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Research Article

BIOBLEACHING OF TEXTILE DYE EFFLUENT BY BACTERIAL LIGNIN PEROXIDASE

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ABSTRACT

Lignin is biologically stabile due to its complex structure, only few microorganisms can degrade it. Biodegradation is caused by the enzymes that initiate its extracellular oxidative depolymerization. These enzymes, responsible for the generation of non-specific free radicals with high reactivity, attack the C-C bonds connecting the structural units of lignin. Lignin-degrading enzymes, lignin peroxidases is a oxidoreductive enzyme, which are useful in the treatment of colored industrial effluents and other xenobiotics as it has bioremediation potential to decolourize the effluents. The present study focused on to isolate and characterizes the lignin degrading bacteria which show the remarkable ability to bleach textile dye effluent. The plant compost soil and cow dung were collected for the isolation of lignin degrading bacteria and tree barks for the preparation of alkali lignin solution. The lignolytic activity of the isolates were studied on LB agar plates containing 0.25% Methylene blue showed zone of lignolysis. The isolates showing lignolytic activity were subjected to lignin peroxidase assay with their crude extracts. The two extracts with highest enzyme activity were used for biobleaching experiment on textile dye effluent. The treated and untreated effluents (control) were characterized by FTIR and the functional groups were determined.The toxicity of the treated and untreated effluents were tested for phytotoxicity with Zea mays seeds and the untreated effluent was found to be toxic to the plants when compared with enzyme treated effluent.

INTRODUCTION

Lignin the major component of plants is the second most abundant aromatic biopolymer in the biosphere (Rahman *et al.,* 2013). Lignin is the most structurally complex carbohydrate possessing a high molecular weight, consisting of various stable linkages and the most recalcitrant, (Perez *et al.,* 2002).

The lignocellulosic waste generated through forestry and agricultural practices, paper-pulp industries, timber industries and many agro-industries pose environmental pollution problems owing to its low biodegradability (Howard *et al.,* 2003). Only few soil microorganisms are capable of degrading complex lignin polymers (Alexander, 1977). However the disadvantages of using fungi are the problems related to fungal protein expression and genetic manipulations and showed unstable under practical treatment condition (Crawford and Muralidhara, 2004). For this reason, studies on the bacterial degradation and bacterial ligninolytic enzymes have been increased in recent year (Renugadevi *et al.,* 2011).

The highly colored effluents from paper and pulp mills, alcohol distilleries and leather industries, textiles and dye-making industries creates unacceptable intense coloring of soil and water bodies (Jin *et al.,* 2007). Moreover they lead to anaerobic conditions in aquatic ecosystems due to blockage of light to lower depths and cessation of photosynthesis. This results in the death of aquatic life. Oxidoreductive enzymes play an important role in degradation and transformation of polymeric substances. Lignin peroxidase (Lip) is a ligninolytic enzyme first discovered in 1983. Lignin peroxidase is useful in the treatment of textile industrial effluents and other recalcitrants to decolorize the effluents (Shi *et al.,* 2013). The partially degraded or oxidized products can easily be taken up by microbial cells where they are completely mineralized (Duran *et al.,* 2000).

The aim of the present study was to isolate and characterize the lignin degrading bacteria which show the remarkable ability to bleach textile dye effluent.

MATERIALS AND METHODS

Alkali Lignin Preparation

The barks used for extraction of lignin were dried and ground to powder (Howard *et al.,* 2003).To the 10g of powdered bark (lignin sources), 5ml of 1% sulfuric acid was added and heated

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in hot air oven at 80°C for 20 mins and allowed to cool. After cooling 100 ml of 4% sodium hydroxide was added and boiled for 30 mins. The dark brown colored alkali lignin was filtered and autoclaved at 15 lbs for 10 mins (Bholy *et al.,* 2012).

Isolation of Lignin Degrading Bacteria

The samples used for the isolation of lignin degrading bacteria were cow dung and plant composting soil. The samples were collected in a sterilize polythene bag and transported to the laboratory and stored at 4°C. The lignin degrading bacteria was enriched using 1% alkaline lignin minimum salt medium (Chandra *et al.,* 2008) and incubated at 120 rpm for 7 days at 37° C. Enriched sample of 1 ml were transferred to 99 mL of sterile 0.9% NaCl. The solution were stirred vigorously and allowed to settle down. Using 1 mL of the liquid mixture, serial dilutions were performed. 0.1 ml of serially diluted sample was spreaded on minimal salt medium agar containing alkaline lignin. The plates were incubated at 30° C for 7 days until colonies developed. The isolated bacteria were plated onto fresh MSM-L agar plates repeatedly to obtain pure cultures (Rahman *et al.,* 2013).

