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Research Article

EFFECT OF INOCULATING LIGNOCELLULOLYTIC FUNGUS AND EXOTIC EARTHWORMS ON MAJOR NUTRIENT CHANGES DURING DEGRADATION OF COIR WASTE AMENDED WITH SUGARCANE BAGASSE

Jayan. R. Krishnan[1] and S. Manivannan[2]*

[1]Department of Zoology, Research and Development Centre, Bharathiar University, Coimbatore, Tamilnadu, India.

[2]Department of Zoology, Annamalai University, Annamalai Nagar, Tamilnadu, India.

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ABSTRACT

Composting and vermicomposting is a widely used method for disposal of organic wastes for nutrients recovery. Application of fresh organic wastes or non-stabilized compost to soil may leads to immobilization of plant nutrients and cause phytotoxicity. The environmental problems associated with raw coir waste (CW) application, such as release of polyphenols could be mitigated by stabilizing its nutrient and organic matter contents by composting/vermicomposting before application to agricultural soils. The objectives of this study was to evaluate the changes in nutrient content of CW amended with sugarcane bagasse (SBG) using different lignocellulolytic fungal inoculations and vermicomposting during over a period of 90 days in order to produce stabilized organic fertilizer. The composts and vermicompost were sampled at 0, 30, 60 and 90 days for the assessment of temporal changes in nutritional properties. Results revealed that nutrient contents during composting and vermicomposting showed a significant variation in all the treatments ($p<0.05$) for all the sampling days. Among the different treatments, vermicomposting and inoculation of fungal consortium, *A. niger* and *T. viridae* in CW and SBG mixed at equal proportion (1:1) produced a superior quality compost with desirable C:N ratio and higher nutritional status than natural composting. Therefore producing nutrient rich stabilized product from CW and SBG useful for sustaining high crop yield and minimizing soil depletion.

Keywords: Composting, Vermicomposting, Coir waste, Bagasse, Nutrients.

INTRODUCTION

Coir waste (pith), 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). 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).

Similarly any other biological method, composting and vermicomposting need some factors to ensure acceptable results. The composition of the fibrous fraction of organic biomass (cellulose, hemicelluloses and lignin) significantly influences the degradation rate of these compounds, mainly when the lignin comprises the major part of the biomass. Therefore, it is clear that the coir waste content possibly will vary significantly according to the initial litter material and the lignin content present in it (Orrico Junior *et al*., 2010). The purpose of this study was to study the role of different inoculating fungal species and two exotic earthworm species on rapid degradation of CW with SBG fractions and the quality of the final compound.

*Corresponding Author: Dr. S. Manivannan, Assistant Professor, Department of Zoology, Annamalai University, Annamalai Nagar-608002, Tamilnadu, India, Email: sarosubamani@rediffmail.com, Mobile: +91 9442378151

MATERIALS AND METHODS

Collection of organic waste and Microbial inoculants

Coir waste (CW) 15days 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. 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).

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: T1: CW + SBG (control - without inoculums and earthworms); T2: CW + SBG + *Aspergillus flavus*; T3: CW + SBG + *Aspergillus niger*; T4: CW + SBG + *Trichoderma viride*; T5: CW + SBG + *Phanerochaete chrysosporium*; T6: CW + SBG + Consortium; T7: CW + SBG + *E. eugeniae* and T8: 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 (T2 – T6). 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). 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 15 days, 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). 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. Samples were collected periodically from each treatment on days 0, 30, 60 and 90 days for compost and vermicompost quality parameters.

Nutrient analysis

Samples were collected periodically from each treatment on days 0, 30, 60 and 90 days for physico-chemical analysis. The pH and electrical conductivity (EC dSm-1) were determined using a double distilled water suspension in the ratio of 1:10 (W/V) using a digital pH and conductivity meter. Total organic carbon (TOC) content in the sample was determined by chromic oxidation method (Walkely and Black, 1934). Furthermore total Kjeldhal nitrogen (TKN) was measured by micro Kjeldhal method (Tiquia, 2005). Total phosphorus (TP) was estimated by vanadomolybdo phosphoric acid yellow colour method using a colorimeter (Model 115, Systronics, India) (Jackson, 1973). While Total potassium (TK) was detected by the method of Jackson (1973) using flame photometer (Model 128, Systronics, India). C: N was considered from the measured value of C and N. Exchangeable elements (Na, Ca, and Mg) were determined after extracting the sample using ammonium acetate extract ion method. Results are the means of the three replicates. Two way analysis of variance (ANOVA) was performed by using the SPSS 10.5 software. The objectives of statistical analysis to determine any significant differences among the parameters

analyzed in different treatments during the composting process.

