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Sita Heris Anita
Research Center for Biomaterials, Indonesian Institute of Sciences (LIPI), Bogor 16911, Indonesia, sita.heris@biomaterial.lipi.go.id

Fahriya Puspita Sari
Research Center for Biomaterials, Indonesian Institute of Sciences (LIPI), Bogor 16911, Indonesia

Dede Heri Yuli Yanto
Research Center for Biomaterials, Indonesian Institute of Sciences (LIPI), Bogor 16911, Indonesia

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Decolorization of Synthetic Dyes by Ligninolytic Enzymes from *Trametes hirsuta* D7

Sita Heris Anita*, Fahriya Puspita Sari, and Dede Heri Yuli Yanto

Research Center for Biomaterials, Indonesian Institute of Sciences (LIPI), Bogor 16911, Indonesia

*E-mail: sita.heris@biomaterial.lipi.go.id

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**Abstract**

The ability of ligninolytic enzymes from *Trametes hirsuta* D7 to decolorize several synthetic dyes was investigated. A crude enzyme powder was produced by fermenting oil palm empty fruit bunch fibers for one month. The dye decolorization by the enzymes occurred at an efficient 0.25 U/mL. The enzymes degraded 100, 200, 300, 400, and 500 ppm Remazol Brilliant Blue R (RBBR) within 7 h by 95.57 ± 0.32%, 93.46 ± 3.09%, 91.84 ± 0.65%, 86.44 ± 0.97%, and 82.14 ± 0.52%, respectively. The enzyme also decolorized anthraquinone (Acid Blue 129), monoazo (Acid Orange 7), diazo (Reactive Black 5), and trimethyl methane (Methyl Violet) dyes within 7 h by 94.59 ± 7.97%, 13.99 ± 0.30%, 7.61 ± 0.01%, and 7.59 ± 0.18%, respectively. Addition of MnSO$_4$, H$_2$O$_2$, and violuric acid enhanced the dye decolorization rate by up to 10-fold. This study shows the potential for application of ligninolytic enzymes from *T. hirsuta* D7 in the treatment of wastewater effluent of textile industries.

**Keywords:** biodecolorization; bioremediation; dye; ligninolytic enzyme; textile waste

**Introduction**

Synthetic dyes are extensively used in the textile, paper, rubber, cosmetics, pharmaceuticals, and food industries. However, the presence of dyes in wastewater from these industries can be particularly problematic. The dyes have a complex chemical structure; therefore, they are resistant to oxidation by light and water. The accumulation of undegradable dyes in surface water can therefore reduce aquatic diversity by blocking the passage of sunlight, thereby affecting the ecological balance of aquatic ecosystems. In addition, many synthetic dyes are toxic, mutagenic, and carcinogenic [1,2].

Dyes can be classified based on their chemical structure (e.g., azo, anthraquinone [AQN], indigo, triphenylmethane, etc.), the method of usage (e.g., direct, reactive, chromic, metal-complexes, disperse, sulfur, vat, pigments, etc.), and their chromogens (e.g., donor, acceptor, cyanine, polyene, etc.) [3]. The major classes of synthetic dyes are azo and AQN dyes, and their complex structure makes decolorization a challenge. Currently available physical or chemical treatments, like adsorption, precipitation, filtration, and chemical transformation, are expensive, environmentally unfriendly, and inefficient. As an alternative to these physicochemical methods, biological treatment of dyes using ligninolytic enzymes provides a lower energy cost and an eco-friendly process [4].

Synthetic dyes are efficiently decolorized by white rot fungi (WRF), which produce one or more extracellular ligninolytic enzymes. The WRF enzymes have a very low substrate specificity; therefore, they can degrade a wide range of highly recalcitrant substances including synthetic dyes, which are structurally similar to lignin. Lignin-modifying enzymes (LMEs) include laccase (LAC) (EC 1.10.3.2), lignin peroxidase (LiP) (EC 1.11.1.14), and manganese peroxidase (MnP) (EC 1.11.1.13). Some WRF produce all these enzymes, while others produce only one or two [4, 5]. Several WRF, such as *Pleurotus sajor-saizu* [6], *Polyporus sp.* [7], *Trametes versicolor* [8], and *Ganoderma lucidum* [9], were reported capable of decolorizing a wide range of dyes. Oktavia and Yanto [10] reported that *T. hirsuta* D7 showed a high dye decolorization efficiency through its laccase enzyme activities.