Lignolytic Activity

The bacterial isolates were further screened for lignolytic activity using media containing methylene blue dye as an indicator. The bacteria possessing lignolytic enzymes undergo oxidation of indicator dye. The isolated bacteria were streaked on LB agar containing 0.25% methylene blue indicator dye. The plates were incubated at 30° C for 72 h. The agar plates were monitored daily for bacterial growth and decolorization of the methylene blue dyes (Bondounas *et al.,* 2011). The decolorized microbial colonies were subjected for identification.

Lignin Peroxidase Enzyme (LiP) Assay

The isolates were assayed for the presence of lignin peroxidase enzyme (LiP). A spectrophotometric assay method for the determination of lignin peroxidase activity is based on the demethylation of the methylene blue dye. The method can be efficiently used for the quantification as its sensitivity is close to veratryl alcohol assay. The enzyme lignin peroxidase demethylates methylene blue, the substrate in the presence of H_2O_2 (inducer). The final product is a tri-demethylated methylene blue derivative and Azure C, the reaction occurs at pH-4. Enzyme activity can be measured as percent decolourization of the methylene blue dye.

All the isolates were freshly inoculated in 100ml of 0.5% lignin broth in 500 ml flask and were incubated at 30°C at 120rpm for 5 days in a shaker incubator to obtain a heavy bacterial growth. About 10 ml of the culture broth of the isolates was taken in cooling centrifuge tubes and the culture broths were centrifuge at 4° C at 7000rpm in cooling centrifuge. After centrifugation the tubes were kept in ice bath without disturbing it and enzyme assay was carried out using the supernatant. Assay protocol- 1ml of 50 mM Sodium Potasium Tartarate (pH-4) buffer, 0.1 ml of 0.1mM H_2O_2 inducer was added to which 32µM methylene blue as substrate and 10 µl of enzyme solution was added. The solution was incubated for 1hr at RT and A_{650} was measured. The results were interpreted as the percent decolourization of the methylene blue dye by the enzyme as compared to the control tube calculated as (A_{650} for control- A_{650} for test/ $A_{650}$for control) X100 (Denise *et al.,*1996).

Characterization of Potent Isolates

The lignin degrading bacterial isolates were characterized and identified. Colony characteristics, Gram staining, motility and biochemical tests were observed to identify the isolates using the Bergey's manual of determinative Bacteriology 9th edition.

Lignin Degrading Products (Phenols)

Lignin is a complex aromatic polymer containing phenyl units and degradation of lignin leads to the accumulation of phenolic components and a number of simple aromatic compounds. Hence estimation of phenol was done by Folin-ciocalteau method (Oliveria *et al.,* 2009). To 1 ml of the centrifuged culture broth, 1ml D/W and 0.5ml folin ciocalteau reagent was added, incubated at RT for 30 mins and A_{615} was measured. The concentrations of phenol present in culture broth were calculated using the standard graph.

Biobleaching of Effluents

Textile dye based industries releases highly colored effluents due to the usage of mixture of azo dyes (Singh and Thakur, 2004). The azo groups have a complex structure and are considered as recalcitrant (Livin *et al.,* 2004). The lignin peroxidase enzymatic system can be applied for the biobleaching of these effluents. The lignin peroxidases is an oxidoreductases enzyme which can oxidize large number of complex structure as it is not specific in action and act on a wide range of substrates. (Abdulla *et al.,* 2000). The diluted (2:1) effluents from respective industries were grouped into six flasks. Fungdid B was added to each flask as an antifungal agent. The crude extract of the lignin peroxidases enzymatic system of the isolates were added to the respective flasks, and a control flask without enzyme was also maintained. The rate of decolorization was monitored. The bleaching activity was calculated using the formula after measuring the absorbance of the respective centrifuged effluents samples for consecutive 5 days. The colour reduction percentage (%) was measured as (Abs on 1st day- Abs on 5th day/ Abs on 1st day) x 100 (Palmieri *et al.,* 2005).

Fourier Transform Infrared Renosance Spectroscopy (FTIR)

The supernatants obtained after biobleaching from the tests and control was characterized by FTIR analysis. The FTIR analysis of extract was performed using standard potassium bromide solution.

