

Full Length Research Paper

Production and optimization of ligninolytic enzymes by white rot fungus *Schizophyllum commune* IBL-06 in solid state medium banana stalks

Muhammad Irshad^{1,2*} and Muhammad Asgher¹

¹Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan.

²Department of Biochemistry, NSMC, University of Gujrat, Pakistan.

Accepted 17 October, 2011

The present study deals with production of ligninolytic enzymes from an indigenous white rot fungus *Schizophyllum commune* IBL-06 by using banana stalk as substrate through the process of solid state fermentation. The production process was further improved by optimizing a number of physical parameters such as incubation time, moisture level, pH, temperature, inoculums size and nutritional factors (carbon and nitrogen ratio, mediators and metal ions). By optimization of different parameters, the maximum activities of enzymes synthesized by *S. commune* IBL-06 were observed as 3745 IU/ml for manganese peroxidase (MnP), 2700 IU/ml for lignins peroxidase (LiP) and 345 IU/ml for laccase after 3 days incubation at pH 4.5 and 35°C temperature with 3 ml inoculum size, 60% moisture content, 20:1 C:N ratio (glucose and ammonium nitrate as carbon and nitrogen supplements), 1ml of 1mM MnSO₄ as mediator, and 1ml of 1mM MgSO₄·7H₂O₂. This High activities of ligninolytic enzymes produced by the fungus suggest its potential for commercial scale production of these enzymes for diverse industrial applications.

Key words: *Schizophyllum commune* IBL-06 (*S. commune* IBL-06), ligninases, manganese peroxidase (MnP), lignin peroxidase (LiP).

INTRODUCTION

White rot fungi (WRF) are capable of degrading recalcitrant environmental pollutants such as textile dyes, polyaromatic hydrocarbons, polychlorophenols and polychlorinated biphenyls (Asghar et al., 2006). White rot fungi have a complex enzymatic machinery that enables the fungi to degrade lignin. This is achieved by excreting extracellular polyphenol oxidases particularly lignin peroxidases, manganese peroxidases and laccases which are effective in degrading lignin (Revankar and Lele, 2006).

Manganese peroxidases have been found in most WRF studied till today. Some WRF even secrete MnP as a sole ligninolytic enzyme for lignin degradation. MnP is a heme containing glycoprotein which requires hydrogen

peroxide (H₂O₂) as well as Mn⁺² ions for its activities (Asghar et al., 2008; Bermek et al., 2004). MnP oxidizes a wide range of substrates, including several phenolic compounds, high molecular weight chlorolignins and nylon, rendering the enzyme an interesting biocatalyst for potential applications in various industries such as pulp and paper pulp and paper industry and textile industry where it is utilized it is utilized to degrade the pollutants (Bermek et al., 2004).

Lignin peroxidases are glycosylated, heme containing enzymes which functionally require H₂O₂ for the oxidation of lignin related aromatic structures (Asgher et al., 2006; Papinutti and Forchiassin, 2007). LiPs are strong oxidizers capable of catalyzing the oxidation of phenols, aromatic amines, aromatic ethers and polycyclic aromatic hydrocarbons (Tien and Kirk, 1998).

Laccase is a dimeric or tetrameric glycoprotein containing four copper atoms which are distributed in redox sites and has the advantage of not needing H₂O₂ for substrate oxidation, which makes the enzyme to have a

*Corresponding author. E-mail: muhammad.irshad@uog.edu.pk

Abbreviations: MnP, Manganese peroxidase; LiP, lignins peroxidases.

broader application spectrum than peroxidases (Mishra and Kumar, 2007).

LiPs, MnPs and laccases have been applied to numerous processes such as pulp delignification, oxidation of organic pollutants, stabilization of fruit juices, biosensor development, biofuels cells, textile biofinishing, environmental protection processes, beverage processing, animal feed stuffs, biobleaching systems, cosmetics, enzyme immunoassays, wastewater detoxification, denim stone washing, detergent manufacturing and transformation of antibiotics and steroids (Tien and Kirk, 1998; Ryan et al., 2003; Boer et al., 2006; Papinutti and Forchassin, 2007; Ravankar and Lele, 2007; Asghar et al., 2008).

Several lignocellulosic materials are efficient substrates for white-rot fungi, which produce industrially important ligninolytic and cellulolytic enzymes (Reddy et al., 2003). Among processes used for enzyme production, solid-state fermentation (SSF) using agro-wastes is an attractive and cost effective option because it presents higher productivity involving a simpler operation, when compared with submerged cultures (SmF) (Pointing, 2001).

One of the important aspects of *Schizophyllum commune* IBL-06 relates to the use of its ligninolytic potential. In this study a lignocellulosic substrate banana stalk was employed as solid substrate for ligninolytic enzymes production by *S. commune* IBL-06 in SSF.

MATERIALS AND METHODS

Inoculum development

To prepare the inoculum, *S. commune* IBL-06 was grown in a liquid medium (pH 4.5) containing (g/L): glucose 2; $MgSO_4 \cdot 7H_2O$, 0.05; $CaCl_2 \cdot 2H_2O$, 0.1; NH_4Cl , 0.12 and thiamine, 0.001 (Asghar et al., 2006). The inoculum flask was autoclaved at 121°C (15 lb) for 15 min and inoculated with a loopful of the fungus transferred aseptically from the PDA slant under sterile conditions.

Solid state fermentation of banana stalk

Erlenmeyer flasks (used in triplicates) contained 5 g of the lignocellulosic substrate banana stalk. The moisture substrates content was increased to 60% (w/w) by adding 7.5 ml of Kirk's basal medium (pH 4.5) containing glucose as carbon source and NH_4NO_3 as nitrogen source in 20:1 C:N ratio; 1 mM $MnSO_4$, 1 ml; and 1.25 mM $MgSO_4 \cdot 7H_2O$, 1 ml (Tien and Kirk, 1988). The flasks were sterilized in a laboratory scale autoclave, allowed to cool at room temperature and inoculated with 3 ml of the homogenous inoculum of *S. commune* IBL-06. The inoculated flasks were allowed to ferment at 35°C for 5 days in an incubator (Sanyo, Japan).