RESULTS AND DISCUSSION

An increase in pH was recorded in all the treatments during initial period of composting of CW with SBG. Initially slight increases in pH was observed up to 30 days but later decreased to almost neutral pH on 60 and 90 days of fungal inoculated composting and vermicomposting (Table 1 and 2). The increase in pH during composting (0-30 day) of this study was attributed to the production of ammonia associated with protein degradation in the raw materials and to the decomposition of organic acids (Warman and Tremmeer, 2005). Moreover, presence of carboxylic and phenolic groups in humic acids caused lowering of pH while ammonium ions increased the pH of the system and combined effect of these two oppositely charged ions actually regulates the pH of compost leading to a shift of pH towards neutrality at the end of (90 days) composting and vermicomposting (Ndegwa and Thompson, 2000).

In the present study, an increase in electrical conductivity (EC) was recorded in all the treatments during microbial composting and vermicomposting (Table 3). The relatively low EC values were recorded in all treatments on 0 days. A gradual increase in EC was observed in all the fungal inoculated treatments (MCT1- MCT6) and vermicomposting treatments (VCT7-VCT8) with increase in decomposition time. However, the EC value was significantly different from control treatment (MCT1) when compared to combinations of fungal consortia inoculated and vermicomposting treatment. In the present study, maximum increase in EC during composting and vermicomposting treatments (MCT3, MCT4, MCT6, VCT7 and VCT8) might have been due to effective composting by inoculated fungal species and earthworms and release of different mineral ions, such as phosphate, ammonium, and potassium during composting and vermicomposting, respectively (Yadav and Garg, 2011; Tamizhazhagan, 2016).

The TOC value for all the treatments varied significantly ($P < 0.05$) on 30^{th} and 60^{th} sampling days as per the analysis of variance. At the end of the composting process, the combinations of fungal consortia inoculated treatment (MCT3, MCT4 and MCT6) and vermicomposting treatment (VCT7 and VCT8) contained maximum reduction of TOC, while the minimum reduction was observed in control and other treatments (Table 4). The results observed in this study are consistent with previous work of Molla *et al.* (2001) and Manivannan *et al*. (2004) and they reported that significant reduction in total organic carbon content after microbial inoculation during composting. Moreover in this study there was a significant difference among the treatments for TOC possibly due to different rate of enzyme activity related to carbon mineralization. A significant increase in TKN content in the compost was observed in all the treatments as compared to the initial material (Table 5). However, in the present study the maximum increase was observed in the combinations of fungal consortia inoculated treatment, *T. viride* inoculated treatment and vermicomposting treatment, but among these treatments values was not statistically significant ($P<0.05$). Increase in nitrogen content in the final product in the form of mucus, nitrogenous excretory substances, growth stimulating hormones and enzymes from earthworms have also been reported (Manivannan *et al.*, 2004).

In the present study, the maximum increase of TP was observed in VCT7 and VCT8 treatments and combinations of fungal consortia inoculated treatments T6 on 60^{th} day and T_6 on 90^{th} day, respectively. While, control treatment (MCT1) showed the minimum increases for TP content at the end (Table 6). The significant increase in TP during experimentation is may be due to mineralization and mobilization of phosphorus by microbes and phosphate activity of microorganisms (Prabhakaran and Manivannan, 2014). Similarly, the maximum TK content was significantly higher in combinations of fungal consortia inoculated treatments MCT6, VCT7 and VCT8 treatments than other treatments on 60^{th} day (Table 7). However, the differences in the TK content of the compost obtained from *T. viride* inoculated treatment and *A. niger* inoculated treatment was not significant ($p<0.05$). The previous studies suggested that microorganism processed waste material contains higher concentration of exchangeable K due to enhanced microbial activity during the microbial composting and vermicomposting process, which consequently enhances the rate of mineralization (Suthar, 2007, Prabhakaran and Manivannan, 2014).

Table 1. Physico-chemical characterizations of the CW and bulking agent SBG used in composting.

Parameters	pH	EC (dSm^{-1})	TOC	TKN	TP	TK
			(%)			
CW	7.9 ± 0.3	2.81 ± 0.5	29.45 ± 0.14	1.42 ± 0.11	1.20 ± 0.09	1.29 ± 0.07
SBG	6.5± 0.8	0.66 ± 0.05	66.17 ± 0.24	1.34 ± 0.03	0.65 ± 0.17	0.79 ± 0.04

CW- Coir waste, SBG-Sugar cane Bagasse, All values are mean and standard deviation of six replicates.

Table 2. Changes of pH during fungal inoculated composting and vermicomposting of CW with SBG in different treatments.

