

BIODEGRADATION OF WATER HYACINTH BY GROWING *PLEUROTUS OSTREATUS* AND *P. SAJOR-CAJU* AND TRIAL FOR USING IN PRODUCTION OF MUSHROOM SPAWN

Y. A.-G. MAHMOUD

Mycology Research Lab., Botany Department, Faculty of Science, Tanta University,
Tanta 31527, P.O. Box 8, Egypt

(Received: 12 March 2005; accepted: 25 November 2005)

Two *Pleurotus* species were used to ferment water hyacinth (*Eichhornia crassipes*) plants without roots. The mushroom delignified the lignocellulose of water hyacinth and produced sugar, protein and organic matter. *Pleurotus ostreatus* and *P. sajor-caju* delignified 26.2±2.0 and 30.4±3.0% of the plants and utilized most of its hemicellulose after 7 weeks under its growing condition more than in solid-state fermentation. The produced biomass was enriched with mycelia protein, organic matter and reducing sugars, which increased gradually with incubation time. The effect of incubation time and moisture contents on spawn production by using chopped water hyacinth biomass was also studied. Seventy percent moisture content and 14 days of incubation are the optimum conditions for spawn production for both tested *Pleurotus* species.

Keywords: water hyacinth, *P. sajor-caju*, *P. ostreatus*, cellulose, protein, solid-state fermentation, spawn

Plant raw material wastes, made up mainly of cellulose, hemicellulose and lignin, are used as a source of nutrition for microorganisms. Fungi able to remove lignin from wood are of interest through bioconversion processes such as microbial pulping, conversion of forest and agricultural residues into animal feed and releasing wood sugar for ethanol production (KIRK et al., 1980).

Various abundantly available lignocellulose wastes may be exploited as cheap substrates for the production of feed materials (FLACHOWSKY et al., 1999; MUKHERJEE & NANDI, 2000). Microbiological delignification is required to be less energy consuming process and the amount of carbohydrate consumed by microorganisms should be minimum in relation to delignification rate. Lignin complex with cellulose and hemicellulose makes the polysaccharides less accessible to ruminal microbial digestion by blocking the access to rumen bacteria and their enzymes (KARUNANANDAA et al., 1995).

Although water hyacinth has become a serious problem due to its rapid spread in waterways, now it is considered as an inexpensive source of animal feed. Water hyacinth contains large amounts of cellulose and hemicelluloses; it could be an excellent food source for ruminants except for its high lignin content. White rot fungi

have been used to increase the digestibility of lignocellulosic biomass (ZADRAZIL, 1985; KARUNANANDAA et al., 1995; ZADRAZIL et al., 1996). White rot fungi have been used to increase the digestibility and the feed value of lignocellulosic biomass as well as for the direct conversion of these biomasses into human food (KAMRA & ZADRAZIL, 1986; GARCHA et al., 1995; ZADRAZIL, 1997; FLACHOWSKY et al., 1999; MUKHERJEE & NANDI, 2001; 2004).

Spawn is needed for mushroom cultivation in a universal demand for maximal production. Mushroom spawn has been defined as a medium impregnated with fungal mycelia and serves as seeds or inoculum starter for mushroom cultivation. KATHE and co-workers (1996) tested various materials for spawn production of *Pleurotus sajor-caju*. However, MATHEW and co-workers (1996) found that sorghum, wheat and paddy grains were equally good for spawn production of *P. ostreatus*, *P. platypus*, *P. sajor-caju* and *P. citrinopileatus*.

In the present study, the ability of two *Pleurotus* spp. to colonize water hyacinth biomass, and to degrade it under solid-state fermentation (SSF) conditions, in addition to conditions favourable for spawn production, were examined.

1. Materials and methods

1.1. Organisms

Two species of *Pleurotus* (Fr.) Kummer, *Pleurotus ostreatus* (HARI7-Hungary) and *P. sajor-caju* (WC 353PSU, USA) were used in this study. They were obtained from the Agricultural Research Center, Cairo, Egypt and maintained on 2% (w/v) malt agar medium at 4 °C.

1.2. Water hyacinth

Water hyacinth (*Eichhornia crassipes*) was collected from water canals in Tanta districts, Tanta, Egypt. Roots of water hyacinth were discarded because they contain heavy metals (CHOCOOJ et al., 1993). The plants were chopped into small pieces, dried in an oven at 60 °C to constant weight. Parts of the dried pieces were used for mushroom spawn production and the rest were ground to powders for solid-state fermentation.