Crude enzyme recovery

Samples were taken after every 48 h. To the fermented solid biomass, 100 ml of 50 mM sodium malonate buffer (pH 4.5) was added and the flasks were shaken (120 rpm) for half an hour. The

contents were filtered through a Whatman filter paper (No.1, 125 mm) and residues were discarded. The filtrates were centrifuged (3,000 ×g, 10 min, 4°C) to remove the fungal pellets and the recovered supernatants were used for quantifying activities of LiP, MnP and laccase.

Ligninolytic enzymes assays

Manganese peroxidase assay

Manganese peroxidase activity was determined by the method of Wariishi et al. (1992). $MnSO_4$ was added to the enzyme extract in sodium malonate buffer in the presence of H_2O_2 . Manganic ions (Mn^{+3}) form a complex with melonate which absorbs at 270 nm (ϵ_{270} 11570 $M\text{cm}^{-1}$).

LiP assay

The lignin peroxidase was assayed by the method of Tien and Kirk (1988). The rate of oxidation of veratryl alcohol to veratraldehyde was monitored in 100 mM tartarate buffer of pH 3 in the presence of H_2O_2 .

Laccase assay

Laccase activity was determined by monitoring the rate of 2,2-azinobis (3-ethylbenzthiazoline)-6 sulphonate (ABTS) oxidation by the culture supernatants (Shin and Lee, 2000) at pH 4.5 and 35°C temperature in 50 mM malonate buffer of pH 4.5. The oxidation was followed at 420 nm (ϵ_{420} 36000 $M\text{cm}^{-1}$).

RESULTS

The indigenous white rot fungus *S. commune* IBL-06 was used for the production of ligninases in solid state fermentation of a lignocellulosic substrate banana stalk. Different experiments were conducted for optimization of parameters such as time period, medium composition, pH, temperature, inoculum size, moisture content, different carbon and nitrogen sources, carbon : nitrogen ratio, surfactants and mediators to maximize ligninases production by *S. commune* using banana stalk as substrate and Kirk's medium in solid state cultures.

Effect of incubation time

After every 48 h, the triplicate SSF flasks were harvested and culture supernatants were analyzed for ligninolytic enzymes and dry weight of biomass (residue) was also recorded. The results of time course study showed that maximum production of MnP (1017 IU/ml), LiP (744 IU/ml) and laccase (75 IU/ml) was achieved in 8 days of SSF of banana stalk by *S. commune* IBL-06. As the incubation time increased, the biomass weight also increased. It was observed that ligninases production steadily increased with an increasing fermentation time and further increase in fermentation time showed a

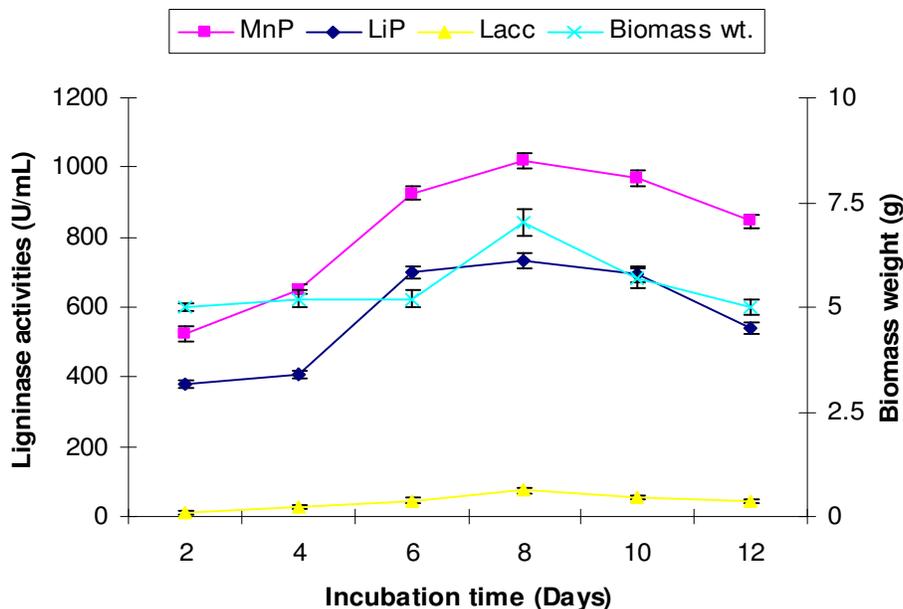


Figure 1. Effect of incubation time on production of ligninases and biomass by *S. commune* IBL-06.

decrease in ligninases activities (Figure 1). It was also important to note that *S. commune* IBL-06 produced MnP (1017 IU/ml) as the major enzyme activity, followed by LiP (744 IU/ml) and laccase (75 IU/ml).

Optimization of initial pH of the medium

The initial time course experiment was run using pH 5 medium of banana stalk. To optimize pH for maximum enzyme production, media adjusted to varying pH were used. The maximum MnP (1125 IU/ml), LiP (783 IU/ml) and laccase (197 IU/ml) activities were observed in the medium of pH 4.5. Consistent with the enzyme activities, the biomass dry weight also increased by increasing the medium pH and reached its maximum (8.99 g) at pH 4.5. It was noted that ligninases production steadily increased with an increase in initial medium pH from 3.0 to 4.5 and further pH increase showed a decrease in ligninase production (Figure 2).

Optimization of incubation temperature

To optimize the temperature for growth and ligninase production by *S. commune* IBL-06, the triplicate flasks of optimum pH were incubated at varying temperatures. The maximum activities of MnP (1445 IU/ml), LiP (987 IU/ml) and laccase (128 IU/ml) were produced in the flasks incubated at 35°C. The dry weight of biomass also increased with the temperature and peaked at 35°C. A further increase in temperature caused a decreased in biomass weight and enzymes synthesis by the fungus (Figure 3).

