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Recycling of Crop Wastes to Compost with a Consortium of Efficient Biodegrading Microorganisms

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Bioconversion of agrowaste is an environment friendly and safe method for the disposal and recycling of organic wastes. Composting process to produce quality compost within short duration may be valuable in recycling agrowaste in organic manure to improve soil fertility. Microbial consortium of efficient biodegrading strains consisting amylolytic (A-6) and proteolytic bacteria (P-3), cellulolytic actinomycetes (C-3) and pectinolytic fungus (PC-15) with and without mustard cake amendment was used for degradation of pigeon pea stover, chickpea stover, mustard stover, sugarcane trash and cotton stover. Microbial inoculation along with mustard cake amendment accelerated composting and increased total nitrogen per cent and humus content of the compost and also decreased the pH value and total organic carbon content in composted crop wastes in 60 days of decomposition.

Key words: Crop waste, Consortium, Compost, Bacteria, Fungi, Actinomycetes.

A large number of crops are grown throughout the world. After using their economic parts, the remaining portion is mostly wasted except for a few crops. Million tonnes of nutrients are wasted through these crop wastes either by burning or by disposal.

The recycling of crop wastes as composts for maintenance of soil health as well as proper use of waste with hygienic methods is of significant advantage for crop production and soil health. Composting technology has been recognized as the most eco-friendly and cost effective alternative to convert agro-wastes into product that conditions soil and nourishes plants. Composting offer several benefits such as enhanced soil fertility and soil health thereby increased agricultural productivity, improved soil biodiversity, reduced ecological risks and better environment. Composting is a biological conversion and stabilization of heterogenous organic substrates under condition that allow development of thermophilic temperature as a result of biologically produced heat. Final product is hygienic, humus rich, relatively stable product that is free from pathogens, conditions soil and nourishes plants. Humus as soil organic matter resides in the aggregates in soil[1]. It exhibits functional groups such as carboxyl, hydroxyl, and carbonyl, strongly binded with clay minerals and oxides and they act as active catalytic centers for binding and decomposition of pesticides and other molecules[2].

Different hydrolytic enzymes are released by the microorganisms which are involved in the depolymerization of different constituents of

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organic wastes[3]. The bioconversion of agricultural wastes is not possible with single microorganism because all the enzymes needed for degradation of starch/protein/lignin/cellulose/hemicellulose are not present in one organism and therefore, a consortium of efficient microbes (fungi, bacteria and actinomycetes) is required for a rapid bioconversion of agricultural wastes.

In this investigation, an attempt was hereby made to isolate microorganisms from natural sources having amylase, protease, cellulase and pectinase enzyme producing ability. A consortium of these microbes was prepared which can hasten the process of decomposition resulting in a C:N ratio in an optimum range.

MATERIALS AND METHODS

Isolation of decomposers and preparation of consortium

Efficient strains of amylolytic and proteolytic bacteria, pectinolytic fungus and cellulolytic actinomycetes were isolated by enrichment technique from natural sources i.e. degraded mustard stover, degraded sorghum stover and soil, collected from the tropical area of Rajasthan. Qualitative screening of the isolates was done for amylolytic activity[4], proteolytic activity[5], cellulolytic activity[6] and pectinolytic activity[7] and codes were assigned as A-1 to A-20 for amylolytic, P-1 to P-23 for proteolytic, C-1 to C-6 for cellullolytic and PC-1 to PC-15 for pectinolytic microbes. After primary screening, quantitative screening was done by enzyme assay. Amylase activity was assayed in the culture filtrate when grown in starch containing broth and incubated for 72 hrs at 30^0C[8]. Isolate A-6 was able to produce high amount of amylolytic enzyme (149.17 IU/ml). Protease activity was assayed by the method of[9] with few modifications described by[10]. Isolate P-3 resulted in production of highest protease enzyme activity (29.16 IU/ml). Cellulolytic isolates were grown under submerged condition with Reese's mineral medium with cellulose as sole carbon source[11] and Filter paperase activity, carboxymethyl cellulose and cellobiase was estimated[12]. Actinomycetes C-3 showed highest FPase activity (21.17 IU/ml), CMCase activity (49.69 IU/ml) and cellobiase activity (132.43 IU/ ml). Pectinase activity was detected by plate assay[13] and the enzyme activity was quantified by the glucose released (mg) in the fermentation broth when pectin was used as sole source of carbon[14]. The enzyme activity was calculated by using the formula (one unit of Pectinase = Glucose released (in mg) x 0.185). Highest pectinase activity (0.457 IU/ ml) was produced by the fungal isolate, PC-15.