The aim of the present study was to evaluate the ability of ligninolytic enzymes from *T. hirsuta* D7 to decolorize various dyes at different concentrations. The effect of enzyme mediators, such as manganese sulfate (MnSO$_4$), hydrogen peroxide (H$_2$O$_2$), and violuric acid (VA), on dye decolorization was also investigated.
Methods

All experiments were carried out in the Laboratory of Biomass Conversion Technology and Bioremediation and the Laboratory of Microbiology, Research Center for Biomaterials. Oil palm empty fruit bunches (OPEFB) were used as a substrate for enzyme production. The test fungus was *T. hirsuta* D7, a white rot fungus isolated from a peat swamp forest area in Riau, Indonesia. The test dye substrates were Remazol Brilliant Blue R (RBBR), Acid Blue 129 (AB129), Acid Orange 7 (AO7), Reactive Black 5 (RB5), and Methyl Violet (MV) were purchased from Sigma-Aldrich. The characteristics of these substrates are shown in Table 1. Malt extract, peptone, glucose, corn steep liquor, malonate buffer (pH 4.5), MnSO₄, 2,6-dimethoxyphenol (DMP), H₂O₂ violuric acid (VA), and acetate buffer (pH 4.5) were purchased from Wako Pure Chemical Industries, Ltd. (Japan).

**Fungal culture.** The *T. hirsuta* D7 strain was grown on malt extract agar and incubated at room temperature (~25–30 °C) for 7 days. The fungus culture was then inoculated in a malt extract-glucose-peptone broth containing 20 g/L malt extract, 20 g/L glucose, and 1 g/L peptone. An inoculum was prepared by inoculating 4 plugs (Ø 8 mm) of the fungal colony into a 300 mL Erlenmeyer flask containing 100 mL medium broth and agitated at 150 rpm at room temperature for 7 days. After incubation, 1% (w/v) corn steep liquor was added and the culture was homogenized with a Waring blender twice for 2 sec. The homogenized mixture was used as the inoculum for enzyme production.

**Substrate preparation and enzyme production.** The enzyme was produced by solid state fermentation (SSF), as follows: 10 meshes of 150 g OPEFB, 150 mL distilled water, and 150 mL malt extract-glucose-peptone broth were combined in a heat-resistant plastic bag. The substrate (53% water content) was sterilized at 121 °C for 15 min and then cooled at room temperature. A 10% (w/v) of *T. hirsuta* D7 inoculum was inoculated into the sterilized substrate and incubated at room temperature for 1 month [14].

**Enzyme extraction.** The crude enzyme was extracted from the substrate by adding 50 mM malonate buffer, pH 4.5, at a ratio of 1:3 to the weight of substrate. The mixture was homogenized using an ACE AM-11 homogenizer (Nissei, Japan) at 10,000 rpm for 10 min on ice and then filtered. The filtrate was centrifuged at 10,380 x g (20 °C) for 20 min. Ammonium sulfate was then added to the supernatant to give a 50% saturated solution. The solution was stirred with a magnetic stirrer for 1 h and then centrifuged at 10,380 x g at 4 °C for 20 min. The pellet was dissolved in 50 mM malonate buffer, pH 4.5 and freeze-dried for 1–2 days to yield a crude enzyme powder.

**Table 1. Characteristics of the Synthetic Dyes used in This Study [3,11,12,13]**

| Dye (C.I.No.) | Molecular structure | Functional Group | Molecular weight | λ<sub>max</sub> (nm) |
|--------------|---------------------|------------------|-----------------|-------------------|
| Remazol Brilliant Blue R (6200) | ![Molecular structure](attachment) | Anthraquinone | 626.54 | 592.5 |
| Acid Blue 129 (62058) | ![Molecular structure](attachment) | Anthraquinone | 460.48 | 629 |
| Acid Orange 7 (15510) | ![Molecular structure](attachment) | Monoazo | 350.32 | 482.5 |
| Reactive Black 5 | ![Molecular structure](attachment) | Diaz | 991.82 | 598 |
| Methyl Violet (42535) | ![Molecular structure](attachment) | Trimethyl methane | 393.5 | 584 |
Enzyme assay. MnP activity was determined using DMP as a substrate. The reaction mixture consisted of 50 mM malonate buffer (pH 4.5), 20 mM manganese sulfate, 2 mM H₂O₂, and 20 mM DMP. The MnP activity was observed at 470 nm with a UV-Vis spectrophotometer and calculated from the molar extinction coefficient (ε) of 49,600 M⁻¹cm⁻¹. One unit of MnP is defined as the amount of enzyme needed to oxidize 1 mmol of Mn (II) to Mn (III) in 1 min. Laccase activity was determined by the laccase assay using syringaldazine as the substrate. The reaction mixture contained 10 mM acetate buffer and 0.5 mM syringaldazine and was monitored at 525 nm with a UV-Vis spectrophotometer. Laccase activity was calculated from the oxidation of syringaldazine to its quinone using a molar absorptivity (ε) of 6,500 M⁻¹cm⁻¹ [15]. Enzyme activity (U/L) was calculated according Eq. (1).

