29 ±0.26^{bc}	4.32 ± 0.34^{d}	3.65 ± 0.18^{cd}	1.19 ± 0.06^{a}
VCT7	1.30± 0.15^{a}	2.18 ± 0.14^{a}	2.27 ±0.27^{bc}	4.15 ± 0.38^{c}	3.58 ± 0.33^{c}	1.21 ± 0.14^{a}
VCT8	1.35± 0.17^{a}	2.19 ± 0.15^{a}	2.35 ±0.25^{bc}	4.55 ± 0.30^{d}	3.68 ± 0.25^{cd}	1.23 ± 0.15^{ab}

All values are mean and standard deviation of six replicates; means in a column followed by the same letter(s) are not significantly different (ANOVA; Tukey's test, $p<0.05$).

Table 5: Changes in the invertase activity during fungal inoculated composting and vermicomposting of CW with SBG in different treatments

Treatments	Invertase activity (mg reducing sugar $g^{-1}2h^{-1}$)					
	Days					
	15	30	45	60	75	90
CT1	4.22 ± 0.47^{a}	4.41 ± 0.63^{a}	3.30 ± 0.29^{a}	4.88 ± 0.63^{a}	3.16 ± 0.21^{a}	3.02 ± 0.50^{a}
MCT2	4.29 ± 0.29^{a}	5.27±0.72^{cd}	3.56±0.37^{bc}	5.83±0.50^{bc}	3.37±0.11^{ab}	3.30 ± 0.53^{ab}
MCT3	4.45±0.42^{ab}	8.27 ± 0.50^{b}	4.87±0.30^{ab}	8.39 ± 0.74^{c}	6.40±0.44^{bc}	6.06 ± 0.62^{b}
MCT4	4.20 ± 0.40^{a}	4.41 ± 0.63^{a}	3.30 ± 0.29^{a}	4.88 ± 0.63^{a}	3.16 ± 0.21^{a}	3.02 ± 0.50^{a}
MCT5	4.38±0.35^{ab}	8.52 ± 0.74^{c}	3.98±0.45^{bc}	5.69±0.77^{cd}	5.25 ± 0.58^{b}	5.17 ± 0.68^{bc}
MCT6	4.35±0.38^{ab}	8.61 ± 0.71^{c}	4.45 ± 0.41^{b}	8.64 ±0.73^{cd}	6.53±0.55^{bc}	6.35 ± 0.62^{bc}
VCT7	4.32±0.54^{ab}	8.29±0.56^{bc}	4.75±0.34^{ab}	8.63 ± 0.61^{b}	6.25 ± 0.26^{a}	6.19 ± 0.51^{ab}
VCT8	4.31±0.50^{ab}	8.39±0.66^{bc}	4.75±0.34^{ab}	8.63 ± 0.61^{b}	6.25 ± 0.26^{a}	6.19 ± 0.51^{ab}

All values are mean and standard deviation of six replicates; means in a column followed by the same letter(s) are not significantly different (ANOVA; Tukey's test, $p<0.05$).

Conclusion

The enzyme activity of the organic substrates increased due to inoculation of microorganisms and earthworms. The results suggested that inoculation of *E. eugeniae*, *E. fetida, T. viride* and *A. niger*, the enzyme activity was recorded more than that of other treatments. Hence, inoculation of *T. viride* and *A. niger* in the initial organic wastes (CW) significantly enhanced activity of the enzyme which was equal to that of earthworm's treatment, which indicated that rapid degradation.

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