1.3. Solid-state fermentation

Four grams of powdered water hyacinth and 0.08 g of limestone dissolved in 16 ml of distilled water were put into 250 ml Erlenmeyer flasks, and were autoclaved at 121 °C for 40 min. The flasks were inoculated with 3 agar disks (10 mm in diameter) from 7 days old culture of either *P. ostreatus* or *P. sajor-caju* and incubated for 3, 5 and 7 weeks at 26 °C. The bioconverted substrates were dried at 60 °C, weighed and finally ground and sieved through 40 mesh for chemical analysis. All experiments were performed in triplicate in our present study.

1.4. Cultivation of *P. ostreatus* and *P. sajor-caju* on water hyacinth dried pieces

The method of MUKHERJEE and NANDI (2004) was followed with some modifications as follows:

1.4.1. Spawn preparation: Spawn was prepared using wheat grains according to the method mentioned by JODON and RAYES (1975). Fifty grams of wheat grains were placed in 250 ml Erlenmeyer flask containing 75 ml distilled water and 1 g limestone chalk. The flasks were sterilized for 45 min at 121 °C and each was inoculated with 2 disks (10 mm diameter) of *P. ostreatus* and *P. sajor-caju*, and incubated at 26 °C for 15 days.

*1.4.2. Growing of *Pleurotus ostreatus* and *P. sajor-caju* on water hyacinth.* Polypropylene bags were loaded with 40 g of water hyacinth dried pieces that were moistened to 70% moisture content (w/w) and sterilized for 45 min at 121 °C. This substrate was inoculated with 4% wheat grain spawn (wet weight basis) by multilayered-spawning. The inoculated bags were perforated for aeration and incubated for three weeks at 26 °C. The bags were removed and the substrate blocks were placed in exposed beds on shelves, which were sprayed with distilled water three times a day to maintain about 95% relative humidity. Flushes of mushrooms were harvested from each bag during the cultivation period of 7 weeks. After 3, 5 and 7 weeks, the remained water hyacinth substrates were dried, weighed and ground separately for analysis.

1.5. Chemical analysis of remained and untreated water hyacinth

Acid-insoluble lignin was estimated quantitatively following the method of SAEMAN and co-workers (1954). Lignocellulosic content was calculated by comparing the ash content of the bioconverted and untreated biomass. Crude protein content of partially bio-converted and untreated biomass was determined by method of LOWRY and co-workers (1951). Reducing carbohydrate of untreated and the remained biomass was estimated according to MILLER (1959). Loss of organic matter was calculated as the difference in weight of the residual and initial organic matter. Hemicelluloses of the ground and bioconverted biomass were extracted according to SEIFERT (1983) and estimated according to DETROY and co-workers (1982). Holocellulose was treated with 4% NaOH solution for 24 h and the total cellulose was isolated as residual on a glass filter. Ash content of the substrate was estimated according to A.O.A.C method (1990).

1.6. Factors affecting spawn production

1.6.1. Effect of moisture content on the spawn production. For every *Pleurotus* species five Erlenmeyer flasks (250 ml) were prepared, each containing 50 g of suitable grains and one g of limestone. Each flask received different quantity of distilled water to achieve 30, 50, 70 and 90% (w/w) moisture contents. The flasks were sterilized for 45 min at 121 °C and each was inoculated with 2 disks (10 mm diameter) of each of the tested *Pleurotus* spp., and incubated at 26±2 °C for 15 days. The growth of *Pleurotus* species was assessed by measuring the total protein content and laccase activity.

1.6.2. Culture age. Five grams of water hyacinth, 15 ml of distilled water and 0.08 g of limestone chalk were added to each Erlenmeyer flask (250 ml), and were

sterilized for 35 min at 121 °C. Each flask was inoculated with 7-day old disks (10 mm diameter) of each of the tested *Pleurotus* spp. The flasks were incubated at 26 °C for 18 days. Samples were drawn in 2-day intervals for determination of laccase activity and total protein concentrations.

