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Microbial activity during Rice Straw Composting under Co-inoculation of Cellulomonas cellulans and Phanerochaete chrysosporium

Brijesh Kumar Mishra[1*], and Lata Nain[2]

[1]National Research Centre on Seed Spices, Tabiji, Ajmer-205306, India
[2]Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110012, India.

***Corres.author: bkmmicro@gmail.com**

Abstract: Rice straw, amended with poultry manure or urea was composted with a co-inoculum of fungi and bacteria in perforated cemented pits. Microbial activity in terms of FDA hydrolysis, alkaline phosphatase and dehydrogenase was measured at monthly intervals. Microbial activity was highest at 2nd month. Carbon content was lowest at 3rd month while nitrogen was higher at 3rd month. pH and electrical conductivity of the compost was found to be within the desirable limits for its use in crop production at the end of 3rd month of composting.
Key words: *Cellulomonas*; compost; dehydrogenase; FDA hydrolysis; microbial activity; *Phanerochaete.*

Introduction

Large amount of agroresidues (388 million tonnes) generated throughout India after crop harvest[1]. All sorts of cereal straw may not be available for composting because of their fodder value but paddy straw has limited use as animal feed because of its high oxalic acid and silica content[2]. On the other hand, composting offers an opportunity to recover and reuse a portion of the nutrient and organic fraction in paddy straw. Composting is the biological decomposition of the organic wastes under controlled aerobic conditions[3]. The formation of compost from the degradation of organic matter depends on the abilities of microflora to produce and excrete specific degradative enzymes. Several reports are available regarding composting of agricultural residues where inoculation with mesophilic fungi improved the quality of compost. During composting, the starting material is transformed through a variety of biological and biochemical processes in which microbial enzymes play a role[4]. Composting is a biological decomposition and stabilization of organic substrates, under conditions that allow development of elevated temperatures as a result of biologically produced heat to produce a final product that is stable, free of pathogens and plant seeds, and can be beneficially applied to land. The complete degradation of complex

agricultural biomass is believed to be the result of cooperative action among fungi, bacteria and other microflora. To successfully integrate composting and use of compost into existing crop production systems, the composting must result in the production of quality compost which is sufficiently matured. Compost maturity, *i.e.* the biological stabilization of compost is recognized as one of the most critical factors in the processing and the agronomic use of compost. However, there are very few studies on microbial activity during the composting process that can be correlated with the compost maturity and other parameters of compost quality. There are certain enzymes such as dehydrogenases, phosphatases and FDA hydrolysis which can be assayed during the composting process to ascertain both, the effect of inoculants and extent of decomposition. However, the evaluation of compost maturity is difficult and no single test has been found to conclusively confirm that compost has reached maturity[5]. Generally, C/N ratio lower than 20 is generally regarded as a benchmark for compost maturity. With this background information present investigation was carried out to understand the effect of co- inoculation of cellulolytic bacteria and white rot fungi in terms of microbial activity during the composting of paddy straw.

Materials and Methods

Collection of substrates

In the present study, rice straw was used as a substrate for composting by cellulolytic bacterium (*Cellulomonas cellulans*) and lignocellulolytic fungus (*Phanerochaete chrysosporium*). Poultry manure and urea was used for supplementation of nitrogen in the rice straw. Rice straw was collected from Indian Agricultural Research Institute (IARI) farm and chopped into small pieces of 2.5 cm for preparation of compost as well as decomposition studies. Poultry manure was procured from a local poultry farm in Delhi.

Chemical composition of paddy straw and poultry manure

Rice straw, poultry manure and compost samples were analyzed for its chemical composition after grinding and passing through sieve of 1 mm. Paddy straw and poultry manure were analyzed for organic carbon, total nitrogen and other chemical parameters and their values are given in Table1.

Table1. Characteristics of different ingredients used in composting process

S. No.	Ingredient	Carbon (%)	Nitrogen (%)	C: N ratio	pH	EC (mS cm^{-1})
1.	Paddy straw	36.8	0.48	76.7	7.9	3.0
2.	Poultry manure	10.0	2.92	3.42	7.6	3.9
3.	Urea	20.0	46.0	0.42	-	-
4.	40 kg Paddy straw + 5 kg poultrymanure @12.5%	33.82	0.75	45.02	8.0	5.10
5.	40 kg Paddy straw + 0.2 kg urea @ 0.5%	36.7	0.70	52.40	8.50	3.75

