

Biodegradation of wheat straw by *Pleurotus ostreatus*

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Abstract

Wheat straw pretreated with chemicals as well as hot water was subjected to degradation by edible mushroom *Pleurotus ostreatus*. Lignin, cellulose and hemicelluloses component of both chemically as well as hot water treated wheat straw was degraded by the fungus and in turn the edible and nutritious fruiting body of the mushroom was produced. Biodegradation of wheat straw in terms of loss of lignin, cellulose and hemicellulose showed positive correlation with cellulases, xylanase, laccase and polyphenol oxidase (PPO) activity of the fungus. During vegetative growth of the fungus, lignin degradation was faster and during fructification, lignin degradation was slower than cellulose and hemicellulose. The carbon content of the wheat straw decreased while, nitrogen content increased during degradation of the waste. Hot water treated wheat straw supported better production of enzymatic activity and degraded more efficiently than chemically sterilized substrate. The cumulative yield and biological efficiency (BE) of the mushroom was maximum on the hot water treated substrate. Degradation of the hot water treated wheat straw was better and faster than chemically treated substrate.

Key words: *Pleurotus ostreatus*, wheat straw, Biodegradation.

Introduction

Wheat straw is an agricultural byproduct generated in large quantity in India. It is lignocellulosic in nature and is formed by three main polymeric constituents - cellulose, hemicellulose and lignin. Major portion of this wheat straw is used as fodder and in Punjab, Haryana, U.P and other states of India after harvesting some portions are burnt in the field leading to severe environmental aggression and wastage of resource. Biodegradation of wheat straw by *Pleurotus ostreatus* is significant as it not only leads to formation of simpler compounds but also results in protein rich food.

Pleurotus ostreatus is an edible mushroom, which confers advantages over other mushrooms for its capability to grow on non-fermented lignocellulosic wastes and produce in turn fruit bodies with higher nutritious content. The aim of present investigation was to study the production of nutritional food through the biodegradation of wheat straw by *Pleurotus ostreatus*.

Materials and methods

The culture and its maintenance

The pure culture of *Pleurotus ostreatus* used in the present work was procured from Indian Agricultural Research Institute (IARI), New Delhi. Throughout the study culture was maintained on both potato dextrose agar and malt extract agar (PDA & MEA) medium at 23-25°C and was sub-cultured at the regular interval of three weeks.

Cultivation

Spawn preparation

Spawn is referred to as the vegetative mycelium of the fungus, which is grown on cereal grains i.e. grains of

wheat. Wheat grain spawn was prepared following the method of Singh *et al* (27). The preparation of spawn involved soaking of wheat grain in water followed by mixing of buffers, sterilization and inoculation with pure culture of *Pleurotus ostreatus* under aseptic conditions. The spawn was prepared in polypropylene bags. After 3-4 days of inoculation fungal mycelium started spreading on the grains. The mycelium was white net web like in appearance. The bags were nearly half filled in 10-12 days and in 18-21 days these were completely filled with white mycelium growth.

Preparation of Substrate

Wheat straw was used for the cultivation of *Pleurotus ostreatus*. This substrate was pretreated and sterilized by (i) hot water treatment and (ii) chemical treatment method.

Substrate Pretreatments

Hot water sterilization

Hot water (Temperature 70-80°C) was used for the sterilization of substrate. The substrate was completely dipped in normal room temperature water (50 liters for every 10 kg dry chopped substrate) in a drum. The substrate was allowed to stay in water for 20 hours. After that the substrate (wheat straw) was taken out of water and was again completely dipped in hot water (temperature 70-80°C) for one hour. Then excess water was drained out and substrate was evenly spread on platform till the cooling of substrate. This hot water treated substrate was ready for spawning.

Chemical sterilization

In the chemical treatment, the substrate was soaked in water (50 liter for every 10 kg dry substrates) containing 200 ppm each of nuvan and bavistin in a drum. The

substrate was allowed to stay in water for 20 hr. After that water was drained out and the substrate was evenly spread on slanted clean platform for about one hour to further remove free water. This chemically sterilized substrate was ready for spawning.

