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DEGRADATION OF AGRO-RESIDUE USING FUNGAL CONSORTIA AND DAP

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Abstract: Recycling of agricultural wastes comprising of garden waste (grass cutting) and leaf litter was carried out by different fungi (*Aspergillus niger* and *Trichoderma reseei*) and using cow dung and Di ammonium phosphate (DAP) (@ 0.1% as activators. The results indicated that organic carbon was decreased from 38.6 % to 24.5% with concomitant increase in available nitrogen content from 84.6 ppm to 456.5 ppm over 90 day of incubation. In garden waste carbon content was decreased by 25 % and in leaf litter (comprising mainly with *Bambusa vulgaris* and *Eucalyptus leaves*) by 27% while the nitrogen content was increased by 76% and 52% respectively when the biomass was treated with fungal consortia with the addition of 0.1% DAP as activator. The results indicate that activators like DAP enhances the rate of decomposition when agricultural wastes are either treated with fungal consortia or cow dung.

Keywords: Garden waste, leaf litter, fungal consortia, cow dung, DAP

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INTRODUCTION

Today the progress of environmental policies and regulations leads to the development of biodegradable processes to turn the organic waste into some valuable resources by potential microbes because only few strains are capable of secreting a complex of cellulose enzymes, which could have practical applications.

Cellulosic biomass constitutes a huge and renewable resource that can be converted to compost and fuel feedstocks. More efficient means for conversion of agricultural and forest waste are sought so that useful biomass-derived products can not only compete with or eventually replace petroleum based products but also supplement and complement the use of petroleum based fuels as additives to promote more efficient burning and lower emissions. Using these cellulosic resources efficiently can thus reduce the disposal problems and pollution resulting from accumulation of these wastes (Edwin, 2001).

The cellulose molecules are composed of long chains of sugar molecules. In the hydrolysis process, these chains are broken down to free the sugar, before it is fermented for alcohol production. There are two major cellulose hydrolysis (cellulolysis) processes: a chemical hydrolysis or an enzymatic hydrolysis.

The chemical hydrolysis includes ozonolysis by using ozone, acid hydrolysis by using Sulphuric acid (H_2SO_4) & Hydrochloric acid (HCl) and oxidative delignification by using peroxidase enzyme in presence of H_2O_2. Enzymatic hydrolysis of cellulose is carried out by celullase enzymes which are highly specific (Béguin and Aubert, 1994). The products of hydrolysis are usually reducing sugars including glucose. Utility cost of enzymatic hydrolysis is low as compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45-50 ºC) and does not have a corrosion problem (Duff and Murray, 1996).

Hydrolysis of cellulose is carried out by cellulase enzymes which are highly specific (Bégiun and Aubert, 1994). The products of the hydrolysis are usually reducing sugars including glucose. Both bacteria and fungi can produce cellulases for the hydrolysis of cellulosic materials. These microorganisms can be aerobic and anaerobic, mesophilic or thermophilic (Bisaria, 1991). Fungi that have been reported to produce cellulases include *Sclerotium rolfsii, Penicillium chrysosporium* and species of *Trichoderma, Aspergillus, Schizophyllum* and *Penicillium* (Fan et al., 1987; Duff and Murray, 1996). Of all these fungal genera, *Trichoderma* has been most extensively studied for cellulase production.

There are a number of ancillary enzymes that attack hemicellulose such as glucuronidase, acetylesterase, xylanase, β-xylosidase, galactomannase and glucomannanase (Duff and Murray, 1996). The addition of β- glucosidases into the *Trichoderma reseei* cellulases system achieved better saccharification than the system without β-glucosidases (Excoffier *et al*., 1991; Xin et al., 1993).

Cow dung manure is a nitrogen rich material and has economic importance as fertilizer, feed supplement or as energy source. Cow dung manure has been collected and used to supply nitrogen, phosphorous, potassium and calcium to the soil for plant production. Cow dung has relatively high carbon to nitrogen ratio.

