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Studies on *in vitro* degradability of mixed crude enzyme extracts produced from *Pleurotus* spp.

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Abstract: *A preliminary investigation was conducted to assess lignocellulolytic efficiency of crude extracts from three white-rot fungi, Pleurotus florida PF05 (PF), Pleurotus sajor-caju PS07 (PS) and Pleurotus eryngii PE08 (PE). The activities of CMC-ase, xylanase, β-glucosidase, β-xylosidase, laccase and Mn peroxidase in extracts were evaluated. PF produced its highest CMC-ase (317 UL^{-1}), β-glucosidase (62 UL^{-1}), β-xylosidase (37 UL^{-1}) and laccase (347 UL^{-1}) activities while, PS produced highest xylanase (269 UL^{-1}) and Mn peroxidase (69 UL^{-1}) activities. In addition, crude extracts extracted were employed for their in vitro degradability assessment; and were evaluated with mono and mixed extracts separately to corn cob substrate. The losses in cell wall components and dry matter during 5 and 10 days incubations were analyzed after treatments of extracts. Maximum 8.2, 4.4 and 2.8% loss were found respectively in hemicellulose (HC), cellulose (C) and lignin (L) with mono extract of PF within 10 days. The influence of mono extract of each strain (PF, PS and PE) and their mixed extracts (PF+PS, PF+PE, PS+PE and PF+PS+PE) on degradation of cell wall constituents were remarkably differed. The mixed extract treatment proved maximum 13.6% HC loss by PF+PS+PE extract, 9.2% loss in C by PF+PS extract and 5.2% loss of L by the PF+PS+PE extract treatment. The highest dry matter loss (8.2%) was recorded with PF+PS+PE mixed extract combination.*

Key words: *Pleurotus spp., Corn cob, Lignocellulolytic enzymes, Degradation, Enzyme extract*
PDF of full length paper is available online

Introduction

Pleurotus is a versatile genus of white-rot basidiomycete fungi and well known for their complexity of the enzymatic system and prominent lignocellulolytic property, member of this genus can colonize a wide range of natural lignocellulosic wastes. Lignocellulosic substrates are generally used as low-grade domestic fuel and burnt negligently or thrown away, which create post disposal problems in our surroundings (Naraian *et al*., 2009a,b). Therefore the development of a biotechnological process to convert agro industrial wastage up to a non-harmful level will have environmental importance (Zadrazil, 2000; Peixoto-Nogueira *et al*., 2009).

In this order of bioconversion the use of lignocellulolytic enzymes from *Pleurotus* spp. is one of the most important aspect for the biodegradation of organo-pollutants, xenobiotics and industrial contaminants (Cohen *et al*., 2002).The white-rot basidiomycetes are strong decomposers of lignocellulosic wastages due to their capability to synthesize the relevant and unique oxidative network of lignocellulolytic extracellular enzymes (Maganhotto de Souza Silva *et al*., 2005; Eichlerova *et al*., 2006). The genus *Pleurotus* includes species that belong to group of white-rot fungi and have unique ability to produce extra cellular lignocellulolytic enzymes including laccase and Mn peroxidase (Stajic *et al*., 2006), xylanase (Elisashvili *et al.*, 2008), CMCase, β-glucosidase and β-xylosidase. These enzymes have shown enormous biotechnological potential as they can be used at a wide level for of lignocellulose degradation (Ren and Buschle-Diller, 2007) and detoxification of agro-industrial residuals with high phenolic contents (Mata *et al*., 2005).

Several studies have been addressed the degradation of lignocellulosics by direct cultivation of fungi (Tellez-Tellez *et al*., 2008; Elisashvili *et al.*, 2008). The lignolytic system of *Pleurotus* spp. has been extensively studied in recent years by fungal cultivation with substrates. These studies have been characterized different lignolytic enzymatic families are peroxidase and laccase (Sania *et al*., 1991; Giardiana *et al*., 2000; Cohen *et al*., 2001), preferentially these enzymes can be used for various biotechnological and environmental applications. *Pleurotus* spp. and their enzymes serve as a proficient substitute for the bioremediation of resistant pollutant (Cohen *et al*., 2001; Koroleva *et al.,* 2002). The efficient and reproducible bioconversion of the agricultural lignocellulosic residues on an industrial scale by using enzymatic preparations directly may have many practical applications like degradation of lignocellulosic and organo-pollutants (Cohen *et al.,* 2002).

The present study was conducted to assess the impact of crude extracts of white-rot basidiomycete fungi *Pleurotus florida* PF05 (PF), *Pleurotus sajor-caju* PS07 (PS) and *Pleurotus eryngii*

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PE08 (PE) on *in vitro* degradation and total dry matter loss of the corn cob substrate.

Materials and Methods

Fungal strains maintenance and preparation of extract: Three white-rot basidiomycete fungi *Pleurotus florida* PF05 (PF), *Pleurotus sajor-caju* PS07 (PS) and *Pleurotus eryngii* PE08 (PE) were procured from the Department of Microbiology, Institute of Bioscience and Biotechnology (IBSBT), Chhatrapati Sahu Ji Mahraj University, Kanpur, India. These were grown on potato dextrose agar and malt extract agar media at 22±1°C and maintained by sub culturing every fortnightly at 4°C.

