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AZO DYE (DIRECT BLUE 14) DECOLORIZATION BY IMMOBILIZED EXTRACELLULAR ENZYMES OF *PLEUROTUS* SPECIES

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Abstract

Four species of *Pleurotus* i.e., *P. florida*, *P. ostreatus*, *P. citrinopileatus* and *P. eryngii* were evaluated for laccase and MnP production in submerged condition. Among these *P. ostreatus* showed highest production of laccase and MnP. Twelve days old culture of *P. ostreatus* produced 1096 U/ml and 693.5 U/ml of the laccase and MnP, respectively. Crude extracts of enzymes from *P. ostreatus* were immobilized in Ca- alginate matrix and tested for decolorization activity of the azo dye (Direct blue; CI 23850) in aerobic and microaerophilic condition for 24h. Treatment of dye with the immobilized enzymes decolorized up to 99% in eighteen hour.

Key words: Decolorization, direct blue 14, immobilized enzymes, *Pleurotus* species.

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INTRODUCTION

Dyes are colored organic compounds having the property of imparting their color to other objects. Natural and synthetic, both types of dyes are used in industrial application. Manufacture and use of dyes and pigments is a multibillion dollar industry and a huge amount of these dyes is released into the environment in the form of industrial effluent (2, 3). Azo dyes account for the majority of all textile dye stuffs produced and have been the most commonly used synthetic dyes in the textile, food, paper making, color paper printing, leather and cosmetic industries (10, 11, 34). World production of azo dyes is annually increasing; presently this is around one million tons (36). A significant fraction of these dyes is discharged as industrial effluent because there is never complete fixation of dyes takes place on fibres or other substances. Dyes, owing to their brilliance, are visible even at the lower concentrations, and their persistence in the environment is deleterious not only for the photosynthetic processes of the aquatic plants but also for all the living organisms. Azo dyes are recalcitrant xenobiotics and therefore, conventional aerobic wastewater treatment processes usually cannot efficiently decolorize and degrade azo dye bearing effluents to the regulatory levels (10). Their persistence is mainly due to sulfo and azo groups, which do not occur naturally, making the dyes xenobiotic and recalcitrant to oxidative biodegradation (29). The chemical structures of the synthetic dye molecules are designed to resist fading on exposure to light or chemical attack, and this renders them recalcitrant (13).

The various physical and chemical methods that can possibly be used for the treatment of industrial effluent containing various dyes are not self-sufficient and effective (50). Treatment of effluent by biological method was found satisfactory to some extent. Among the biological treatment, aerobic bacteria are incapable of degrading these dyes, but the chromophoric group of azo dyes (the azo bond) can be acted upon by anaerobic bacteria, thus decolorizing the dyes (12). However, by the action of anaerobic bacteria, the azo bond is reduced to amines, which are potentially carcinogenic (5) and due to larger size of dyes; bacteria are unable to degrade these dyes efficiently. To overcome above-mentioned problems associated with bacterial systems various workers utilize fungal systems including brown rot and white rot fungi (21,33,38,49,56). White rot fungi, by virtue of their ability to degrade lignin in nature, produce enzymes such as laccases (EC 1.10.3.2), Manganese peroxidases (MnPs; E.C 1.11.1.13), lignin peroxidases (LiPs; E.C 1.11.1.14) and these enzymes are able to carry out oxidative decolorization of dyes thus bypassing the danger of formation of carcinogenic amines. Laccases seem to be most promising candidates for enzyme-mediated remediation processes because of their broad substrate specificity, easy production, and rapid action at milder pH and temperature. These are multicopper oxidases, which catalyze one electron oxidation of a wide range of inorganic and organic substances, coupled with four-electron reduction of oxygen to water. The free radicals formed, due to laccase action, bypass the step involving the formation of carcinogenic amines (14) and, hence, can decolorize a wide range of industrial dyes. Laccases can not act on the nonphenolic components of aromatic compounds because of their low redox potential (0.5–0.8 V). Moreover, the complex high molecular substrates cannot penetrate the active site of the enzyme. However, small organic compounds (mediators) having high redox potentials (>0.9 V) can be oxidized and activated by laccases, and these enable degradation of the substrate (8,9). Laccases from different basidiomycete strains differed remarkably in their dye-decolourising efficiency. According to Meyer (31), because of the structural variety of azo dyes, they are not uniformly susceptible to biodegradation. It was demonstrated that substituent groups such as nitro and sulpho are frequently recalcitrant to biodegradation, whereas 2-methyl, 2-methoxy, 2, 6-dimethyl and 2, 6-dimethoxy-substituted 4-(4-sulfophenylazo)-phenol were preferred for azo-dye degradation by peroxidase from *Streptomyces* spp and *Phanaerochaete chrysosporium* (46).