1.6.3. Laccase assay. At the end of the incubation period, flasks containing spawn were extracted with succinate buffer (pH 4.5) overnight at 4 °C. The extract was filtered and the filtrate obtained was dialyzed against the same buffer. The dialyzed preparation was used as the crude laccase enzyme. Laccase activity was determined by method of PRILLING (1975) modified by HAARS and HUTTERMAN (1983). The assay mixture contained 0.2 mM of 2, 6 dimethoxyphenol (substrate) in 50 mM citric acid phosphate buffer at pH 4.5 in a total volume of one ml, and was incubated at 37 °C for 10 min. The reaction was terminated by adding one ml dimethylsulfoxide and the absorbance was recorded at 468 nm.

1.6.4. Determination of protein. Protein concentration was estimated by the BRADFORD method (1976). Bovine serum albumin was used as standard protein in order to determine the protein concentration of unknown sample.

2. Results and discussion

2.1. Water hyacinth biodegradation

The biodegradation of treated and untreated water hyacinth using *P. ostreatus* and *P. sajor-caju* spawn grains is shown in Table 1. Cellulose is the major component of plant (36±3.10%) and lignin (15±1.30%) of dry matter. Treatment of water hyacinth with *Pleurotus* spp. insignificantly increased the components of the analyzed water hyacinth, and the reducing carbohydrate and total proteins. Degradation of lignin and cellulose of water hyacinth biomass was increased under normal growth conditions of *P. ostreatus* and *P. sajor-caju* compared to solid-state fermentation conditions.

Table 1. Analysis of water hyacinth (WH)

Constituents	Dry matter (%)		
	<i>Pleurotus sajor-caju</i> treated plants	<i>Pleurotus ostreatus</i> treated plants	Untreated plants (Control)
Lignin	15.6±2.00	16.0±1.30	15±1.30
Cellulose	37.5±2.60	36.0±3.20	36±3.10
Hemicellulose	23.7±2.00	25.0±2.30	22±2.20
Reducing carbohydrate	0.90±0.07	0.90±0.06	0.3±0.02
Total crude protein	3.00±0.20	2.70±0.40	2.0±0.30
Ash	2.70±0.20	2.70±0.20	2.5±0.20

Lignin conversion by *P. sajor-caju* was $30.4 \pm 3.0\%$ under normal growth conditions and $19.0 \pm 2.0\%$ under solid-state fermentation (Tables 2 and 3). *P. ostreatus* exhibited less efficiency for lignin degradation compared to *P. sajor-caju*, under both conditions. Hemicellulose conversion by *P. ostreatus* was more than that of cellulose and lignin and reached a maximum (45.5 ± 4.0) after 7 weeks. TSANG and co-workers (1987) found that *Pleurotus* spp. degraded hemicellulose of wheat straw to 50%, cellulose to 20% and lignin to 11% under fungal growing conditions. In accordance with our results, ligninolysis was higher for *Pleurotus* species under mushroom growing conditions than in solid-state fermentation. Many researchers (FAISON & KIRK, 1983; KAMRA & ZADRAZIL, 1986) have attributed the increased ligninolysis to be connected to the increase in co-substrate consumption under 100% O₂ availability, and stated that 50% O₂ availability in solid-state fermentation also induces H₂O₂ production in the system, indicating that the phenomenon is oxidative. Lignin degradation was low in the primary growth of *Pleurotus* spp. due to the presence of high concentration of cellulose and lignin components that act as barriers for the fungal degradation (REID, 1995).

Table 2. Biomass conversion of water hyacinth in solid-state fermentation by *Pleurotus ostreatus* and *P. sajor-caju*

Constituents	Conversion (%)		
	3 weeks	5 weeks	7 weeks
<i>Pleurotus sajor-caju</i>			
Lignin	6.5 ± 0.6	13.5 ± 1.2	19.0 ± 2.0
Cellulose	15 ± 1.5	19.0 ± 2.0	28.7 ± 2.5
Hemicellulose	24 ± 2.0	28.0 ± 2.5	31.0 ± 3.0
Reducing carbohydrate	0.5 ± 0.04	1.00 ± 0.1	1.20 ± 1.1
Total crude protein	5.5 ± 0.50	11.0 ± 1.0	9.00 ± 0.8
Total organic matter (OM) ^a	14 ± 1.4	26.0 ± 3.0	30.0 ± 3.0
Ligninolysis ^b	0.46 ± 0.04	0.52 ± 0.05	0.63 ± 0.05
<i>Pleurotus ostreatus</i>			
Lignin			
Cellulose	5.00 ± 0.04	11.0 ± 1.0	18.7 ± 1.6
Hemicellulose	15.8 ± 1.4	22.0 ± 2.2	30.2 ± 3.0
Reducing carbohydrate	28.0 ± 2.5	32.6 ± 3.0	38.1 ± 3.1
Total crude protein	0.60 ± 0.05	1.40 ± 0.1	1.50 ± 0.1
Total organic matter (OM) ^a	4.30 ± 0.3	8.20 ± 0.7	7.00 ± 0.6
Ligninolysis ^b	18.0 ± 1.40	28.0 ± 2.4	32.2 ± 0.3
	0.28 ± 0.02	0.39 ± 0.02	0.58 ± 0.04