For preparation of fungal inocula the cultures were initially grown in Petri plates. Fungal disks were made from the edge of actively growing fungal colony by using sterile cork borer measuring 7 mm in diameter. Two disks were transferred per 250 ml Erlenmeyer conical flask containing sterile 100 ml nutrient rich culture media [glucose (10.0 gl^{-1}), NH_4NO_3 (2.0 gl^{-1}), KH_2PO_4 (0.8 gl^{-1}), $Na_2HPO_4.7H_2O$ (0.75 g l^{-1}), $MgSO_4.7H_2O$ (0.5 gl^{-1}) and yeast extract (2.0 g l^{-1})] and these conical flasks were incubated at 22±1°C in a rotatory incubator shaker (150 rpm). Thus obtained fungal broth culture was employed as inoculum for further studies.

Submerged cultivation for each fungus was carried out in 250 ml sized Erlenmeyer conical flasks. Tien and Kirk medium and malt extract broth medium having 4% corn cob powder were used for submerged cultivation to produce enzymes. The liquid medium was inoculated with 4% (v/v) of liquid inoculum. The inoculated conical flasks were then incubated in a rotatory shaker at 22±1°C and 150 rpm.

For preparation of enzyme extract, 100 ml of each broth culture was separately centrifuged at 8000 × g for 10 min in a cooling centrifuge (Remi, India). The mycelium was discarded while the supernatant was mixed with 50 ml of 0.1M phosphate buffer (pH 6.8) and used as crude enzyme extract for further studies.

Enzyme assay: Manganese peroxidase (MnP) activity (EC 1.11.1.13) was determined spectrophotometrically with 3 mM phenol sulfonpthalein (Merck) as substrate. The reaction mixture contained 0.2 ml buffer enzyme preparation, 2 mM H_2O_2 and phenol sulfonpthalein, it was incubated for 5 min at 22±1°C. The reaction was terminated by using 2M NaOH (Glenn and Gold, 1985). The assay of laccase (EC 1.10.3.2) activity (Sandhu and Arora, 1985) was carried out of 5.0 ml reaction mixture with 2 mM guaicol (Merck) prepared in 10 mM sodium acetate buffer (pH 5.0) and by adding 0.2 ml of enzyme extract. Assay was performed in small culture tubes at 22±1°C. For carboxymethyl cellulase activity (Miller, 1959), assay mixture was prepared by adding 0.5 ml culture filtrate and 0.5 ml of 1%(w/v) carboxymethyl cellulose (Merck) solution to 1 ml of 100 mM phosphate buffer and followed by 3.0 ml 3, 5-dinitrosalycylic acid reagent. The mixture was incubated at 22±1°C for 30 min and 1 ml of 40% (w/v) Rochelle salt (sodium-potassium tartrate) solution was added to stop reaction and kept in boiling water bath for 15 min to develop colour. The intensity of red colour was measured spectrophotometrically. For xylanase (EC 3.2.1.8) activity (Miller, 1959) assay mixture contained 0.5 ml enzyme extract to 0.5 ml of 0.25 % (w/v) xylan (Oat spelt, Sigma) solution to 1ml of 100 mM of phosphate buffer and 3.0 ml DNS reagent and incubated at 22°C. Reaction was stopped by Rochelle salt and intensity of colour was measured. The β-glucosidase (EC 3.2.1.21) and β-xylosidase (EC 3.2.1.37) activities were assayed (Panbangred *et al*., 1983) by measuring the amount of p-nitrophenol liberated from p-nitrophenyl β-glucopyranoside (PNPG) (Sigma) and p-nitrophenyl β-xylopyranoside (PNPX) (Sigma) substrates respectively. The assay mixture containing 0.9 ml of 0.1% (w/v) PNPG and 0.1ml enzyme extract was incubated at 22±1°C for 10 min. The reaction was stopped by adding 1ml of 2% sodium carbonate solution and the liberated p-nitrophenol was measured spectrophotometrically.

***In vitro* degradation study:** Corn cob substrate was obtained locally after removal of the grains. It was collected; oven dried (60°C for over night) and then milled into small pieces (< 1mm) for further extract treatments studies. 15 g of powdered corn cob substrate was primarily soaked and saturated with 35 ml of distilled water up to a level to make substrate composite, porous and heterogeneous. It was performed separately in 250 ml conical flask and sterilized in autoclave. Furthermore, after cooling different sets were treated with equal volume (7ml) of either mono extract (PF, PS and PE) or mixed extracts (PF+PS, PF+PE, PS+PE and PF+PS+PE) preparations, thrice during complete period of incubation. To run the optimal enzymatic activity all experimental sets were incubated at 22±1°C in a BOD incubator (Yarco, India) up to 10 days.