On the basis of quantitative assay, best strains of amylolytic (A-6) and proteolytic bacteria (P-3), cellulolytic actinomycete (C-3) and pectinolytic fungus (PC-15) were selected for preparation of consortium for the bioconversion of crop wastes. All these strains were also tested for their compatibility and were found to have no antagonistic effect against each other. A consortium was prepared by growth of individual microbial strain in nutrient broth as per standard protocol and then a homogenous mixture was prepared by all the strains in equal ratio before inoculation. Pectinolytic fungus (PC-15) was grown on sorghum grain.

Evaluation of efficiency of microbes to decompose agro wastes and preparation of compost

For Evaluation of efficiency of microbes to decompose agrowaste and for the preparation of compost, an experiment was conducted under completely Randomized Design (CRD-factorial). Three treatments namely, Uninoculated+amended, Inoculated and Inoculated + amended were applied in five kinds of agro-wastes i.e. pigeonpea stover, chickpea stover, mustard stover, sugarcane trash and cotton stover. These treatments were replicated four times in plastic containers (90 L capacity)

Ten Kg straw, each of these agro-wastes were filled in separate plastic containers. These were coinoculated with mycelium and spore mixture (grown on grains) of pectinolytic isolates at the rate of 1.0 per cent (w/w) and bacterial culture of amylolytic, proteolytic and cellulolytic isolates @ 1.0 per cent (v/w) as per treatments. These agrowastes were amended with calculated quantity of mustard cake to lower C:N ratio to 50:1 wherever needed as per treatments. The moisture was maintained at 60 per cent (w/w) throughout the experiment by addition of water. The substrate was allowed to decompose for 60 days. The composted samples were taken at 20, 40 and 60 days interval and dried in an oven at 90^0C for 24 hrs. The chemical analysis was done to monitor the changes in pH by pH-meter[15], total organic carbon[16], total nitrogen content by micro-kjeldahl method[17], and humus content[18]. Agrowastes were also analyzed for their initial chemical composition (Table 1).

This experiment was carried out at the Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India during 2005 to 2006.

RESULTS AND DISCUSSION

The interactive effect of microbial inoculation and plant biomass on pH was non significant up to 40 days of decomposition (Table 2 and Table 3); where as the same was significant at 60 days of decomposition (Table 4). The decrease in pH from initial alkaline value may be due to the action of microbes on the most labile fraction (carbohydrates) of crop wastes leading to the release of organic acids[19]. In case of total carbon content, the interaction effect of microbial inoculation and plant biomass was found non significant at 20 days of decomposition (Table 2), but it was observed significant at 40 and 60 days of decomposition (Table 3 and table 4). At 60 days of decomposition, the lowest organic carbon content (23.78%) was recorded in pigeon pea stover with inoculated + amended which was significantly lower than any other treatment combinations. Highest total organic carbon content (43.26%) was recorded in cotton stover with inoculation only. Soluble carbohydrates and amino acids are the major sources of carbon for the microorganisms involved in the decomposition of crop residues[20]. The extent and the rate of C mineralization is reduced as the proportion of lignin making up the organic matter increases., since this is the most resistant fraction to microbial decomposition[21]. Total nitrogen content and C:N ratio of the agrowastes were significantly influenced throughout the period of decomposition by the interaction effect of microbial inoculation and plant biomass (Table 2, Table 3 and Table 4). Maximum N content was recorded in pigeon pea stover under inoculated + amended (1.28%) which was at par with that of chick pea stover (1.25%) and mustard stover (1.22%). Minimum N content was recorded in cotton stover under inoculation (1.2%) at 60 days of decomposition (Table 4).

