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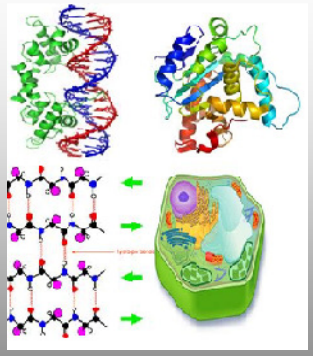

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RESEARCH PAPER

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Effect of co-Fungal Cultures on Fibre Biodegradation of Coffee Pulp Waste

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ABSTRACT

In our study, pH of the coffee pulp colonized by the white rot, brown rot and soft rot fungal monocultures and their corresponding co-cultures continuously decreased during the course of biodegradation of the coffee pulp substrate to a very low level of 5.2 - 5.5 from the initial 7.2. pH of coffee pulp colonized by the co-cultures containing Phanerochaete chrysosporium with a brown rot fungus or a soft rot fungus or by the antagonistic co-culture of P.eous + F. badius decreased by a minimum value (final pH 5.9 – 6.2) indicating the lower efficiency of these co-cultures in this group in coffee pulp biodegradation. The combination P.eous + P.flabellatus gave the maximum lignin loss 73.4% which was statistically significant when compared to the lignin loss caused by either of the two organisms in monoculture. The lignin loss caused by the association between the different soft rots and white rots was significantly higher when compared to the lignin loss caused by the soft rot fungal monocultures.

Keywords: White rotters, Brown rotters, Soft rotters, coffee pulp degradation

INTRODUCTION

Coffee pulp is one of the most abundant agro industrial wastes and pollutants generated by the coffee industry. The utilization of coffee pulp as food, animal feed and compost has been investigated for several years (Pandey *et al.,* 2000), but its chemical composition is a great limiting factor due to the presence of anti-physiological factors such as caffeine, poly phenolic compounds and tannins (Hakil *et al.,* 1998) which include tannic acid in high concentrations (3-4%).

Later it was proposed that desirable biochemical changes in terms of higher nitrogen content and degradation of cellulose, hemi cellulose, lignin and other toxic components can be effected in these substrates through mushroom growth (Arora and Kahlon , 1992; Ramamoorthy *et al.,* 1999). Early reports on ligno cellulosic conversion into fungal food and feed indicated *Chaetomium cellulolyticum* as the most promising organism of choice (Chahal *et al.,* 1983). Later, several workers demonstrated the lingo cellulose degradation potential of *Phanerochaete chrysosporium* and *Pleurotus sp.* which have been found to be the most efficient lignocellulose decomposing types of the white rot fungi (Kannan *et al.,* 1990; Masaphy and Levanon, 1992).

The study on degradation of lignin from fibres by *Chrysosporium pruinosum* (Rosenberg, 1980) and from wheat straw by *Pleurotus ostreatus* (Detroy and Rhodes, 1980) indicated that it took a long time (30-60 days) to obtain about 40% degradation of lignin. Lignin degradation was found to be inhibited by CO_2 and increasing air or oxygen flow over the substrate reversed the inhibition (Zadrazil and Kamra, 1989).

Lignin is found in all vascular plants, a major fraction being distributed throughout the secondary walls of woody cells and also in the middle lamella between the secondary cell walls (Eriksson *et al.,* 1990). Whilst cellulose and hemi cellulose are the supporting components of plants, lignin provides the essential rigidity and durability. Lignin is a natural polymer with a high molecular mass of up to 100 KDa or more (Kastner, 2000) and can make up 20-30% of the lingo cellulose in trees (Kuhad *et al.,* 1997). It is the most abundant aromatic carbon form and after cellulose, the second most abundant natural organic compound on earth. Lignin is deposited as an encrusting and protecting material on the cellulose or hemi cellulose matrix and acts as a kind of glue that cements the fibrous cell walls together. Due to its unique structure, lignin is highly resistant and forms a barrier to microbial attack. In general, only white rot fungi are considered to be efficient degraders of lignin (Griffin, 1994).

Most biodegradation studies have been carried out by using monocultures though a few workers have shown that synthetic lignins or their model compounds are best degraded by consortia (Federle and Vestal, 1980). The variation in degradation success can perhaps be explained by the fact that under field conditions the fungus must compete with the indigenous soil microflora. On the one hand, degradation of contaminants may be enhanced by synergistic interaction between the inoculated wood-rotting fungus and the native soil microflora (in der Wiesche *et al.,* 1997) while on the other hand, degradative activity could be negatively influenced by soil microorganisms antagonistic to the fungus (Ali and Wainwright, 1994).