Present study was aimed at finding out the best combination of cellulosic agricultural waste amended with different components, viz. cow dung, fungal consortia and DAP for its rapid degradation to a nutrient rich compost.

MATERIALS AND METHODS

Agricultural Wastes

The experiment was set to find the best combination of cellulosic agricultural waste amended with different components, viz. cow dung, fungal consortia and DAP, two agricultural wastes ie., Garden waste and leaf litter were collected from the field of Hindustan College of Science & Technology, Mathura; and were overnight oven dried at 105˚C. After complete drying the waste material was crushed using a mixer grinder. Cow dung was collected from the dairy farm from the campus itself and was dried at 105˚C in a hot air oven to maintain the moisture content around 30%.

Fungal Consortia

The fungi used in the study were *Aspergillus niger* and *Trichoderma reseei.* A piece of mycelia of each of the fungi was subcultured in Potato Dextrose Broth (PDB) for mass cultivation and incubated at 28 ± 2 ºC and 120 rpm for 48 hours. After 48 hours of growth, the inoculums (10 ml) was mixed to the waste.

Bioconversion of Agricultural Wastes

After the processing (chopping) of agricultural waste, cow dung, fungal consortia and DAP were mixed to the waste in different proportions (w/w) in a conical flask of 500 ml capacity in triplicates as follows:

- In control Garden waste (GW)(25 gm);
- Garden waste + cow dung (CD)(2:1);
- Garden waste + cow dung (2:1) + DAP (0.1 gm);
- Garden waste + fungal consortia (FC)(2.5:1);
- Garden waste + fungal consortia + DAP (0.1 gm)

The same treatments were set for leaf litter too.

The mouths of the flasks were plugged with cotton plugs to maintain the aerobic conditions and were kept at 30 ± 0.2 ºC in an incubator.
The samples were withdrawn from the flasks and stored in poly-bags at 15 days interval from day 0 to day 90.

Analytical Methods

Moisture was maintain at 50 %, estimated by gravimetric method and pH was measured potentiometrically in a supernatant liquid that was in equilibrium with waste sample suspension of a 1:10 waste to distilled water. A digital pH/mV meter with a combination electrode and an automatic compensator made by EUTECH Instruments was used for pH measurement.

The carbon % was estimated by the method as prescribed by Walkley and Black (1934), available nitrogen was estimated as per the protocol given by Jackson (1967), at an interval of 15 days upto 90 days. The concentration of cellulose and reducing sugars were estimated as per the protocol described by Updegraff and Ghose, (1987) respectively.

Enzyme Preparation

For the reducing sugars estimation the enzyme was extracted from fungi *A. niger* and *T. reseei* , for this the fungal cultural were inoculated on Potato Dextrose media and kept at 28 ± 2ºC for 48 hours. For the suspension of fungal spores 0.1% Tween-80 solution was used. Spore count of the fungal suspensions was set to approximately 5 x 10^6 spores/ml using haemocytometer. 1 ml of fungal suspension was inoculated in 100 ml of Duff's medium and incubated at varying temperatures viz 20º, 30°, 37° and 45° for 48 hrs with shaking at 120 rpm and enzymes were extracted by filtration (Ghose et al*., 1987)*.

RESULTS AND DISCUSSION

pH

Initially pH was found 8.3 in garden waste (grass cutting) and 7.95 in leaf litter, it was varied with the different combinations such as 7.82 in garden waste (control), 7.98 and 8.08 in garden waste with cow dung and with fungal consortia respectively, while 7.03, 7.35 and 7.41 in leaf litter (control), leaf litter amended with cow dung and leaf litter with fungal consortia respectively.