It can be inferred from Table 2, Table 3 and Table 4 that the variation in the humus content of different crop wastes was non significant at initial stage i.e. at 20 days of decomposition, but it was significant afterwards i.e. at 40 and 60 days of decomposition. This is because of the gradual advancement of the decomposition of lignin compounds of crop wastes. Composting can not be related directly to chemical parameters such as C:N ratio, pH, EC and amount of extractable C because these parameters depend apon the composition of starting material[22]. Stability is directly related to microbial activity. Amount of humic substances and their fractions can be used as indexes of maturity of compost[23]. Lignocellulose degradation demands the cooperative activity of hydrolytic and oxidative enzymes. Fungi are the most active primary lignocellulose degrader but actinomycetes and bacteria are also being recognized as efficient cellulolytic degraders. All these facts discussed are supported by the trend observed in humus content at 60 days of decomposition of the crop wastes, pigeon pea stover showing the highest humus content (11.4%), and the cotton stover having the lowest value thereof (7.3%). The C:N ratio was decreasing with time as evident from Table 2 to 4.

Data in Table 2 indicate that at 20 days of decomposition the C:N ratio of chick pea stover was the minimum (32.83) in case of

Table 1. Chemical composition of different crop residues/ agrowaste used for decomposition

S.No	substrate	C%	N%	P%	K%	C/N Ratio
1	Sugar cane trash	43.3	0.41	0.09	0.98	117.8:1
2	Cotton stover	51.00	0.98	0.41	0.53	52.04:1
3	Pigeonpea stover	53.30	0.60	0.18	0.57	89:10
4	Chickpea stover	53.4	1.01	0.24	1.92	52.87
5	Mustard stover	53.70	0.81	0.22	0.76	66.3:1
6	Mustard cake	54.10	4.80	1.50	1.30	11.27:1

Table 2. Interaction effect of microbial inoculation and plant biomass on pH, carbon content(C), C/N ratio and humus content at 20 days of decomposition

Plant Biomass	Uninoculated+Amended					Inoculated					Inoculated+Amended				
	pH	C%	N%	C/N ratio	Humus content (%)	pH	C%	N%	C/N ratio	Humus content (%)	pH	C%	N% ratio	C/N content (%)	Humus
Pigeonpea stover	7.83	44.63	1.01	44.42	6.41	7.65	41.42	0.9	46.02	6.3	7.75	39.18	1.02	38.61	6.93
Chickpea stover	7.5	37.43	1.14	32.83	6.51	7.63	40.3	1.13	36.04	6.68	7.53	37.7	1.14	33.2	7.05
Mustard stover	7.5	45.82	1.09	42.87	6.68	7.38	46.93	0.95	49.41	6.23	7.18	41.91	1.03	40.68	6.88
Sugarcane trash	7.83	40.08	1.05	38.21	5.18	7.43	42.33	0.73	58.58	5.28	7.6	39.23	1.09	36.21	5.58
Cotton stover	7.68	48.15	1.12	43.24	5.15	7.8	49.43	0.97	51.29	4.95	7.6	46.68	1.11	41.95	5.25

CD at 5% (Plant Biomass×Microbial Inoculation) pH= NS, C%= NS, N%= 0.06, C/N ratio= 3.29, Humus content= NS

Table 3. Interaction effect of microbial inoculation and plant biomass on pH, carbon content(C), C/N ratio and humus content at 40 days of decomposition

Plant Biomass	Uninoculated+Amended					Inoculated					Inoculated+Amended				
	pH	C%	N%	C/N ratio	Humus content (%)	pH	C%	N%	C/N ratio	Humus content (%)	pH	C%	N% ratio	C/N content (%)	Humus
Pigeonpea stover	7.4	37.26	1.06	35.17	7.93	7.5	36.43	0.93	39.15	7.63	7.33	30.92	1.12	27.55	8.63
Chickpea stover	7.3	34.37	1.16	29.9	7.95	7.48	35.15	1.16	30.4	7.65	7.3	30.55	1.18	25.94	9.18
Mustard stover	7.2	37.17	1.08	34.58	7.73	7.28	41.5	1.01	41.22	7.48	7.03	35.19	1.13	31.2	8.45
Sugarcane trash	7.33	36.15	1.09	33.24	6.43	7.38	39.08	0.78	50.51	6.2	7.33	34.23	1.16	29.75	6.9
Cotton stover	7.48	44.37	1.05	42.18	6.08	7.43	46.13	0.92	50.34	5.78	7.48	37.24	1.14	32.67	6.25

