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Efficacy of ligno-cellulolytic microbial consortia on biodegradation of paddy residues and its effect on biological properties of soil

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Abstract

In rice cultivation, rice straw is produced as a by products, which is composed largely of ligno-cellulolytic components which makes it high C:N ratio. Ligno-cellulolytic fungus, bacteria and actinomycetes can be used as potential agents for rapid composting of bulky rice residues. Four species of fungus (*Aspergillus spp.*) bacteria (*Bacillus spp.*) and actinomycetes (*Streptomyces spp.*) were used for the decomposition of paddy straw both individually as well as in various combinations for analyzing their potential for the degradation of lignin and cellulose content of paddy straw. Among eight treatments, the microbial consortia (*Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.*) were showed maximum activity. Inoculation of these ligno-cellulolytic microbial consortia in rice residues accelerated the process of decomposition when compared to control. A significant increase was also observed in total NPK content. Soil microbial biomass carbon (242.91mg CO₂-C kg⁻¹) and nitrogen and particulate organic carbon (1.34 g kg⁻¹) was maximum in soil + paddy straw + *Aspergillus spp.*+ *Bacillus spp.*+ *Streptomyces spp.* treatments. Hence we conclude that combination of ligno-cellulolytic microbial consortia viz., *Aspergillus spp.* + *Bacillus spp.*+ *Streptomyces spp.* can be recommended for the degradation of rice residues and enhance the soil properties.

Keywords: Soil microbial biomass carbon, *Aspergillus spp.*, *Streptomyces spp.*, ligno-cellulolytic and total nutrients

Introduction

Cultivation of paddy generates huge amount of rice straw as by product which is not recycled in the soil due to its huge quantity. Slow degradation rate causes short-term negative effect of nitrogen immobilization as well as poor yield. Cultivation of paddy generates huge amount of rice straw as by product which is not recycled in the soil due to its bulky volume. Slow degradation rate causes short-term negative effect of nitrogen immobilization as well as poor yield. Hence, the farmers usually dispose huge amount of straw by burning into the field. Burning of rice residues leads to emission of green-house gases. It's also contributes to emission of harmful air pollutants, (Polycyclic aromatic hydrocarbons) (Korenaga *et al.*, 2001) [17]. Poly-chlorinated dibenzofurans and poly-chlorinated dibenzodioxins (Gullette and Touati, 2003; Lin *et al.*, 2007) [19]. Keeping in mind the harmful effects of open field burning of rice residues as well as an economical and ecofriendly approach should be adopted for effective utilization of rice residues. It is a potential food source for microorganism and used as compost in to the soil it maintain the nutrient availability and fertility of the soil. The compost serves as an excellent source of nutrient in organic farming and mitigates the ill-effects of chemical fertilizers. In soil micro-biota, fungi are an important component, constituting more of the soil biomass than bacteria and population of fungus is lower but it dominates the soil biomass when the soil is not disturbed. (Ainsworth and Bisby, 1995) [2]. A large number of bacteria in the soil exist, but they have a lesser biomass due to their small size. Actinomycetes are one tenth in number but are larger in size so they are similar in biomass to bacteria. Bacteria, actinomycetes and protozoa are tolerate in tilled soil but fungi and nematode can tolerate in non-tilled soil. (Ainsworth and Bisby, 1995) [2]. Many microorganisms have been reported with cellulolytic activities both aerobic and anaerobic condition including many bacteria (*Trichonympha*, *Clostridium*, *Actinomycetes*, *Bacteroides succinogenes*, *Butyrivibrio fibrisolvans*, *Ruminococcus albus*,) and fungal strains (*Fusarium Myrothecium*, *Trichoderma*, *Penicillium*, *Aspergillus* play an important role in biodegradation of the lignocelluloses in organic waste and colonize very quickly on substrate. (Schwarz, 2001 [31]. Milala *et al.*, 2005 [21]. Hudson, 1972) [13]. It Increase microbial density, biomass and enzyme activities in the soil through biodegradation (Hayano *et al.*, 1995) [12].

In the present study, an attempt was made to evaluate the effect of use of ligno-cellulolytic microbial consortia on biodegradation of paddy residues and its effect on biological properties of soil.

Method and Material

Substrates

The pot experiment was conducted in a Vertisols at screen house of Indian Institute of Soil Science (IISS), Bhopal. The Vertisol was clayey in texture with pH 7.5. The mean maximum and minimum temperature from July to October were 30.6°C and 29.9°C, respectively. Paddy straw used as organic amendments was obtained from farmer's field, Sukhisewania Tehsil, Bhopal. Straw was chopped and prepared for pot experiment. The analytical characterization of soil and paddy straw used as amendments are given in Table 1. There is wide variation in all the parameters studied starting with pH to C/N ratio. Soil and paddy straw was tended to have pH near neutrality.