Inoculum preparation for composting

Phanerochaete chrysosporium (MTCC 787 and NCIM 1073) and one bacterial strain of *Cellulomonas cellulans* (MTCC 23) was selected for composting experiment on the basis of their lignocellulytic potentials[6]. Two kg of sorghum (*Sorghum bicolor*) grains was soaked for 3 h in water. After soaking, water was decanted out. The grains were boiled in 0.2 per cent dextrose solution for 15 minutes. Sorghum grains were transferred into conical flasks (250 ml) at the rate of 70 grams each. $CaSO_4$ and $CaCO_3$ were added at the rate of 2% and 4%, respectively and autoclaved at 15 lbs for 15 minutes. Flasks containing grains were inoculated with five 6 mm discs of 5 days old cultures of *Phanerochaete chrysosporium* from potato dextrose agar (PDA) medium and mixed by hand shaking. The flasks were incubated at 37^0C for two weeks. Inoculum of bacterial culture *Cellulomonas cellulans* was grown in beef extract (0.5%) nutrient broth at 28^0C for three days on a rotary shaker.

Preparation of compost

The 40 kg paddy straw was weighed and filled in the perforated pits co-inoculated with mycelium and spore mixture (grown on grains) of *Phanerochaete chrysosporium* at the rate of 1.0 per cent (w/w) and bacterial culture at the rate of 1.0 per cent (v/w). The inoculum was mixed in the pits. Paddy straw was amended with poultry manure (12.5% w/w) and urea (0.5% w/w) individually. The moisture was maintained at 60 per cent (w/w) throughout the experiment. The experiment was done under natural conditions in during winter months. The mean day temperature

varied from 21 to 28 ^{0}C and night temperature ranged from 7 to 13 ^{0}C during the composting experiment. The substrate mixture was allowed to decompose for three months. The following substrate mixtures were maintained in experiment:

1. Paddy straw + Poultry manure (12.5% w/w)
2. Paddy straw + Urea (0.5% w/w)
3. Paddy straw (Control)

Following strains were used in inoculation of substrate mixture for composting

1. Co-inoculum A+B (*P. chrysosporium* MTCC 787 + *C. cellulans* MTCC 23)
2. Co-inoculum B+C (*P. chrysosporium* NCIM 1073 + *C. cellulans* MTCC 23)

Determination of microbial activity in compost

Microbial activity in terms of fluorescein diacetate (FDA) hydrolysis, dehydrogenase and alkaline phosphatase activity were monitored at monthly interval.

Fluorescein diacetate (FDA) hydrolysis

For Microbial activity estimation in terms of Fluorescein diacetate (FDA) hydrolysis[7], one g compost sample was incubated in a test tube containing 5 ml of 60 mM sodium phosphate buffer (pH 7.6) and 20 µl Fluorescein diacetate solution(2 mg ml^{-1}) at ambient temperature. Reaction was terminated after 45 minutes by adding 0.2 ml acetone. Absorbance of samples was recorded at 490 nm. FDA hydrolysis was calculated in terms of A_{490} unit h^{-1}g^{-1} of compost sample.

Dehydrogenase activity

For estimation of Dehydrogenase activity of compost samples one g fresh air-dried compost sample was saturated with 1.0 ml TTC (3% w/v) solution in a screw-capped test tube. Care was taken that no air bubble remains during packing of compost sample[8]. These test tubes were incubated at 28 ± 1^{0}C for 24 h. Methanol (10 ml) was added to these test tubes and shaken vigorously. Supernatant was taken out carefully after allowing to stand for six hours. Absorbance of supernatant was recorded at 485 nm. A standard curve was prepared with TPF (0-50 µg ml^{-1}). Concentration of TPF in sample was calculated with standard curve. Dehydrogenase activity was expressed in terms of µg TPF g^{-1}h^{-1}.

Alkaline phosphatase activity

Alkaline phosphatase activity of compost samples was determined by standard method[9]. One g fresh air-dried compost sample was added in 100 ml Erlenmeyer flask in which 0.2 ml toluene and 1.0 ml of 25 mM p-nitrophenyl phosphate was added. After swirling the flask for one min, it was incubated at 37^{0}C for 1 h. Then 0.5 ml $CaCl_2$ and 4.0 ml 0.5 N NaOH was added to this flask. A control was also prepared with similar way without adding p-nitrophenyl phosphate. After swirling the flasks for few seconds, 1 ml of p-nitrophenyl phosphate was added to control. All samples were filtered through Whatman No. 1 filter paper. Absorbance of yellow colour developed was recorded at 440 nm. A standard curve was prepared with p-nitrophenol (0-50 µg ml^{-1}) and amount of p-nitrophenol liberated was calculated with standard curve. Alkaline phosphatase activity was calculated in terms of µg p-nitrophenol (PNP) g-^{1} h^{-1}.