Optimization of moisture level

Substrate was moistened with different volume of selected M-II medium to varying moisture levels before inoculation. With an increase in the moisture content, up to 60% (w/w), the fungal growth and ligninase production by *S. commune* IBL-06 increased. With 60% moisture, optimum activities of MnP (1531 IU/ml), LiP (1109 IU/ml) and laccase (176 IU/ml) were produced. A further increase in the moisture content of banana stalk repressed fungal growth and enzyme production (Figure 4).

Optimization of carbon : nitrogen ratio

After selection of best carbon and nitrogen sources, the effect of varying C : N ratio on ligninase production by *S. commune* IBL-06 in banana stalk medium under optimum conditions was studied. Maximum activities of LiP (2087 IU/ml), MnP (2917 IU/ml) and laccase (340 IU/ml) were produced in the medium having C:N ratio of 20:1. It was observed that ligninases production steadily increased with an increase in C : N ratio from 5:1 to 20:1 and with further increase after optimum point, there was a decrease in enzyme activities and fungal biomass weight (Figure 5).

Effect of mediators

Veratryl alcohol, MnSO₄, oxalate, ABTS and H₂O₂ act as mediators of different enzymes of white rot fungi. The mediators were used to enhance ligninase production by

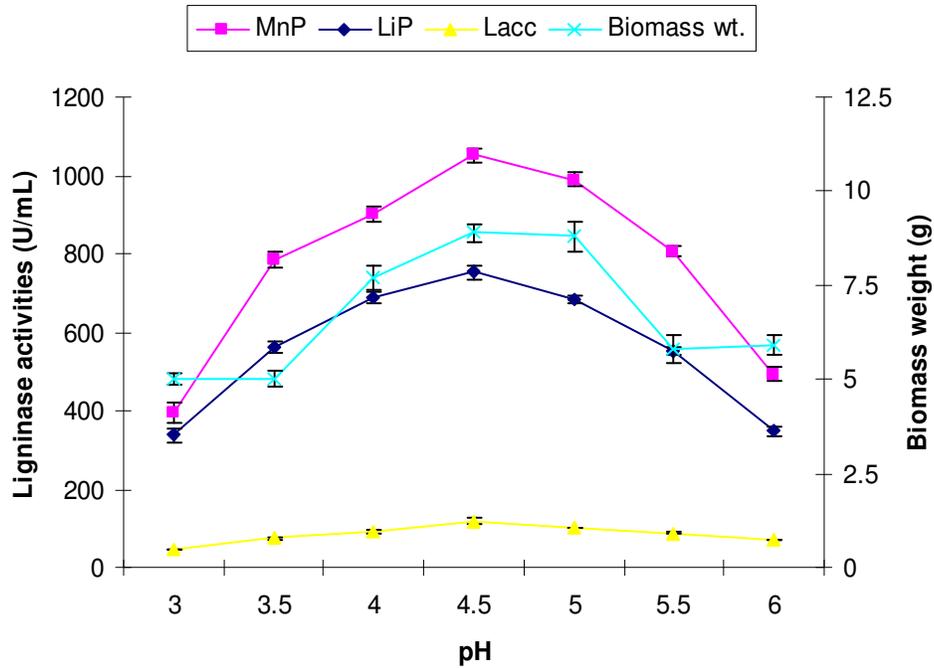


Figure 2. Effect of pH on ligninase production and biomass weight produced by *S. commune* IBL-06.

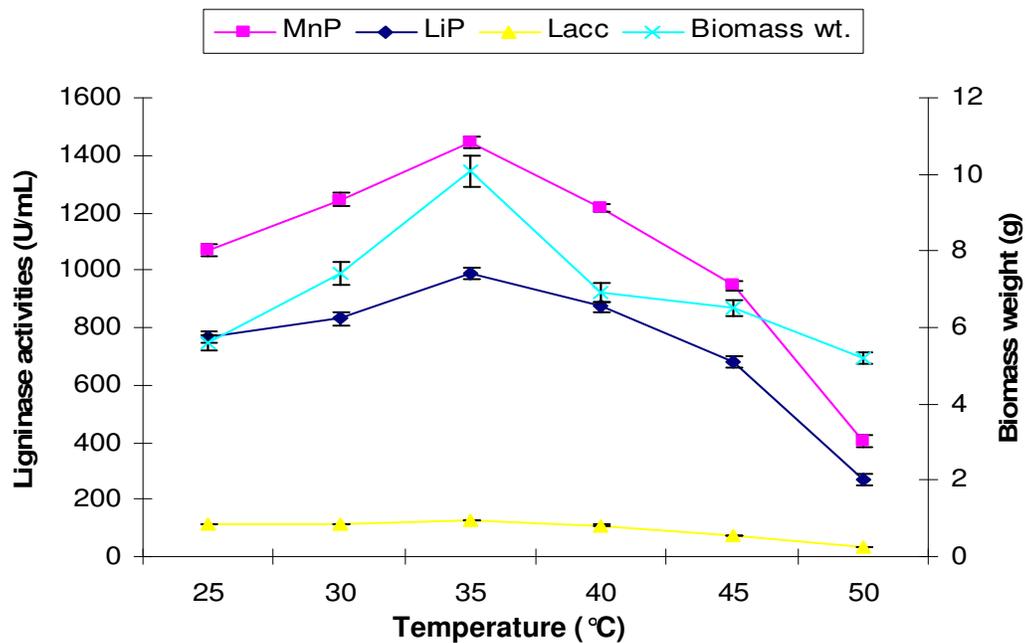


Figure 3. Effect of varying temperature on ligninase production and biomass weight produced by *S. commune* IBL-06.