\[
\text{Enzyme activity} = \left( \frac{\text{Abs}}{t} \right) \times (V_{\text{mix}} \mu\text{L} : 10^6) \times 10^6 \times \left( \frac{60}{t} \right) : \left( V_{\text{enzyme}} \mu\text{L} : 10^3 \right)
\]

(1)

Influence of enzyme activity on RBBR dye decolorization. Six different concentrations of enzyme (0.025, 0.05, 0.10, 0.25, 1.00, and 4.00 U/mL) were used to decolorize 100 ppm RBBR dye (3 mL).

Influence of RBBR dye concentration on decolorization. Crude enzyme at optimum activity was added to 100, 200, 300, 400, and 500 ppm RBBR dye.

Decolorization of various dyes. Crude enzyme at optimum activity was added to 3 mL of 100 ppm dye: RBBR, AB129, AO7, RB5, or MV.

Influence of mediators on decolorization of dyes. Mediators (1.0 mM MnSO₄ and 0.1 mM H₂O₂) were added to 3 mL of 100 ppm dye. In a separate experiment, 2 mM VA was added to activate the laccase.

Decolorization experiment and assay. All reaction mixtures were incubated at room temperature for 7 h. The decolorization process was measured at an interval of 1 h with UV-Vis spectrophotometer at the λ_max of each dye. Each decolorization experiment was performed in triplicate and the mean of the decolorization percentages was reported. The percentage of decolorization was calculated using Eq. (2).

\[
\text{Decolorization(%)} = \left( \frac{A_i - A_f}{A_i} \right) \times 100
\]

where A_i and A_f are the initial and final absorbances of the mixture, respectively.

Results and Discussion

The enzymatic activities of MnP and laccase were 0.038 ± 0.001 U/mg and 0.314 ± 0.010 U/mg, respectively, in the crude powder.

Effect of enzyme loading. Enzyme loading is one of the reaction parameters required to achieve maximum dye degradation. The decolorization of RBBR dye at various enzyme concentrations during the 7 h reaction time is shown in Figure 1a. The dye degradation was more efficient at 0.25–4.00 U/mL, with maximum decolorization of 95.57–100% after a 7 h reaction time (Fig. 1b). In general, increasing the enzyme concentration enhanced the decolorization activity. This result agrees with the findings of Ang et al. [6], who reported that the initial decolorization efficiency of RBBR increased with the increase of enzyme concentration. However, an excessively high enzyme concentration would increase the cost of the treatment process [16]. Therefore, an enzyme concentration of 0.25 U/mL was presumed adequate for use in further decolorization experiments in this study.

Figure 1. Percentage of RBBR Decolorization at Various Enzyme Concentrations (a) During the 7 h Reaction Time, (b) at Maximum Decolorization

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Effect of dye concentration. The RBBR dye concentrations at 100, 200, 300, 400, and 500 ppm were treated with a constant enzyme concentration of 0.25 U/mL for a 7 h reaction time (Figure 2a). Maximum decolorization (95.57 ± 0.32%) was found at a lowest dye concentration (100 ppm). The decolorization activity by the enzyme decreased with increasing dye concentration (Fig. 2b). This is possibly related to the maximum capacity of enzymes at a certain concentration for bleaching the dyes [17]. Sabarathinam et al. [16] also reported that the maximum decolorization of 96.90% was achieved at 25 ppm of RBBR and the decolorization was reduced to 53.21% at 250 ppm of RBBR.

Decolorization of various dyes. Synthetic anthraquinone, azo, and trimethyl methane dyes are common dyes used in the textile industry. In this study, AB129, AO7, RB5, and MV were tested in the decolorization process using the crude ligninolytic enzymes extracted from T. hirsuta D7. The enzyme was most effective at decolorizing anthraquinone AB129 (94.59%) in a 7 h reaction period, followed by monoazo AO7, diazo RB5, and trimethyl methane MV (13.99%, 7.61% and 7.59% decolorization, respectively) (Figure 3). Ratanapongleka and Phetson [18] noted that the efficiency of decolorization depended on the dye structure.