Phytotoxicity Study

The effect of treated and untreated effluent on seed germination was analyzed by irrigating seeds with treated, untreated effluent and tap water under static condition. Seeds of *Zea mays* were surface sterilized by 0.1% w/v Hg Cl_2 solution and rinsed with in sterile distilled water to remove residual Hg Cl_2. The seeds (ten numbers each) were incubated in sterile tubes and irrigated with treated, untreated effluent and tap water. After 24 hrs the seeds were sown in soil and watered every day. Length of plumule (shoot), radicle (root) and germination (%) were recorded after 15 days.

RESULTS AND DISCUSSION

Alkali Lignin Preparations

The crude lignin was extracted from tree barks to alkali lignin solution. The alkaline delignification method was done to extract lignin using 4% sodium hydroxide, a dark color alkali lignin was obtained which was stored in an air tight bottle at 4^{o} C and used for further experiments.

Isolation and Acclimatization

Totally 13 lignolytic degrading isolates with different colony morphology were obtained from the MSM-L plate containing compost soil, cow dung and their lignolytic activity was checked. The lignin agar plates showed decolourization of lignin after incubation as shown in Figure 1&2. The capacity to decolourize ligninolytic indicator dyes is a common method to demonstrate lignin-degrading ability in fungi (Kiiskinen *et al*., 2004; Field *et al*., 1993). Based on their decolourization ability 6 isolates were selected for the further studies.

Lignolytic Activity

The decolourisation of lignin by the isolates was significantly enhanced after acclimatization of the isolates. Among all the isolates highest activities were obtained for PCS3 and CD 2.

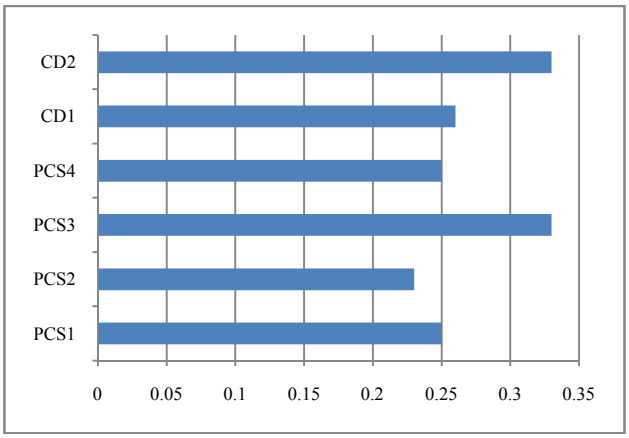
Figure 1 Zone of decolourization

Figure 2 Absence of decolourization (control)

Enzyme Assay

Lignin Peroxidase Enzyme Assay

The lignin peroxidase enzyme activity was increased in A_{670} per minute per milliliter of the isolates was in the range of 0.23 to 0.33. Among all the isolates highest activities were obtained for PCS3 and CD2 as shown in Graph 1.



Graph 1 Lignin peroxidase activity of the isolates

Characterization of Potent Isolates

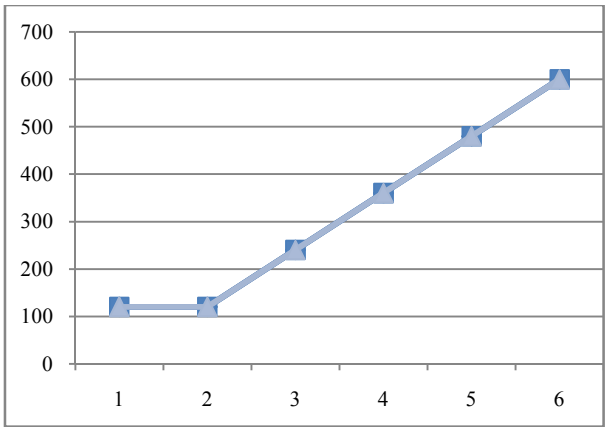
Among the 6 isolates 2 bacterial isolates PCS 3 and CD 2 showed highest results which were selected for characterization and for further application. As per Bergey's manual of Determinative Bacteriology 9th edition and comparing with standard microorganisms, it was indentified that the isolate PCS 3 and CD2 were *Providencia rettgeri* and *Serratia marcescens* respectively (Table 1).