Table 1: Characteristics of various amendments used

Parameters	Soil	Paddy straw
pH	7.59	7.1
Carbon%	0.62	40.78
Nitrogen%	0.037	0.52
C/N	16.75	78.42

Preparation of inoculums

Aspergillus spp., *Bacillus* spp. and *Streptomyces* spp. was obtained from the sample collected from waste dumping area, Bhanpura, Bhopal. Bacteria, fungi and actinomycetes were isolated from the soil sample collected from the Bhanpura sewage dumping area of Bhopal. In this ways, cellulose and lignin degrading microbes were isolated and screened from these two positive samples and identified as 4 species of *Bacillus*, 4 species of *Aspergillus* and 4 species of *Streptomyces* and total 12 species was identified.

Experimental design

The experiment was laid out with completely randomized design (CRD) with three replications comprising of 8 treatments. In a pot experiment, paddy straw and microbial inoculants were applied. The treatments were designed as follows, T1, control (Soil, 5kg); T2, soil (5kg) + paddy straw (7.6g) + Fungal consortia (4 species of *Aspergillus*); T3, soil (5kg) + paddy straw (7.6g) + Bacterial consortia (4 species of *Bacillus*); T4, soil (5kg) + paddy straw (7.6g) + Actinomycetes consortia (4 species of *Streptomyces*); T5, soil (5kg) + paddy straw (7.6g) + Fungal + Bacterial consortia (4 species of *Aspergillus* + 4 species of *Bacillus*); T6, soil (5kg) + paddy straw (7.6g) + Bacteria + Actinomycetes consortia (4 species of *Bacillus* + 4 species of *Streptomyces*); T7, soil (5kg) + paddy straw (7.6g) + Fungal + Actinomycetes consortia (4 species of *Aspergillus* + 4 species of *Streptomyces*) and T8, soil (5kg) + paddy straw (7.6g) + Fungal + Bacteria + Actinomycetes consortia (4 species of *Aspergillus* + 4 species of *Bacillus* + 4 species of *Streptomyces*).

Soil Sampling and analysis

Soil samples were taken manually, two times during the experiment at 30 and 60 days after decomposition, to assess the effects of paddy straw decomposition with microbial consortia on different soil parameters. Three sub samples

taken from three replications. Moist samples were stored in refrigerator, for biological study.

These soil samples were examined for changes in biological properties of soil in response to organic addition.

Total nitrogen was estimated by Kjeldahl's method (Jackson, 1973) [15]. Total phosphorus and Potash was estimated the following method of Olsen *et al.*, (1954) [26], and Black *et al.*, 1965 [5]. Microbial biomass of soil was determined by fumigation incubation method in which two sets of 10 g incubated soil were taken; one set in a beaker and was subjected to chloroform fumigation until the chloroform in vacuum desiccator boiled for 2 minutes and another set kept in fridge. The desiccator was then incubated at 25° C for 24 h. After 24 hrs fumigation, the both fumigated and non-fumigated sample was incubated in conical flask with vial keep 0.5 N NaOH. These allow trapping of CO₂ for 10 days. Taken 150 conical flask and transfer 0.5 N NaOH after 10 days from vial to the flask. Added 4 ml saturated BaCl₂ and 2-3 drops of phenolphthalein indicator. The excess NaOH is titrated against 0.5N HCl until the pink colour disappeared. The volume of NaOH that absorb by CO₂ is taken for calculation for amount of CO₂ evolved. (calculation 1ml of 1N HCl is equal to 0.022g CO₂). Soil Microbial Biomass Carbon was calculated in terms of mg CO₂-C kg⁻¹. (Jenkinson and Powlson, 1975) [16]. Soil Microbial Biomass Nitrogen was analysed by taken 10g incubated soil in 2 sets, one taken in a beaker and another keep as a non-fumigated. The sample was fumigated with ethanol free chloroform. After 24hrs the fumigated soil samples was extracted with 40ml of 0.5M K₂SO₄ and shaking was done for 30 mint. Filtration was done by whatman-42 paper. Unfumigated control soils were also extracted with K₂SO₄ at the time fumigation commenced. Taken 30 ml aliquot and added 0.6ml 0.19M CuSO₄, 10 ml of conc. H₂SO₄ and anti-bumping granules in a 250 ml digestion tube and refluxed for 3 hrs. After cooling, 20ml water was cautiously added the digestion tube, then cooled again. 25ml of 10 M NaOH was slowly added to the tube. The digestion tube then was attached to a steam-digestion unit, a further aliquot of 25ml NaOH was added to render the solution alkaline and the mixture steam-distilled into a titration vessel containing 5ml of 2% boric acid. The distillation was continued until 40ml of distillate had been collected, then titrated to 50 mM H₂SO₄ (Brookes *et al.*, 1985) [6].