An immobilized enzyme is an enzyme that is attached to an inert, insoluble material such as calcium alginate (produced by reacting a mixture of calcium alginate solution and enzyme solution with calcium chloride). This can provide increased resistance to changes in conditions such as pH or temperature. It also allows enzymes to be held in place throughout the reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so is widely used in industry for enzyme catalyzed reactions.

MATERIALS AND METHODS

Cultures and their maintenance

The pure culture of *Pleurotus* species i.e. *P. florida*, *P. ostreatus*, *P. citrinopileatus* and *P. eryngii* were procured from Directorate of Mushroom Research, Solan (H.P.), India. Throughout the study, cultures were maintained on malt extract agar medium at 28^{0}C and subcultured at the regular interval of three weeks.

Production of enzymes

The medium for enzyme production contained 2% wheat bran and 2.5% malt extract. Double distilled water was used for preparation of the medium and the pH was adjusted to 6.0 by using NaOH or HCl. Incubation was carried out at 28^{0}C in BOD incubator in cotton plugged 250 ml Erlenmeyer flasks containing 50 ml of media. Flasks were inoculated with 1 cm^2 agar pieces from actively growing fungus on malt extract agar plate.

Extraction of Extracellular enzymes

Samples of substrate were collected at regular interval of 3 days and extracted in phosphate buffer (pH 6.0) for lignolytic enzymes. Filtrate of extraction was used for enzyme assay.

Enzyme assay

Laccase (E.C. 1.10.3.2) activity was determined using o- methoxyphenol catechol monomethylether (guaiacol) as substrate. The reaction mixture contained 1mM substrate and crude enzymes. The oxidation of substrate was followed spectrophotometrically (A_{495}) (20).

Manganese dependent peroxidases (MnP) (E.C. 1.11.1.13) activity was determined using guaiacol as substrate. The reaction mixture contained 0.5 M Na- tartrate buffer (pH 5.0), 1mM $MnSO_4$, 1mM H_2O_2, 1mM substrate and crude enzymes. The oxidation of substrate at 30^{0}C was followed spectrophotometrically at (A_{465}) (24).

Immobilization of crude enzymes

Crude enzyme extracts of *P. citrinopileatus* were mixed with 3% (w/v) Ca- alginate. The mixture was introduced into chilled 0.2 M $CaCl_2$ solution to form beads of 3.0- 4.0 mm in diameter. The beads were suspended in 0.2 M $CaCl_2$ for 24 hrs, to enhance the mechanical stability.

Decolorization of Direct blue 14

Decolorization assays were carried out under static and agitated (150 rpm) conditions with 200 mg/l dye and immobilized enzymes. The samples were incubated at 30^{0}C for 24 h. Dye decolorization was measured spectrophtometrically (A_{595}) for the microaerophilic and aerobic stages, and the percentage of effluent decolorization was calculated.

RESULTS AND DISCUSSION

The time course of MnP and laccase activity was followed in the wheat and rye bran supplemented liquid cultures over a period of one month. Initially, it was verified that amongst the four species of *Pleurotus; P. ostreatus* showed highest laccase activities on all days evaluated, reaching maximum levels of 1096 U/ml in up to 12 days of culture on wheat bran containing media. This was followed by *P. citrinopileatus* which showed maximum laccase activity (910.3 U/ml) in 12 days. Subsequently *P. eryngii* and *P. florida* showed maximum laccase activity i.e. 741.1 U/ml and 353.3 U/ml in 18 days and 21 days, respectively.

MnP activities were detected at levels of up to 693.5 U/ml by *P. ostreatus* in 12 days old culture followed by *P. eryngii*, *P. citrinopileatus* and *P. florida* i.e. 678.5 U/ml, 580.5 U/ml and 329 U/ml on 21, 12 and 9 days of culture, respectively.

With immobilized enzymes of *Pleurotus ostreatus*, decolorization of direct blue was recorded. In this experiment decolorization of direct blue 14 was done by crude extracellular enzymes of *P. ostreatus* immobilized on Ca-alginate matrix. Decolorization experiment was performed in two conditions i.e. static and agitated. In agitated condition on 150 rpm it was observed that 99.32% of direct blue 14 containing media was decolorized in 18 hrs whereas in static condition it was 97.04% in 24 hrs. Initially, decolorization was slow but with time attained its maximum.