Table 1 Morphological, physiological and biochemical observations of the microbial colonies isolated

Parameters	PCS3	CD2
Color	White	yellow
Gram	G-ve	G-ve
Motility	Motile	Motile
Oxidase	Negative	Negative
Catalase	Positive	Positive
Indole	Positive	Negative
Methyl red	Positive	Negative
Voges praskeur	Negative	Positive
Citrate	Positive	Positive
Growth at Temp	37ºC	37ºC

Lignin Degrading Products (Phenol)

The concentration of phenol was almost same till 48 hours but increased after 48 hours as per the lignolytic activity of the isolates. The early increase showed that the lignin was degraded into various phenolic compounds. The concentration of phenol at 1st day for *Providencia rettgeri* was 120µg/ml and 600 µg/ml at the final day shown in graph 2. The enzymatic decomposition of the phenolic compounds in lignin leads to effective degradation and this is only possible due to the hydrophilic attack at the arene substituents (Srebotnik and Hammel, 2000; Christopher *et al*., 2014).Consequently, application of such enzyme system in the decolourization of dye would only be effective if the arene substituents of the dye are susceptible to hydrolization (Goszczynski *et al*., 1994).



Graph 2 Concentration of phenol

Biobleaching of Effluents

The application of the present research of the lignin peroxidase enzymes as crude extracts of the culture broth was studied on biobleaching of textile dye-based effluents. The enzyme extracts of *Providencia rettgeri* and *Serratia marcescens* were individually applied. The results for the individual isolates were recorded. The λmax of the controls for textile dye-based effluent was also recorded. The absorbance from 1st day to 5th day decreased and the decolourization efficiencies were calculated for each isolate.

Treatment of textile dye-based effluent gave 57% decolorization with the crude extract of the enzyme obtained from *Providencia rettgeri* (PCS3) and 80% decolourization with the crude extract of the enzyme obtained from *Serratia marcescens* (CD2). It was concluded that the treatment of the effluent by using *Serratia marcescens* (CD2) gave maximum decolourization.