Biological delignification has been targeted towards production of animal feeds (Al-Ani and Smith, 1986), edible mushrooms (Tautorus, 1985) and composting processes (Peerally, 1981). A major constraint to commercial exploitation of biological systems for delignification is the time factor required to achieve appreciable degradation. Earlier efforts concentrated on the use of monocultures for delignification (Al-Ani and Smith, 1986). However, lingo cellulose de ploymerization using monocultures may not be a perfect simulation of natural lignocellulose fermentation, which may explain reasons for poor feed conversion ratios with such processes.

Mixed cultures of aerobic fungi are known to perform certain catabolic operations not known to occur with monocultures in laboratory fermentations (Asiegbu *et al.,* 1996). Keeping these in mind, the present study focused on the effect of co-fungal cultures on fibre biodegradation of coffee pulp waste.

MATERIAL AND METHODS

Substrate

Coffee pulp, the solid waste of coffee industry, processing the coffee beans by wet processing method was used as the substrate for biodegradation studies.

Organisms

The selected white rot fungi were *Phanerochaete chrysosporium, Pleurotus eous, Pleurotus flabellatus*; brown rot fungi namely *Ganoderma luciderm* and *Fomes badius*; soft rot fungi namely *Chaetomium globosum* and *Aspergillus terreus* are used for biodegradation of coffee pulp. These fungal cultures were maintained on malt extract (2%) agar medium.

Biodegradation Studies

Biodegradation of coffee pulp was studied in solid state in Erlenmeyer flasks (250ml) using the selected mushroom fungi and their fungal associations. Ten g of coffee pulp containing 60% moisture was taken in individual Erlenmeyer flasks (250ml). The flasks were plugged with cotton and autoclaved at 121°C for 15 min. Single mycelial agar block (8 mm) from seven days-old cultures of the selected fungi was used as inoculum for monoculture experiments. For co culture studies, two agar blocks of the test white rot fungus and its respective fungal association were used as inocula. The conical flasks were incubated at 28 $\pm$ 2°C for a period of 40 days in the culture room. At each ten days interval of study, the entire content of each flask was withdrawn, dried at 60°C overnight and was used in the analyses for measuring pH and fiber by the gravimetric method of Chesson (1978). All the experiments were carried out in triplicates and were replicated twice.

RESULTS AND DISCUSSION

Solid state fermentation has scored high among other fermentation types used in large scale biodegradation and bioconversion processes, due to its economically viable and practically acceptable design (Pandey *et al*., 2001). Garcha *et al.* (1995) have proposed solid state fermentation as a promising technology for bio-processing of agricultural residues into quality feed. One of the current approaches in improving the efficiency of biodegradation and bioconversion of agricultural or agro-industrial residues in SSF is the use of co cultures or mixed cultures of lignocellulolytic microorganisms.

Many of these co cultures or mixed cultures were reportedly more efficient in lingo cellulolytic biodegradation in producing high activity enzymes due to their synergistic action (Ahlawat and Verma, 2001; Bajpai et *al*., 2001).

pH

pH of the coffe pulp colonized by the white rot, brown rot and soft rot fungal monocultures and their corresponding co cultures continuously decreased during the course of biodegradation. Co cultures involving the white rot or mushroom fungi brought down the pH of the coffee pulp substrate to a very low level of 5.2 - 5.5 from the initial 7.2. A second group of co cultures comprising the mushroom fungi, *Pleurotus eous* or *Pleurotus flabellatus* with the selected brown rot or soft rot fungi brought down the pH to an intermediate range of 5.5 - 5.8.

pH of coffee pulp colonized by the co cultures containing *Phanerochaete chrysosporium* with a brown rot fungus or a soft rot fungus or by the antagonistic co culture of *Pleurotus eous* + *Fomes badius* decreased by a minimum value (final pH 5.9 – 6.2) indicating the lower efficiency of these co cultures in this group in coffee pulp biodegradation.