Water soluble organic carbon was extracted by using the method of McGill *et al.*, (1986) [22], from incubated soil sample by shaking 10g soil with 20 ml of deionized water for 60 min. Followed by centrifugation at 10000×g at 4°C for 30 min. The supernatant was filtered with suction through a 47mm diameter, 0.2µm metricel membrane filter previously washed with 150 ml deionized water. Filtrates were stored at -10 °C until analyzed. Duplicate analyses were within 5% of each other. The concentrations of water-soluble carbon were estimated by the wet oxidation method using potassium dichromate (Walkley and Black, 1934) [33]. Particulate organic carbon (POC) was examined by using the method of Cambardella and Elliott, 1992) [7]. A portion of the dried soil sample (10 g) was dispersed in 30 ml of sodium hexameta phosphate (5 gl⁻¹) and placed on a reciprocating shaker (90 r min⁻¹) for 18 h. The soil suspension was decanted over a 53 µm sieve under a flow of distilled water to ensure separation. The remaining soil on the sieve was transferred to a glass dish, dried at 55–60 °C for 48 h and ground to powder by a ball mill. The concentrations of organic carbon in POC were estimated by the wet oxidation method using potassium dichromate (Walkley and Black, 1934) [33].

Results and Discussion

The total nitrogen of soil increased significantly with the use of soil and straw along with *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.* consortia. The content of total nitrogen (Table 2). Showed a significant increase in different treatments as compared to all over treatments except soil + paddy straw + *Aspergillus spp.* + *Streptomyces spp.* It was found in the range of 0.035 – 0.048% in pot soils after 60 days of decomposition and minimum in control i.e. 0.035%. It is apparent from result that decomposition of straw with consortia resulted significantly higher total nitrogen percent in soil as compared to control. This finding is corroborated with (Abdulla *et al.*, (2007) [1]. total nitrogen content has significantly increased in straw + microbial consortia inoculated treatments and improved decomposition process and also increases total nitrogen percentage. Thus, actinomycetes of the current study may represent important agents for enhancing decomposition of rice straw and humification of its residue in soil. With the progression of paddy straw decomposition, nitrogen content increased due to concentration effect which resulted in decreased C: N ratio (Lee *et al.*, 2002) [18]. The maximum P increase was observed in paddy straw inoculated with the consortia of lignocellulolytic fungal, bacterial and actinomycetes species. The highest total phosphorus content was recorded in paddy straw + *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.* i.e. 0.029% and lowest was found in control i.e. 0.021%. Viji and Neelanarayanan (2015) [32]. reported that application of paddy straw with inoculation of three lignocellulolytic fungal strains i.e. *Rhizopus oryzae* + *Aspergillus oryzae* + *Aspergillus fumigatus* increases significantly the total phosphorus content. A significant increase in total K content was registered in the treatment soil + paddy straw + *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.* treated plot. It was found in the range of 0.660 – 0.750% after 60 days of composting. Maximum, however was in soil + paddy straw + *Aspergillus sp.* + *Bacillus sp.* + *Streptomyces sp.*) and minimum in control respectively. Total potassium plays an important role where its function is to increase the elongation of the root, control ion balance, improve protein synthesis, encourage enzyme reaction and improve the photosynthesis process and food development in plant growth (Md Sabiani *et al.*, (2004). Soil Microbial Biomass Carbon (SMBC) is the most active fraction in soil organic matter. In pot culture the treatment paddy straw + *Aspergillus sp.* + *Bacillus spp.* + *Streptomyces spp.* showed highest SMBC value and this was statistically different from the other i.e. 242.91 mg CO₂-C kg⁻¹ which was at par with treatments of paddy straw + *Bacillus spp.* + *Streptomyces spp.* (239.48 mg CO₂-C kg⁻¹) (Table 3). The change of SMBC reflects the process of microorganism propagation and degradation, utilizing soil carbon. In general, residue incorporation produced more microbial activity than the residue removal or burning in-situ into the soil.

