Use of Common Reed (*Phragmites communis* Trin.) as Raw Material in the Cultivation of *Pleurotus ostreatus*

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Abstract: This paper describes the cultivation of *Pleurotus ostreatus* (Jack.ex.Fr.) Kummer on common reed (*Phragmites communis* Trin.). In this study, common reed materials were prepared by hand chopping, roughly chopping and fine chopping. Yield values, diameters and the numbers of the fruit bodies obtained from the cultivation of *P. ostreatus* mushrooms were determined. In addition, changes in the components of common reed after inoculation with *P. ostreatus* mycelium were determined.

Key Words: *Pleurotus ostreatus*, common reed, yield, fruit body, lignin, cellulose

1 Introduction

All over the world, especially in developing countries, there is a problem of shortage of protein. Producing cultured mushrooms can be one suitable solution to this problem. Nowadays, because of rapid industrialization, the amount of waste materials increases excessively. Utilization of the wastes in different fields is very important for government economy and natural balance.[1] The ability of fungi to colonize wood and wood wastes and produce edible reproductive structures has been exploited for centuries in Asia for the production of mushrooms like Shiitake (*Lentinula edodes*) and the oyster mushroom (*Pleurotus ostreatus*).[2,3] *P. ostreatus* growing wild in nature, is well known for its culinary taste and flavour.[4] Because of their rich mineral contents and medicinal properties, short life cycle, reproducibility in the recycling of certain agricultural and industrial wastes, low demand on resources and technology, several species of *Pleurotus* can be cultivated commercially in different parts of the world. *P. ostreatus* is a prospective source of valuable food protein, and an organism with the ability to effectively various lignocellulosic materials.[5] In addition, the used substrate, following the harvesting of the mushrooms, is valuable as a fertilizer and a soil conditioner for the growth of plants.[6] Additionally, fermented residues could be used as animal feed after mushroom cultivation.[7] It is possible to provide additional income to people living in the rural areas particularly working on wheat, hazelnut and rice agriculture. The utilization of waste paper for the production of cultured mushroom can solve one of the most important problems in solid waste disposal. This will provide an economical gain and protect the environment while giving people a nutritious food source such as mushrooms.

Previous researches have shown great potential for using some lignocellulosic materials as raw material for the production of *P. ostreatus*.[3, 8-12] However, every kind of lignocellulosic substances is likely be used as substrate for *Pleurotus* sp. cultivation, the main and co-substrate differ among countries and even regions on available abundant and cheaper ones.[13-16]

This study dealt with the cultivation of *P. ostreatus* (Jack.ex.Fr.) Kummer on Common Reed (*Phragmites communis* Trin.). P. Trin. Common Reed, is a tall (to 4 m) perennial grass that grows in dense monotypic thickets (to 400 stems/m2) in wetlands. *Phragmites* spreads rhizomatously, can start from a single fragment of rhizome, and can spread to cover hundreds of hectares. *Phragmites* is of concern to wetland managers because it grows rapidly, excludes other plant species, provides little wildlife habitat, and is of little aesthetic or recreational value. Because of these undesirable characteristics, many attempts have been made to control the spread of this plant, but with little success. Growing mushroom on this invaluable (common reed) raw material which can be grown and obtained easily may offer an alternative solution to utilize common reed.

The Eastern Black Sea Region has suitable natural climate properties, especially in terms of relative humidity and temperature, for the mushroom cultivation.

The objectives of the study were to experimentally determine the using common reed on the productivity of *P. ostreatus* cultivation and the changes in the chemical constitutions of common reed.

2 Materials and Methods

2.1 Mushroom strain and cultivation

The substrate used in this study was common reed obtained from a pulp and paper factory in Afyon, Western Turkey. In order to find suitable substrates and suitable ratios for the cultivation of *P. ostreatus*, various wastes materials and combinations were tested. In this study, common reed materials were prepared by hand chopping, roughly chopping and fine chopping. Mycelium of *P. ostreatus* was obtained from Faculty of Agriculture of Ankara University.

Mushroom cultivation was accomplished in the Experimental Mushroom House of the Forest Industry Engineering Department, Karadeniz Technical University. The temperature, ventilation and relative humidity were accurately controlled. The following procedures for growing *P. ostreatus* were based on those of Stamets and Chilton[17] and Kurtzman.[18]

Chopped common reed materials were moistened with water until 70-80% moisture content levels were attained and then placed in nylon bags of two kg. Ten replicate nylon bags were used for each substrate medium. The nylon bags were sterilized with direct steam at 65-70°C for 12 h. After cooling the substrates to 20°C, they were inoculated by spreading spawn on the surface of the substrate with a weight percentage of about 4% of the wet weight of compost. Inoculated blocks were incubated at 25-28°C. The lights were kept on 12 hrs per day. After 15 days, the substrates were completely colonized by the mycelium. The blocks were then shocked at 4-5°C for 48 h to stimulate fruit body production. The bags were then incubated at 21°C and 80-90% relative humidity until fruit bodies developed. The room was ventilated with fresh air moving at 2 m/s and illuminated 9-12 h/d until primordia formed. Harvests were started within two weeks after the first primordia had emerged. Ecological conditions in the harvesting period were the same as for primordium formation.[19, 20]