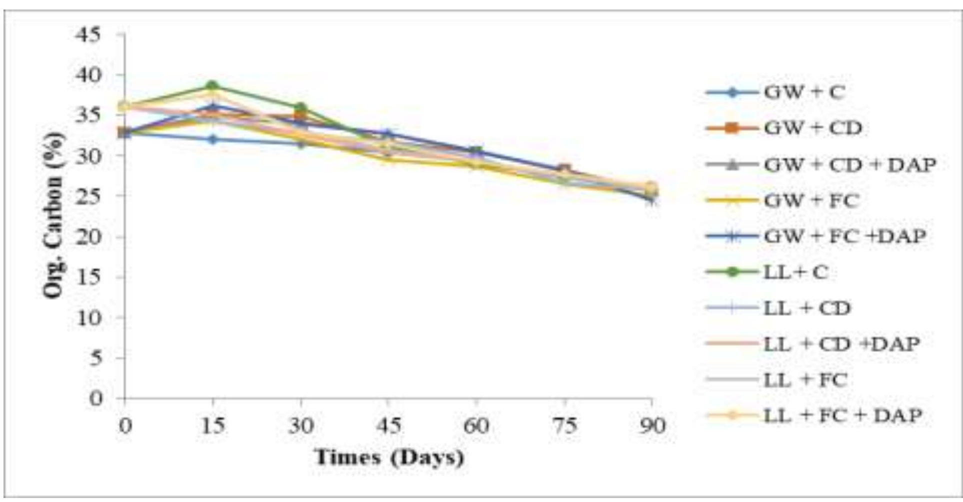
Rahman et al. 2011, worked on kitchen waste and found *Trichoderma* was found to have adapted to an acidic habitat and effective in decomposition. It provided the highest volume (31.80%) and weight (30.80%) losses in waste treated with spore suspension.

The pH was found to be decreased as the decomposition proceeded because of the reaction of carbon dioxide and water resulting in formation of carbonic acid (Rahman et al. 2011).

Organic Carbon

Biodegradation is the general term used for all biologically mediated breakdown processes for chemical compounds and complete biodegradation leads to mineralization (Bennett 1988). The process of decomposition of organic materials is chiefly microbial and fungi play an important role, as they are more effective in carbon assimilation than bacteria and actinomycetes (Saritha and Maruthi, 2010).

At the beginning of the experiment, the organic carbon was found 32.8 % in garden waste and 36.1 % in leaf litter, at day 15 organic carbon was found minimum in garden waste (control) i.e. 32.1 % and 34.3 % in leaf litter amended with cow dung. After 15 days, the results showed a gradual decrease in carbon from 32.1 % to 25.9 % for garden waste (control) and 34.3 % to 26.1 % for leaf litter (control) over a period of 90 days. After completion of 90 days the minimum carbon was found in garden waste with fungal consortia and DAP i.e. 24.5 % and maximum 26.3 % in leaf litter with fungal consortia. The initial organic carbon in different combinations varied from 32.8 % to 36.1 %. At the end of the experiment i.e. day 90, the lowest carbon was found in garden waste with fungal consortia & DAP and maximum in leaf litter with fungal consortia (Table 1).



Legends: GW: Garden Waste; LL: Leaf Litter; CD: Cow Dung; FC: Fungal Consortia; DAP: Di Ammonium Phosphate

Conditions: Temperature during dark incubation: 30ºC. Moisture content: 50%.

Figure 1. Organic Carbon (%) in various treatments at different time of interval.

The initial organic carbon in different combinations varied from 22.8 % to 26.1 %. The content of organic carbon was decreased as the decomposition processed but in early stages of the experiment the carbon percentage was increased due to the initiation of some microbial activities. At the end of the experiment i.e. day 90, the lowest carbon was found in garden waste with fungal consortia & DAP and maximum was found in leaf litter with fungal consortia. As the decomposition progressed due to losses of carbon mainly as carbon dioxide, the carbon content of the agricultural waste decreased with time and nitrogen content per unit material increased, which resulted in decrease of C:N ratio (Goyal et al., 2005).