In recent years there is a substantial interest in harnessing degradative capabilities of fungi for the treatment of contaminated wastewaters and some authors have highlighted decolorization efficiency of various eco-physiological groups of basidiomycetes (26,27,47,51). Immobilization of enzyme on inert supports showed it to be useful tool; it actually represents several applicative advantages, such as long time use (either in batch or continuous mode), treatment of large volumes of wastewater, possibility to refresh cultures between different cycles, and allowing the persistence in competition with faster growing species (4,28,52).

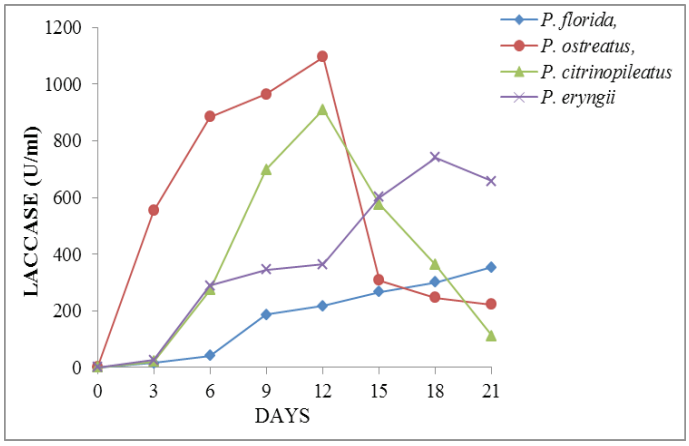

Figure 1. Laccase production of different *Pleurotus* species in submerged condition.

In the present investigation four species of *Pleurotus* i.e., *P. florida*, *P. ostreatus*, *P. citrinopileatus* and *P. eryngii* were tested for enzyme production. It is evident from figure 1 and 2 that among all four species *P. ostreatus* showed maximum laccase and MnP activity i.e., 1096 and

693.5 U/ml respectively after 12 days. In comparison to *P. ostreatus* and *P. citrinopileatus, P. florida* and *P. eryngii* showed low enzyme activity. Every species showed maximum enzyme activity at 12th day of incubation and thereafter; it might be due to occurrence of initial lag phase when species try to establish it in new medium. When culture established itself in the culture medium, it enters into log phase and metabolically this is most active phase where species showed maximum enzymatic activity. Cereal bran was reported to increase ligninolytic enzyme production of the white-rot fungi *Coriolopsis gallica* and *Bjerkandera adusta* (37). In the beginning of the experiment on day 3, different *Pleurotus* species showed low enzymatic activities. This was followed by sharp increase up to 12 days in *P. ostreatus* and *P. citrinopileatus* whereas in case *P. eryngii* and *P. florida* it took 18 and 21 days and for laccase and for MnP it took 18 and 9 days, respectively. Laccase and MnP both are oxidative enzyme and having broad range of substrate specificity.

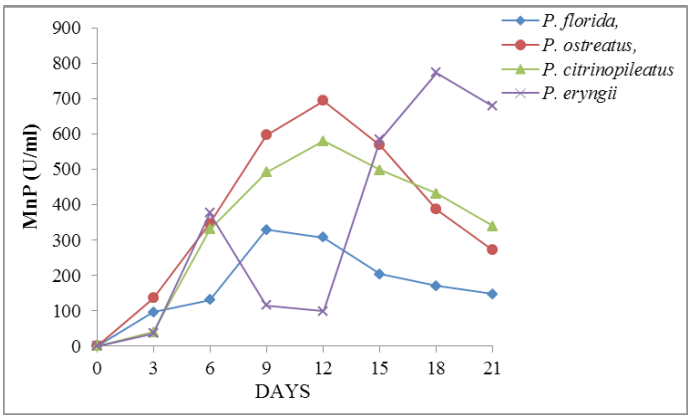

Figure 2. MnP production of different *Pleurotus* species in submerged condition.

Several authors have discussed the role of enzymes in the decolorization activity of lignicolos fungi (1, 30, 33, 42, 43, 54, 55, 57). Different aromatic compounds (2, 5- xylidine, vanillic acid, guaiacol, gallic acid) have been tested for their effects on laccase production by basidiomycetes (16, 17, 22). Though hundreds of azo dyes are in industrial use, their environmental fate is not well understood. Laccases are copper- dependent enzymes produced by a number of fungi and plants, and they oxidize phenols and anilines in the presence of oxygen (6, 7, 23, 53).

The introduction of covalent bonds during immobilization usually enhances stabilities of enzymes due to the limitation of conformational changes (1). Immobilization of fungal laccases on various carrier materials such as activated carbon (19), agarose (40), Eupergit C (18), Sepharose (31), and porosity glass (41,42), has been shown to increase stabilities of the enzyme at high pH and tolerance to elevated temperatures and to make the enzyme less vulnerable to inhibitors, such as Cu chelators. Previously, it was found that a considerable number of textile wastewaters reacted toxically and mutagenically (25, 32).