Estimation of cell wall components: Various cell wall components *viz*., neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose (C) and lignin (L) were estimated as per standard methods (Van-Soest and Wine, 1967). The hemicellulose (HC) content was calculated by deducting ADF from NDF value. The per cent loss in each cell wall component was determined from their initial (before treatment) and final (after treatment) values.

Determination of total dry matter loss: To determine the per cent dry matter loss, weighed substrate was kept in an Erlenmeyer conical flask of known weight. The conical flask having treated substrate was oven dried at 60°C up to complete removal of moisture and weighed promptly. After enzymatic treatments these conical flasks were again oven dried and weighed to calculate total dry matter loss.

Statistical analysis: Statistical analysis of all data was performed by the analyses of variance through one way ANOVA and post hoc Tukey's test, employing probability level below 5% ($p<0.05$).

Results and Discussion

Enzyme activity of different enzymes *viz*., carboxymethyl cellulase (CMC-ase), xylanase, β-glucosidase, β-xylosidase,

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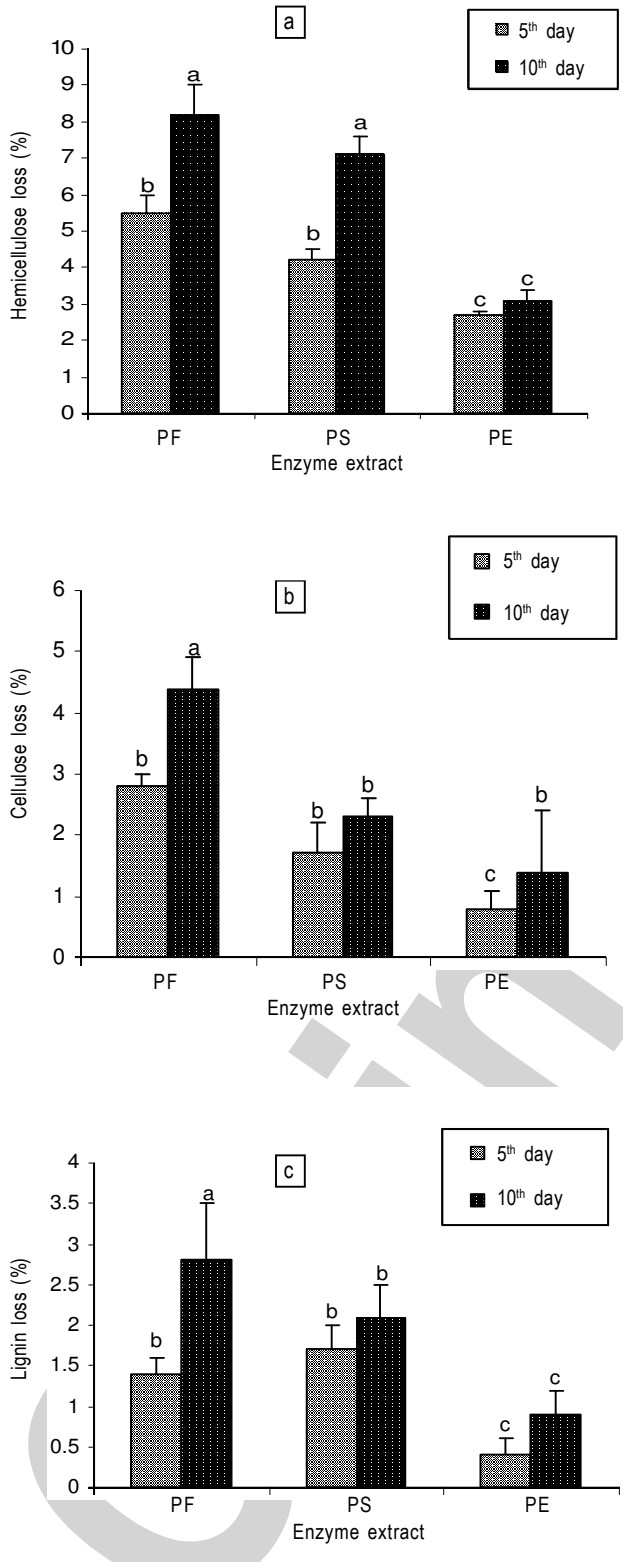

Fig. 1: Effect of different mono fungal culture extracts on total loss of hemicellulose (a) cellulose (b) and lignin (c) content of corn cob substrate. Treatments having different letters at error bars are statistically different at 5% probability level ($p<0.05$)