Application of only chemical fertilizer resulted in little decrease in SMBC concentration in surface 0-15 cm soil layer whereas, the combined application of chemical fertilizer and straw significantly increased soil MBC concentration over the control (Nie *et al.*, 2007) [25]. According to Esther *et al.*, (2013) [9]. In-situ incorporation of wheat straw was showed maximum amount SMBC in soil and increased their content on soil over the control treatment.

There was a significant difference found in pot experiment in Soil Microbial Biomass Nitrogen (SMBN) among various treatments (Table 3). The increase in SMBN ranged from 22.25 to 32.42 mg kg⁻¹ soil after 30 days and 23.28 to 33.82 mg kg⁻¹ soil after 60 days of decomposition. After 60 days, the maximum content of SMBN was recorded in soil + paddy straw + *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.* treatment i.e. 33.82 mg kg⁻¹ soil and lowest content i.e. 23.28 mg kg⁻¹ soil of SMBN was associated with control treatment. The application of inorganic fertilizer and increased carbon inputs into the soil result in increased SMBN. (Adeboye *et al.*, 2011 [3]. Ocio *et al.*, 1991; Singh and Singh, 1992) [27].

There was greater response of crop residues on particulate organic carbon (POC) content in after 60 days of decomposition (Table 3). The maximum content of POC was recorded in soil + paddy straw + *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.* treatment i.e. 1.34 g kg⁻¹ soil overall of the treatments except soil + paddy straw + *Aspergillus spp.* + *Bacillus spp.* and soil + paddy straw + *Aspergillus spp.* + *Streptomyces spp.* and lowest content i.e. 1.18 g kg⁻¹ soil of POC was associated with control treatment. POM responded rapidly and selectively to management practices (Franzluebbers & Stuedemann, 2002) [10]. and has been suggested as a sensitive indicator of SOM turnover changes (Cambardella & Elliott, 1994) [8]. This is a readily decomposable substrate for soil microorganisms and a short-term reservoir for plant nutrients (Mrabet *et al.*, 2001) [23].

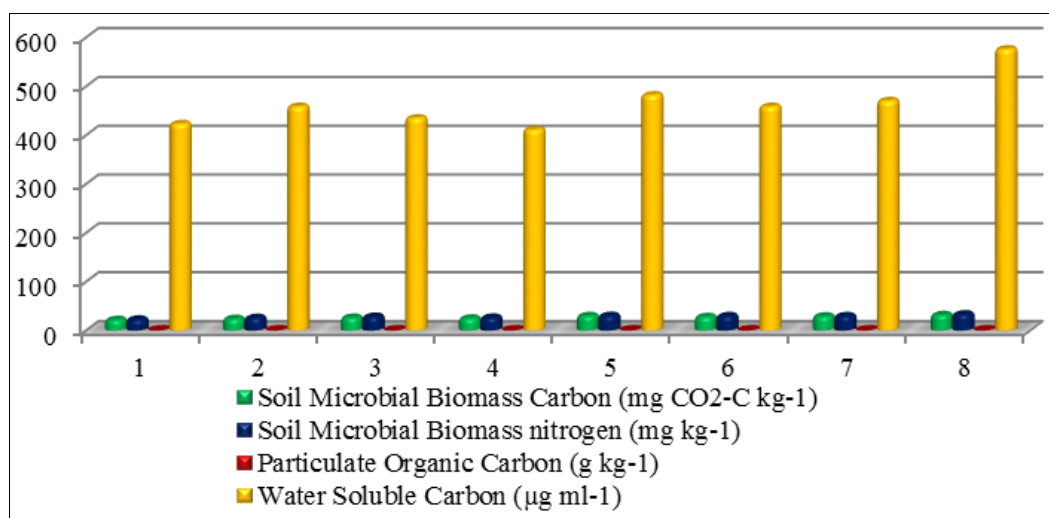
Water soluble carbon (WSC) is the most mobile and reactive soil carbon source available. It plays an important role in many biogeochemical processes. Lu *et al.*, (2011) [20]. The WSC was significantly higher in paddy straw + *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.* than the other treatments. The maximum WSC value was i.e. 576.46 µg ml⁻¹ and lowest was i.e. 411.77 µg ml⁻¹ in T₄. The above findings showed that the decomposition of paddy straw with microbial consortia capable of activating microbial population might have resulted in increased WSC. In most soils, the majority of organic carbon is in insoluble form except for a small fraction that is water soluble and not yet leached out so, this fraction of organic carbon is known as the water soluble organic carbon (WSOC). It is the most important carbon source for soil microorganisms (Schnabel *et al.*, 2002 [29]. Marschner and Kalbitz, 2003 [24]. Sparling *et al.*, 1998) [28].