Available Nitrogen

At the beginning of the experiment, available nitrogen was found 1008 ppm in garden waste and 765.3 ppm in leaf litter, at day 15 it was found maximum in garden waste amended with cow dung & DAP i.e. 522.6 ppm and leaf litter amended with fungal consortia i.e. 578.6 ppm. After day 15 it showed gradual increase in concentration from 522.6 ppm to 1717.3 ppm in garden waste amended with cow dung & DAP and in leaf litter amended with fungal consortia, available nitrogen increased from 578.6 ppm to 1222.7 ppm over a period of 90 days.

Table 1. Changes in Organic Carbon (%) at different time of interval

Sample name	0 day	15 days	30 days	45 days	60 days	75 days	90 days
GW + C	32.8±0.14	32.1±0.1	31.5±0.1	30.4±0.2	29.4±0.1	27.4±0.3	25.9±0.1
GW + CD		35.1±0.4	34.8±0.1	31.8±0.1	30.4±0.4	28.3±0.4	25.6±0.1
GW + CD + DAP		34.9±0.1	33.9±0.2	32.7±0.1	30.5±0.1	28.2±0.5	25.7±0.2
GW + FC		34.3±0.1	32.1±0.3	29.5±0.09	28.7±0.5	26.6±0.1	25.1±0.1
GW + FC +DAP		36.2±0.05	34.1±0.3	32.7±0.2	30.6±0.1	28.1±0.1	24.5±0.1
LL+ C	36.1±0.14	38.6±0.09	35.9±0.1	31.1±0.1	28.9±0.1	27.6±0.3	26.1±0.3
LL + CD		34.3±0.1	32.7±0.1	31.8±0.7	29.7±0.7	26.7±0.3	25.8±0.2
LL + CD +DAP		35.1±0.1	32.6±0.2	30.4±0.1	29.2±0.1	27.5±0.3	25.9±0.2
LL + FC		37.6±0.1	33.1±0.5	30.7±0.3	29.3±0.3	27.5±0.1	26.3±0.1
LL + FC + DAP		37.6±0.1	32.8±0.1	31.5±0.8	29.2±0.7	27.7±0.2	26.1±0.1

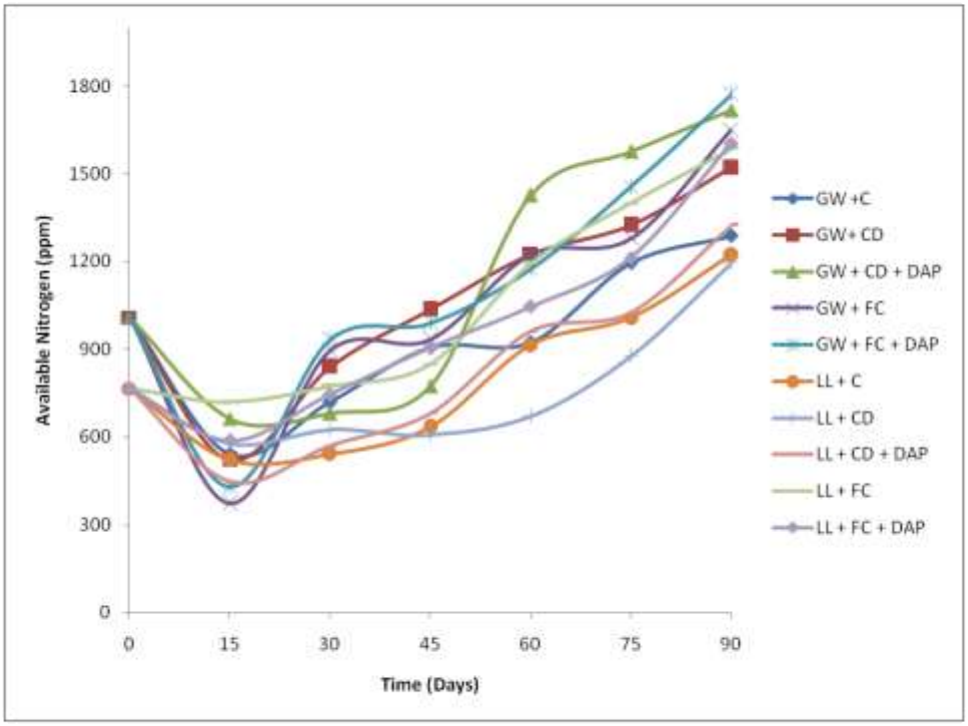
Legends: GW: Garden Waste; C: Control; CD: Cow Dung; FC: Fungal Consortia; DAP: Di Ammonium Phosphate; LL: Leaf Litter. Conditions: Temperature during dark incubation: 30ºC. Moisture content: 50%