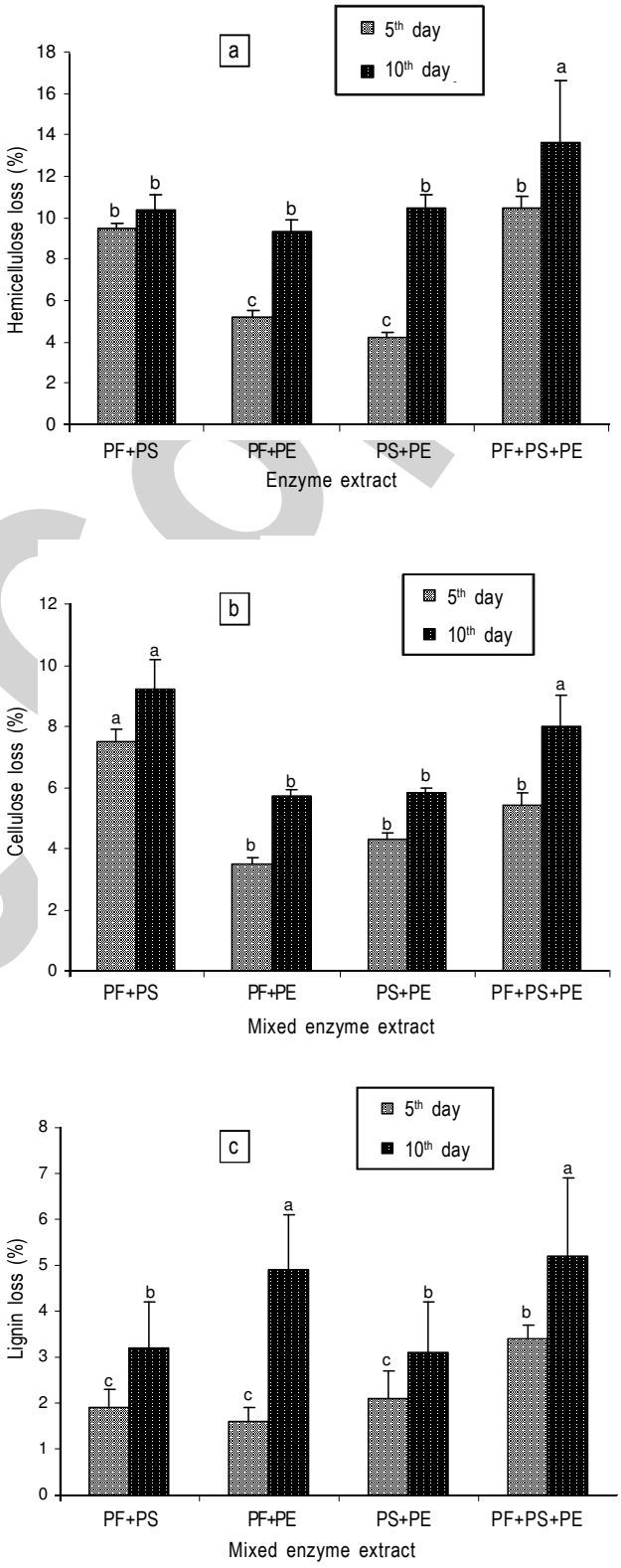

Fig. 2: Effect of different fungal mixed extracts on total loss of hemicellulose (a), cellulose (b) and lignin (c) content of corn cob substrate. Treatments having different letters at error bars are statistically different at 5% probability level ($p<0.05$)

Table - 1: Enzyme profile of crude extracts produced by three different white-rot fungi

Enzyme extracts	Enzyme activities (U L^{-1})					
	CMC-ase	Xylanase	β-glucosidase	β-xylosidase	Mn peroxidase	Laccase
PF	317 ±8[a]	256 ±10[a]	62±4[a]	37±3[a]	68 ±5[a]	347±10[a]
PS	198 ±6[c]	269 ±8[a]	56±5[c]	28±2[a]	69 ±7[a]	278±8[b]
PE	267 ±9[b]	178 ±7[b]	48±3[a]	19±1[b]	52 ±4[b]	261±6[b]

PF=*Pleurotus florida*, PS=*Pleurotus sajor-caju*, PE=*Pleurotus eryngii,* Mean ± Standard deviations (n=3), Means in a column having different superscript letters are significantly different (p<0.05)

Table - 2: Composition of corn cob substrate before treatment

Components	Amount (%)
NDF	93.75±2.67
ADF	46.87±1.32
Hemicellulose (HC)	46.88±2.11
Cellulose (C)	36.68±1.41
Lignin (L)	10.19±0.76

ADF= Acid detergent fibre, NDF= Neutral detergent fibre, ±, Standard deviations (n=3)

manganese peroxidase (MnP) and laccase were investigated and data are represented in Table 1. The highest activity (317 U L^{-1}) of CMC-ase was found in culture extract of PF it was followed by 267 and 198 U L^{-1} in extracts of PE and PS respectively. The highest xylanase activities noted were 269, 256 and 178 U L^{-1} in PS, PF and PE culture extracts respectively. However, PF extract produced highest 37 and 62 U L^{-1} of β-xylosidase and β-glucosidase activities respectively. The PE extract represented lowest activities (48 and 19 U L^{-1}) for β-xylosidase and β-glucosidase enzymes. In addition, the highest (69 U L^{-1}) peroxidase activity was investigated in PS and lowest (52 U L^{-1}) in PE extract. The highest laccase activities found are 347, 278 and 261 U L^{-1} respectively in the extracts of the PF, PS and PE.