^a Loss of total organic matter is calculated as the difference in weight of initial and residual organic matter.

^b Ligninolysis is the sum of dividing lignin content with total organic matter.

Organic matter conversion increased steadily with the time for both tested *Pleurotus* under solid-state fermentation and under normal growing conditions. The total organic matter conversion was the maximum after the third flush. Organic matter loss percentage after 7 weeks was 30.0 ± 3.0 in case of *P. sajor-caju* and 32.2 ± 0.3 in case of *P. ostreatus* under solid-state fermentation conditions (Table 2). Organic matter conversion was enhanced under growing conditions for both fungi. ZADRAZIL and PUNIYA (1994) reported a low rate of organic matter loss reaching 14.5% after 40 days in solid-state fermentation of wheat straw by *P. sajor-caju*. Evolution of gaseous metabolites from fungal activities that accumulated to higher concentrations on the surface during solid-state fermentation may explain the lower fermentation rate of organic matter during solid-state fermentation.

Total crude protein and reducing carbohydrates increased after inoculating the biomass with *Pleurotus* spp. This may be due to higher rate of cellulose-hemicellulolysis rather than the utilization of the breakdown products (MUKHERJEE & NANDI, 2001). The crude content of treated mustard biomass increased under both conditions of treatments with a maximum concentration after 5 weeks. However, crude protein content decreased after 7 weeks of biomass treatment, which might be due to proteolysis, and higher increase of the biological consequences through cultivation process as mentioned by DHANDA and co-workers (1996).

Table 3. Biomass conversion of water hyacinth under normal growth conditions and inoculation by *Pleurotus ostreatus* and *P. sajor-caju*

Constituents	Conversion (%)		
	3 weeks	5 weeks	7 weeks
<i>Pleurotus sajor-caju</i>			
Lignin	13.8 $\pm$ 1.2	26.0 $\pm$ 2.5	30.4 $\pm$ 3.0
Cellulose	19.5 $\pm$ 2.0	26.2 $\pm$ 2.0	35.1 $\pm$ 2.5
Hemicellulose	28.5 $\pm$ 3.0	30.3 $\pm$ 2.8	34.7 $\pm$ 3.4
Reducing carbohydrate	1.00 $\pm$ 0.1	2.10 $\pm$ 0.2	2.50 $\pm$ 0.24
Total crude protein	7.00 $\pm$ 0.6	5.90 $\pm$ 0.4	3.20 $\pm$ 0.31
Total organic matter (OM) ^a	18.0 $\pm$ 1.7	27.9 $\pm$ 2.5	31.0 $\pm$ 0.30
Ligninolysis ^b	0.76 $\pm$ 0.06	0.93 $\pm$ 0.08	0.98 $\pm$ 0.08
<i>Pleurotus ostreatus</i>			
Lignin			
Cellulose	12.3 $\pm$ 1.3	22.5 $\pm$ 2.0	26.2 $\pm$ 2.0
Hemicellulose	21.0 $\pm$ 2.0	26.5 $\pm$ 3.0	37.1 $\pm$ 3.5
Reducing carbohydrate	31.5 $\pm$ 3.1	39.0 $\pm$ 4.0	45.5 $\pm$ 4.0
Total crude protein	1.50 $\pm$ 0.14	2.00 $\pm$ 0.21	3.00 $\pm$ 0.3
Total organic matter (OM) ^a	6.30 $\pm$ 0.54	6.00 $\pm$ 0.5	5.00 $\pm$ 0.5
Ligninolysis ^b	21.4 $\pm$ 0.21	32.0 $\pm$ 0.3	34.5 $\pm$ 3.2
	0.57 $\pm$ 0.04	0.70 $\pm$ 0.05	0.76 $\pm$ 0.06

^a Loss of total organic matter is calculated as the difference in weight of initial and residual organic matter.

