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Evaluation of Lignocellulosic Wastes for Production of Edible Mushrooms

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Abstract The degradation of lignocellulosic wastes such as paddy straw, sorghum stalk, and banana pseudostem was investigated during solid-state fermentation by edible mushrooms *Pleurotus eous* and *Lentinus connotus*. Biological efficiency of 55–65% was observed in paddy straw followed by sorghum stalk (45%) and banana pseudostem (33%) for both fungal species. The activity of extracellular enzymes, namely cellulase, polyphenol oxidase, and laccase, together with the content of cellulose, lignin, and phenols, was studied in spent substrates on seventh, 17th, and 27th days of spawning, and these values were used as indicators of the extent of lignocellulosic degradation by mushroom. Both the mushroom species proved to be efficient degraders of lignocellulosic biomass of paddy straw and sorghum stalk, and the extent of cellulose degradation was 63–72% of dry weight (d.w.), and lignin degradation was 23–30% of the d.w. In banana pseudostem, the extent of the degradation was observed to be only 15–22% of the d.w. for both lignin and cellulose. Preferential removal of cellulose during initial growth period and delayed degradation of lignin were observed in all three substrates. This is associated with decrease in activity of cellulase and polyphenol oxidase and increase in laccase activity with spawn aging in spent substrates. Thus, bioconversion of lignocellulosic biomass by *P. eous* and *L. connotus* offers a promising way to convert low-quality biomass into an improved human food.

Keywords Agricultural waste recycling · Edible mushrooms · *Lentinus connotus* · *Pleurotus eous* · Biological efficiency · Lignocellulolytic activity

Introduction

Cultivation of edible mushroom with agricultural residues is a value addition process to convert these materials, which are otherwise considered to be wastes into human food. It represents one of the most efficient biological ways by which these residues can be recycled

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[1]. Mushroom can flourish successfully on a wide variety of inexpensive substrates such as cereal straws, banana leaves, sawdust, tea leaves, peanut hulls, coffee pulp, soybean stalk, cotton stalk, and almost any agricultural waste that has a substantial cellulose component [2, 3]. Paddy straw, sorghum stalk, and banana pseudostem are the populous and readily available lignocellulosic residues for mushroom cultivation in many parts of South India. More than 70 million tons of paddy straw are produced every year, and its disposal pose many problems. Even if one fourth of the paddy straw burnt is used to grow edible mushrooms, approximately 319 million metric tons of it can be produced annually [4]. At present, paddy straw is mainly used for commercial mushroom cultivation [5]. Often, sorghum stalks are thrown away or burnt in the fields after the harvest, leading to pollution and respiratory problems. If the sorghum stalks are allowed to remain in the field, they might harbor several insects and pests, which may damage the next crop [6]. Similarly, banana is produced in large quantities in tropical and subtropical areas, and the pseudostem is traditionally wasted after harvest and is usually left in the soil to be used as an organic material. Alternatively, these agricultural residues can be used as substrate for mushroom cultivation.

Lentinus species, known as Shiitake in Japan, is the second most important edible mushroom in the world from the standpoint of total population. *Pleurotus* species also known as Dhingri or oyster mushroom is the fourth important mushroom in the world. These wood-degrading saprophytic fungi occur widely and are differently colored. Cultivation of *Pleurotus* species and, presently, *Lentinus* species is becoming important throughout the world because of their ability to grow on a wide range of temperature and on different lignocellulosic residues in a short cultivation time [7].

The efficiency of mushroom species in producing food protein from agro-wastes lies in their extensive ability to secrete a variety of hydrolyzing and oxidizing enzymes that aid in the degradation of lignocellulosic wastes. The enzyme systems of *Pleurotus* and *Lentinus* species that are involved in the degradation of lignocellulose substrates are endoglucanase, laccase, and polyphenol oxidase [8, 9].

In the present study, an attempt has been made to use edible mushrooms—*P. eous* (APK-I) and *L. connotus* (VM-I)—as potent biological agents for organic recycling of agricultural wastes like paddy straw, sorghum stalk, and banana pseudostem.

Materials and Methods

Sources of Spawn and Substrates

The spawn of *L. connotus* (VM-I) and *P. eous* (APK-I) were obtained from the Regional Research Station, Tamilnadu Agricultural University, Viruthachalam, Tamilnadu, India. Paddy straw, sorghum stalks, and banana pseudostem were collected from agricultural fields in Coimbatore, Tamilnadu.