The dye decolorization by fungal cultures is often correlated to ligninolytic enzyme activities (39,48). Using a respiration-inhibition test, it has been found that anaerobic degradation of azo dyes rendered the effluents more toxic by generating amines, while a second aerobic treatment eliminated this toxicity (35). Immobilization of enzymes on Ca- alginate matrix enhanced the efficiency of enzymes. Decolorization experiment with immobilized enzymes was run for 24 h. Figure 3 demonstrates that in agitated condition decolorization of direct blue 14 was maximum in 18 h i.e. 99.32%, whereas in static condition it took 24 h to reach up to 97.04% decolorization. From figure it is clear that decolorization in agitated condition is more efficient and significant than static condition with respect to time.

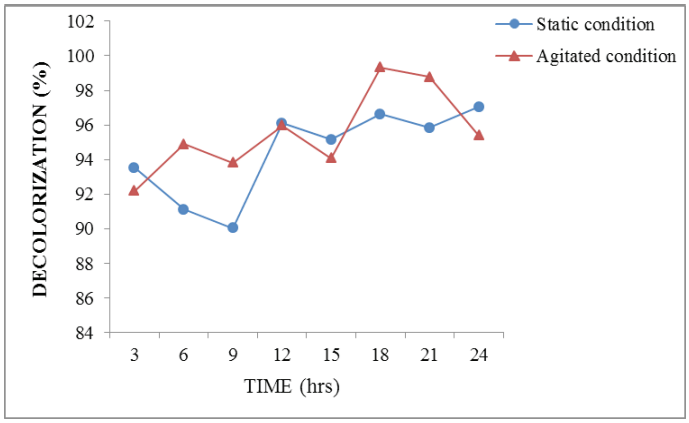

Figure 3. Decolorizatioon of Direct blue 14 by immobilized extracellular enzymes of *P. ostreatus* in static and agitated conditions.

Other articles in this theme issue include references (59-86).

REFERENCES

1. Abadulla, E., Tzanov, T., Costa, S., Robra, K.H., Paulo, A.C. and Gubitz, G.M., Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsuta. Appl. Environ. Microbiol.* 2000, **66(8)**: 3357-3362.

2. Ahlawat, O.P., Gupta, P., Raj, D. and Vijay B., Dye decolorization potential of spent substrates from *Agaricus bisporus* and *Pleurotus* sp. - a laboratory study. *Mush. Res*. 2006, **15(1)**: 75-82.

3. Anliker, R., Eco-toxicological assessment of dyes with particular reference to ETAD's activities, *J. Soc. Dyers Color*. 1979, **95**: 317–326.

4. Bailey, J. E. and Olis, D.F., Biochemical Engineering Fundamentals, 2nd Ed., McGraw-Hill, Singapore, 1986, pp. 984.

5. Benigni, R., Giuliani, A., Franke, R. and Gruska, A., Quantitative structure- activity relationships of mutagenic and carcinogenic aromatic amines. *Chem. Rev*. 2000, **100**: 3697-3714.

6. Bollag, J.M., Enzymes catalyzing oxidative coupling reactions of pollutants. *Metal Ions Biol. Sys*. 1992, **28**: 205-217.

7. Bollag, J.M., K.L. Shuttleworth and Anderson, D.H., Laccase mediated detoxification of phenolic compounds. *Appl. Environ. Microbiol*. 1988, **54**: 3086-3091.

8. Bourbonnias, R., Paice, H.G., Freiermuth, B., Bodie, E. and Borneman, S., Reactivities of various mediators and laccase with kraft pulp and lignin model compounds. *Appl. Environ. Microbiol*. 1997, **63**: 4627-4632.

9. Camarero, S., Ibarra, D., Martinez, M.J. and Martinez, A.T., Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. *Appl. Environ. Microbiol*. 2005, **71**: 1775-1784.

10. Carliell, C.M., Barclay, S.J., Naidoo, N., Buckley, C.A., Mulholland, D.A. and Senior, E., Microbial decolorization of a reactive azo dye under anaerobic conditions. *Water SA*. 1995, **21**: 61-69.

11. Chang, J.S., Chou, C., Lin, Y.C., Ho, J.Y. and Hu, T.L., Kinetic characteristics of bacterial azo-dye decolorization by *Pseudomonas luteola. Water Res*. 2001, **35**: 2841-2850.

12. Chen, K.C., Wu, J.Y., Liou, D.J. and Hwang, S.C.J., Decolorization of the textile dyes by newly isolated bacterial strains. J. Biotechnol. 2003, **101(1)**: 57–68.