Spawning

Spawning is the process of mixing spawn in the sterilized substrate. 3% wet weight basis spawn grain was mixed with the substrate and filled into polythene bags. The mouth of the bag was tied with rubber band and 12 holes of about 1cm diameter was made two at each corner at the base, four each on the broader area and one each on the narrow, rectangular side to drain out extra water and for proper aeration. 60 bags of each, treatments was filled and kept in mushroom house on the iron racks on the bricks.

Biological efficiency

At the stage of pinhead (primordia) appearance, perforation was made to facilitate the formation of full-fledged fruit body. The pinheads were allowed to grow their full size and the mature fruit bodies were picked up before the edge of the cap started curling. The fruit bodies were harvested by twisting them so that broken pieces of mushroom did not remain in the substrates and adjacent smaller fruit bodies was not disturbed. After first harvest the polythene was cut open and the substrates were sprayed with water according to the atmospheric conditions. The yield was expressed as of fresh fruit bodies produced per bag. Biological efficiency was calculated as the percentage conversion of dry substrates to fresh fruit bodies following Chang *et al.* (2) i.e.

$$\text{Biological efficiency} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

Sample collection

After every 5 days interval three bags for each treatment were removed for enzyme assay. The content of a set of three bags were mixed uniformly. 10 gram sample was homogenized in 100 ml of 50mM sodium acetate buffer (pH 5.0) for cellulose and hemicellulose assay, while 10 gram sample was homogenized in 50mM phosphate buffer (pH 6.0) for laccase and PPO assay. Homogenized samples were filtered through whatman No 1 filter paper and filtrate was used for enzymatic study.

Cellulose, Hemicellulose and Lignin Estimation

The method of Jayme and Lang (10) was followed for cellulose, hemicellulose and lignin estimation. It included two major steps: (a) Digestion of sample and (b) estimation of protein by Bradford method (1). For digestion, acid detergent solution (0.5M H₂SO₄ + 2% cetyl trimethyl ammonium bromide (CTAB) and 72% H₂SO₄) was used.

Hemicellulose

Dried sample (0.5 gm of 20 mesh powder of the substrate under estimation) was digested with acid detergent solution. The digested sample was filtered with Glass micro fiber filter (GF/C). Filtrate was analyzed by

Bradford method (1) to calculate protein. Then a residue was dried at 105°C and its weight was deducted from 0.5 gm (Initial weight of lignocellulose).

Lignin and cellulose determination

Two hundred mg of sample (left after filtration) was mixed with 2 ml of 72% H₂SO₄ and the mixture was placed in water bath at 30°C for 1 h, and made up 30ml with distilled water and then hydrolyzed in autoclave for 1hour. The hot solution was filtered through GF/C and lignin residues were washed with hot water. The GF/C was then dried at 105°C and finally lignin was deducted from 200 mg. The remaining was cellulose.

Extraction of Extracellular enzyme

Samples of substrate were collected at regular interval of 5 days and extracted in acetate buffer (pH=5.0) for cellulolytic and hemicellulolytic enzymes and in phosphate buffer (pH=6.0) for lignolytic enzymes. Filtrate of extraction was used for enzyme assays.