S. commune IBL-06 in pre-optimized fermentation medium of banana stalk. It was observed that all mediators had stimulatory effects of ligninase production by the fungus but their effects on different enzymes were highly variable. MnSO₄, vartraryl alcohol and ABTS were

more effective for MnP, LiP and laccase production, respectively. Oxalate and H₂O₂ had almost similar impact on LiP and MnP production (Figure 6). However, as MnP was the enzyme produced in higher activities as compared to other two enzymes, MnSO₄ was selected as

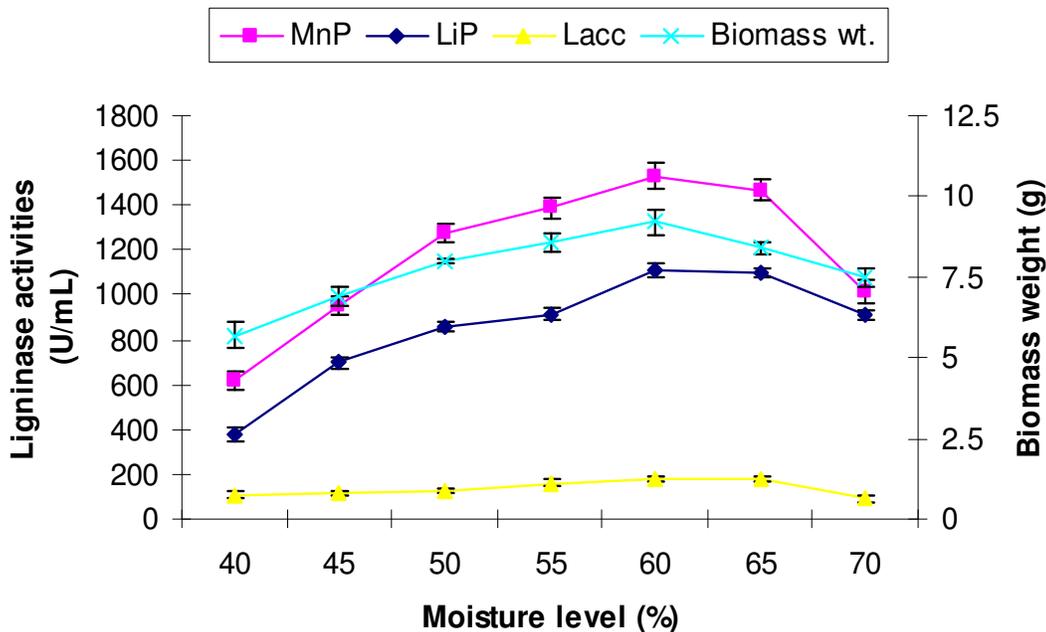


Figure 4. Effect of varying moisture level on ligninases and biomass production by *S. commune* IBL-06.

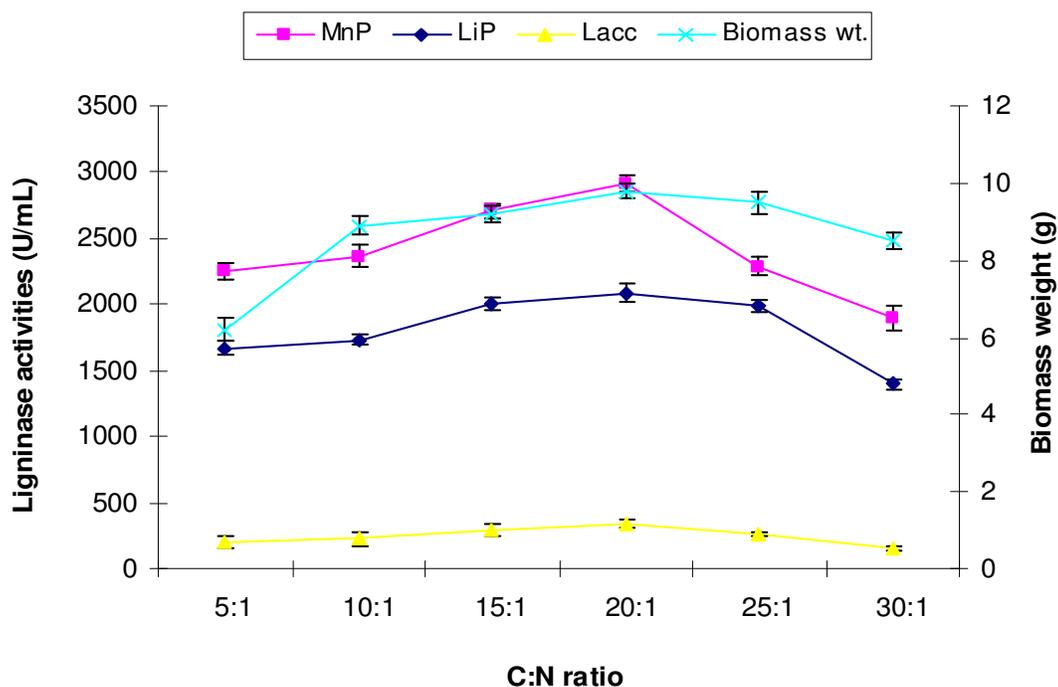


Figure 5. Effect of varying C : N ratios on ligninase and biomass production by *S. commune* IBL-06.

the best mediator for ligninase production.

Effects of metals ions

Various metal ions were added in the form of their salts

into the optimum banana stalk medium to investigate their influence on ligninase production under optimum conditions (moisture, 60%; pH, 4.5; temperature, 25°C; inoculum size, 3 ml; carbon source, glucose; nitrogen source, (NH₄)₂NO₃; incubation time, 3 days; C : N ratio, 20:1; mediator, 1 mM MnSO₄ (1 ml).