Mushroom Cultivation

The mushrooms were cultivated by the perforated polythene bag method given by Bano and Srivastava [10] with minor modifications. Dried paddy straw and sorghum stalk were chopped into 5- to 7-cm length and soaked in water for 12 h, and excess water was drained off. Fresh banana pseudostem was also chopped into 5-cm length. All these substrates were sterilized in hot water at 80 °C for 60 min. One kilogram of each substrate was placed in

25- to 35-cm-sized polyethylene bag and spawned with 8% mushroom mycelia grown on sorghum grains. Spawning was done in five layers at the rate of 2% of net substrate. The bags were subsequently placed into spawn running room at 25 °C±2 °C under dark conditions. After the completion of spawn running, polythene bags were placed into a room at 15 °C and 80–90% relative humidity. The bags were cut open on the sides without disturbing the beds, and water was sprayed twice a day for maintaining moisture.

Three replicates were used for each growing trial for each substrate. The data concerning spawn running and fruiting body formations were observed.

Mushroom Harvesting

When the fruiting body begins to flatten, they were harvested, and the fresh weight of the mushrooms were determined. At the end of the harvesting period, the biological efficiency (BE) was calculated. BE is the ratio of kilogram of fresh mushroom weight per kilogram dry substrate and counted as percentage [11].

Measurements of Substrate Utilization

Substrate utilization was evaluated by measuring the cellulose, lignin, and total phenol contents of substrates before spawning and at different days of spawning. Three replicates were used for each growing trial for each substrate. At the end of the seventh, 17th, and 27th days of spawning, spent substrates were totally removed from all the three replicates for each substrate and analyzed for cellulose, lignin, and phenols.

Substrate samples were dried for 24 h at 105 °C, powdered, and stored in airtight bottles in a refrigerator until analysis. The lignin and cellulose content of the substrate were determined according to the methods of Browning [12] and Updegroff [13], respectively. The total phenol and protein were determined according to Bray and Thorpe [14] and Lowry et al. [15], respectively.

Enzyme Extraction

Spent substrates taken from different days of spawning were used to extract extra cellular enzymes. The extraction was performed according to Criquet et al. [16] using 10 g of lyophilized substrate in a 200-ml of the extraction solution containing polyvinyl polypyrolidone at 5.7 g, $CaCl_2$ at 0.2 M, and Tween 80 (0.05%). Samples were subjected to shaking for 1 h at 120 rpm, and solids were eliminated by filtration through nylon screen, and filtrates were centrifuged at 10,000×*g* for 15 min. The supernatant were dialyzed against phosphate buffer (0.01 M, pH 6.0) and used for enzyme assay.

Enzyme Activity Measurements

Cellulase activity was determined by measuring the amount of reducing sugar released in the reaction mixture containing 1.0 ml of 50 mM acetate buffer (pH 5.0) with 1% carboxymethylcellulose (CMC) and 1.0 ml of appropriately diluted enzyme solution. The mixture was maintained at 40 °C for 2 h, and the reducing sugar released was determined by using a dinitrosalicylic acid reagent [17]. Laccase activity was determined by guaiacol oxidation in a reaction mixture containing 3.0 ml of 0.1 M phosphate buffer (pH 6.0) with 20 mM guaiacol and 1 ml enzyme source [18]. The activity of polyphenol oxidase activity was measured by catechol oxidation [19] in a reaction mixture containing 2.5 ml of 0.1 m phosphate buffer (pH 6.5), 0.3 ml of catechol solution (0.01 M), and 0.2 ml of enzyme.

Statistical Analysis

Data were subjected to ANOVA according to the method of Snedecor and Cochran [20].

Results

Growth of Spawn Running and Fruiting Body Formation

In the present study, both *P. eous* and *L. connotus* were cultivated on different substrates spawned with 8% mushroom mycelia. Spawning was done in five layers at the rate of 2% of net substrate. Time duration taken for the mycelia to cover the entire bed was observed for the three substrates and was found to be around 15 days for the paddy straw and sorghum stalk, and around 20 days for banana pseudostem. The first bud formation was noticed on the 15th day for paddy straw, on the 20th for sorghum stalk, and the 26th day for banana pseudostem.

Total Yield and Biological Efficiency

For both the mushroom species studied, totally five to six flushes were obtained from paddy straw, and three to four flushes were obtained from sorghum stalk and banana pseudostem. The yield of mushroom was affected by different substrates for both the species of mushrooms. Maximum biological efficiency of 68.75% for *L. connotus* and 55.49% for *P. eous* were observed with paddy straw followed by sorghum stalk (45–47%). When banana pseudostem was used as substrate, the yield was only 30–35% (Table 1).