Treatments	pH			
	Days			
	0	30	60	90
MCT1	6.8 ± 0.04^{ab}	8.2 ± 0.02^{ab}	7.5 ± 0.03^{c}	7.5 ± 0.05^{bc}
MCT2	6.8 ± 0.02^{ab}	8.6 ± 0.04^{c}	7.1 ± 0.05^{a}	7.2 ± 0.02^{b}
MCT3	6.7 ± 0.03^{a}	8.0 ± 0.03^{a}	7.3 ± 0.02^{b}	7.2 ± 0.03^{b}
MCT4	6.8 ± 0.02^{ab}	8.0 ± 0.03^{a}	7.5 ± 0.05^{c}	$7. 2 \pm 0.02^{b}$
MCT5	6.7 ± 0.02^{a}	8.2 ± 0.04^{ab}	7.0 ± 0.02^{a}	7.0 ± 0.04^{a}
MCT6	6.7 ± 0.03^{a}	8.4 ± 0.05^{b}	7.3 ± 0.02^{b}	7.2 ± 0.03^{b}
VCT7	6.7 ± 0.04^{a}	8.3 ± 0.04^{ab}	7.1 ± 0.02^{a}	7.1 ± 0.04^{a}
VCT8	6.7 ± 0.03^{a}	8.4 ± 0.05^{ab}	7.0 ± 0.02^{a}	7.1 ± 0.03^{a}

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 of EC during fungal inoculated composting and vermicomposting of CW with SBG in different treatments.

Treatments	EC (dsm^{-1})			
	Days			
	0	30	60	90
MCT1	2.29 ± 0.02^{a}	3.05 ± 0.04^{a}	3.73 ± 0.07^{a}	3.62 ± 0.06^{a}
MCT2	2.29 ± 0.03^{b}	3.15 ± 0.05^{ab}	3.86 ± 0.06^{ab}	3.85 ± 0.06^{ab}
MCT3	2.32 ± 0.06^{bc}	3.05 ± 0.04^{a}	3.94 ± 0.08^{b}	3.90 ± 0.09^{b}
MCT4	2.30 ± 0.05^{bc}	3.25 ± 0.02^{b}	3.91 ± 0.06^{b}	3.90 ± 0.07^{b}
MCT5	2.31 ± 0.04^{b}	3.13 ± 0.03^{ab}	3.84 ± 0.09^{ab}	3.82 ± 0.07^{ab}
MCT6	2.33 ± 0.05^{bc}	3.30 ± 0.03^{bc}	3.98 ± 0.09^{bc}	3.92 ± 0.07^{b}
VCT7	2.32 ± 0.05^{bc}	3.31 ± 0.02^{bc}	3.97 ± 0.04^{bc}	3.93 ± 0.06^{b}
VCT8	2.33 ± 0.06^{bc}	3.33 ± 0.04^{bc}	3.96 ± 0.05^{bc}	3.95 ± 0.08^{b}

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. Loss of TOC during fungal inoculated composting and vermicomposting of CW with SBG in different treatments.

Treatments	TOC (%)			
	Days			
	0	30	60	90
MCT1	47.60 ± 0.19^{a}	$43.85 \pm 0.23b^{c}$	29.37 ± 0.20^{c}	23.20 ± 0.15^{c}
MCT2	47.49 ± 0.26^{a}	42.70 ± 0.22^{b}	23.38 ± 0.16^{b}	19.47 ± 0.05^{b}
MCT3	47.21 ± 0.16^{a}	32.38 ± 0.27^{ab}	18.97 ± 0.19^{ab}	15.33 ± 0.12^{ab}
MCT4	47.39 ± 0.17^{a}	33.25 ± 0.24^{ab}	17.62 ± 0.21^{ab}	15.41 ± 0.14^{ab}
MCT5	48.49 ± 0.11^{a}	42.76 ± 0.18^{b}	20.71 ± 0.25^{b}	18.59 ± 0.11^{bc}
MCT6	48.21 ± 0.29^{a}	32.42 ± 0.28^{a}	17.22 ± 0.15^{a}	15.02 ± 0.12^{a}
VCT7	49.25 ± 0.23^{a}	31.53 ± 0.20^{a}	16.12 ± 0.13^{a}	14.81 ± 0.11^{a}
VCT8	48.51 ± 0.09^{a}	31.42 ± 0.11^{a}	16.81 ± 0.10^{a}	14.92 ± 0.09^{a}

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 TKN during fungal inoculated composting and vermicomposting of CW with SBG in different treatments.