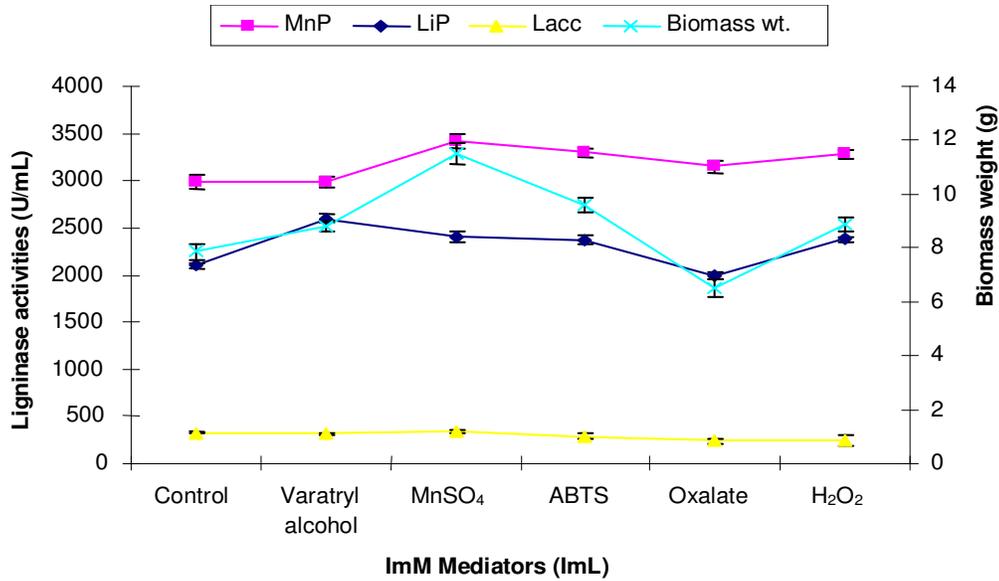


Figure 6. Effect of different mediators on ligninases and biomass production by *S. commune* IBL-06.

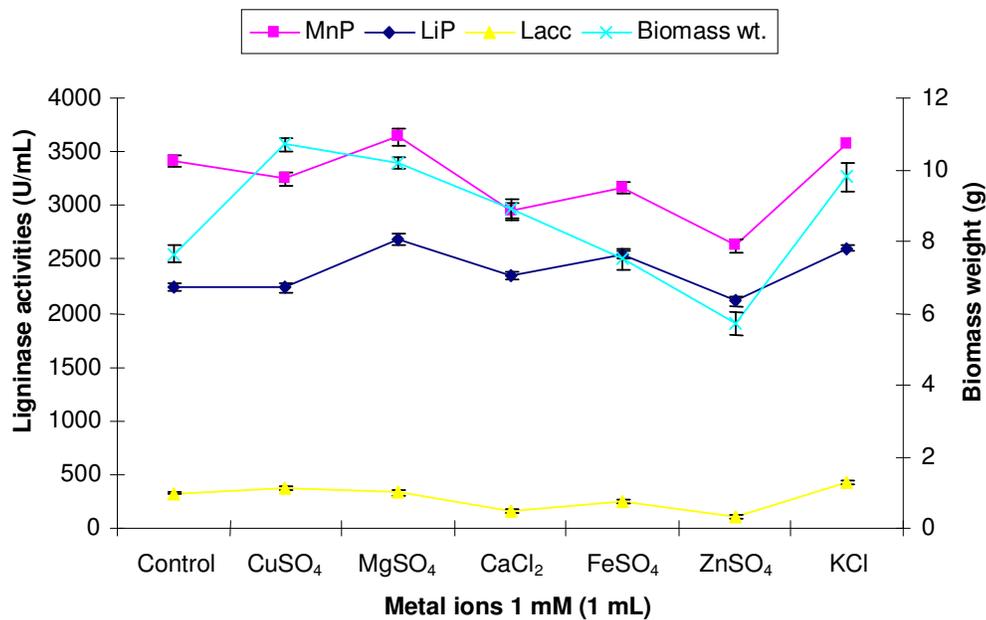


Figure 7. Effect of metal ions on ligninases and biomass production by *S. commune* IBL-06.

The optimum production of MnP (3637 IU/ml), LiP (2688 IU/ml) and laccase (331 IU/ml) was noted in the medium that received 1 ml of 1 mM MgSO₄ as shown in Figure 7.

06. It was noted that 1.25 mM MgSO₄.7H₂O showed maximum production of MnP (3714 IU/ml), LiP (2745 IU/ml) and laccase (376 IU/ml) in 3 days under all optimum conditions (Figure 8).

Optimization of magnesium sulphate concentration

Different concentrations of magnesium sulphate were used to select the most suitable concentration for enhanced production of ligninases by *S. commune* IBL-

DISCUSSION

Different white rot fungi have been reported to produce maximum ligninolytic enzymes after different time periods

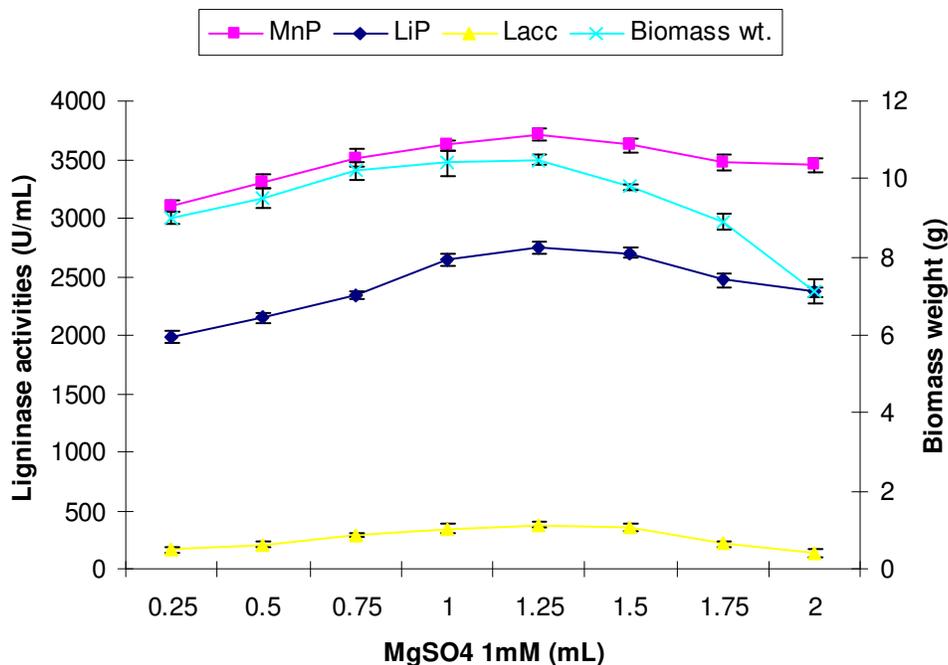


Figure 8. Effect of varying concentrations of MgSO₄ on ligninases and biomass production by *S. commune* IBL-06.