Effect of mono and mixed extracts on cell wall components: The loss of cell wall constituents was increased significantly (p<0.05) from 5th day of incubation onwards up to the 10th day (Fig. 1a,b,c). All three separate mono extracts tested have reduced hemicellulose, cellulose and lignin contents independently. It was noted that extract from PF was more efficient and significantly (p<0.05) degraded highest 5.5% hemicellulose after 5 days of incubation; it was followed by 4.2% (PS) and 2.7% (PE). After 10 days of incubation the same PF extract degraded highest 8.2% hemicellulose significantly (p<0.05); followed by 7.1 and 3.1% loss by the extracts of PS and PE respectively. Furthermore, the highest loss in cellulose content was noted in the treatment of PF extract that was 4.4% on 10th day and 2.7% on 5th day. However, lowest losses in cellulose were recorded with the treatment of PE extract these were 0.8 and 1.4% reduction after 5th and 10th day respectively. In comparison to the other cell wall components (hemicellulose and cellulose), a very little reduction in the lignin content was observed. The PF extract have degraded highest 2.8% lignin after 10 days of incubation that was followed by PS extract treatment (2.1%) on 10th day. As it was compared amongst different extracts used separately PF extract was most effective that have degraded each cell wall component significantly (p<0.05).

The reduction in cell wall components were also assessed with the treatment of mixed fungal extracts in different probable combinations (PF+PS, PF+PE, PS+PE and PF+PS+PE). As it was observed that hemicellulose was the major cell wall component that was remarkably influenced with the mixed extract treatments. The significant (p<0.05) 10.5 and 13.6% reduction in hemicellulose were observed with the treatment of mixed extracts of PF+PS+PE after 5th and 10th day of incubation. The PS+ PE and PF+PS extracts combinations also significantly (p<0.05) reduced 10.5 and 10.4 % hemicellulose in their respective incubation of 10th day (Fig.2a). Cellulose was the next cell wall component that was degraded by the mixed extracts. The highest 9.2% loss in cellulose was recorded in the PF+PS extract treatment after 10th day of incubation; it was followed by 8.2% PF+PS+PE extract combination on 10th day (Fig. 2b). A reduction of 5.2% in the lignin content was also noted with the PF+PS+PE mixed extract treatment during 10 days (Fig. 2c). Comparing the efficiency of degradation the PF+PS+PE combination of three extracts was most effective amongst the extracts used that have significantly (p<0.05) reduced to each cell wall component.

Total dry matter loss by mono and mixed extracts: Total dry matter loss of the substrate after mono extracts treatment have been presented in Fig. 3a. The highest 4.5% *in vitro* dry matter loss was noted in PF extract treatment on 10th day which was followed by 4.1% in PE extract treatment after 10 days of incubation. The lowest 1.9% dry matter loss was recorded with PF extract on 5th day of incubation. Amongst several extracts tested, mono extract of PF was found to be most effective for dry matter reduction.

The substrate when treated with mixed extracts of different combinations have been remarkably reduced the dry matter in comparison to the individual extract treatments. A combination of three extracts of PF+PS+PE has reduced highest 8.5% dry matter during 10 days of incubation; it was followed by 6.9% loss with PS+PE extract combination during same period of incubation. However, the lowest 3.7% loss was analyzed during 5th day by PF+PS extract combination (Fig. 3b).

A range of both hydrolytic and oxidizing enzymes are excreted into the lignocellulosic substrate (Wood, 1985), acting to depolymerise the lignocellulosic polymers into compounds of lower molecular weight which can be assimilated by the fungus. The

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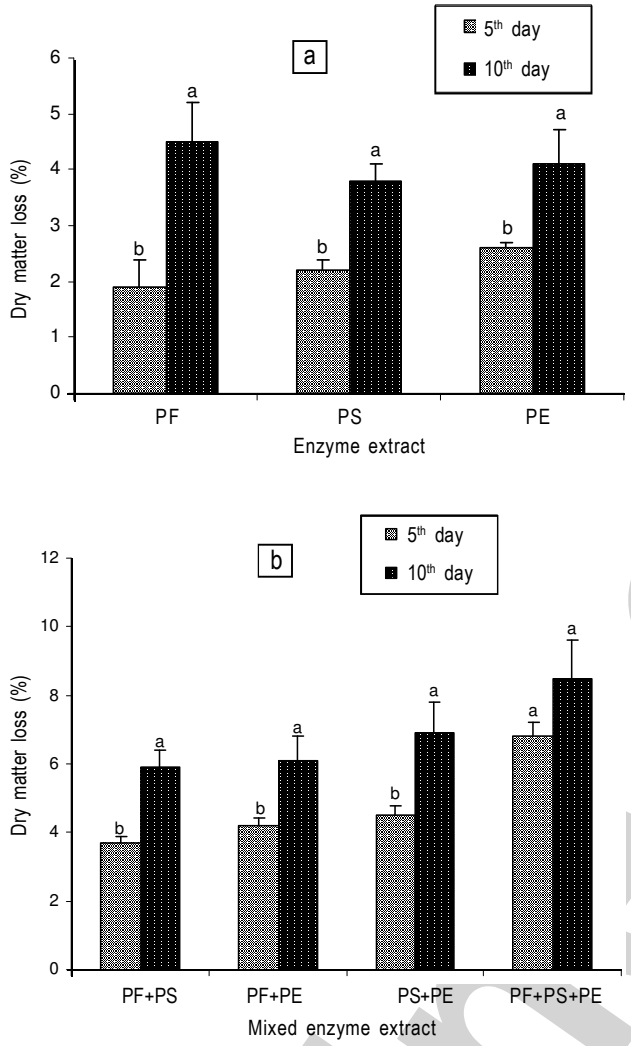