Enzyme assays

Cellulases and xylanase were assayed by the method of Sandhu and Kalra (17). 0.5 ml enzyme extract was mixed in 0.5 ml of substrate. Substrates used for exoglucanase (FPase, EC 3.2.1.91), endoglucanase (CMCase, EC 3.2.1.4) and xylanase (EC 3.2.1.8) were Whatman filter paper no.1, carboxymethyl cellulose (CMC) and xylan respectively, prepared in 0.1 M acetate buffer separately. For exo-1,4 β-glucanase, 8 disc of 0.6 cm diameter Whatman filter paper No.1 was used as substrate and 0.5 ml of 0.1 M acetate buffer was mixed. All solutions were taken in triplicate. Solutions were kept in the water-bath at 45°C for 6 hrs and then 1 ml of alkaline CuSO₄ was added in each test tube and again kept in boiling water-bath at 100°C for 20 minutes. Then solutions were taken out of water-bath and 1ml of arsenomolybdate solution was mixed in each test tube. Final volume was made up 10 ml of each test tube with the help of distilled water. After cooling for 30 minutes at room temperature, absorbance was read at 540 nm using digital spectrophotometer. For β -glucosidase (E.C. 3.2.1.21) 0.5 ml of appropriate dilution of culture filtrate and 0.5ml of p-nitro phenyl-β-d- glucopyranoside (PNPG) in 0.1 M acetate buffer pH 5.0 was added. The reaction mixture was incubated at 45°C for 1 hour. After incubation period 1.5ml of 10 % sodium carbonate solution was added to each test tube, and absorbance was read at 425 nm. The amount of reducing sugars released was estimated using glucose standard. Laccase (EC 1.10.3.2) was assayed following by Dhaliwal *et al.* (5) using a reaction mixture consisting of 1ml of enzyme filtrate and 3ml of guaicol substrate prepared in 0.1M sodium phosphate buffer (pH 6.0), while PPO (EC 1.10.3.1) was assayed using the methodology of Rai and Saxena (13) consisting of 1ml of enzyme extract and 3 ml of catechol prepared in 0.1 M sodium phosphate buffer (pH 6.0). Change in absorbance was observed at 495 nm. The units used for cellulases and xylanase is μ mole glucose release h⁻¹ml⁻¹ and for laccase and PPO change in absorbance by 0.001 min⁻¹ml⁻¹.

Carbon and Nitrogen estimation

Carbon was determined by Walkley and Black (26)

Table 1. Biodegradation of cellulose, hemicellulose and lignin of wheat straw by *Pleurotus ostreatus*.

Treatment	CELLULOSE				HEMICELLULOSE				LIGNIN			
	A	B	C	D	A	B	C	D	A	B	C	D
Hot water	36.8	33.12	31.2	21.64	30.73	29.83	24.33	22.21	24.3	15.14	12.12	12.10
Chemicals		(10.0)	(15.21)	(41.20)		(2.93)	(20.82)	(27.65)		(37.69)	(50.13)	(50.20)
		33.44	31.72	24.5		29.82	25.32	22.76		16.32	13.23	12.87
		(9.23)	(13.80)	(33.42)		(2.93)	(17.60)	(25.93)		(36.91)	(45.55)	(47.03)

A=Untreated substrate, B= At the completion of spawn run, C= After fructification, D= Spent compost figure in bracket shows Percent loss

Table 2. Percent carbon, nitrogen and their ratio in wheat straw during growth of *Pleurotus ostreatus*.

Treatment	At Completion											
	First Day			of spawn run			After First flush			Spent compost		
	C	N	C: N	C	N	C: N	C	N	C: N	C	N	C: N
Hot water	36.26	0.54	67.14	30.16	0.63	47.87	26.32	1.16	22.68	18.13	1.93	9.39
Chemicals	36.31	0.58	62.60	28.76	0.69	32.31	22.13	0.98	22.58	18.24	1.36	13.41

C = Carbon percent, N = Nitrogen percent, C/N = Carbon and nitrogen ratio

and nitrogen of lignocellulosic wastes was determined by Microjeldal method from oven dried powdered samples of zero days, at completion of spawn run, after first flush and after cropping (spent compost).

Carbon

0.5 gm crushed and dried sample was taken in 500 ml flask. two blanks were included to standardized FeSO_4 solution. 15 ml $\text{K}_2\text{Cr}_2\text{O}_7$ solution was added. This was followed by rapid addition of 20 ml of concentrated H_2SO_4 , swirling of flask 2 to 3 times and allowed to stand for 30 minutes. 200 ml distilled water was added then 10 ml conc. Phosphoric acid and 1ml indicator was added and titrated against FeSO_4 .

Nitrogen

Digestion of sample

2.0 gm dried and crushed sample was taken in 500 ml Kjeldahl flask. 10 ml of digestion mixture and 20 ml of concentrated sulphuric acid was added. The flask was heated for 4 to 6 hour in a digestion fume hood until clean solution is obtained. The solution was made up to 100 ml with distilled water.

Distillation

Dry distillation assembly was used. 10 ml of digested aliquots was taken in a modified Markman apparatus along with an equal volume of 45% NaOH. Hot steam was allowed to pass through mixture for 5-10 minute and distillate was collected in 150 ml conical flask containing 20 ml of 4% Boric acid with 1 drop of mixed indicator (0.5% Bromocresol green and 0.1% methyl red in 95% ethyl alcohol). The colour of solution was changed greenish blue to green.