^b Ligninolysis is the sum of dividing lignin content with total organic matter.

2.2. Spawn production for mushroom culture

In this study, the possibility of using water hyacinth for mushroom production was tested. Therefore, the effect of fungal age (Fig. 1) and moisture content (Table 4) was studied.

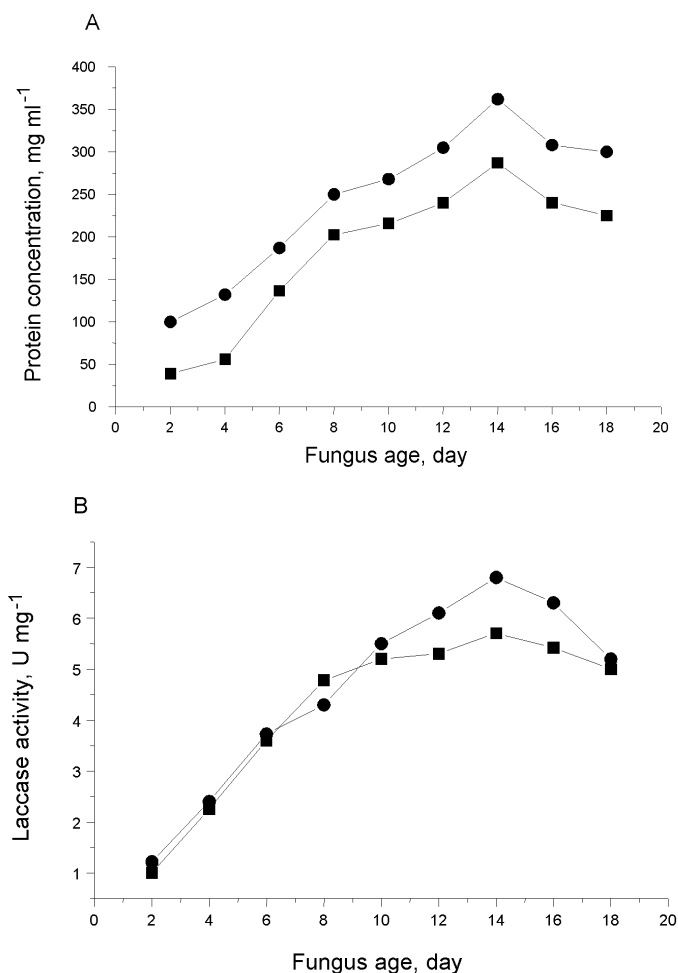


Fig. 1. Effect of incubation periods on spawn production total protein (A) and laccase activity (B) of spawn by *Pleurotus ostreatus* and *P. sajor-caju* grown on water hyacinth with moisture content of 70% (w/w) as substrate. ■: *P. ostreatus*; ●: *P. sajor-caju*

Seventy percent of moisture content was the best for cultures of *P. ostreatus* and *P. sajor-caju* (laccase activity 4.4 ± 0.4 , 4.0 ± 0.3 units/mg and 185 ± 18 , 173 ± 16 $\mu\text{g}/\text{mg}$ total protein, respectively). In accordance with our results, ZADRAZIL and BRUNNERT (1982) reported that water content of 65–70% was the optimum for degradation of cellulose by white rot fungi, low moisture content inhibits the fungal growth and high moisture content favours the spread of bacteria and inhibits gas circulation.

Table 4. Effect of moisture contents on growth of *Pleurotus ostreatus* and *P. sajor-caju* on water hyacinth for production of spawn

Moisture content percentage (w/w)	<i>Pleurotus ostreatus</i> ^a		<i>Pleurotus sajor-caju</i> ^a	
	Laccase activity (U mg ⁻¹)	Total protein concentration ($\mu\text{g}/\text{mg}$)	Laccase activity (U mg ⁻¹)	Total protein concentration ($\mu\text{g}/\text{mg}$)
30	1.7 ± 0.1	147 ± 15	1.4 ± 0.1	145 ± 14
50	2.8 ± 0.3	170 ± 16	2.3 ± 0.2	168 ± 15
70	4.4 ± 0.4	185 ± 18	4.0 ± 0.3	173 ± 16
90	2.4 ± 0.2	167 ± 15	2.2 ± 0.2	165 ± 16

^a The fungal inoculum was 7 days old.