Analysis of Compositional Changes of Substrates

Enzymatic Activities

Both the fungi exhibited significant cellulase activity in all the three substrates, the activity ranging 1.5–2.5 units (Table 2). For both the species of mushroom, maximal activity was observed on the seventh day in paddy straw, and the activity decreased by 30–45% with spawn aging. Similar trend was also observed in sorgum stalk, but the decrease was 16–30% for *P. eous* and 40–60% for *L. connotus*. Cellulase activity increased linearly with

Table 1 Biological efficiency of *L. connotus* and *P. eous* on selected substrates.

Substrate	Biological efficiency (%)	
	Lentinus connotus	*Pleurotus eous*
Paddy straw	68.75±2.024**c**	55.49±1.005**c**
Sorghum stalk	46.67±1.319**b**	45.10±1.868**b**
Banana pseudostem	30.10±0.655**a**	33.00±3.539**a**
	SED=0.75, CD (5%)=1.59, CD (1%)=2.20	SED=1.23, CD (5%)=2.62, CD (1%)=3.62

All parameters are expressed as means of three replicates of the sample. In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Table 2 Enzymatic activities of *P. eous* and *L connotus* in spent substrates on different days of spawning.

Species	Days of spawning	Cellulase activity (mg glucose released per h/g dm)			Laccase activity (0.01 OD change per min/g dm)			Polyphenol oxidase activity (0.001 OD change per min/g dm)		
		Paddy straw	Sorghum stalk	Banana pseudostem	Paddy straw	Sorghum stalk	Banana pseudostem	Paddy straw	Sorghum stalk	Banana pseudostem
Pleurotus eous	7th	1.188**a**±0.052	1.548**a**±0.004	0.886**a**±0.019	11.70**c**±0.072	14.77**c**±0.139	8.44**a**±0.330	139.3**a**±0.78	105.0**a**±0.67	129.07**c**±3.790
	17th	0.972**a**±0.067	1.296**ab**±0.081	1.506**b**±0.031	12.24**b**±0.053	15.30**b**±0.080	10.34**b**±0.122	120.0**b**±0.57	72.9**b**±0.67	106.87**b**±3.302
	27th	0.666**a**±0.073	1.080**b**±0.289	2.385**c**±0.011	12.78**a**±0.076	15.95**a**±0.127	11.93**c**±0.101	83.3**c**±0.62	58.8**c**±0.70	84.57**a**±1.792
Lentinus connotus	7th	1.278a±0.040	3.096**a**±0.175	0.721**a**±0.012	13.08**c**±0.063	13.82**c**±0.071	7.02**a**±0.023	129.8**a**±1.09	94.1**a**±0.43	126.01**c**±2.261
	17th	1.134**a**±0.029	1.890**b**±0.052	1.202**b**±0.018	13.96**b**±0.084	14.77**b**±0.071	9.52**b**±0.180	85.9**b**±1.20	79.2**b**±0.50	100.32**b**±2.190
	27th	0.774**a**±0.043	1.224**c**±0.097	2.121**c**±0.010	14.61**a**±0.095	14.47**a**±0.094	11.27**c**±0.132	75.2**c**±0.78	51.2**c**±0.43	90.51**a**±1.931
		F=<1 ns	*F*=27.09[a]	*F*=17.01[a]	*F*=7.47[a]	*F*=12.33[a]	*F*=6.54[a]	*F*=282.54[a]	*F*=248.97[a]	*F*=168.27[a]
			SED=0.008	SED=0.018	SED=0.84	SED=0.81	SED=0.17	SED=0.87	SED=0.58	SED=2.52
			LSD (5%)=0.017	LSD(5%)=0.044	LSD (5%)=1.82	LSD (5%)=1.76	LSD(5%)=0.43	LSD (5%)=1.90	LSD (5%)=1.26	LSD(5%)=6.16
			LSD (1%)=0.023	LSD(1%)=0.067	LSD (1%)=2.56	LSD (1%)=2.46	LSD(1%)=0.65	LSD (1%)=2.67	LSD (1%)=1.76	LSD(1%)=9.33

[a] Significant at 1% level. All parameters are expressed as means of three replicates of the sample. In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

ns not significant

spawn runtime in banana pseudostem, and the activity as such was more in this substrate than the other two substrates for both the fungal species.

In all the three substrates, while the laccase activity increased marginally from 5% to 15%, the activity of polyphenol oxidase was shown to decrease with spawn aging for both of the mushroom species (Table 2). The decrease in polyphenol oxidase activity was around 15–45% for both *P. eous* and *L. connotus*.