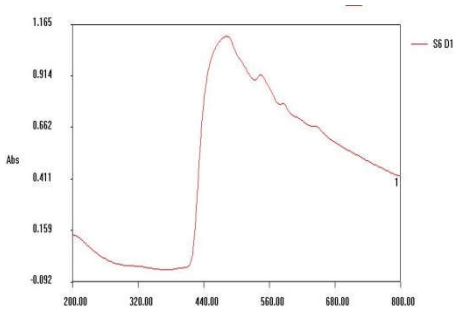


Figure 3 UV- Visible analysis of S6 on day 1

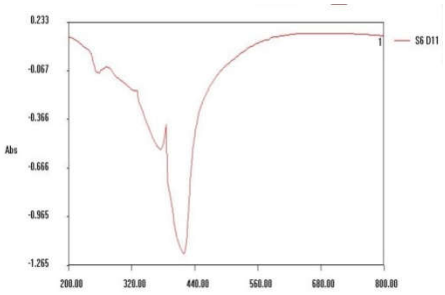


Figure 4 UV- Visible analysis of S6 on day 11

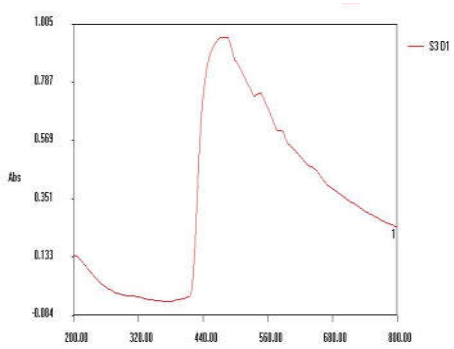


Figure 5 UV- Visible analysis of S3 on day 1

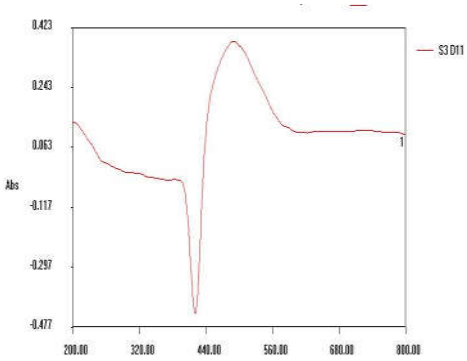


Figure 6 UV- Visible analysis of S3 on day 11

FTIR

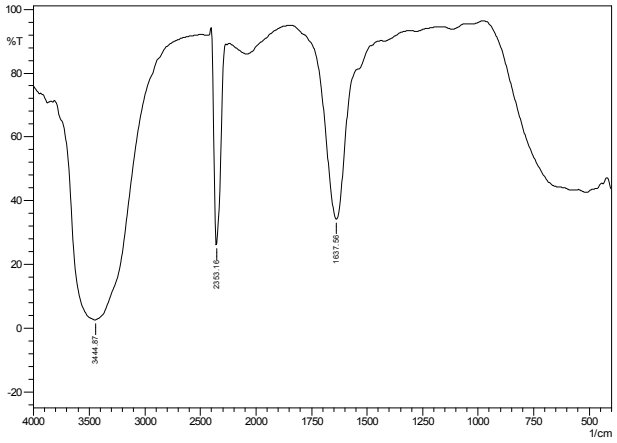


Figure 7 FTIR analysis of effluent (Control)

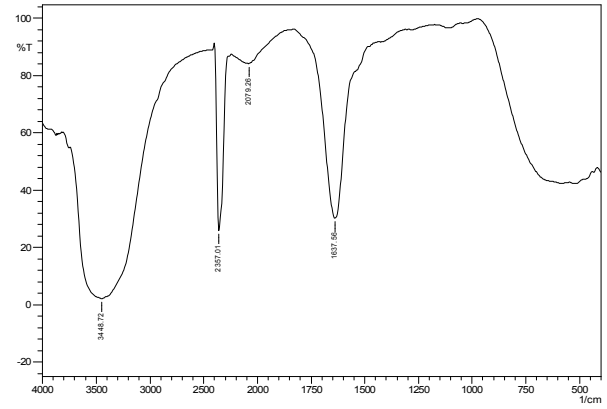


Figure 8 FTIR analysis of S6 treated effluent

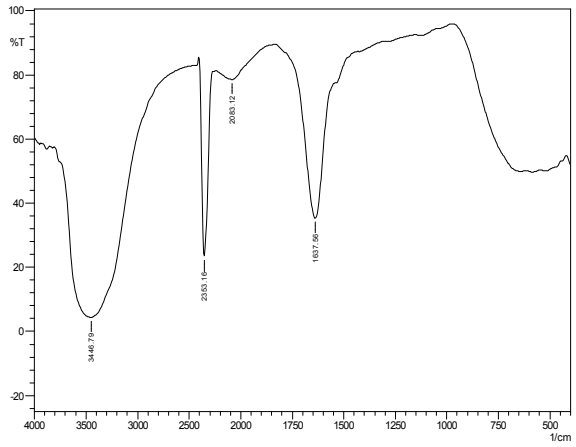


Figure 9 FTIR analysis of S3 treated effluent

The FTIR analysis showed the various functional groups present in the control and in both the treated samples an additional functional group alkyene with medium intensity was observed.

Phytotoxicity Test

Results from phytotoxicity studies showed that the enzyme produced by the individual strain *Providencia rettgeri* did not inhibit germination of plants and found to be nontoxic to the plant growth (Table 2).

Table 2 Percentage of seed germination and plant growth of *Zea mays*

Parameters studied	Zea mays seeds irrigated with		
	Normal water	Enzyme Treated effluent	Untreated effluent
Germination (%)	40%	40%	No germination
Stem(cm)	20	6	-
Root(cm)	16.5	6.5	-

CONCLUSION

The application of lignin peroxidases from *Providencia rettgeri* and *Serratia marcescens* as an enzymatic method for biobleaching of textile dye based effluents showed significant colour removal. Therefore, this treatment can be adopted and applied at industrial level for decolourisation of textile dye effluents. The toxicity of the textile dye effluent on seed germination and growth was found to be less in lignin peroxidase treated effluent than in raw untreated. This suggests the use of lignin peroxidase in the treatment of effluents before disposal. Further study on mechanism(s) of action and characterization of this enzyme is crucial prior to commercial application in future.