Treatments	TKN (%)			
	Days			
	0	30	60	90
MCT1	1.38 ± 0.06[a]	1.65 ± 0.08[a]	1.88 ± 0.14[a]	1.76 ± 0.14[a]
MCT2	1.38 ± 0.03[a]	1.82 ± 0.04[ab]	2.17 ± 0.15[b]	2.05 ± 0.06[b]
MCT3	1.41 ± 0.07[a]	2.01 ± 0.05[b]	2.52 ± 0.09[c]	2.48 ± 0.12[c]
MCT4	1.40 ± 0.02[a]	2.05 ± 0.09[b]	2.49 ± 0.12[cd]	2.39 ± 0.10[cd]
MCT5	1.38 ± 0.06[a]	1.86 ± 0.08[ab]	2.09 ± 0.06[b]	2.01 ± 0.12[b]
MCT6	1.40 ± 0.05[a]	2.20 ± 0.06[c]	2.69 ± 0.10[d]	2.66 ± 0.14[d]
VCT7	1.41 ± 0.04[a]	2.25 ± 0.07[c]	2.72 ± 0.08[d]	2.70 ± 0.11[d]
VCT8	1.40 ± 0.03[a]	2.24 ± 0.05[c]	2.69 ± 0.09[d]	2.66 ± 0.10[d]

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 6. Changes in TP during fungal inoculated composting and vermicomposting of CW with SBG in different treatments.

Treatments	TP (%)			
	Days			
	0	30	60	90
MCT1	0.93 ± 0.04[a]	1.17 ± 0.13[a]	1.58 ± 0.25[a]	1.43 ± 0.33[a]
MCT2	0.96 ± 0.02[a]	1.38 ± 0.18[b]	1.81 ± 0.27[b]	1.69 ± 0.28[ab]
MCT3	0.98 ± 0.14[a]	1.55 ± 0.23[c]	2.60 ± 0.46[c]	2.51 ± 0.27[c]
MCT4	0.97 ± 0.11[a]	1.57 ± 0.19[c]	2.60 ± 0.49[c]	2.52 ± 0.46[c]
MCT5	0.95 ± 0.13[a]	1.32 ± 0.14[b]	1.85 ± 0.23[b]	1.77 ± 0.31[b]
MCT6	0.98 ± 0.08[a]	1.72 ± 0.15[d]	2.69 ± 0.37[d]	2.53 ± 0.41[d]
VCT7	0.98 ± 0.07[a]	1.74 ± 0.11[d]	2.70 ± 0.27[d]	2.55 ± 0.32[d]
VCT8	0.97 ± 0.05[a]	1.72 ± 0.09[d]	2.68 ± 0.25[d]	2.53 ± 0.18[d]

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 7. Changes in TK during fungal inoculated composting and vermicomposting of CW with SBG in different treatments.

Treatments	TK (%)			
	Days			
	0	30	60	90
MCT1	1.10 ± 0.15[a]	1.23 ± 0.28[a]	1.41 ± 0.24[a]	1.36 ± 0.12[a]
MCT2	1.09 ± 0.09[a]	1.38 ± 0.18[ab]	1.53 ± 0.20[ab]	1.43 ± 0.16[a]
MCT3	1.09 ± 0.17[a]	1.51 ± 0.28[b]	1.89 ± 0.19[bc]	1.80 ± 0.15[b]
MCT4	1.08 ± 0.11[a]	1.47 ± 0.12[ab]	1.76 ± 0.23[bc]	1.70 ± 0.09[b]
MCT5	1.13 ± 0.06[a]	1.35 ± 0.17[ab]	1.61 ± 0.11[b]	1.57 ± 0.20[ab]
MCT6	1.12 ± 0.18[a]	1.62 ± 0.22[bc]	1.92 ± 0.15[c]	1.88 ± 0.06[bc]
VCT7	1.11 ± 0.21[a]	1.65 ± 0.26[bc]	1.95 ± 0.22[c]	1.90 ± 0.03[bc]
VCT8	1.13 ± 0.20[a]	1.64 ± 0.21[bc]	1.94 ± 0.11[c]	1.90 ± 0.07[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, $p<0.05$).

CONCLUSION

This study suggests that microbial inoculation and selective earthworms effectively degraded these waste in the treatments, however, the highest degradation was recorded when inoculation of fungal consortium in to the treatments and vermicomposting treatments followed by inoculation of *T. viride* and *A.niger*. According to the experimental results, the highest values of nutrients were obtained in VCT7, VT8, MCT6, MCT3 AND MCT4 treatments. Therefore, the mixture ratio of CW to SBG in 1:1 with inoculation *E. eugeniae*, *E. fetida, T. viride* and *A. niger* was better for nutrient rich compost/vermicompost production.

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