The drop in pH by two degrees of magnitude as observed in the white rot + white rot co cultures indicated the higher rate of biodegradation achieved by these co cultures through the efficient production of hydrolytic enzymes and the acids (Kumaran *et al*., 1997). Similar views in support of our results on the better performance of co cultures involving white rot fungi have been given by Friteg and Marrell (1992), who suggested that enzyme activities of white rot fungi might be responsible for regulating the pH of the substrate at a suitable range for fungal colonization. Similar results were reported by Safari Sinegani *et al*. (2000) who found that *Aspergillus terreus* produced the highest biomass utilizing various aromatic compounds resulting in a significant reduction in the pH of the culture media. Ofuya and Nwajuba (1990) recorded a similar drop in pH from 5.6 to 4.4 in cassava peel degraded by *Rhizopus sp,* and they associated this drop in pH with increasing concentration of soluble reducing sugars and crude protein of the peel indicating a high degree of biodegradation achieved by this fungus than the other organisms.

A significant drop in pH of coffee pulp caused by the white rot + white rot co cultures compared very well with the higher crude protein content of the substrate. Similar growth associated decrease in pH was observed by Singh *et al*. (1989) during degradation of wheat straw by *Coprinus fimetarius* monocultures. Further co cultures of *Coprinus fimetarius* with *Azotobacter chroococum* reportedly resulted in a steeper decrease in pH and a higher degree of degradation (Singh *et al*., 1994).

Fibre

The fibre content of coffee pulp was 17.3% dry weight. Among the different fungal monocultures, *Phanerochaete chrysosporium* degraded the maximum 50.2% fibre from coffee pulp in 40 days followed by *Pleurotus flabellatus* and *Pleurotus eous* (48.0% and 46.2% fibre loss respectively).

Comparatively, the brown rot fungi *Fomes badius* and *Ganoderma lucidum* caused only 36.4% and 38.7% loss in fibre content respectively during the same period. The fibre degrading potential of *Chaetomium globosum* was higher than that of the *Ganoderma lucidum* (40.4% loss in fibre content), while *Aspergillus terreus* had the least fibre degrading potential showing the minimum 23.1% loss in fibre content (Table 1).

Basidiomycetous were reported to be the most efficient lignin degraders, but other ascomycetes and deuteromycetes also were reported to degrade lignin slowly (Rai *et al.,* 1993). Significant fibre degradation observed in *Chaetomium globosum* was in agreement with findings of other workers (Arora and Kahlon, 1992). The results agreed well with the ligninase positive character and laccase activity of the individual organisms on plate assays. *Aspergillus terreus,* the poor lignin degrader neither showed the brown zone in MEA – tannic acid medium nor the puple blue color in the MEA – lignin medium treated with α - naphthol.

Singh *et al*. (1992) attributed the decrease in neutral detergent fibre of wheat straw to the degradation of cell wall constituents by co cultures and mixed cultures. Choudhary *et al*. (1998) and Ramamoorthy *et al.* (1999) found that different *Pleurotus spp.,* were able to decrease the lignin content of coir pith by 62.0 to 78.0% over control. Our results on fibre degradation by mushroom monocultures corroborate with this report. Further the results showed that the coffee pulp fibre degradation potential of both the brown rot fungi was considerably less than that of the white rot fungi and *Chaetomium globosum.* Even though brown rot fungi are known colonizers of wood, the percentage of lignin degradation by the brown rot and white rot fungi was found to vary in different substrates by Ortega *et al.* (1992) and Saravanan *et al*. (2002).

Fibre loss in coffee pulp caused by the white rot and brown rot monocultures was low at initial stages of incubation, but was found to increase gradually with time. The results agree favorably with those of Ghosh and Nandhi (1995). Different schools of research reported that the maximum lignolysis occurred between 21 and 28 days of incubation (Choudhary *et al*., 1998; Arora and Kahlon, 1992) or between 30 and 40 days of incubation (Ramamoorthy *et al.,* 1999; Garcha *et al*., 1995; Ghosh and Nandi, 1995). It was found in this study that maximum fibre loss occurred between 20-30 days in white rot monocultures and between 30-40 days in brown rot monocultures.

The fungi with longer life duration and higher biological efficiency (*P.flabellatus, F.badius* and *G.lucidum*) continued to degrade the various components of coffee pulp with greater efficiency during the experimental period and finally ended up with achieving higher mineralization of the substrate. Lignin degradation had frequently been associated with biomass production. *Phanerochaete sp*. which degraded raw fibres of apple distillery sample by 20% was found to have increased the crude protein content of the filter cake by 17-20% (Kahlon *et al*., 1990). Substantial reduction in lignin and phenols through solid state fermentation by *Pleurotus ostreatus* was reported by Martinez *et al*. (1985). Variable responses were observed in the extent of ligninolysis in the different combinations tested. The combination *P.eous* + *P.flabellatus* gave the maximum lignin loss 73.4% (Table 1 & 2).

which was statistically significant when compared to the lignin loss caused by either of the two organisms in monoculture. The lignin loss caused by the association between the different soft rots and white rots was significantly higher when compared to the lignin loss caused by the soft rot fungal monocultures. The results are in agreement with those of Adhikary *et al*. (1992) who reported that mixed cultures of fungi, actinomycetes and bacteria could substantially improve lignin degradation compared with the control fungal monocultures. The reason for the enhanced degradation by mixture of organisms could be due to their synergistic activity through utilization of intermediate degradation products.