13. Chhabra, M., Mishra, S. and Sreekrishnan, T.R., Mediator-assisted

decolorization and detoxification of textile dyes/dye mixture by *Cyathus bulleri* laccase. *Appl. Biochem. Biotechnol*. 2008, **151**: 587–598.
14. Chivukula, M. and Renganathan, V., Phenolic azo dye oxidation by laccase from *Pyricularia oryzae*. *Appl. Environ. Microbiol*. 1995, **61**: 4374–4377.
15. Chung, K.T. and Cerniglia, C.E., Mutagenicity of azo dyes: structure activity relationships. *Mutat. Res*. 1992, **277**:201–220.
16. Couto, S.R, Sanroman, M.A., Hofer, D. and Gubitz, G.M., Production of laccase by *Trametes hirstla* grown in an immersion bioreactor and its application in the decolorization of dyes from a leather factory. *Eng. Life Sci.* 2004, **4**: 233-238.
17. Couto, S.R., Gundin, M., Lorenzo, M. and Sanroman, M.N., Screening of supports and inducers for laccase production *Trametes versicolor* in semi-solid-state conditions. *Proc. Biochem.* 2002, **311**: 249-255.
18. D'Annibale, A., Rita Stazi, S., Vinciguerra V. and Giovannozzisermanni G., Oxirane-immobilized Lentinula edodes laccase: stability and phenolics removal efficiency in olive mill wastewater. *J. Biotechnol*. 2000, **77**:265–273.
19. Davis, S. and Burns, R.G., Covalent immobilization of laccase on activated carbon for phenolic effluent treatment. *Appl. Microbiol. Biotechnol*. 1992, **37**: 474–479.
20. Dhaliwal, R.P.S., Garcha, H.S. and Khanna, P.K., Regulation of lignocellulotic enzyme system in *Pleurotus ostreatus*. *Indian J. Microbiol.* 1991, **31(2)**: 181-184.
21. dos Santos, A.Z., Neto, J.M.C., Traver, C.R.G. and da Costa, S.M.G., Screening of filamentous fungi for the decolorization of a commercial dye. *J. Basic Microbiol.* 2004, **44**(**4**): 288-295.
22. Galhaup, C., Wagner, D., Hinlerstoisser, B. and Haltrich, D., Increased production of laccase by the wood-degrading basidiomycete *Trametes pubescells. Enzyme Microb. Technol.* 2002, **30**, 529-536.
23. Hoff,T., Liu, S.Y. and Bollag, J.M.. Transformation of halogen, alkyl and alkoxy-substituted anilines by a laccase of *Trametes versicolor*. *Appl. Environ. Microbiol*. 1985, **49**: 1040–1045
24. J. Putter, Peroxidases, In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis, vol.2, Academic Press, NewYork, 1974, pp. 685-690.
25. Jaeger, I., Gartiser, S. and Willmund, R., Testing effluents of the textile refining industry with biological methods. *Acta Hydrochim. Hydrobiol*. 1996, **24:** 22-30.
26. Jaouani, A., Sayadi, S., Vanthournhout, M. and Penninckx, M.J., Potent fungi for decolorization of olive oil mill wastewaters. *Enzyme Microb. Technol.* 2003, **33**(**6**): 802-809.
27. Jarosz-wilkolazka, A., Kochmanska-rdest, J., Malarczyk, E., Wardas W. and Leonowicz, A., Fungi and their ability to decolorize Azo and anthraquinonic dyes. *Enzyme Microb. Technol*. 2002, **30**: 566-572.
28. Kasinath, A., Novotny, C., Svobodova, K, Patel, K.C. and Sasek, V., Decolorization of synthetic dyes by *Irpex lacteus* in liquid cultures and packed-bed bioreactor. *Enzyme Microb. Technol* 2003, **32**: 167-173.
29. Kulla, H.G., Klausener, F., Meyer, U., Ludeke, B. and Leisinger, T., Interference of aromatic sulfo groups in the microbial degradation of the azo dyes Orange I and Orange II. *Arch. Microbiol*. 1983, **135**: 1-7.
30. Liu, W.X., Chao, Y.P., Yang, X.Q., Bao, R.B. and Qian, *S.I.,* Biodecolorization of azo, anthraquinonic and triphenyl methane dyes by white rot fungi and a laccase secreting engineered strain. *J. lndustr. Microbiol. Biotechnol.* 2004, **31**: 127-132.
31. Meyer, U., Biodegradation of synthetic organic colorants. *FEMS Symp*. 1981, **12**: 371-385.
32. Milstein, O., Huttermann, A., Majcherczyk, Schulze, A.K., Fruend, R. and Luedermann, H.D., Transformation of lignin-related compounds with laccase in organic solvents. *J. Biotechnol*. 1993, **30**: 37-47.
33. Nerud, F., Baldrian, P., Eichleror, I., Merhautov,V., Gabriel, J. and Homolka, L., Decolorization of dye by using white rot fungi and radical generating reactions. *J. Biocat. Biotrans.* 2004, **22**(**56**): 325-330.