due to genetic variation among the strains as well as nature and composition of the substrates used (Heinzkill et al., 1998; Giardina et al., 2000; Patel et al., 2009). *Termitomyces clypeatus*, a white rot fungus was found to produce maximum MnP activity after 6 days of incubation using lignocellulosic substrates (Bose et al., 2007). MnP enzyme produced by *Phanerochaete chrysosporium* peaked on the 7th day of cultivation (Zahamatkesh et al., 2010) and white-rot fungus *Datronia* sp. KAPI0039 produced maximum laccase and MnP after 4 and 8 days of cultivation, respectively (Vaithanomsat et al., 2010).

Different microbial strains show maximum cell growth and metabolic activities under different pH conditions. Optimal pH was found between 4.5 and 5.0 for production of ligninolytic enzymes by *Phanerochaete sordida* grown in milled wood lignins extracted in solid state medium (Ruttimann et al., 2008). The lignolytic enzymes production (LiP and MnP) by *Pleurotus ostreatus* in the medium containing liquid culture aflatoxin as substrate was also maximum at pH 4 (Motomura et al., 2003). WRF in most of the cases have shown optimum mycelial growth and had higher activities of ligninolytic enzymes at pH 3 to 6 (Xu, 1996; Radha et al., 2005; Shin and Lee, 2000; Motomura et al., 2003; Yamanaka et al., 2008).

A variation in incubation temperature has a significant influence on synthesis of ligninolytic enzymes and their activities. The temperatures ranging from 25 to 37°C have been found to be optimum for ligninase production by different WRF (Zadrazil et al., 1999; Arora and Gill 2001; Tekere et al., 2001; Tripath et al., 2008). Higher

temperatures denature the metabolic enzymes of microorganisms leading to inhibition of growth and enzyme formation. The change in temperature may also affect fatty acid synthesis and membrane fluidity (Vyas et al., 1994; Tripath et al., 2008). Shin et al. (1997) observed rapid enzyme inactivation when *P. ostreatus* was grown at above 35°C temperatures.

In SSF, microbial growth occurs on or near the surface of the solid and optimum moisture level in SSF is governed by the water holding capacity of the substrate and water requirements of the fungus (Asgher et al., 2006). Higher and lower water contents adversely affect the primary metabolic activities of microbes causing lower ligninase production in secondary growth (Rodriguez et al., 1998; Raghavarao et al., 2003; Regina et al., 2008). Low moisture contents may also result in reduced solubility of nutrients, lower substrate swelling and higher water tension (Lonsane et al., 1992). In a recent study, the maximum laccase yield was obtained by *Ganoderma* sp. using wheat bran as substrate with 70% initial moisture content (Revankar et al., 2007).

The effects of C : N ratio was more pronounced as compared to carbon and nitrogen sources. Increase in C : N ratio caused decrease in biomass weight suggesting inhibition of fungal growth by increasing the nitrogen content. Carbon and nitrogen are critical nutritional variables in the production of LiP and MnP by WRF. Excess carbon and nitrogen repress ligninolytic activities of WRF (Pascal et al., 1991). At low C : N ratios, the fungi are carbon starved and under high-nitrogen conditions, the ligninase production is considerably reduced

(Xiaoping and Xin, 2008). On the other hand, at higher C : N ratios, an imbalance between very high carbon and very low nitrogen content of the medium, leads to fungal growth inhibition.

The natural fungal secondary metabolites veratryl alcohol (VA) acts as redox mediator of LiP (Christian et al., 2005) that can also enhance its production by WRF. However, the main function of veratryl alcohol is protection of LiP from inactivation by hydrogen peroxide and veratryl alcohol is not an inducer of the ligninolytic system (Kapich et al., 2004). Enzyme activities produced by *P. chrysosporium* cultures in the absence of veratryl alcohol were lower than in the presence of veratryl alcohol (Ferrara et al., 2002). Mn²⁺ performs the role of mediator for MnP. MnP catalyzes the oxidation of Mn²⁺ to Mn³⁺ that forms complex with oxalate and other chelators that enhance the activity of MnP (Makela et al., 2005; Sundramoorthy et al., 2005). MnP production by *Lentinula edodes* was enhanced by supplementing the on corn cob solid state cultures with 5 mM MnSO₄ (Boer et al., 2006). MnSO₄ has also previously been reported to increase the production of MnP by 2304 nkat/L, 1972 U/ml and 356 U/L (Rogalski et al., 2006; Asgher et al., 2010; Urek and Pzarlioglu, 2007) using different substrates in LSF and SSF. ABTS performs the role of laccase mediator in degradation of phenolic components of lignin and a range of phenolic pollutants

Different metal ions can enhance or inhibit the growth, cause morphological and physiological changes and may affect the reproduction of WRF (Wuyep et al., 2003). Different strains and species of WRF differ in their sensitivity towards metals during their growth on lignocellulosic substrates (Sathiya-Moorthi et al., 2007). In a recent study, the MnP production by the WRF strain *L-25* was enhanced by the addition of Mn⁺² (Fujihara et al., 2010).