Fig. 3: Effect of different mono culture extracts (a) and different mixed (b) extracts on total dry matter loss of corn cob substrate. Treatments having different letters at error bars are statistically different at 5% probability level ($p<0.05$)

types of the extracellular enzymes produced include manganese peroxidase, laccase, cellulase, hemicellulases (Cohen *et al*., 2002) β-glucosidase and β-xylosidase which are responsible for biodegradation of different lignocellulosics.

The release of numerous extracellular enzymes under the conditions of submerged fermentation can be used in the hydrolysis of water insoluble cell wall components, namely lignin, cellulose and hemicellulose. It is also known that plant cell wall degradation requires the synergistic action of several enzymes. A number of extracellular enzymes capable of cellulolytic, hemicellulolytic and lignolytic activities have been reported (Rodriguez *et al*., 2008; Zyani *et al*., 2009; Naraian *et al.,* 2009b). Several of these enzymes may act synergistically in producing high reducing sugars (Garcia and Wood, 1993). Zadrazil (2000) noted that most of the fungi produced cellulase (CMC-ase) and hemicellulase (xylanase and β-xylosidase) for degradation which is quite obvious in our study.

Owing to the occurrence of the several isoenzymes, enzyme activity in crude supernatants is an effective reflection and role of the specific isoenzymes (Cohen *et al*., 2001). In the present study PF produced its highest CMC-ase and laccase activities while, PS produced highest xylanase and peroxidase activities. The results are in the agreement of Stajic *et al*. (2006) during production of laccase and peroxidase by selected *Pleurotus* spp. The production of higher laccase and MnP was also reported by Arora and Gill (2005) which has been attributed due to the presence of complete pool of amino acids in the medium.

Although our study resulted the low degradation of cell wall components but it can be correlated with the enzyme activities of the extracts. Similar attributions were also made by Novotny *et al*. (2004). As it was observed mono culture and mixed extracts used contained their highest CMC-ase, xylanase, Mn peroxidase and laccase activities. The extracts were employed for degradation studies having highest activities of these enzymes. Although these were different for different fungi, the mixed extracts were more effective to degrade cell wall components in comparison to the mono extracts. This might be due to the presence of various active isoenzymes in the extracts from different cultures working synergistically that has been previously suggested by the other workers also (Mansur *et al*., 2003; Thurston, 1994). To the date, five peroxidase genes have been isolated from *Pleurotus* spp. synthesizing different isoenzymes with multifunction (Cohen *et al*., 2001). Synergistic effects between enzymes are considered essential for efficient degradation of cell wall polysaccharides, and examples of such relationships have also been found by De Vries *et al*. (2000) and Bhat and Hazlewood (2001). Some enzymes systems contain a number of individual enzyme species which co-operate to degrade hydrogen bond ordered cellulose (Wood, 1992). Das *et al*. (2001) have reported the production of two laccase enzymes (L_1 and L_2) by *P. florida.* Our results suggest significant variability in the degradation of the cell wall components contrary to other observations (Camarero *et al*., 1994; Karunananda and Varga, 1996).

As compared with other cell wall components hemicellulose was one of the major components which were highly influenced with the mixed extract treatments. Although no alternate direct with enzyme extract treatments have been reported preferably for lignocellulosics, but this can be suggested that it could be due to the structural simplicity of the hemicellulose in comparison to cellulose and lignin. Moyson and Verachtert (1991) reported that *Pleurotus pulmonarius* and *Pleurotus sajor-caju* degraded hemicellulose simultaneously with lignin. The degradation of lignocellulosic structures is probably mediated by small, diffusible agents like the Fenton's reagents *viz*., lipid peroxides or Mn complexes formed by MnP (Wood, 1994; Valaskova and Baldrian, 2006).

Although lignin degradation mechanisms are relatively well known in several white-rot fungi cultivated as monocultures either in liquid cultures or solid substrate fermentations. However, very little is known about the biochemistry and enzymology of mixed fungal

cultures during wood decay (Chi *et al*., 2007).There are no published results till date regarding to the degradation of lignocellulosic substrates with enzyme extract rather direct decomposition with cultivation of fungi. Moreover, these enzymes are responsible for initial attack on the aromatic ring and resulted in the opening of ring which is a necessary step leading the mineralization of aromatic ring (Cohen *et al*., 2002).

Mixed extracts of different combinations in the study has effectively reduced the dry matter in comparison to the individual. Chi *et al*. (2007) postulated that co-cultivation of fungal species in three possible combinations enhanced weight loss of wood blocks; this effect was significant only in the co-culture of *C. subvermispora* with *P. ostreatus*, when compared to the respective monocultures.