The chemical structure of dyes generally consists of a conjugated system of double bonds and aromatic rings. The major classes of dyes are composed of anthraquinoid, indigoid, and azo aromatic structures [3]. AQN is the basic unit of dye classes, as its quinonoid system acts as a chromophore. AB129 is an acidic dye or an AQN-type dye that has groups containing nitrogen and sulfur (amine and sulfonate, respectively). It has two structural units, AQN and 1,3,5-trimethylbenzylamine, which constitute the chromophore core [3]. The azo dye class is the largest group of dyes, with -N=N- as a chromophore in an aromatic system [19]. The azo groups are generally connected to benzene and naphthalene rings, but they can also be attached to aromatic heterocyclic or enolizable aliphatic groups [20]. AO7 is mono-azo dye that has three aromatic rings and one sulfonate group. RB5 is diazo dye that contains stringent aromatic molecules and bis-azo bonds and is difficult to degrade due to its complex structure [21]. MV belongs to a class of intensely colored organic compounds called triphenylmethane dyes, due to the presence of three aryl groups, each of which is bonded to a nitrogen atom that interacts with one or two methyl groups [22].

Dyes with simple structures exhibit high rates of decolorization. Lavanya et al. [19] and Lade et al. [23] showed that the color removal rate is faster for monoazo dyes than for diazo and triazo dyes. Azo compounds with hydroxyl or amino groups are more easily degraded than are those with methyl, methoxy, sulfo, or nitro groups. In this study, decolorization proceeded in the order of AB129 > AO7 > RB5 > MV. Anthraquinone dye is more susceptible to laccase enzymatic activities [24]. This was proven in this study, where AB129 and RBBR dyes were more susceptible to the crude ligninolytic enzyme activity, while the triphenyl methane dye (MV) was resistant to the enzyme activity.
A mixture of MnSO₄ and H₂O₂ enhanced the efficiency of dye decolorization (Figure 4). A previous study also found that industrial dyes, such as RBBR, Congo red, trypan blue, methylene blue, MV, methyl green, and brilliant cresyl blue, were decolorized when the reaction medium contained an enzyme as well as 1 mM MnSO₄ and 0.1 mM H₂O₂ [25]. Asgher et al. [26] reported that MnP requires hydrogen peroxidase as well as Mn²⁺ for its activity. The addition of manganese (MnSO₄) at a concentration of 0.1–1 mM also can enhance the MnP activity. Mn is a specific mediator in the MnP catalytic mechanism [27], while hydrogen peroxidase acts as a co-substrate for MnP. H₂O₂ is also accelerates the MnP reaction [28] through a Fenton reaction, where H₂O₂ generates hydroxyl radicals (•OH) and/or superoxide radicals (O₂⁻) to bleach the dyes [29].

In a separate experiment, the addition of 2 mM VA led to a greater decolorization of the dyes than was achieved with addition of MnSO₄ and H₂O₂ (Figure 4). A previous study showed that the addition of 2 mM and 5 mM VA could enhance the decolorization of the diazo dye Acid Red (AR97) by 3-fold. VA was reported as one of the most effective redox mediators for laccase oxidation when compared to the natural mediators [30]. In the present study, the addition of mediators such as MnSO₄, H₂O₂, and VA enhanced the decolorization rate of the dyes up to 10-fold when compared to the rate without the mediator.

In the present study, the dye decolorization was a result of a combination of MnP and laccase enzyme activity. The MnP oxidizes Mn (II) to Mn (III), and Mn (III) then oxidizes many phenolic compounds [17]. The laccase decolorizes azo dyes by catalyzing the oxidation of phenolic compounds and aromatic amines through the reduction of molecular dioxygen to water. The enzymes attack the double bonds or aromatic rings of the dyes and change the configuration of the dye structure. Disruption of the dye structure results in a reduction in the color intensity. Changes in the absorption spectra of the dyes (AB 129, AO 7, RB 5, and MV) as a result of the enzymatic reaction are shown in Figure 5.