CD at 5% (Plant Biomass×Microbial Inoculation) pH=NS, C%= 0.91, N%=0.05, C/N ratio=2.28, Humus content=0.28

Table 4. Interaction effect of microbial inoculation and plant biomass on pH, carbon content(C), C/N ratio and humus content at 60 days of decomposition

Plant Biomass	Uninoculated+Amended					Inoculated					Inoculated+Amended				
	pH	C%	N%	C/N ratio	Humus content (%)	pH	C%	N%	C/N ratio	Humus content (%)	pH	C%	N% ratio	C/N content (%)	Humus
Pigeonpea stover	7.25	31.75	1.1	28.83	9.8	7.25	29.63	1.01	29.5	9.76	7.05	23.78	1.28	18.61	11.36
Chickpea stover	7.2	31.33	1.19	26.32	9.76	7.3	31.4	1.17	26.9	9.26	7.08	27.26	1.25	21.91	11.14
Mustard stover	7.1	29.83	1.13	26.52	8.71	7.2	27.18	1.05	25.91	8.36	7.03	24.26	1.22	20.15	10.82
Sugarcane trash	7.33	33.26	1.12	29.71	7.18	7.1	35.19	0.88	40.24	7.08	7.13	32.49	1.2	27.19	7.81
Cotton stover	7.45	38.57	1.14	34	6.81	7.25	43.26	1.02	42.64	6.7	7.3	34.61	1.17	29.68	7.3

CD at 5% (Plant Biomass×Microbial Inoculation) pH=0.116, C%=0.72, N%= 0.05, C/N ratio= 1.28, Humus content=0.36

Uninoculated+amended whereas that of mustard stover, cotton stover, pigeonpea stover were at par; the C:N ratio of chickpea stover was minimum, that of sugarcane trash was highest in inoculated treatment (58.58); the C:N ratio of chick pea stover was at par with sugarcane trash and the later was at par with pigeonpea stover while cotton stover having the highest value thereof.

Table 3 indicates that at 40 days of decomposition, the C:N ratio of chickpea stover was the minimum (29.9,30.4 and 25.94) under all the treatment conditions whereas cotton stover having the highest C:N ratio under uninoculated+amended conditions (42.18), that of cotton stover (50.34) and sugarcane trash(50.51) were at par under inoculated treatment conditions, cotton stover having the highest C:N ratio (at par with that of mustard stover) under the Inoculated +amended treatment conditions.

Consortium of microorganisms with mustard cake amendment decreased the C:N ratio of Pigeon pea stover to a minimum value of 18.61 at 60 days of decomposition (Table 4). This may be due to the fact that pigeon pea stover having the higher initial C:N ratio (89:1) followed by mustard stover (66.3:) followed by chick pea stover (53:1) as it is known that greater the C:N ratio, the more will be the mineralization, thereby leading to lowering of C:N ratio. However sugarcane trash, having the highest C:N ratio (117.8:1) could not follow this trend because of its higher lignin content. For any given compost, the C:N ratio was highest in inoculated treatment at all the stages of decomposition and it was lowest under inoculated+amended treatment conditions thereby indicating the role of microbes in hastening the decomposition.

CONCLUSIONS

From present study, it can be inferred on the basis of decrease in C:N ratio that this consortium [Amylolytic (A-6) and proteolytic (P-3) bacteria, cellulolytic (C-3) actinomycetes and pectinolytic (PC-15)) fungus] with mustard cake amendment is able to decompose pigeon pea stover rapidly followed by other agrowastes used in the present investigation. Pigeon pea stover, mustard stover and chick pea stover were effectively decomposed within 60 days with the proposed consortium. It was also able to increase humus content and nitrogen content in these three agrowastes. On the basis of narrow C:N ratio, high humus content and high N content of the resultant composts from agrowastes can be incorporated in soil for soil fertility improvement and crop production.

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