Table 1. Percent degradation of fibre in coffee pulp during solid state biodegradation of selected fungal monocultures.

S. No	Organisms	Fibre – Percent degradation			
		Degradation (Days)			
		10	20	30	40
1.	*P. chrysosporium*	11.5	28.9	41.6	50.2
2.	*P. eous*	9.8	27.1	42.1	46.2
3.	*P. flabellatus*	7.5	17.3	28.9	47.9
4.	*F. badius*	0	5.7	15.6	36.4
5.	*G. lucidum*	5.7	9.8	21.3	38.7
6.	*A. terreus*	0	13.2	21.3	23.1
7.	*C. globosum*	4.1	27.1	38.7	40.4
	LSD (0.05) = 1.57				

Initial fibre content: 17 .3 % dry wt

In co cultures, depending upon the type of association, the maximum fibre loss was found to be induced earlier. Similar promotion in substrate lignin degradation due to the synergistic lingo cellulolytic activity of the organisms in a co culture had been reported by Asiegbu *et al*. (1996) in sawdust colonized by co-fungal cultures. The results of our studies corroborate with earlier findings of Arora (1995) and Puniya and Singh (1995) where lignin degradation was better in co cultures and mixed cultures. These studies showed that certain associations particularly between white rot fungi can be useful in enhancing the biodegradation of coffee pulp.

Table 2. Percent degradation of fibre in coffee pulp during solid state biodegradation of white rot and their fungal associations.

S. No	Organisms	Degradation (days)			
		10	20	30	40
I.	White rot + White rot				
1	*P. chrysosporium + P. eous*	15.6	39.8	67.6	69.3
2	*P. chrysosporium + P. flabellatus*	19.0	40.4	57.8	71.0
3	*P. eous + P. flabellatus*	19.0	38.7	57.8	73.4
II	White rot + Brown rot				
1	*P. chrysosporium + F. badius*	15.6	38.7	52.0	53.7
2	*P. eous + F. badius*	5.7	15.6	34.6	34.6
3	*P. flabellatus + F. badius*	15.6	27.1	46.2	63.5
4	*P. chrysosporium + G. lucidum*	17.3	44.5	59.5	61.8
5	*P. eous + G. lucidum*	15.6	28.4	59.5	61.8
6	*P. flabellatus + G. lucidum*	15.6	30.6	52.0	65.3
III	*White rot + Soft rot*				
1	P. chrysosporium + A. terreus	17.3	34.1	53.7	57.8
2	*P. eous + A.. terreus*	5.7	17.3	47.9	49.7
3	*P. flabellatus + A. terreus*	5.7	17.3	28.9	52.0
4	*P. chrysosporium + C. globosum*	19.0	34.6	59.5	63.5
5	*P. eous + C. globosum*	15.6	27.1	50.2	59.5
6	*P. flabellatus + C. globosum*	13.2	28.9	59.5	61.8
	LS D (0.05) = 1.252				

Initial fibre content: 17:3 % dry wt

The suppression in fibre loss caused by *Pleurotus eous* + *Fomes badius* association was significant in comparison with that caused by *Pleurotus eous* and *Fomes badius* monocultures. Asiegbu *et al*. (1996) working with cocultures involving *P.chrysosporium*, *P.sajor-caju* and *T.versicolor* found that coculture of *P.chrysosporium* + *P.sajor-caju* similarly had only a lower potential for delignification. Co cultures in which the mushroom fungus overgrew its partner at a later stage (*P.flabellatus* + *A. terreus*; *P.eous* + *A.terreus*; *P.flabellatus* + *F.badius* and *P.flabellatus* + *G.lucidum*) showed higher lignin removal after this stage. The nitrogen from the mycelial biomass of the coculture partner might have kept the synthesis of lignin degrading enzymes under a de-repressed state as reported by Bisaria *et al*. (1997) and thus could have aided in enhancing lignin degradation.