Figure 4. Decolorization of AB129 (A), RB5 (B), AO7 (C), and MV (D) by Crude Enzyme D7 only, ( ), with the Addition of 1 mM Mn²⁺ and 0.1 mM H₂O₂ ( ) and with the Addition of 2 mM VA ( )
Conclusion

The ligninolytic enzyme from \textit{T. hirsuta} D7, at 0.25 U/mL, can effectively decolorize 100 ppm of RBBR dye. The decolorization activity of this enzyme decreases with increasing dye concentration. Dyes with simpler structure show higher rates of decolorization than do dyes with a more complex structure. The addition of mediators, such as MnSO\textsubscript{4}, H\textsubscript{2}O\textsubscript{2}, and VA enhances the dye decolorization rate.

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References

\[1\] Özdemir, M., Durmuş, Ö., Şahin, Ö., Saka, C. 2015. Removal of methylene blue, methyl violet, rhodamine B, alizarin red, and bromocresol green dyes from aqueous solutions on activated cotton stalks. \textit{Desalin. Water Treat.} 57(38): 1-11, http://dx.doi.org/10.1080/19443994.2015.1085916.

\[2\] Przystas, W., Zablocka-Godlewska, E., Grabinska-Sota, E. 2015. Efficacy of fungal decolorization of a mixture of dyes belonging to different classes. \textit{Braz. J. Microbiol.} 46(2): 415-424, DOI: http://dx.doi.org/10.1590/S1517-838246246220140167.

\[3\] Palencia, M., Martinez, J.M., Arrieta, A. 2017. Removal of acid blue 129 dye by polymer-enhanced ultrafiltration (PEUF). \textit{J. Sci. Technol. Appl.} 2: 65-74.

\[4\] Cuoto, S.R. 2009. Dye removal by immobilised fungi. \textit{Biotechnol. Adv.} 27: 227-235, http://dx.doi.org/10.1016/j.biotechadv.2008.12.001.

\[5\] Vrsanska, M., Buresova, A., Damborsky, P., Adam, V. 2015. Review: influence of different inducers on ligninolytic enzyme activities. \textit{J. Met. Nano.} 3: 64-70.

\[6\] Ang, T.N., Ngoh, G.C., Chua, A.S.M., Ismail, I. 2014. Remazol brilliant blue R dye decolourization by lacase produced by \textit{Pleurotus sajor-caju} via solid state fermentation. Proceed. the Regional Conference on Chemical Engineering, 1-6.

\[7\] Sukarta, I.N., Sastrawidana, I.D.K. 2014. The use of agricultural waste to increase the production ligninolytic enzyme by fungus \textit{Polyporus sp}. \textit{Open Acess Library J.} 1: 1-7, http://dx.doi.org/10.4236/oalib.1100642.
[8] Asgher, M., Noreen, S., Bilal, M. 2016. Enhancing catalytic functionality of Trametes versicolor IBL-04 laccase by immobilization on chitosan microspheres. Chem. Eng. Res. Des. 119: 1-11, http://dx.doi.org/10.1016/j.cherd.2016.12.011.

[9] Shaheen, R., Asgher, M., Hussain, F., Bhatti, H.N. 2017. Immobilized lignin peroxide from Ganoderma lucidum IBL-05 with improved dye decolorization and cytotoxicity reduction properties. Int. J. Biol. Macromol. 103: 57-64, http://dx.doi.org/10.1016/j.ijbiomac.2017.04.040.

[10] Oktaviani, M., Yanto, D.H.Y. 2016. Bio-decolorization of textile dye by isolated tropical fungi. Proceeding of International Conference of Indonesia Forestry Researchers III-2015, Bogor, IPB, p 276-285.

[11] Pogni, R., Brogioni, B., Baratto, M.C., Sinicropi, A., Giardina, P., Pezzella, C. et al. 2007. Evidence for a radical mechanism in biocatalytic decoloration of synthetic dyes by fungal laccases mediated by violuric acid. Biocatal. Biotransform. 25(2-4): 269-275, https://doi.org/10.1080/10242420701422757.

[12] Nabil, G.M., El-Ham, N.M., Mahmoud, M.E. 2014. Enhanced decolorization of reactive black 5 dye by active carbon sorbent-immobilized-cationic surfactant (AC-CS). J. Ind. Eng. Chem. 20(3): 994-1002.

[13] Hameed, B.H. 2008. Equilibrium and kinetic studies of methyl violet sorption by agricultural waste. J. Hazard. Mater. 154(1-3): 204-212, doi:10.1016/j.jhazmat.2007.10.010.

[14] Hermiati, E., Anita, S.H., Risanto, L., Styarini, D., Sudiyani, Y., Hanafi, A., Abimanyu, H. 2013. Biological pretreatment of oil palm frond fiber using white rot fungi for enzymatic saccharification. Makara Seri Tekonologi. 17(1): 39-43, doi:10.7454/mst.v17i1.1926.