Based on the results of the present study it is quite obvious that production of lignocellulolytic enzymes depends upon species. It has been observed that all the strains used were capable for producing major lignocellulolytic enzymes. In the present study *P. florida* PF05 was one of the species amongst the fungi tested, have produced highest enzyme activities and also efficiently degraded all cell wall components of the corn cob substrate. The mixed co culture extracts of three different species were most effective than monoculture extracts. The hemicellulose was the most affected cell wall component.

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References

Arora, D.S. and P.K. Gill: Production of lignolytic potential enzymes by *Phlebia floridensis*. *World J. Microbiol. Biotechnol.,* **21**, 1021-1028 (2005).

Bhat, M .K. and G.P. Hazlewood: Enzymology and other characteristics of cellulases and xylanases. *In*: Enzymes in farm animal nutrition (*Eds*.: M.R. Bedford and G.G. Partridge). CABI Publishing, Wallingford, Oxon, UK. pp. 11-60 (2001).

Camarero, S., G.C. Galleti and A.T. Martinez: Preferential degradation of phenolic lignin units by two white-rot fungi. *Appl. Environ. Microbiol*., **60**, 4506-4516 (1994).

Chi, Y., A. Hatakka and P. Maijala: Can co-culturing of two white-rot fungi increase lignin degradation and the production of lignin-degrading enzymes. *Int. Biodet. Biodeg*., **59**, 32-39 (2007).

Cohen, R., Y. Hadar and O.Yarden: Transcript and activity levels of different *Pleurotus ostreatus* peroxidases are differently affected by Mn^{2+}. *Environ. Microbiol*., **3**, 312-322 (2001).

Cohen, R., L. Persky and Y. Hader: Biotechnological applications and potential of wood degrading mushrooms of the genus *Pleurotus*. *Appl. Microbiol., Biotechnol*., **58**, 582-594 (2002).

Das, N., T.K. Chakraborty and M. Mukherjee: Purification and characterization of a growth-regulating laccase from *Pleurotus florida*. *J. Basic Microbiol*., **41**, 261-267 (2001).

De Vries, R.P., H.C.M. Kester, C.H. Poulsen, J.A.E. Benen and J. Visser: Synergy between accessory enzymes from *Aspergillus* in the degradation of plant cell wall polysaccharides. *Carbohydr. Res*., **327**, 401-410 (2000).

Eichlerova, I., L. Homolka and F. Nerud: Ability of industrial dyes decolorization and ligninolytic enzymes production by different Pleurotus species with special attention on *Pleurotus calyptratus*, strain CCBAS 461. *Process Biochem*., **41**, 941-946 (2006).

Elisashvili, V., E. Kachlishvili and M. Penninckx: Effect of growth substrate, method of fermentation and nitrogen source on lignocellulose-degrading enzymes production by white-rot basidiomycetes. *J. Ind. Microbiol. Biotechnol.,* **35**, 1531-1538 (2008).

Garcia, C.V. and T.M. Wood: Purification and characterization of a beta-D-xylosidase from the anaerobic rumen fungus *Neocallimastix frontalis*. *Carbohydr. Res*., **242**, 229-245 (1993).

Giardiana, P., G. Palmieri, B. Fontanella, V. Rivieccio and G. Sania: Mangnese peroxidase isoenzymes produced by *Pleurotus ostreatus* grown on wood sawdust. *Arch. Biochem. Biophys*., **376**, 171-176 (2000).

Glenn, J.K. and M.H. Gold: Purification and characterization of an extracellular Mn(II)-dependendent peroxidase from the lignin degrading basidiomycete *Phanerochaete chrysosporium*. *Arch. Biochem. Biophys*., **242**, 329-341 (1985).

Karunananda, K. and G.A. Varga: Colonization of crop residues by white-rot fungi (*Cyathus stercoreus*): Effect on retinal fermentation pattern, nitrogen metabolism, and fibre utilization during continuous culture. *Anim. Feed Sci. Technol*., **61**, 1-16 (1996).

Koroleva, O.V., E.V. Stepanova, V.P. Gavrilova, N.S. Yakovleva, E.O. Landesman, I.S. Yavmetdinov and A.I. Yaropolov: Laccase and Mn-peroxidase production by *Coriolus hirsutus* strain075 in a jar fermenter. *J. Biosci. Bioengg*., **93**, 449-455 (2002).

Maganhotto de Souza Silva, C.M., I. Soares de Melo and P. Roberto de Oliveira: Lignolytic enzyme production by *Ganoderma* species. *Enz. Microbiol. Technol*., **37**, 324-329 (2005).

Mansur, M., M.E. Arias, J.L. Copa-Patino, M. Flardh and A.E. Gonzalez: The white-rot fungus *Pleurotus ostreatus* secretes laccase isozymes with different substrate specificities. *Mycologia*, **95**, 1013-1020 (2003).

Mata, G., D.M. Murrieta Hernandez and L.G. Iglesias Andreu: Changes in lignocellulolytic enzyme activities in six *Pleurotus* spp. strains cultivated on coffee pulp in confrontation with *Trichoderma* spp. *World J. Microbiol. Biotechnol*., **21**, 143-150 (2005).