Mushroom yield (g) was calculated by division of fresh weight of fruit bodies obtained from each one bag to dry weight of compost. Biological efficiency is defined as the percentage ratio of the fresh weight of harvested mushrooms over the dry weight of substrate.[21]

2.2 Chemical analayses

Before the chemical analyses, control and decayed common reed samples were chopped to a length of 1-2 cm and ground in a Wiley mill to a homogeneous meal. Holocellulose analysis was made according to the sodium chlorite method of Wise,[22] cellulose by the nitric acid method of Kurschner-Hoffner,[23] and the lignin content was determined as acid-insoluble Klason lignin.[24] Hemicellulose content was determined by subtracting the cellulose content from holocellulose content.

3 Results

3.1 Morphological properties and yield values

The effects of different chopping methods on productivity were determined. Morphological properties and yield values of harvested fruit bodies are shown in Table 1.

Table 1. Morphological properties and yield values of *P. ostreatus* grown on common reed substrates

Substrates (Common reed)	B.E (%)	Yield (%)			Number of fruit Bodies			Diameter of fruit bodies (cm)		
		Av*	Sd**	Hg***	Av	Sd	Hg	Av	Sd	Hg
Hand- chopped	40.0	13.0	3.3	a	12.7	4.2	a	4.9	0.5	a
Roughly-chopped	68.9	22.4	4.3	bc	29.0	2.3	b	6.7	1.3	b
Fine-chopped	82.1	26.7	5.8	c	42.5	3.4	c	5.3	0.8	ab

Av : Average; ** Sd : Standard deviation; ***Hg : Homogeneous groups; same letters denotes insignificant statistical differences (P≤0.05).

The results showed that the highest yield was obtained when common reed material was prepared by fine chopping method while the lowest yield was obtained when the substrate was prepared by hand chopping. This can be explained by the additional space among the pieces of common reed when the substrate is prepared either by hand or roughly chopping. Therefore, it is more difficult and takes more time for mycelium of *P. ostreatus* to cover the substrate. The greatest number of sporophores was seen in the substrates where the yield was the best in general. Similarly, the least number of fruit bodies were seen in the substrate where the yield was the lowest. The best results for diameters of fruit body were obtained on substrates prepared by roughly and fine chopping methods.

3.2 Changes in the chemical components

3.2.1 Holocellulose

Changes in the holocellulose content of common reed after inoculation with *P. ostreatus* mycelium are shown in Table 2. The first sample for holocellulose analysis was taken after the substrate was completely covered by mycelium. The holocellulose content for fine chopped material decreased from 83.69% to 71.33%, and from 83.69% to 74.87% and 83.69% to 73.53% for hand chopped and roughly chopped, respectively at the end of the 14-week incubation period. The results showed that dramatic changes in holocellulose content occurred just after one or two weeks after the substrates were completely covered by mycelium.

3.2.2 Lignin

Changes in the lignin content of common reed after inoculation with *P. ostreatus* mycelium are shown in Table 3. The first sample for lignin analysis was taken after the substrate was completely covered by mycelium. The lignin content for fine chopped material decreased from 21.90% to 17.01%, and from 21.90% to 18.32% and 21.90% to 18.09% for hand chopped and roughly chopped, respectively at the end of the 14-week incubation period. The results showed that the dramatic changes in lignin content occurred just after one or two weeks after the substrates were completely covered by mycelium.

Table 2. Changes in the holocellulose content of common reed after inoculated with *P. ostreatus* mycelium

Incubation period (day)	Hand chopped		Roughly chopped		Hand chopped	
	Average (%)	Std	Average (%)	Std	Average (%)	Std
0 (control)	83.69	0.17	83.69	0.17	83.69	0.17
21	—	—	—	—	77.11	0.22
28	—	—	—	—	76.58	0.09
35	—	—	—	—	76.44	0.66
42	—	—	81.77	0.31	76.31	0.21
49	—	—	81.09	0.23	75.82	0.45
56	—	—	80.38	0.44	75.68	0.44
63	81.56	0.12	77.57	0.33	75.33	0.29
70	79.38	0.67	77.24	0.16	74.74	0.10
77	76.40	0.32	75.93	0.19	73.96	0.24
84	75.30	0.06	74.90	0.21	72.87	0.54
91	75.11	0.22	74.21	0.17	72.11	0.23
98	74.87	0.32	73.53	0.33	71.33	0.44

Table 3. Changes in the lignin content of common reed after inoculated with *P. ostreatus* mycelium