The most suitable period for fungi to produce spawn (after 14 days) is indicated by the protein content and laccase activity. In other studies, RANDHIR and co-workers (1999) revealed that the optimum time for good mycelial growth was 12–13 days to produce spawn of *P. sajor-caju*. SERRA and co-workers (1999) stated that the age of spawn significantly affected the yield of *Pleurotus* species, and SHEIKH and co-workers (1988) found that the growth of *Volvariella volvaceae* was affected by spawn age.

3. Conclusion

Treating water hyacinth with *P. ostreatus* and *P. sajor-caju* led to increasing protein, organic matter, and carbohydrate amounts of water hyacinth biomass. Also, lignin, cellulose and hemicellulose contents of the plant were considerably degraded and enriched the nutritional value of the plant biomass, which might be used for animal feeding.

*

Author thanks Prof. Dr. ABDEL-RAHEEM EL-SHANSHOURY for critical reading of the manuscript and his valuable advises.

References

- A.O.A.C. (1990): *Official Methods of Analysis*. 15th ed. Association of Official Analytical Chemists, Washington, DC.
- BRADFORD, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72, 218–254.
- CHOCOOJ, R.D., SOTO-VELAZO, C., GUZMAN-DAVALOS, L. & GUZMAN, G. (1993): Cultivation of *Pleurotus* on water hyacinth and determination of the heavy metals in Mexico. *Mushroom Res.*, 2, 37–40.
- DETROY, R.W., CUNNINGHAM, R.I., BOTHAST, R.J., BAGBY, M.O. & HERMAN, A. (1982): Bioconversion of wheat straw cellulose and hemicellulose to ethanol by *Saccharomyces uvarum* and *Pachysolen tannophilus*. *Biotechnol. Bioengng.*, 24, 1105–1113.
- DHANDA, S., GARCHA, H.S., KAKKAR, V.K. & MAKKAR, G.S. (1996): Improvement in feed value of paddy straw by *Pleurotus* cultivation. *Mushroom Res.*, 5, 1–4.
- FAISON, B.D. & KIRK, T.K. (1983): Relationship between lignin degradation and production of reduced oxygen species by *Phanerochaete chrysosporium*. *Appl. environ. Microbiol.*, 46, 1140–1143.
- FLACHOWSKY, G., MAMRA, D.N. & ZADRAZIL, F. (1999): Cereal straws as animal feed-possibilities and limitation. *J. appl. Animal Res.*, 16, 105–118.
- GARCHA, H.S., KHANNA, P.K. & DHANDA, S. (1995): Improvements in delignification and digestibility of paddy straw by SSF using hyperlaccase mutants of *Pleurotus florida*. *Mushroom Res.*, 4, 59–64.
- HAARS, A. & HUTTERMANN, A. (1983): Laccase induction in white rot fungus *Heterobasidium annosum* (Fr.) Bref (*Fomes anosum* Fr.Cooke). *Archive Microbiol.*, 134, 309–313.
- JODON, M.H. & RAYES, D.J. (1975): *Care and handling of cultures of cultivated mushroom*. Pennsylvania Agric. Expt. Sta. Bull. No. 258, p. 4.
- KAMRA, D.N. & ZADRAZIL, F. (1986): Influence of gaseous phase, light and substrate pretreatment on fruit body formation, lignin degradation and *in vitro* digestibility of wheat straw fermented with *Pleurotus* spp. *Agricult. Wastes*, 18, 1–17.
- KARUNANANDAA, K., VARGA, G.A., AKIN, D.E., RIGSBY, L.I. & ROYSE, D.L. (1995): Botanical fractions of rice straw colonized by white rot fungi: Changes in chemical composition and structure. *Animal Feed Sci. Technol.*, 55, 179–199.
- KATHE, A.A., BALASUBRAMANYS, R.H. & KHANDEPARKAR, V.G. (1996): Cotton stalk spawn of *Pleurotus sajor-caju* and the yield of mushroom. *Mushroom Res.*, 5, 5–8.
- KIRK, T.K., HIGUCHI, T. & CHANG, H.-M. (1980): Lignin biodegradation: summary and perspective. -in: KIRK, T.K., CHANG, H.-M. & HIGUCHI, T. (Eds) *Lignin biodegradation: microbiology, chemistry and potential applications*. Vol. 2. CRC Press Boca Raton, Florida, USA, pp. 236–243.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951): Protein measurements with the Folin phenol reagent. *J. biol. Chem.*, 193, 265–275.
- MATHEW, A.V., MATHAI, G. & SUHARBAN, M. (1996): Performance evaluation of five species of *Pleurotus* (Oyster mushroom) in Kerala. *Mushroom Res.*, 5, 9–12.
- MILLER, G.L. (1959): Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, 31, 426–428.
- MUKHERJEE, R. & NANDI, B. (2000): Effect of pH on the submerged mycelial production in two *Pleurotus* spp. in the medium with lignocellulose biomass. *J. mycopathol. Res.*, 38, 65–69.
- MUKHERJEE, R. & NANDI, B. (2001): Changes in dry matter digestibility and composition of bioconverted mustard biomass by two *Pleurotus* spp. under mushroom growing conditions. *J. sci. ind. Res.*, 60, 405–409.
- MUKHERJEE, R. & NANDI, B. (2004): Improvements of *in vitro* digestibility through biological treatment of water hyacinth biomass by two *Pleurotus* species. *Int. Biodeterior. Biodegrad.*, 53, 7–12.
- PRILLING, H. (1975): Genetische Kontrolle der Phenoloxidase, Laccase des Ascomyceten. *Podospora anserina*. Diss. Uni. Bochum. Bibl. Myco. 51.
- RANDHIR, S., SINGH, D.P., YADAV, K.S. & SINGH, R. (1999): Use of agrocellulosic by products for spawn production of edible mushroom (*Pleurotus sajor-caju*). *Environ. Ecol.*, 17(1), 199–206.
- REID, I.D. (1995): Biodegradation of lignin. *Can. J. Bot.*, 73 (suppl. 1), 1011–1028.