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References

1. Rahman NHA, Rahman NAA, Surainiabdaziz M, Hassan M. Production of ligninolytic enzymes by newly isolated bacteria from palm oil plantation soils. Bioresour. 2013; 8(4):6136-6150.
2. Perez J, Munoz-Dorado J, de la Rubia T, Martinez J. Biodegradation and biological treatments of cellulose, hemicelluloses and lignin: an overview. Int Microbial. 2002; 5(2): 53-63.
3. Howard RL, Abotsi E, Rensburg EL, Howard S. Lignocellulose biotechnology: Issues of bioconversion and enzyme production: Riview. *Afri.J.Biotechnol* 2003; 2(12):602-619.
4. Alexander M. Introduction to Soil Microbiology. Krieger Publishing company. 1977; Edition 2.
5. Crawford DL and Muralidhara PJ. Bacterial extracellular lignin peroxidase. Unites States Patent. 2004. 5200338.
6. Renugadevi R, Aryyappadas MP, Preethy PH, Savetha S. Isolation, screening and induction of mutation in strain for extracellular lignin peroxidase producing bacteria from soil and its partial purification. J. Res. Biol. 2011; 4:312-318.
7. Jin XC, Liu GQ, Xu ZH, Tao WY. Decolorization of a dye industry effluent by *Aspergillus fumigates* XC6. Applied Microbial Biotechnol. 2007; 74(1).
8. Shi Y, Chai L, Tang C, Yang Z, Zheng Y, Chen Y. Biochemical investigation of Kraft lignin degradation by *Pandoraea* sp.B-6 isolated from bamboo slips. Bioproc .Biosyst. Engg. 2013; 36:1957-1965.
9. Duran N and Esposito E.Potential applications of oxidative enzymes and phenoloxidases-like compounds in wastewater and soil treatment. *Applied catalysis B: Environmental* 2000; 83-99.
10. Bholy AD, Bhavna VB, Priyanka UJ, Kaveri SP, Mayuri VD, Nalawada PM. Bacterial lignin peroxidase: biobleaching and biodegradation of industrial effluent. Univ. J. Environ. Res. Technol. 2012; 2(1):58-64.
11. Chandra R, Raj A, Porohit HJ, Kapley A. Characterization and optimization of three potential aerobic bacterial strains for kraft lignin degradation from paper pulp waste. Chemosphere. 2008; 67(9):839-846.
12. Boundounas L, Nick JP, Wierck, Winde JH, Ruijssenaarsm HJ. Isolation and characterization of novel bacterial strain exhibiting lignolytic potential. *BMC* Biotechnol. 2011; 11(94):1-11.
13. Denise BM, Maria LA, Elba B, Julio SAN, Sergio HK. Colorimetric assay for lignin peroxidase activity determination using methylene blue as substrate. Biotechnological Techniques. 1996; 10: 273-276.
14. Oliveria PL, Marta CT, Ponezi AN, DurrantLR.Use of *Bacillus pumilus* CBMAI 0008 and *Paenibacillus* sps.CBMAI 868 for color removal from paper mill effluent. Brazilian Journal of Microbiology. 2009; 40:354-357.
15. Singh P and Thakur IS. Removal of colour and detoxification of pulp mill effluent by microorganisms in two step bioreactor. Journal of Scientific and Industrial research. 2004; 63 (11): 944-948.
16. Levin L, Papinutti L , Forchiassin F. Evaluation of Argentinean White Rot Fungi for their ability to produce lignin-modifying enzymes and decolourize industrial dyes. Bioresource Technol. 2004; 94:169-176.
17. Abadulla E, Robra KH, George M, Gubitz, Luisa M, Silva, Artur Cavaco-paulo. Enzymatic decolorization of

Textile Dyeing effluents. Textile Research Journal.2000; 70(5):409-414.
18. Palmeiri G, Cennamo G and Sannia G. Remazol brilliant blue R decolourisation by the fungus *Pleurotus ostreatus* and its oxidative enzymatic system. Enzyme Microb. Technol 2005; 36:17-24.
19. Kiiskinen LL, Ratto M, Kruus K.Screening for novel laccase producing microbes. Journal of Applied Microbiology. 2004; 97(3): 640-646.
20. Field JA, de Jong E, Feijoo-Costa G, de Bont JM. Screening of lignolytic fungi applicable to the biodegradation of xenobiotics. Trends in Biotechnology. 1993; 11: 44-49.
21. Srebotnik E, Hammel KE. Degradation of nonphenolic lignin by the lactate/1.hydroxybenzotriazole system. J. Biotechnol. 2000; 81: 179-188.
22. Christopher LP, Yao B, Ji Y. Lignin biodegradation with laccase mediator systems. Front. Energy Res. 2014; 2:12
23. Goszczynski S, Paszczynski A, Grigsby-Pasti MB, Crawford RL, Crawford DL. New pathway for degradation of sulfonated azo dyes by microbial peroxidases of *Phanerochaete chrysosporium* and *Streptomyces chromofuscus*. J.Bacteriol. 1994; 176: 1339-1347.

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