Statistical analysis

The data recorded in triplicate for the various observations was subjected to statistical analysis by the method of completely randomised block design using MSTAT-C statistical package and CD (Critical Difference) values were calculated at 0.05 P-level[10].

Results and Discussion

Microbial activity during paddy straw composting

Microbial populations change during the composting process, progressing from mesophilic stage into the thermophilic stage and then a gradual cooling period in final stabilization stage.

Table 2 Effect of amendment and inoculation on microbial activity in terms of FDA hydrolysis (A_{490} g^{-1} h^{-1}) during paddy straw composting

Amendment	Period of composting											
	30 days				60 days				90 days			
	Uninoculated	Inoculated (A+C)	Inoculated (B+C)	Mean	Uninoculated	Inoculated (A+C)	Inoculated (B+C)	Mean	Uninoculated	Inoculated (A+C)	Inoculated (B+C)	Mean
Poultry manure	1.39	1.48	1.70	1.52	1.53	2.20	1.92	1.88	1.31	1.41	1.62	1.45
Urea	1.27	1.45	1.32	1.35	1.39	1.82	1.92	1.71	1.19	1.37	1.31	1.29
Mean	1.33	1.46	1.51	1.43	1.46	2.01	1.92	1.80	1.25	1.39	1.46	1.37
Control	0.85				0.92				1.14			
Interaction				SEm ±					CD at 5%			
Days x Amendments				0.012					0.034			
Inoculation x Days				0.015					0.041			
Inoculation x Amendments				0.012					0.034			
Control vs Rest				0.023					0.063			

A= *P. chrysosporium* MTCC 787, B= *P. chrysosporium* NCIM 1073, C= *C. cellulans* MTCC 23

Table 3 Effect of amendment and inoculation on dehydrogenase («g TPFg^{-1} h^{-1}) during paddy straw composting

Amendment	Period of composting											
	30 days				60 days				90 days			
	Uninoculated	Inoculated (A+C)	Inoculated (B+C)	Mean	Uninoculated	Inoculated (A+C)	Inoculated (B+C)	Mean	Uninoculated	Inoculated (A+C)	Inoculated (B+C)	Mean
Poultry manure	1561.35	3786.50	2377.95	2575.27	2811.88	4095.77	3554.59	3487.41	2380.73	3578.40	3051.88	3003.67
Urea	621.11	3466.92	2733.89	2273.97	1094.08	3983.80	3466.19	2848.03	2242.00	3205.75	3027.34	2825.03
Mean	1091.23	3626.71	2555.92	2424.62	1952.987	4039.79	3510.39	3167.72	2311.36	3392.07	3039.61	2914.35
Control	568.03				1101.91				2020.30			
Interaction				SEm ±					CD at 5%			
Days x Amendments				12.37					34.28			
Inoculation x Days				12.37					41.99			
Inoculation x Amendments				12.37					34.28			
Control vs Rest				13.14					64.14			

A= *P. chrysosporium* MTCC 787, B= *P. chrysosporium* NCIM 1073, C= *C. cellulans* MTCC 23

Table 4 Effect of amendment and inoculation on alkaline phosphatase («g pNP g^{-1} h^{-1}) during paddy straw composting

Amendment	Period of composting											
	30 days				60 days				90 days			
	Uninoculated	Inoculated (A+C)	Inoculated (B+C)	Mean	Uninoculated	Inoculated (A+C)	Inoculated (B+C)	Mean	Uninoculated	Inoculated (A+C)	Inoculated (B+C)	Mean
Poultry manure	865.29	1725.76	1746.72	1445.92	1014.69	2242.53	2273.22	1842.81	816.26	1108.44	894.53	939.74
Urea	751.30	1527.04	1511.68	1263.34	900.56	2009.72	2015.98	1668.75	917.00	807.39	966.86	897.08
Mean	808.29	1626.40	1629.20	1354.63	997.62	2126.13	2143.60	1755.78	866.63	957.91	930.70	918.41
Control	533.387				697.86				893.83			
Interaction				SEm ±				CD at 5%				
Days x Amendments				0.69				1.92				
Inoculation x Days				0.85				2.36				
Inoculation x Amendments				0.69				1.92				
Control vs Rest				1.30				3.90				