WRF require essential metal ions such as Mg²⁺, Ca²⁺, Mn²⁺, Zn²⁺ or Cu³⁺ as cofactors/prosthetic groups of different metabolic enzymes but these metals are toxic when present in excess (Srinivasan and Murthy, 2000). Ligninase production by *Lentinus squarrosulus* and *Psathyrella atroumbonata* was enhanced by 2 to 12 fold after addition of Mn⁺² and Ca⁺² to the lignocellulosic waste medium (Wuyep et al., 2003).

Conclusions

S. commune IBL-06 produced high activities of MnP and LiP with lower laccase activities. as compared to previously reported for different white rot fungi.

In future studies, the activities and thermo stabilities of the enzymes can be improved by immobilization of individual enzymes using different solid supports or by entrapment in hydrophobic gels, making them more suitable catalysts for industrial applications. Immobilization is preferred than all other techniques because immobilization modifies the activity, selectivity and

equipped permanence of enzymes.

ACKNOWLEDGEMENTS

The manuscript is based on findings of a research project funded by Higher Education Commission, Islamabad, Pakistan. The financial support provided by HEC and UAF for completion of the PhD research is highly acknowledged.

REFERENCES

- Arora SD, Gill KP (2001). Effects of various media and supplements on laccase production by some white rot fungi. *Biores. Technol.* 77: 89-91.
- Asghar M, Asad MJ, Legge RL (2006). Enhanced lignin peroxidase synthesis by *Phanerochaete chrysosporium* in solid state bioprocessing of a lignocellulosic substrate. *World J. Microbiol. Biotechnol.* 22: 449-453.
- Asgher M, Kausar S, Bhati HN, Shah SAH, Ali M (2008). Optimization of medium for decolorization of Solar golden yellow R direct textile dye by *Schizophyllum commune* IBL-06. *Int. Biodeter. Biodegr.* 61(2): 189-193.
- Asgher M, Sharif Y, Bhati HN (2010). Enhanced Production of Ligninolytic Enzymes by *Ganoderma lucidum* IBL-06 using Lignocellulosic Agricultural Wastes. *Inter. J. Chem. Reactor Eng.* 8: 1-17.
- Bermek H, Yazici H, Ozturk H, Tamerler C, Jung HLiK, Brown KM, Ding H, Xu F (2004). Purification and characterization of manganese peroxidase from wood-degrading fungus *Trichophyton rubrum*. *Enz. Microbiol. Technol.* 35: 87-92.
- Boer GC, Obici L, De Souza CGM, Peralta RM (2006). Purification and some properties of Mn peroxidase from *Lentinula edodes*. *Proc. Biochem.* 41: 1203-1207.
- Bose S, Mazumder S, Mukherjee M (2007). Laccase production by the white-rot fungus *Termitomyces clypeatus*. *J. Basic Microbiol.* 47: 127-131.
- Christian V, Shrivastava R, Shukla D, Modi H, Rajiv B, Vyas M (2005). Mediator role of veratryl alcohol in the lignin peroxidase-catalyzed oxidative decolorization of Remazol Brilliant Blue R. *Enzyme Microb. Technol.* 36: 426-431.
- Ferrara MA, Bon EP, Araujo Neto JS (2002). Use of steam explosion liquor from sugar cane bagasse for lignin peroxidase production by *Phanerochaete chrysosporium*. *Appl. Biochem. Biotechnol.* 98: 289-300.
- Fujihara S, Hatashita M, Sakurai A, Sakakibara M (2010). Production of manganese peroxidase by white rot fungi from potato-processing wastewater: Role of amino acids on biosynthesis. *Afr. J. Biotechnol.* 9(5): 725-731.
- Giardina P, Palmieri G, Fontanella B, Rivieccio V, Sannia G (2000). Manganese peroxidase isoenzymes produced by *Pleurotus ostreatus* grown on wood sawdust. *Arch. Biochem. Biophys.* 376: 171-179.
- Heinzkill M, Bech L, Halkier T, Scheider F, Anke T (1998). Characterization of laccases and peroxidases from wood rotting fungi. *Appl. Environ. Microbiol.* 64: 1601-1606.
- Kapich AN, Prior BA, Botha A, Galkin S, Lundell T, Hatakka A (2004). Effect of lignocellulose-containing substrate on production of ligninolytic peroxidase in submerged cultures of *Phanerochaete chrysosporium* ME-446. *Enzyme Microb. Technol.* 34: 187-195.
- Lonsane BK, Ghildyal NP, Budiartman S, Ramakrishna SV (1992). "Engineering aspects of solid state fermentation." *Enzyme Microb. Technol.* 7: 258-265.
- Makela MR, Galkin S, Hatakka A, Lundell TK (2005). Production of organic acids and oxalate decarboxylase in lignin-degrading white rot fungi. *Enzyme Microb. Technol.* 30: 542-549.
- Motomura K, Toyomasy T, Mizuno K, shinozawa T (2003). Purification and characterization of an aflatoxin degradation enzyme from