Table 2. Changes in Available Nitrogen (ppm) at different time of interval

Sample name	0 day	15 days	30 days	45 days	60 days	75 days	90 days
GW + C	1008±16.1	541.3±9.3	718.6±9.3	905.3±18.6	924±16.1	1194.6±24.6	1288±16.1
GW + CD		522.6±9.3	840±0	1036±0	1222.7±18.1	1325.3±9.3	1521.3±9.3
GW + CD + DAP		662.6±9.3	681.3±18.6	774.6±24.6	1428±16.1	1577.3±9.3	1717.3±18.6
GW + FC		373.3±9.3	896±16.1	933.3±8.5	1222.7±18.6	1278.6±9.3	1652±16.1
GW + FC +DAP		429.3±9.3	933.3±18.6	989.3±4.9	1176±2.8	1456±3.2	1773±9.3
LL+ C	765.3±9.3	522.6±9.3	541.3±9.3	634.6±9.3	914.6±9.3	1008±16.1	1222.7±18.6
LL + CD		578.6±9.3	625.3±18.6	606.6±24.6	672±16.1	877.3±9.3	1194.7±18.6
LL + CD +DAP		448±16.1	569.3±9.3	681.3±18.8	961.3±9.3	1026.6±4.9	1325.3±9.3
LL + FC		718.6±18.6	774.6±9.3	849.3±3.7	1194.7±18.6	1400±3.2	1586.7±18.6
LL + FC + DAP		588±16.1	746.6±9.3	905.3±3.7	1045.3±18.6	1213.3±8.1	1605.3±9.3

Legends: GW: Garden Waste; C: Control; CD: Cow Dung; FC: Fungal Consortia; DAP: Di Ammonium Phosphate; LL: Leaf Litter. Conditions: Temperature during dark incubation: 30 ºC. Moisture content: 50%.

At day 15, the minimum concentration was found in leaf litter amended with cow dung and DAP i.e. 448 ppm and Garden waste amended with fungal consortia i.e. 373.3 ppm. At day 90, the available Nitrogen was found maximum in Garden waste with fungal consortia and DAP i.e. 1773 ppm and minimum in Leaf litter with cow dung i.e. 1194.7 ppm. Finally, lowest nitrogen was found in leaf litter with cow dung and maximum in garden waste with fungal consortia & DAP (Table 2).



Legends: GW: Garden Waste; LL: Leaf Litter; CD: Cow Dung; FC: Fungal Consortia; DAP: Di Ammonium Phosphate.

Conditions: Temperature during dark incubation: 30ºC. Moisture content: 50%.

Figure 2. Available Nitrogen (ppm) in various treatments at different time of interval.

The decrease in available nitrogen in early stages of experiment was due to losses of N in the form of ammonia which in turn depends upon with the type of material and its CN ratio (Sanchez-Monedero *et al.,* 2001).

CONCLUSION

In conclusion, the data has shown that fungi *Aspergillus niger* and *Trichoderma reseei* along with DAP are capable of doing faster degradation when are cultivated in different agricultural wastes. It also reveals that of the two types of waste materials studied, garden waste (grass cutting) is better to degrade because of its low carbon and high nitrogen contents. The use of the fungi and DAP in the degradation of the agricultural wastes will ultimately bring down the pollution level which is due to these waste materials and also a better use of resources to get some valuable products from the waste material.

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