- SAEMAN, J.F., MOORE, W.E., MITCHELL, R.L. & MILLET, M.A. (1954): Techniques for determination of pulp constituency by quantitative paper chromatography. *Techn. Assoc. Pulp and Paper Ind.*, 37, 336–346.
- SEIFERT, K. (1983): Decay of wood by *Dacrymycetales*. *Mycologia*, 75, 1011–1018.
- SERRA, M.D.A., KIRBY, R. & SERRA, M. (1999): Development of (oyster mushroom) production in Southern Africa using alien wood species as lignocellulose substrate. *Int. J. Mushroom Sci.*, 2 (3), 49–55.
- SHEIKH, M.I., KHAN, B.A. & KAN, N.A. (1988): Effect of spawn age and substrates (compost) on yield of Chinese mushroom. Recent advances in biotechnology and applied biology. *Proceedings of the eighth International Conference on Global Impact of Applied Microbiology and International Conference on Applied Biology and Biotechnology*. August 1988, Hong Kong, pp. 565–569.
- TSANG, L.J., REID, I.D. & COXWORTH, E.C. (1987): Delignification of wheat straw by *Pleurotus* spp. under mushroom growing conditions. *Appl. environ. Microbiol.*, 53, 1304–1306.
- ZADRAZIL, F. (1985): Screening of fungi for lignin decomposition and conversion of straw into feed by basidiomycetes. *Eur. J. appl. Microbiol. Biotechnol.*, 59, 433–452.
- ZADRAZIL, F. (1997): Changes in in vitro digestibility of wheat straw during fungal growth and after harvest of oyster mushroom (*Pleurotus* spp.) on laboratory and industrial scale. *J. appl. Animal Res.*, 11, 37–48.
- ZADRAZIL, F. & BRUNNERT, H. (1982): Solid-state fermentation of lignocellulose containing plant residues with *Sporotrichum pulverulentum* Nov. and *Dichomitus squalens* (Karst.) Reid. *Eur. J. appl. Microbiol. Biotechnol.*, 16, 45–51.
- ZADRAZIL, F. & PUNIYA, A.K. (1994): Influence of carbon dioxide on lignin degradation and digestibility of lignocellulosic treated with *P. sajor-caju*. *Int. Biodeterior. Biodegrad.*, 33 (3), 237–244.
- ZADRAZIL, F., KAMRA, D.N., ISIKHUMHEN, O.S., SCHUCHARDT, F. & FLACHOWSKY, G. (1996): Bioconversion of lignocellulose into ruminant feed with white rot fungi. *J. appl. Animal Res.*, 10, 105–124.