Incubation period (day)	Hand chopped		Roughly chopped		Hand chopped	
	Average (%)	Std	Average (%)	Std	Average (%)	Std
0 (control)	21.90	0.54	21.90	0.54	21.90	0.54
21	—	—	—	—	19.09	0.62
28	—	—	—	—	18.73	0.49
35	—	—	—	—	18.51	0.66
42	—	—	21.34	0.54	18.34	0.51
49	—	—	20.96	0.51	18.11	0.47
56	—	—	20.53	0.56	17.97	0.43
63	20.81	0.52	18.43	0.47	17.91	0.49
70	20.66	0.67	18.31	0.60	17.80	0.60
77	19.12	0.32	18.24	0.39	17.77	0.54
84	18.43	0.56	18.17	0.51	17.75	0.44
91	18.38	0.52	18.11	0.57	17.22	0.53
98	18.32	0.47	18.09	0.43	17.01	0.41

3.2.3 Cellulose

Changes in cellulose content of common reed after inoculated with *P. ostreatus* mycelium are shown in Table 4. The first sample for cellulose analysis was taken after the substrate was completely covered by mycelium. As is seen in table, the cellulose content for fine chopped material decreased from 52.06% to 42.56%, and from 52.06% to 47.66% and 52.06% to 46.11% for hand chopped and roughly chopped, respectively at the end of the 14-week incubation period. The results showed that the dramatic changes in cellulose content occurred just after the substrates were completely covered by mycelium.

Table 4. Changes in cellulose content of common reed after inoculated with *P. ostreatus* mycelium

Incubation	Hand chopped		Roughly chopped		Hand chopped	
period (day)	Average (%)	Std	Average (%)	Std	Average (%)	Std
0 (control)	52.06	0.26	52.06	0.26	52.06	0.29
21	—	—	—	—	45.74	0.54
28	—	—	—	—	44.33	0.54
35	—	—	—	—	44.11	0.27
42	—	—	51.22	0.41	43.52	0.55
49	—	—	50.44	0.33	43.26	0.28
56	—	—	49.73	0.28	43.21	0.53
63	51.66	0.27	49.24	0.55	43.04	0.32
70	51.18	0.28	48.73	0.36	42.98	0.42
77	49.50	0.56	47.64	0.53	42.96	0.31
84	48.87	0.57	47.43	0.28	42.91	0.56
91	47.91	0.29	46.87	0.33	42.88	0.29
98	47.66	0.46	46.11	0.41	42.56	0.44

3.2.4 Solubility in 1% NaOH

Changes in the solubility of common reed in 1% NaOH after inoculation with *P. ostreatus* mycelium are shown in Table 5. The degree of solubility in 1% NaOH indicates the amount of rotted materials available in the substrate. The first samples for analysis were taken after the substrate was completely covered by mycelium. Solubility in 1% NaOH for fine chopped increased from 29.40% to 44.11%, and from 29.40% to 37.47% and 29.40% to 39.49% for hand chopped and roughly chopped, respectively at the end of the 14-week incubation period.

4 Discussion and Conclusions

This study showed that although no activator materials (e.g. ammonium phosphate, ammonium citrate, urea) were used, the performance of the study (yield) was found to be generally satisfactory compared with literature reports. The results showed that the best way to prepare common reed material was the fine chopping method. In addition, changes in the chemical components of common reed were determined. Since cellulose, hemicellulose and lignin are utilized during the growth of spawn and during fructification, this information can provide a better understanding of how *P. ostreatus* is using the substrate. This data also helps to find out the optimum incubation period for bio-pulp production.

The remaining degraded substrate has tremendous potential for several applications including implications such as an upgraded form of ruminant feed, production of biogas, garden manure, or recycling for Agaricus cultivation, and production of saccharification enzymes.

Table 5. Changes in the solubility in 1% NaOH of common reed after inoculated with *P. ostreatus* mycelium

Incubation	Hand chopped		Roughly chopped		Hand chopped	
period (day)	Average (%)	Std	Average (%)	Std	Average (%)	Std
0 (control)	29.40	0.67	29.40	0.67	29.40	0.67
21	—	—	—	—	30.97	0.51
28	—	—	—	—	33.40	0.28
35	—	—	—	—	33.75	0.33
42	—	—	30.07	0.67	36.20	0.45
49	—	—	31.54	0.38	37.42	0.28
56	—	—	32.03	0.21	40.45	0.43
63	29.94	0.33	33.66	0.42	42.75	0.37
70	30.66	0.49	34.57	0.31	43.11	0.29
77	31.93	0.26	36.44	0.28	43.44	0.37
84	33.21	0.27	37.11	0.48	43.77	0.54
91	36.75	0.34	38.17	0.53	43.98	0.27
98	37.47	0.21	39.49	0.57	44.11	0.56

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