Substrate Utilization

The cellulose content decreased as the days of spawning increased, and the decrease was statistically significant ($P<0.01$) for both *P. eous* and *L. connotus* in all the three substrates. The extent of cellulose degradation being 63–72% of the d.w. in paddy straw and sorghum stalk, whereas it was only 15–25% in banana pseudostem.

The lignin content was more in sorghum stalk (135.60 mg/g d.w.) than in paddy straw (100.90 mg/g d.w.) and banana pseudostem (118.0 mg/g d.w.; Table 3). For both the mushroom species, the percentage of lignin degradation varies from 5–10% on the seventh day and around 20–30% on the 27th day when compared to the initial lignin content for all the substrates.

Irrespective of the species of mushroom, total phenols increased significantly ($P<0.01$) as the days of spawning increased in the paddy straw and in the banana pseudostem. In sorghum stalk, an increasing trend was noted in total phenol in both the species, but the increase was statistically insignificant.

Discussion

In the present study, of the three substrates used, mushroom mycelia colonized rapidly in paddy straw and were associated with early pinhead formation. These results are in accordance with Garcha et al. [21], who has also reported early spawn run and pinhead formation in paddy straw compared to other cereal straws.

For both species of the mushroom studied, the yield was affected by different substrates. Maximum yield and biological efficiency (55–65%) was observed for paddy straw followed by sorghum stalk. When banana pseudostem was used as substrate, the yield was only 30–35%. Marimuthu and Krishnamoorthy [4] have also recorded high yield and biological efficiency of mushroom from paddy straw compared to other substrates. Similar results were also reported by Singh et al. [22] and Bisaria et al. [23].

Biological efficiency of 15.21% was reported for *Volvariella volvacea* when it was grown on banana leaves [24].

The slow rate of spawn running and low mushroom yield observed in the banana pseudostem could be attributed to a high moisture-holding capacity and a high susceptibility to other fungal contaminants and improper aeration. Banana pseudostem in combination with other substrates or supplemented with nitrogenous sources viz. soybean meal, alfalfa meal, etc. could be used to increase the mushroom yields. For the mushroom species used in the present study, different spawn level has to be tested on different substrates to accelerate spawn running and to maximize the mushroom yield.

It has also been reported that the optimal spawn level for maximum mushroom yield depends on the type of substrate, mushroom species, spawn quality, cultivation conditions, etc. For the cultivation of oyster mushroom, *P. sajor-caju* on rice and wheat straw, spawn level was tested between 12–18%, and 16% was recommended as optimum to achieve

Table 3 Cellulose, lignin, and total phenol contents in spent substrates on different days of spawning.

Species	Days of spawning	Cellulose content (mg/g dm)			Lignin content (mg/g dm)			Total phenol content (mg/g dm)		
		Paddy straw	Sorghum stalk	Banana pseudostem	Paddy straw	Sorghum stalk	Banana pseudostem	Paddy straw	Sorghum stalk	Banana pseudostem
Pleurotus eous	Initial	364**a**±4.32	500**a**±15.12	328**d**±13.428	100.9**a**±1.51	135.6**a**±0.59	118**d**±3.00	1.35**c**±0.081	1.05**a**±0.053	1.29**a**±0.035
	7th	280**b**±3.74	308**b**±2.94	289**c**±4.725	90.6**a**±0.43	128.7**b**±0.57	112**c**±1.53	1.40**c**±0.036	1.14**a**±0.004	1.36**a**±0.056
	17th	240**c**±4.55	222**c**±4.32	274**b**±4.041	83.7**a**±1.00	124.0**c**±0.57	106**b**±1.00	1.52**b**±0.022	1.60**a**±0.036	1.62**b**±0.036
	27th	108**d**±2.16	140**d**±3.74	254**a**±2.516	80.4**a**±0.70	120.6**d**±0.57	100**a**±2.08	1.63**a**±0.046	1.53**a**±0.440	1.83**c**±0.100
Lentinus connotus	Initial	364**a**±4.32	500**a**±15.12	328**d**±10.810	100.9**a**±1.51	135.6**a**±0.59	118**d**±2.98	1.00**c**±0.053	1.23**a**±0.098	1.29**a**±0.042
	7th	308**b**±2.16	320**b**±4.32	296**c**±4.801	91.6**a**±1.30	129.1**b**±0.64	107**c**±1.82	1.11**c**±0.037	1.35**a**±0.051	1.42**b**±0.015
	17th	246**c**±4.32	266**c**±2.94	279**b**±5.161	85.2**a**±1.02	116.2**c**±0.51	102**b**±1.12	1.70**b**±0.029	1.41**a**±0.029	1.72**c**±0.031
	27th	134**d**±4.97	156**d**±3.56	265 a±4.950	79.1**a**±0.57	94.4**d**±0.59	98**a**±1.96	1.92**a**±0.036	1.76**a** ±0.036	1.90**d**±0.042
		F=12.75[a]	F=5.15[a]	F=4.87[a]		F=461.74[a]	F=91.87[a]	F=81.87[a]		F=61.42[a]
		SED=3.95	SED=8.21	SED=6	F<1, ns	SED=0.58	SED=2	SED=0.035	F<1, ns	SED=0.05
		LSD(5%)=8.37	LSD(5%)=17.40	LSD(5%)=14		LSD (5%)=1.23	LSD(5%)=4	LSD(5%)=0.077		LSD(5%)=0.12
		LSD(1%)= 11.53	LSD(1%)=23.97	LSD(1%)=21		LSD (1%)=1.69	LSD(1%)=6	LSD(1%)=0.107		LSD(1%)=0.17