34. O'Neill, C., Lopez, A., Esteves, S., Hawkes, F., Hawkes, D.L. and Wilcox, S., Azo-dye degradation in an anaerobic-aerobic treatment system operating on simulated textile effluent. *Appl. Biochem. Biotechnol*. 2000, **53**: 249-254.
35. Odeigah, P. G., Genotoxic effects of 2 industrial effluents & EMS in Clarias lazera. *Food Chem*. Toxic. 1995, **33**: 501-505.
36. Pandey, A., Singh, P. and Iyengar, L., Bacterial decolorization and degradation of azo dyes. *Int. Biodeteriorat. Biodegrad*. 2007, **59**: 73–84.
37. Pickard, M.A., Vandertol, H., Roman, R. and Vazquez-Duhalt, R., High production of ligninolytic enzymes from white rot fungi in cereal bran liquid medium. *Can. J. Microbiol*. 1999, **45**: 627–631.
38. Platt, M.W., Hader, Y. and Chet, I., The decolorization of Poly Blue (Polyvinalamine sulfonate-anthroquinone) by lignin degrading fungi. *Appl. Microbiol. Biotechnol.* 1985, **21**(**6**): 394-396.
39. Pointing, S.B., Feasibility of bioremediation using white-rot fungi. *Appl. Microbiol. Biotechnol.* 2001, 57: 20–33.
40. Powell, R., Murray, M., Chen, C., and Lee, A., EPA Report Environmental Protection Agency, Washington, DC, USA. 1979, **560**(**13**): 79–105.
41. Reyes, P., Pickard, M.A., and Vazquez- Duhalt, R., Hydroxybenzotriazole increases the range of textile dyes decolorized by immobilized laccase. *Biotechnol. Lett*. 1999, **21**: 875-880.
42. Robinson, T., McMullan, G., Marchant, R., and Nigam, P., Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Biores. Technol.* 2001, **77**: 247–255.
43. Rodriguez, E., Nuero, O., Guillen, F., Martinez, A.T. and Martinez M.J., Degradation of phenolic and non-phenolic aromatic pollutants by four *Pleurotus* species: the role of laccase and versatile peroxidase. *Soil Biol. Biochem*. 2004, **36**: 909-916.
44. Rogalski, J., Dawidowicz, A. L., Jozwik, E. and Leonowicz A., Immobilization of laccase from *Cerrena unicolor* on controlled porosity glass. J. *Mol. Catal. B: Enzym*. 1999, **6**: 29–39.
45. Rogalski, J., Jozwik, E., Hatakka, A. and Leonowicz, A., Immobilization of laccase from Phlebia radiata on controlled porosity glass. *J. Mol. Catal. A: Chem*. 1995, **95**: 99–108.
46. Salony, Mishra, S., and Bisaria, V.S., Production and characterization of laccase from *Cyathus bulleri* and its use in decolorization of recalcitrant textile dyes. *Appl. Microbiol. Biotechnol*. 2006, **71**: 646–653.
47. Sasek, V., Vitasek, J., Chromokova, D., Prokopova, I., Brozek Nahlik, J., Biodegradation of synthetic polymers by composting and fungal treatment. *Folia Microbiol.* 2006, **51**: 425-430.
48. Selvam, K., Swaminathan, K. and Chae, K.S., Decolorization of azo dyes and a dye industry effluent by a white rot fungus *Thelephora sp*. *Biores. Technol*. 2003, **88**: 115-119.
49. Singh, M.P., Rastogi, P.C., Srivastava, A.K. and Vishwakarma, N.K., Decolorization of azo dyes by white rot fungi *Pleurotus* species. *Poll. Res.* 2008, **27(3)**: 356-369.
50. Sun, Q.Y., Hong, Y.Z., Xiao, Y.Z., Fang, W. and Fang, J., Decolorization of textile reactive dyes by the crude laccase produced from solid-state fermentation of agro-byproducts. *World J. Microbiol. Biotechnol.* 2009, **25**:1153–1160.
51. Svobodova, K., Erbanova, P., Sklenar and J. Novotny C., Role of Mn-dependent peroxidase in dye decolorization by static and agitated cultures of *Irpex lacteus. Folia Microbiol.* 2006, **51**: 573-578.
52. Tavcar, M., Svobodova, K., Kuplenk, J., Novotny, C. and Pavko, A., Biodegradation of azo dye RO16 in different reactors by immobilized *Irpex lacteus*. *Acta Chim. Slov*. 2006, **53**: 338–343.
53. Thurston, C.F., The structure ands function of fungal laccases. *Microbiol*. 1994, **140**: 19- 26.
54. Verma, P. and Madamwar, D., Decolorization of synthetic textile dyes by lignin peroxidase of *Phanerochaete chrysosporium. Folia Microbiol.* 2002, **47**:283-286.
55. Wesenberg, D., Kyriakides, L. and Agalhos, S.N., White-rot and