Titration

The distillate was titrated against 0.1 N HCl. One blank was run without sample. One standard solution of ammonium chloride was also titrated against 0.1 N HCl. Throughout the experiments three replicates of each analysis was used and their average was taken as quantitative measures for determining percentages of cellulose, hemicellulose, carbon, nitrogen and biological efficiency as well as activities of extracellular enzymes.

Results

The biodegradation of cellulose, hemicellulose and lignin content of wheat straw at mycelial growth, during fructification and after harvesting (spent compost) is given in table 1. Cellulose, hemicellulose and lignin content of untreated wheat straw were estimated at 36.8, 30.73 and 24.3 percent, respectively. The rate of degradation of cellulose and hemicellulose of hot water as well as chemically treated wheat straw during vegetative growth of *P. ostreatus* was slower than lignin. The degradation of cellulose, hemicellulose and lignin during vegetative phase was observed as 10.0, 2.93, 37.69 percent in hot water treated substrate and 9.23, 2.93, 36.91 percent in chemically treated substrates in the given order. The rate of degradation of cellulose and hemicellulose increased sharply during fruit body development. Contrary to this, lignin degradation was faster during vegetative phase and slower during fructification. Cellulose and hemicellulose content of hot water treated substrate degraded more efficiently and effectively by the *P. ostreatus* in comparison to chemically treated substrate. However there was no appreciable difference in the rate of degradation of lignin in the differently treated substrate.

Table 3 illustrates the activities of cellulolytic, hemicellulolytic and lignolytic enzymes produced by *P. ostreatus* on wheat straw during cultivation. The result showed that the activity of cellulase and xylanase was lower during vegetative phase and higher during fruit body formation. CMCase (EC 3.2.1.4) activity was more than FPase (EC 3.2.1.91) at all the stages of growth of *P. ostreatus*. β -glucosidase (E.C. 3.2.1.21) acmeyed later than CMCase and FPase. However laccase (EC 1.10.3.2) and PPO (EC 1.10.3.1) appeared and peaked earlier than the cellulases and xylanase. Their activities were higher during vegetative phase and lower during fructification stage. The activity of laccase was more than the PPO at all the stages of growth of the fungus. Hot water treated substrate supported production of more enzymes than chemically sterilized substrate.

The percentage of carbon, nitrogen and their ratio in the substrate at different stage of growth of the fungus is presented in table 2. Carbon content and C/N ratio

Table 4. Yield performance and biological efficiency (B.E) of *Pleurotus ostreatus* on wheat straw.

Substrate and treatment		Dry weight	Wet Weight	Iflush	IIflush	IIIflush	Total weight	B.E %
W	H	112	500	50	40	20	110	98.21
	Chemical	112	500	25	23	17	65	58.03

Table 3. Activity of cellulases (U h⁻¹ml⁻¹), xylanase (U h⁻¹ml⁻¹), laccase (U min⁻¹ml⁻¹) and PPO (U min⁻¹ml⁻¹) by *Pleurotus ostreatus* on wheat straw.

Enzyme	Treatment	D				A			Y		S		
		5	10	15	20	25	30	35	40	45	50	55	60
FPase	H	1.91	3.92	4.51	8.25	11.35	8.75	7.47	6.55	4.61	2.69	2.51	1.39
	C	1.36	7.36	6.20	8.23	10.63	9.12	8.75	6.07	4.67	2.67	2.55	1.92
CMCase	H	2.27	4.59	9.47	15.53	24.73	21.43	12.37	10.03	6.32	3.76	3.16	2.63
	C	8.13	12.36	13.75	20.37	20.43	20.78	10.16	8.75	6.27	4.76	4.56	3.37
β-glucosidase	H	0.94	1.55	2.25	3.08	4.96	3.69	2.54	1.33	0.79	0.27	0.19	0.15
	C	1.73	2.91	3.58	4.08	4.55	4.13	4.06	3.84	2.16	1.96	0.93	0.32
Xylanase	H	3.25	4.0	4.88	8.31	8.85	8.13	7.55	6.34	4.76	3.13	2.65	2.21
	C	1.75	2.91	3.88	5.08	7.75	4.13	4.56	3.84	2.28	2.39	1.41	0.91
Laccase	H	1.10	5.37	8.75	10.12	9.23	8.75	7.31	5.48	3.44	1.36	1.21	1.18
	C	0.92	1.51	3.95	7.07	2.35	2.23	1.76	0.69	0.58	0.32	0.21	0.17
PPO	H	0.80	3.81	2.28	2.18	1.06	0.78	0.42	0.22	0.18	0.16	0.12	0.10
	C	0.93	2.89	1.18	1.06	1.01	0.87	0.66	0.68	0.51	0.29	0.18	0.07