[a] Significant at 1% level. All parameters are expressed as means of three replicates of the sample. In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

ns not significant

maximum mushroom yield [1]. Supplementation of main substrates with nutrient or the combination of two or more substrates were reported to accelerate spawn running, pin-head formation, fruit-body formation, and mushroom yield [3, 25]. Various legume crop wastes including groundnut straw and seed husk [26], cowpea and guar straw [27], and green gram and gram straw [3] were found to be effective in cultivating *Pleurotus* spp.

For both *P. eou* and *L. connotus*, maximal cellulase activity was observed on the seventh day in paddy straw and sorgum stalk, and the activity decreased with spawn aging. Whereas in banana pseudostem, the activity increased linearly with spawn runtime, and the activity as such was more in this substrate than in the other two substrates on the 27th day of spawning. The delay in activity peaking in banana pseudostem is also associated with delay in fruiting body formation.

The highest activity of cellulase observed during the initial period may be attributed to the need for carbohydrates by the fungal mycelia for sporophore and fruiting body formation. When the flush is harvested, there may be less demand for the supply of carbohydrates, hence the decrease in activity on the 17th and 27th days of spawning. The increase in the cellulase activity during fruiting was thus in accordance with other reports [28, 29].

The increase in cellulase activity was associated with decreased cellulose content in spent substrates. The extent of cellulose degradation was threefold higher in paddy straw and sorghum stalk than in banana pseudostem. This indicates that both species of mushroom were more effective in degrading cellulosic content of paddy straw and sorghum stalk rather than banana pseudostem. The degradation of lignocellulosic biomass of banana pseudostem by *P.ostrreatus* and *P. sajor-caju* [30] indicated that both organisms degraded hemicellulose better than cellulose.

In all the three spent substrates, laccase activity increased, whereas the activity of polyphenol oxidase decreased with spawn aging for both the mushroom species. This was correlated with decrease in lignin and increase in total phenols observed in spent substrates.

Maximum activity of laccase after the 24th day of incubation is also reported for two *Pleurotus* species when banana pseudostem is used as biomass [30]. Whereas during the mycelial growth of *P. sajor-caju* on paddy straw, laccase and polyphenol oxidase activity appeared and peaked earlier than endoglucanase [9]. A decline in laccase activity with an increase in the number of days of spawning was reported by Platt et al. [31] during the degradation of cotton straw by *P. florida*.

Production of extracellular laccase and polyphenol oxidase by *Pleurotus* species in paddy straw and their relationship with lignin degradation has been extensively studied. Laccase activity in different *Pleurotus* species and the rate of lignin degradation for decomposing coconut coir pith were reported by Theradi Mani and Marimuthu [32]. Kirk et al. [33] also reported a correlation between laccase production by fungi and lignin decomposition. However, there are conflicting opinions as to the direct relationship between laccase and lignin degradation. Increase in total phenol content during active mycelia growth was also reported by Natarajan et al. [34]. Active mycelia growth might increase the carbon dioxide concentration in the substrate, which would have suppressed the other organism that is involved in the further degradation of phenolic derivatives, so the degraded total soluble phenol might have accumulated in substrate without undergoing any further degradation.

The present study points out a decline in cellulose and lignin content of the spent substrates with increase in cellulase and laccase activity, indicating the lignocellulolytic nature of *P. eous* and *L. connotus*. The efficient degrading capacity of the fungi demonstrated their potential use in the conversion of agricultural wastes into commercially more valuable biomass like mushrooms.

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