REFERENCES

Al - Ani, F. and J.E.Smith, 1986. The effect of different nitrogen supplementation on fermentation of bagasse by *Sporotrichum pulverulentum* wild type and mutants. *Trans. Br. Mycol. Soc*., 86:165 - 168.

Ali, A.T. and M. Wainwright, 1994. Growth of *Phanerochaete chrysosporium* in soil and its ability to degrade the fungicide benomyl. *Biores. Technol*., 49: 197- 201.

Ahlawat, O.P. and R.N.Verma, 2001. *In-vitro* interaction studies between bacteria and *Pleurotus*: A tool for distinguishing different *Pleurotus sp*. and selecting fruiting inducing bacterium. In:*International Conference on New Horizons in Biotechnology (Abs.)*, Trivandrum (India): April, 18-21, p.78.

Arora, M. and S.S. Kahlon, 1992. Biological processing of maize cobs with fungi to improve its feed value. *Indian J. Microbiol*., 32: 81-84.

Asiegbu, F.O., A.Paterson and J.E.Smith, 1996. The effects of co-fungal cultures and supplementation with carbohydrate adjuncts on lignin biodegradation and substrate digestibility. *World J.Microbiol.Biotechnol*., 12:273-279.

Arora, D.S.1995. Biodelignification of wheat straw by different fungal associations. *Biodegradation*, 6: 57-60.

Bisaria, R., M. Madan and P.Vasudevan, 1997. Utilisation of agro residues as animal feed through bioconversion. *Biores. Technol*., 59: 5 - 8.

Bajpai, P. S.P.Mishra, S.Kumar, O.P.Mishra and P.K.Bajpai, 2001. Biopulping of Eucalyptus. In: *National Symposium on Lignocellulose Biotechnology; Present and future Prospects* (Abs.), University of Delhi, New Delhi, Dec 10-11, p.48.

Chahal, D.S. 1983. Growth characteristics of microorganisms in solid state fermentation for upgrading of protein value of lignocelluloses and cellulase production. In: *Foundations of Biochemical Engineering Kinectics and Thermodynamics in Biological Systems.* ACS Symposium Series no. 207, pp.421 – 442.

Chaudhary, S., R.Rohella, M.Mantlian and N. Sahoo, 1998. Decolourization of kraft paper mill effluent by white rot fungi. *Ind. J. Microbiol*., 38: 221-224.

Chesson, A., 1978. The maceration of linen flax under anaerobic condition. *J. Appl. Bacteriol*., 45: 219 - 230.

Detroy, R.W. and R.A.Rhodes, 1980. Biological conversion of agricultural lignocellulosics to ethanol and digestible feedstuffs. Organization for ethanol

Eriksson, K.E., R.A.Blanchette and P.Ander, 1990. Microbial and enzymatic degradation of wood and wood components, *Springer series in Wood Science*. Springer Verlag, Berlin.

Frietag, M. and J.J.Morrell, 1992. Changes in selected enzyme activities during growth of pure and mixed cultures of the white-rot decay fungus *Trametes versicolor* and the potential biocontrol fungus *Trichoderma harizianum*.*Can.J.Microbiol*., 38: 317-323.

Garcha, H.S., P.K.Khanna and S.Dhanda, 1995. Improvements in delignification and digestibility of paddy straw by solid state fermentation (SSF) using hyper-laccase mutants of *Pleurotus florida*. *Mush. Res*., 4: 59 - 64.

Ghosh, M.and B. Nandi, 1995. Dynamics of extracellular enzymes during lignocellulose degradation of water hyacinth biomass by *Pleurotus spp*. *Mush. Res*., 4: 53 - 58.

Griffrin, D.H., 1994. Fungal Physiology, Wiley – Liss, New York.

Hakil, M., S.Denis, G.Viniegra Gonzale, C.Augur, 1998. Degradation and product analysis of caffeine and related dimethylxanthines by filamentous fungi. *Enz. Microbiol. Technol*., 22: 355 - 359.

In der Wiesche, C., R.Martens and F.Zadrazil, 1997. Two-step degradation of pyrene by white rot fungi and soil micro organisms. *Appl. Microbiol. Biotech*., 46: 653 - 659.

Kastner, M.2000. "Humification" process or formation of refractory soil organic matter. In: *Environmental processes* II, J. Klein, (ed.) Wiley- VCH, Weinheim, Germany, pp.89 - 125.