of wheat straw decreased while nitrogen content increased. The decrease in carbon content and increase in nitrogen content was slightly more in hot water treated substrate than chemically sterilized substrate. The mean yield of *Pleurotus ostreatus* from three flushes (fresh weight) on different lignocellulosic wastes and their biological efficiency is given in table 4. The mean yield of *P. ostreatus* during first flush per 112g of dry weight of wheat straw was observed to be 50 and 25g, second flush was recorded as 40g and 23g and third flush was found to be 20 and 17g on hot water and chemically treated wheat straw, respectively. The biological efficiency of *P. ostreatus* 98.21% and 58.03% on hot water treated and chemically treated wheat straw, respectively.

Discussion

During vegetative growth of *Pleurotus ostreatus* the fast depletion of lignin and slow degradation of cellulose and hemicellulose and during fructification slow degradation of lignin and fast depletion of cellulose and hemicellulose in the present investigation revealed the differential requirement of the fungus during different phase of its growth. Same pattern of biodegradation of lignocellulosic wastes by various species of *Pleurotus* have been reported (4, 7, 12, 18, 19, 20, 24, 25). These observations suggest that the cellulose and hemicellulose serve as energy source for the formation of fruit bodies. In the present investigation hot water treated wheat straw degraded more efficiently by *P. ostreatus* than chemically treated wheat straw. The probable reason is

that under high temperature hydrogen bond of cellulose got disrupted leading to formation of amorphous cellulose which are more susceptible to fungal attack and thereby degradation. Similarly disruption in some of the bonds of lignin and hemicelluloses under aforesaid condition could have made the substrate vulnerable to fungal attack.

After the completion of the spawn run and in the spent compost the decrease in carbon content of the substrate in the present study could probably because of bioconversion of organic compounds. The increase in nitrogen content during growth of the mushroom may be either because of its ability to fix atmospheric nitrogen or due to the presence of some nitrogen-fixing bacteria in the compost. Other workers (3, 8, 9, 15, 16) also reported an increase in nitrogen content of the residues in *Pleurotus* bed and suggested that *Pleurotus* have the ability of to fix atmospheric nitrogen. It is noted that the increase in the nitrogen content of the substrates is due to presence of nitrogen fixing bacteria in the bed (11). However contrary to this observed that nitrogen content of rice straw decreased during cultivation of *Pleurotus flabellatus* (14).

In the present investigation activity of cellulolytic enzymes (FPase, CMCase and β-glucosidase) along with xylanase showed gradual increase in vegetative phase and sharp increase during fructification. This can be correlated with slow depletion in cellulose and hemicellulose component in the vegetative phase and fast depletion in reproductive phase. This further support the view that cellulose serve as an energy source for the

formation of fruit bodies in *Pleurotus* species. Similar results have also been reported in many other species of *Pleurotus* on various lignocellulosic substrates (2, 22, 23, 24, 25, 26). Maximum activities of laccase and polyphenol oxidase during vegetative phase of growth of *Pleurotus ostreatus* can be directly correlated with degradation of lignin in this stage. Elisashvili et al (6), Chang et al (2), Singh et al (21, 22, 24,) and Pandey et al (27) also reported high activity of these enzymes during the colonization stage and declined activity during primordia formation. This was because of the presence of more amorphous lignocellulosic material which is easy to be attacked by the fungus mycelia. Hence better spawn run, yield and biological efficiency of *Pleurotus* species remain higher on hot water treated substrate than chemically treated substrates.

Other articles in this theme issue include references (28-43).

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