[15] Srinivasan, C., D’Souza, T.M., Boominathan, K., Reddy, C.A. 1995. Demonstration of laccase in the white rot basidiomycete BKM-F1767. Appl. Environ. Microbiol. 61(12): 4274-4277.

[16] Sabarathinam, S., Priydharsini, U., Savitha, S., Sivakumar, E., Swaminathan, R., Subramanian, S. 2017. Trichoderma asperellum laccase mediated crystal violet degradation-optimization of experimental conditions and characterization. J. Environ. Chem. Eng. 5: 222-231, http://dx.doi.org/10.1016/j.jece.2016.11.04.

[17] Sneha, U., Poornima, R., Sridhar, S. 2013. Decolorization of synthetic textile dyes by using Pseudomonas putida. J. Chem. Pharm. Res. 5(5): 219-225, www.jocpr.com.

[18] Ratanapongleka, K., Phetsom, J. 2014. Decolorization of synthetic dyes by crude laccase from Lentinus polychlorus Lev. Int. J. Chem. Eng. Appl. 5(1): 26-30, doi: 10.7763/IJCEA.2014.V5.345.

[19] Lavanya, C., Dhankar, R., Chikara, S., Sheoran, S. 2014. Degradation of toxic dyes: a review. Int. J. Curr. Microbiol. Appl. Sci. 3(6): 189-199, http://www.ijcmas.com.

[20] Zille, A. 2005. Laccase reactions for textile applications. Universidade do Minho. pp. 1-151. http://hdl.handle.net/1822/4899.

[21] Sharma, P., Singh, L., Dilbaghi, N. 2009. Biodegradation of Acid Orange 7 dye by Phanerochaete chrysosporium in simulated waste water. J. Sci. Ind. Res. 68: 157-161, http://hdl.handle.net/123456789/2914.

[22] Bonetto, L.R., Ferrarini, F., De Marco, C., Crespo, J.S., Guéran R., Giovanela, M. 2015. Removal of methyl violet 2B dye from aqueous solution using a magnetic composite as an adsorbent. J. Water Process. Eng. 6: 11-20, https://doi.org/10.1016/j.jwpe.2015.02.006.

[23] Lade, H., Kadam, A., Paul, D., Govindwar, S. 2014. Biodegradation and detoxification of textile azo dyes by bacterial consortium under sequential microaerophilic/aerobic process. EXCLI J. 14: 158-174, http://dx.doi.org/10.17179/excli2014-642.

[24] Wong, Y., Yu, J. 1999. Laccase-catalyzed decolorization of synthetic dyes. Wat. Res. 33(16): 3512-3520, doi: 10.1016/S0043-1354(99)00664-4.

[25] Boer, C.G., Obici, L., Marques de Souza, C.G., Peralta, R.M. 2004. Decolorization of synthetic dyes by solid state cultures of Lentinula (Lentinus) edodes producing manganese peroxidase as the main ligninolytic enzyme. Bioresour. Technol. 94: 107-112, doi:10.1016/j.biortech.2003.12.015.

[26] Asgher, M., Bhatti, H.N., Ashraf, M., Legge, R.L. 2008. Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. Biodegradation. 19: 771-783, doi:10.1007/s10532-008-9185-3.

[27] Rajan, A., Kurup, J.G., Abraham, T.E. 2010. Solid state production of manganese peroxidases using arecanut husk as substrate. Braz. Arch. Biol. Technol. 53(3): 555-562. http://dx.doi.org/10.1590/S1516-89132010000300008.

[28] Hoshino, F., T. Kajino, H. Sugiyama, O. Asami, H. Takahashi. 2002. Thermally stable and hydrogen peroxide tolerant manganese peroxidase (MnP) from Lentinus betulinus. FEBS J. 530: 249-252, https://doi.org/10.1016/S0014-5793(02)03454-3.

[29] Couto, S.R., Sanroma, M.A. 2007. The effect of violuric acid on the decolorization of recalcitrant dyes by laccase from Trametes hirsuta. Dyes. Pigments. 74: 123-126, doi:10.1016/j.dyepig.2006.01.021.

[30] Hu, M.R., Chao, Y.P., Zhang, G.Q., Xue, Z.Q., Qian, S. 2009. Laccase- mediator system in the decolorization of different types of recalcitrant dyes. J. Ind. Microbiol. Biotechnol. 36(1): 45-51, doi:10.1007/s10295-008-0471-1.