Kuhad, R.C., A. Singh and K.E.Eriksson, 1997. Microorganisms and enzymes involved in the degradation of plant fiber cell walls. In: *Advances in Biochemical Engineering Biotechnology*, K. E. Eriksson (ed.), Springer Verlag, Germany, pp. 46- 125.

Kannan, K., G.Oblisami and B.G.Loganathan, 1990. Enzymology of lignocellulosic degradation in *Pleurotus sajor-caju* during growth on paper-mill sludge. *Biol. Wastes,* 33:1 - 8.

Kumaran, S., C.A.Sastry and S.Vikineswary, 1997. Laccase, cellulase and xylanase activities during growth of *Pleurotus sajor-caju* on sago hampas. *World J.Microbiol. Biotechnol*.,13 : 43 - 49.

Martinez, D., G.Guzman and C.Soto, 1985. The effect of fermentation of coffee pulp in the cultivation of *Pleurotus ostreatus* in Mexico. *MUSH. Newslett. Tropics*, 6: 21-28.

Masaphy, S. and D. Levanon, 1992. The effect of lignocellulose on lignocellulolytic activity of *Pleurotus pulmonarius* in submerged culture. *Appl. Microbiol.Biotechnol*., 36 : 828.

Ofuya, C.O and C.J. Nwajiuba, 1990. Microbial degradation and utilization of cassava peel. *World J. Microbiol. Biotechnol*., 6: 144 -148.

Ortega, G.M., E.O. Martinez, D.Betancourt, A.E. Gonzalez and M.A.Otero, 1992. Bioconversion of sugarcane crop residues with white-rot fungi *Pleurotus sp*. *World J. Microbiol. Biotechnol*., 8 : 402 - 405.

Pandey, A., C.R.Soccol, P.Nigam, D.Brand, R.Mohan and S.Roussos, 2000. Biotechnological potential of coffee pulp and coffee husk for bioprocesses. *Biochem. Engg. J*., 6 : 153 – 162.

Pandey, A., C.R.Soccol, J.A.Rodriguezleon and P.Nigam, 2001. Solid state fermentation in Biotechnology Fundamentals and Applications. Asia Tech. Publishers INC, New Delhi, India, pp. 90-96.

Puniya, A.K. and K. Singh, 1995. Determination of enzyme activities of lignocelluloytic strains in solid substrate fermented products. *Ind*. *J.Anim. Nutri*., 12: 205 - 212.

Rai, R.D., B.Vijay and S.Saxena, 1993. Extracellular cellulase and laccase activity of the fungi associated with *Pleurotus sajor-caju* culture. *Mushroom Res*., 2: 49 -52.

Ramamoorthy, V., B.Meena, M.Muthusamy, K.Seetharaman and D. Alice, 1999. Composting coir pith using lignocellulolytic fungi for the management of root rot of blackgram. *Mush. Res*., 8:13 - 17.

Rosenberg, S.L., 1980. Fermentation of pentose sugars to ethanol and other neutral products by microorganisms. *Enz. Microbiol .Technol*., 2:185 - 193.

Safari Sinegani, A.A., G.Emtiazi and S.Hajrasuliha, 2000. Production of laccase by *Aspergillus terreus* and some basidiomycetes in contaminated media with aromatic compounds. *Asian J. Microbiol. Biotech. Envi.Sc*i., 2:1 - 4.

Singh, K., S.N. Rai, G.P.Singh and B.N.Gupta., 1989. Solid state fermentation of urea – ammonia treated wheat and rice straw with *Coprinus fimetarius. Ind. J. Microbiol*., 29: 371 - 376.

Singh, K., G.P.Singh and B.N.Gupta 1992. Biochemical studies on *Coprinus fimetarius* inoculated straws. *Ind. J. Microbiol*., 32: 473 - 477.

Singh, K., A.K. Puniya and S.Neelakantan, 1994. Degradation of wheat straw components during two stage solid substrate fermentation with *Coprius fimetarius* and *Azotobacter chroococcum*. *Ind. J. Diary Sci.*, 47: 314 - 318.

Tautorus, T.E. 1985. Mushroom fermentation. In: *Advances in Biotechnological Processes.* A. Mizrai, and A.L.Van Wezel (eds.), Alan R. Liss Inc., New York, pp. 227 – 273.

Zadrazil, F. and D.N.Kamra, 1989. Influence of air and oxygen supplies on lignin degradation and its relation with *in vitro* digestibility of wheat straw fermented with *Stropharia rugosoannulata, Pleurotus eryngii* and *Pleurotus sajor-caju. Mush. J. Tropics*, 9:79 - 88.

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