Table 2: Effect of decomposition of paddy residue with ligno-cellulolytic microbes on total N, P and K in pot culture experiment.

Treatments	Total Nitrogen (%)		Total Phosphorus (%)		Total Potassium (%)	
	Days		Days		Days	
	30	60	30	60	30	60
Control	0.035	0.035	0.021	0.021	0.677	0.660
Soil + paddy straw + <i>Aspergillus spp.</i>	0.036	0.038	0.022	0.024	0.693	0.703
Soil + paddy straw + <i>Bacillus spp.</i>	0.037	0.040	0.022	0.025	0.687	0.693
Soil + paddy straw + <i>Streptomyces spp.</i>	0.036	0.036	0.022	0.024	0.680	0.693
Soil + paddy straw + <i>Aspergillus spp.</i> + <i>Bacillus spp.</i>	0.038	0.041	0.024	0.025	0.713	0.720
Soil + paddy straw + <i>Bacillus spp.</i> + <i>Streptomyces spp.</i>	0.037	0.040	0.024	0.024	0.687	0.700
Soil + paddy straw + <i>Aspergillus spp.</i> + <i>Streptomyces spp.</i>	0.041	0.043	0.025	0.026	0.707	0.723
Soil + paddy straw + <i>Aspergillus spp.</i> + <i>Bacillus spp.</i> + <i>Streptomyces spp.</i>	0.044	0.048	0.026	0.029	0.717	0.750
SE(m)±	0.001	0.002	0.001	0.001	0.005	0.006
CD(P=0.05)	0.003	0.006	0.003	0.003	0.016	0.017

Table 3: Effect of decomposition of paddy residue with ligno-cellulolytic microbes on soil microbial biomass carbon and nitrogen, particulate organic carbon and water soluble carbon in pot culture experiment.

Treatments	Soil Microbial Biomass Carbon (mg CO ₂ -C kg ⁻¹)		Soil Microbial Biomass nitrogen (mg kg ⁻¹)		Particulate Organic Carbon (g kg ⁻¹)		Water Soluble Carbon (µg ml ⁻¹)	
	Days		Days		Days		Days	
	30	60	30	60	30	60	30	60
Control	201.33	22.25	22.25	23.28	1.17	1.18	355.24	423.56
Soil + paddy straw + <i>Aspergillus spp.</i>	205.96	24.39	24.39	26.51	1.20	1.21	356.36	458.82
Soil + paddy straw + <i>Bacillus spp.</i>	210.33	26.83	26.83	28.83	1.20	1.28	362.80	435.29
Soil + paddy straw + <i>Streptomyces spp.</i>	218.22	25.23	25.23	27.09	1.18	1.2	361.99	411.76
Soil + paddy straw + <i>Aspergillus spp.</i> + <i>Bacillus spp.</i>	222.11	30.37	30.37	30.68	1.29	1.32	364.20	482.35
Soil + paddy straw + <i>Bacillus spp.</i> + <i>Streptomyces spp.</i>	224.58	28.21	28.21	30.18	1.28	1.3	362.09	458.82
Soil + paddy straw + <i>Aspergillus spp.</i> + <i>Streptomyces spp.</i>	232.47	29.65	29.65	30.19	1.27	1.31	366.70	470.58
Soil + paddy straw + <i>Aspergillus spp.</i> + <i>Bacillus spp.</i> + <i>Streptomyces spp.</i>	237.51	32.42	32.42	33.82	1.30	1.34	370.34	576.46
SE(m)±	0.849	0.423	0.423	0.521	0.009	0.009	2.130	19.948
CD(P=0.05)	2.568	0.128	0.128	1.576	0.028	0.026	6.442	60.318

**Fig 1:** Soil microbial properties after 60 days decomposition with effective microbial consortia.

Conclusion

It is concluded that Incorporation of paddy straw with effective microbial consortia (*Bacillus spp.* + *Aspergillus spp.* + *Streptomyces spp.*) was found best among all the treatments. Availability of soil nutrients also increased in treatment where straw was applied with microbial consortia compared to control. Among the biological properties, microbial biomass carbon and nitrogen increased significantly in microbial consortia treated plots. Application of paddy straw in soil in conjunction with ligno-cellulolytic microbes *Aspergillus spp.*, *Bacillus spp.* and *Streptomyces spp.* may be used as an effective measure for improving soil health and managing nutrient in the soil. Pot culture composting study showed that it could play important role in the large-scale composting of paddy straw.

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