their enzymes for the treatment of industrial dye effluents. *Biotechnol. Adv.* 2003, **22**: 161-187.
56. Yesilada, O. and Ozcan, B., Decolorization of Orange II dye with the crude culture filtrate of white rot fungus *Coriolus versicolor*. *Turk. J. Biol.*1998, **22**: 463-476.
57. Zhao, X.H., Lu, Y.P. and Hardin I., Determination of biodegradation products from sulfonated dyes by *Pleurotus ostretusl* using capillary electrophoresis coupled with mass spectrometry. *Biotechnol. Lett.*2005, **27**: 69-72.
58. Zollinger, H., Color chemistry- synthesis, properties and applications for organic dyes and pigments. New York, N.Y.: VCH Publishers, Inc., 1987, pp. 92-102.
59. Singh, M. P., and Kumar, V., Biodegradation of vegetable and agrowastes by *Pleurotus sapidus*: A noble strategy to produce mushroom with enhanced yield and nutrition. *Cell. Mol. Biol.* 2012, **58** (1): 1-7.
60. Pandey, V. K., Singh, M.P., Srivastava, A. K., Vishwakarma S. K., and Takshak, S., Biodegradation of sugarcane bagasse by white rot fungus *Pleurotus citrinopileatus. Cell. Mol. Biol.* 2012, **58** (1): 8-14.
61. Ruhal, A., Rana, J. S., Kumar S., and Kumar, A., Immobilization of malate dehydrogenase on carbon nanotubes for development of malate biosensor. *Cell. Mol. Biol.* 2012, **58** (1): 15-20.
62. Dash, S. K., Sharma, M., Khare, S. and Kumar, A., *rmpM* gene as a genetic marker for human bacterial meningitis. *Cell. Mol. Biol.* 2012, **58** (1): 26-30.
63. Bertoletti, F., Crespan, E. and Maga, G., Tyrosine kinases as essential cellular cofactors and potential therapeutic targets for human immunodeficiency virus infection. *Cell. Mol. Biol.* 2012, **58** (1): 31-43.
64. Sandalli, C., Singh, K., and Modak, M. J., Characterization of catalytic carboxylate triad in non-replicative DNA polymerase III (pol E) of *Geobacillus kaustophilus* HTA. *Cell. Mol. Biol.* 2012, **58** (1): 44-49.
65. Kaushal, A., Kumar, D., Khare, S. and Kumar, A., *speB* gene as a specific genetic marker for early detection of rheumatic heart disease in human. *Cell. Mol. Biol.* 2012, **58** (1): 50-54.
66. Datta, J. and Lal, N., Application of molecular markers for genetic discrimination of *fusarium* wilt pathogen races affecting chickpea and pigeonpea in major regions of India. *Cell. Mol. Biol.* 2012, **58** (1): 55-65.
67. Siddiqi, N. J., Alhomida, A. S., Khan, A. H. and Onga, W.Y., Study on the distribution of different carnitine fractions in various tissues of bovine eye. *Cell. Mol. Biol.* 2012, **58** (1): 66-70.
68. Ong, Y. T., Kirby, K. A., Hachiya, A., Chiang, L. A., Marchand, B., Yoshimura, K., Murakami, T., Singh, K., Matsushita, S. and Sarafianos, S. G., Preparation of biologically active single-chain variable antibody fragments that target the HIV-1 GP120 v3 loop. *Cell. Mol. Biol.* 2012, **58** (1): 71-79.
69. Singh, J., Gautam, S. and Bhushan Pant, A., Effect of UV-B radiation on UV absorbing compounds and pigments of moss and lichen of Schirmacher Oasis region, East Antarctica. *Cell. Mol. Biol.* 2012, **58** (1): 80-84.
70. Singh, V. P., Srivastava, P. K., and Prasad, S. M., Impact of low and high UV-B radiation on the rates of growth and nitrogen metabolism in two cyanobacterial strains under copper toxicity. *Cell. Mol. Biol.* 2012, **58** (1): 85-95.
71. Datta, J. and Lal, N., Temporal and spatial changes in phenolic compounds in response *Fusarium* wilt in chickpea and pigeonpea. *Cell. Mol. Biol.* 2012, **58** (1): 96-102.