- pleurotus ostreatus*. Microbiol. Res. 158: 237-242.
- Papinutti VL, Forchiassin F (2007). Lignocellulolytic enzymes from *Fomes sclerodermeus* growing in solid-state fermentation. J. Food Eng. 8: 54-59.
- Pascal B, Perez J, Jeffries TW (1991). Regulation of Ligninase Production in White-Rot Fungi. Enzymes in biomass conversion, Proceedings of a symposium. Boston, MA. ACS symposium series 460. Am. Chem. Soc. p. 6.
- Patel H, Gupte A, Gupte S (2009). Effect of different culture conditions and inducers on production of laccase by a basidiomycete fungal isolate *Pleurotus ostreatus* HP-1 under solid state fermentation. Bioresource, 1: 268-284.
- Pointing SB (2001). Feasibility of bioremediation by white-rot fungi. Appl. Microbiol. Biotechnol. 57: 20-33.
- Radha KV, Regupathi I, Arunagiri A, Murugesan T (2005). Decolorization studies of synthetic dyes using *Phanerochaete chrysosporium* and their kinetics. Proc. Biochem. 40: 3337-3345.
- Raghavarao KSMS, Ranganathan TV, Karanth NG (2003). Some engineering aspects of solid-state fermentation. Biochem. Eng. J. 13: 27-135.
- Reddy GV, Babu PR, Komaraiah P, Roy KR, Kothari IL (2003). Utilization of banana waste for the production of lignolytic and cellulolytic enzymes by solid substrate fermentation using two *Pleurotus species* (*P. ostreatus* and *P. sajor-caju*). Proc. Biochem. 38: 1457-1462.
- Regina M, Broetto, Giovannozzi-Sermanni FG, Marabotini R, Peranni C (2008). "Influence of stationary and bioreactor cultivation on *Lentinula edodes* (Berk) Pegler lignocellulolytic activity." Braz. Arch. Biol. Technol. 51: 223-233.
- Revankar M, Desai S, Lele KM (2007). Solid-state fermentation for enhanced production of laccase using indigenously isolated *Ganoderma* sp. Appl. Biochem. Biotechnol. 143: 16-26.
- Rodriguez A, Falcon MA, Carnicero A, Perestlo F, Fuent GD, Trojanowski J (1998). Laccase activities of *Penicillium chrysogenum* in relation to lignin degradation, Appl. Microbiol. Biotechnol. 45: 399-403.
- Rogalski J, Szczodrak J, Janusz G (2006). Manganese peroxidase production in submerged cultures by free and immobilized mycelia of *Nematoloma frowardii*. Bioresour. Technol. 97(3): 469-476.
- Ruttimann JC, Salas L, Vicuna R, Kirk TK (2008). Extracellular enzyme production and synthetic lignin mineralization by *Ceriporiopsis subvermispora*. Appl. Environ. Microbiol. 59: 1792-1797.
- Ryan S, Schnitzhofer W, Tzanov T, Cavaco-Paulo A, Gubitzl GM (2003). An acid-stable laccase from *Sclerotium rolfsii* with potential for wool dye decolorization. Enzyme Microbiol. Technol. 33: 766-774.
- Sathiyamoorthi P, Periyar-selvam S, Sasikalaveni A, Murugesan K, Kalaichelvan (2007). Decolorization of textile dyes and their effluents using white rot fungi. Afr. J. Biotechnol. 6(4): 424-429.
- Shin KS, Lee YJ (2000). Purification and characterization of a new member of laccase family from the white-rot basidiomycete *Coriolus hirsutus*. Arch. Biochem. Biophys. 384: 109-115.
- Srinivasan SV, Murthy DVS (2000). Removal color from waste water using *Trametes versicolor*. J. IAEM. 27: 260-264.
- Sundramoorthy M, Yougs HL, Gold MH, Poulos TL (2005). High resolution crystal structure of manganese peroxidase substrate and inhibitor complexes. Biochemistry, 44: 6463-6470.
- Tekere M, Zvauya R, Read JS (2001). University of Zimbabwe, Biochem. Basic. Microbiol. 41(2): 115-129.
- Tien M, Kirk TK (1988). Lignin degradation *Phanerochaete chrysosporium*, characterization and catalytic properties of unique H₂O₂ requiring oxygenase. Proc. Nat. Acad. Sci. USA. 81: 2280-2284.
- Tien M, Kirk TK (1998). Lignin peroxidase of *Phanerochaete chrysosporium*. Methods Enzyme 161: 238-248.
- Tripathi M, Mishra KAS, Misra AS, Vaithyanathan S, Prasad R, Jakhmola RC (2008). Selection white rot basidiomycetes for biocconversion of mustard (*Brassica campestris*) straw under SSF into energy substrate for rumen microorganism. Lett. Appl. Microbiol. 46(3): 364-370.
- Urek RO, Pzarlioglu NK (2007). Enhanced production of manganese peroxidase by *Phanerochaete chrysosporium*. Braz. Arch. Boil. Technol. 50: 522-528.
- Vaithanomsat P, Apiwatanapiwat W, Petchoy O, Chedchant J (2010). Production of Ligninolytic Enzymes by White-Rot Fungus *Datronia* sp. KAPI0039 and their application for reactive Dye Removal. Int. J. Chem. Eng. 162(504): 1-6.
- Vyas BRM, Volc J, Sasek V (1994). Effects of temperature on the production of manganese peroxidase and lignin peroxidase by *Phanerochaete chrysosporium*. Folia Microbiol. 39: 19-22.
- Wariishi H, Valli K, Gold MH (1992). Manganese (II) oxidation by manganese peroxidase from basidiomycete *Phanerochaete chrysosporium*. J. Biol. Chem. 26: 23688-23695.
- Wuyep P, Khan A, Nok AJ (2003). Production and regulation of lignin degrading enzymes from *Lentinus squarrosulus* (mont) Singer and *Psathyrella atroumbonata*. Afr. J. Biotechnol. 2(11): 444-447.
- Xiaoping XU, Xin W (2008). Effects of culture conditions on ligninolytic enzymes and protease production by *Phanerochaete chrysosporium* in air. Chinese Acad. Sci. Beijing, China. 19: 17-25.
- Xu F (1996). Oxidation of phenols, anilines, and benzenethiols by fungal laccases, correlation between activity and redox potentials as well as halide inhibition. Biochemistry, 35: 7608-7614.
- Yamanaka R, Soares CF, Matheus DR, Machado KMG (2008). Lignolytic enzymes produced by *Trametes villosa* CCB176 under different culture conditions. Braz. J. Microbiol. 39: 78-84.
- Zadzil F, Karma DN, Isikuemhen OS, Schuchardt F (1999). Bioconversion of lignocellulose into ruminant feed with white rot fungi. J. Appl. Anim. Res. 10: 105-124.
- Zahamatkesh M, Tabandeh F, Ebrahimi S (2010). Biodegradation of synthetic dyes by *Phanerochaete chrysosporium* fungi; Application in a fluidized bed bioreactor. Iran. J. Environ. Health Sci. Eng. 7(5): 277-282.