A= *P. chrysosporium* MTCC 787, B= *P. chrysosporium* NCIM 1073, C= *C. cellulans* MTCC 23

Maximum microbial activity occurs during mesophilic phase[11]. Oxidoreductases and hydrolases act during basic processes of organic matter decomposition and can be used as biomarkers for the reactions which occur in soil[12]. Dehydrogenase activity is widely used in evaluating the metabolic activity of soil and compost microorganisms[13.] Significant correlation between microbial biomass and dehydrogenase/phosphatase activity of manured soils has been reported earlier[14]. FDA hydrolysis is indicative of living microbial cells in compost. In present investigation, highest FDA hydrolysis was found at 60 days of composting in poultry manure amended and co-inoculated samples of paddy straw (Table 2). The mean value of FDA hydrolysis of co-inoculated paddy straw samples increased from 1.43 to 1.80 A_{490} g^{-1} h^{-1} (unit absorbance at 490 nm) at 60 days of composting which declined to 1.37 A_{490} g^{-1} h^{-1}at 90 days of composting. It has been reported that the FDA hydrolysis increased during the first part of composting period and was highest on day 83, when flourescein production rate in samples ranged between 3640 to 8037 µg g^{-1} h^{-1} which declined sharply to values mainly below 2000 µg g^{-1} h^{-1} when vegetable and fruit waste was subjected to simple composting process. Decline in microbial activity may occur due to depletion of easily available substrates and is indicative of compost maturity[15].

Dehydrogenases was assayed in the compost samples to monitor the biological activity during composting (Table3). Highest dehydrogenase activity (4095.77 µg TPF g^{-1} h^{-1}) was in *P. chrysosporium* MTCC 787 and *C. cellulans* MTCC 23 inoculated and poultry manure amended paddy straw after 60 days of composting. This higher dehydrogenase activity under dual inoculation and poultry manure amended paddy straw may be due to microbial growth and/or addition of microbial cells or enzymes with the poultry manure[16]. The maximum dehydrogenase activity can be correlated to static respiration index during the maturation mesophilic stage and can be a method to describe the biological activity of the thermophilic and mesophilic stages of composting[13].

Similar pattern was also observed for alkaline phosphatase (Table 4). The level of alkaline phosphatase activity depends on the quantity of microbial biomass contained in the composting material and on the amount of synthesized enzymes[12]. The lowest alkaline phosphatase activity (918 µg PNP $g^{-1}h^{-1}$) recorded after 90 days of composting period may be due to change in microbial profile or adsorption of the enzymes in the compost humic matrix[4]. Phosphatases are considered key enzymes in phosphorus cycling in soil. Variations in phosphatase activity apart from indicating changes in the quantity and quality of soil phosphorated substrate[17] are also good indicators of soil biological status[18].

Characterization and quantification of enzymatic activity during composting can reflect the dynamics of the composting process in terms of the decomposition of organic matter and nitrogen transformation. It may also provide information about the maturity of the composted product[19]. Microbial activity in terms of FDA hydrolysis, dehydrogenase and alkaline phosphatases activity (Table 2, 3, 4) during paddy straw composting indicate a similar pattern. There was highest microbial activity after 60 days of composting which coincides with typical end of mesophilic phase of composting. The pattern of microbial activity in terms of FDA hydrolysis (Table 2) dehydrogenase (Table 3) and alkaline phosphatase activity (Table 4) during paddy straw composting are similar to those reported by earlier workers[15, 16].

The C/N ratio is one of the main characteristics that describe the composting process. The C/N ratio in the solid phase can not be used as an absolute indicator of compost maturation due to the large variation that is dependent on the starting material[20]. In the present study, lowest mean C/N ratio (10.3) was observed after 90 days of composting period, irrespective of amendment and inoculums. Poultry manure amendment was better than urea with respect to C: N ratio of compost after 90 days of composting. After 90 days of composting, pH and electrical conductivity of bio-supplemented samples were found to be within desirable limits (data not shown) for application in soil.

Conclusions

Lignocellulolytic microorganisms are essential for rapid decomposition of agricultural residues. Artificial supplementation of cellulolytic bacteria and fungi enhanced the composting process because of their hydrolytic enzymes. Besides traditional methods of monitoring composting process based on physico-chemical characters, microbiological properties can also be used as an effective indicator of composting process. This microbial activity needs to be correlated with compost maturity for development of compost maturity kits.

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