Miller, G. L.: Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*., **31**, 426-428 (1959).

Moyson, E. and H. Verachtert: Growth of higher fungi on wheat straw and their impact on the digestibility of the substrate. *Appl. Microbiol. Biotechnol.,* **36**, 421-424 (1991).

Naraian, R., R.K. Sahu, S. Kumar, S.K. Garg, C.S. Singh and R.S. Kanaujia: Influence of different nitrogen rich supplements during cultivation of *Pleurotus florida* on corn cob substrate. *Environmentalist*, **29**, 1-7 (2009a).

Naraian, R., N.K. Arora and S.K. Garg: Improved submerged fermentation of corn cob with mechanically broken oil seed cakes and decolorisation of textile dyes by enzyme extract of *Pleurotus florida* PF05. *Res. Environ. Life Sci*., **2**, 83-90 (2009b).

Novotny, C., K. Svobodova, P. Erbanova, T. Cajthaml, A. Kasinath, E. Lang and V. Sasek: Lignolytic fungi in bioremediation: Extracellular enzyme production and degradation rate. *Soil Biol. Biochem*., **36**, 1545-1551 (2004).

Panbangred, W., A. Shinmayo, S. Kinoshita and H. Okada: Purification and properties of endoxylanase produced by *Bacillus pumilus*. *Agric. Biol. Chem*., **47**, 957-963 (1983).

Peixoto-Nogueira, S.C., M. Michelin, J.H.A. Betini, J.A. Jorge, H.F. Terenzi and M.L.T.M. Polizli: Production of xylanase by aspergilli using alternative carbon sources: Application of crude extract on cellulose pulp biobleaching. *J. Ind. Microbiol. Biotechnol*., **36**, 149-155 (2009).

Ren, X. and G. Buschle-Diller: Oxidoreductases for modification of linen fibers, *Colloids Surf. A: Physicochem. Eng. Aspects*, **299**, 15-21 (2007).

Rodriguez, E., F.J. Ruiz-Dueans, R. Kooistra, A. Ram, A.T. Martinez and M.J. Martinez: Isolation of two laccase genes from the white-rot fungus *Pleurotus eryngii* and hetrologous expression of the pel3 encoded protein. *J. Biotechnol*., **134**, 9-19 (2008).

Online Copy

Sandhu, D.K. and D.S. Arora: Laccase production by *Polyporus sanguineus* under different nutritional and environmental conditions. *Experientia*, **41**, 355-356 (1985).

Sania, G., P. Limongi, E. Cocca, F. Buonocore, G. Nitti and P. Giardian: Purification and characterization of a varatryl alcohol oxidase enzyme from the lignin degrading basidiomycete *P. ostreatus*. *Biochem. Biophys. Acta*, **73**, 114-119 (1991).

Stajic, M., L. Persky, D. Friesem, Y. Hadar, S.P. Wasser, E. Nevo and J. Vukojevic: Effect of different carbon and nitrogen sources on laccase and peroxidases production by selected *Pleurotus* species. *Enzyme Micrbiol. Technol.*, **38**, 65-73 (2006).

Tellez-Tellez, M., F.J. Fernandez, A.M. Montiel-Gonzalez, C. Sanchez and G. Diaz-Gondinez: Growth and laccase production by *Pleurotus ostreatus* in submerged and solid state fermentation. *Appl. Microbiol. Biotechnol.*, **81**, 675-679 (2008).

Thurston, C.F.: The structure and function of fungal laccases. *Microbiol.*, **140**, 19-26 (1994).

Valaskova, V. and P. Baldrian: Degradation of cellulose and hemicelluloses by the brown rot fungus *Piptoporus betulinus*-production of extracellular enzymes and characterization of the major cellulases. *Microbiol.*, **152**, 3613-3622 (2006).

Van-Soest, P. J. and R.H. Wine: Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *J. Ass. Offic. Anal. Chem.*, **50**, 50-56 (1967).

Wood, T.M.: Properties of cellulolytic enzyme systems**.** *Biochem. Soc. Trans.,* **13**, 407-410 (1985).

Wood, T.M.: Microbial enzymes involved in the degradation of the cellulose component of plant cell walls. *In*: Rowett Research Institute. Annual Report., 10-24 (1992).

Wood, T.M.: Enzymatic conversion of cellulose into D-glucose. *In*: Methods in Carbohydrate Chemistry (*Ed.*: N.B. James). John Wiley and Sons, New York, USA. pp. 219-230 (1994).

Zadrazil, F.: Is conversion of ligninocellulosics into feed with white-rot fungi realizable? Practical problems of scale-up and technology transfer. *Mushroom Sci.*, **15**, 919-928 (2000).

Zyani, M., D. Mortabit, M. Mostakim. M. Iraqui, A. Haggoud, M. Ettayebi and S.I. Koraichi: Cellulolytic potential of fungi in wood degradation from an old house at the Medina of Fez. *Annal. Microbiol.*, **59**, 699-704 (2009).

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