72. Sharma, R. K., JAISWAL, S. K., Siddiqi, N. J., and Sharma, B., Effect of carbofuran on some biochemical indices of human erythrocytes *in vitro. Cell. Mol. Biol.* 2012, **58** (1): 103-109.
73. Singh, A. K., Singh, S. and Singh, M. P., Bioethics A new frontier of biological Science. *Cell. Mol. Biol.* 2012, **58** (1): 110-114.
74. Adedeji, A. O., Singh, K. and Sarafianos, S. G., Structural and biochemical basis for the difference in the helicase activity of two different constructs of SARS-CoV helicase. *Cell. Mol. Biol.* 2012, **58** (1): 115-121.
75. Singh, S., Choudhuri, G., Kumar, R. and Agarwal, S., Association of 5, 10-methylenetetrahydrofolate reductase C677T polymorphism in susceptibility to tropical chronic pancreatitis in North Indian population. *Cell. Mol. Biol.* 2012, **58** (1): 122-127.
76. Sharma, R. K., Rai, K. D. and Sharma, B., *In* vitro carbofuran induced micronucleus formation in human blood lymphocytes. *Cell. Mol. Biol.* 2012, **58** (1): 128-133.
77. Naraian, R., Ram, S., Kaistha S. D. and Srivastava J., Occurrence of plasmid linked multiple drug resistance in bacterial isolates of tannery effluent. *Cell. Mol. Biol.* 2012, **58** (1): 134-141.
78. Pandey, A. K., Mishra, A. K., And Mishra, A., Antifungal and antioxidative potential of oil and extracts, respectively derived from leaves of Indian spice plant *Cinnamomum tamala. Cell. Mol. Biol.* 2012, **58** (1): 142-147.
79. Mishra, N., and Rizvi, S. I., Quercetin modulates na/k atpase and sodium hydrogen exchanger in type 2 diabetic erythrocytes. *Cell. Mol. Biol.* 2012, **58** (1): 148-152.
80. Kumar, A., Sharma, B. and Pandey, R. S., Assessment of stress in effect to pyrethroid insecticides, λ-cyhalothrin and cypermethrin in a freshwater fish, *Channa punctatus* (Bloch). *Cell. Mol. Biol.* 2012, **58** (1): 153-159.
81. Srivastava N., Sharma, R. K., Singh, N. and Sharma, B., Acetylcholinesterase from human erythrocytes membrane: a screen for evaluating the activity of some traditional plant extracts. *Cell. Mol. Biol.* 2012, **58** (1): 160-169.
82. Singh, M.P., Pandey, A. K., Vishwakarma S. K., Srivastava, A. K. and Pandey, V. K., Extracellular Xylanase Production by *Pleurotus* species on Lignocellulosic Wastes under *in vivo* Condition using Novel Pretreatment. *Cell. Mol. Biol.* 2012, **58** (1): 170-173.
83. Kumar, S., Sharma, U. K., Sharma, A. K., Pandey, A. K., Protective efficacy of *Solanum xanthocarpum* root extracts against free radical damage: phytochemical analysis and antioxidant effect. *Cell. Mol. Biol.* 2012, **58** (1): 174-181.
84. Shukla, A., Singh, A., Singh, A., Pathak, L.P., Shrivastava, N., Tripathi, P. K., Singh, K. and Singh, M.P., Inhibition of *P. falciparum* pfATP6 by curcumin and its derivatives: A bioinformatic Study. *Cell. Mol. Biol.* 2012, **58** (1): 182-186.
85. Michailidis, E., Singh, K., Ryan, E. M., Hachiya, A., Ong, Y. T., Kirby, K. A., Marchand, B., Kodama, E. N., Mitsuya, H., Parniak, M.A. and Sarafianos, S.G., Effect of translocation defective reverse transcriptase inhibitors on the activity of n348i, a connection subdomain drug resistant HIV-1 reverse transcriptase mutant. *Cell. Mol. Biol.* 2012, **58** (1): 187-195.
86. Parveen, A., Rizvi, S. H. M., Gupta, A., Singh, R., Ahmad, I., Mahdi, F., and Mahdi, A. A., NMR-based metabonomics study of sub-acute hepatotoxicity induced by silica nanoparticles in rats after intranasal exposure. *Cell. Mol. Biol.* 2012, **58** (1): 196-203.