



Research Article – Environmental Science

Enzymatic decolorization of Remazol Brilliant Blue Royal (RB 19) textile dye by white rot fungi

Hamisu Afiya¹, Erkurt Emrah Ahmet², M. Manjur Shah^{1*}

¹Department of Biological Sciences, Yusuf maitamaSule University, kano, Nigeria

²Department of Environmental Science, Cyprus International University, Turkish Republic of Northern Cyprus (TRNC)

(Received: 18-11-2018; Accepted 24-01-2019; Published Online 27-01-2019)

* Corresponding author (E-mail: mmanjurshah@gmail.com)

Abstract

Synthetic dyes are widely used by different industries with over 7×10^5 metric tons produced globally each year. Dyes pose adverse effects including chemical oxygen demand, visual pollution, cytotoxicity, genotoxicity, mutagenicity and carcinogenicity on various types of living organisms. The versatile white rot fungi (basidiomycetes fungi) have developed specialized ligninolytic enzymes for reductive cleavage of dyes and xenobiotics. The present study optimized the decolorization of Remazol brilliant blue royal (RBBR) dye by enzymatic extracts of *Coriolus versicolor* and *Pleurotus ostreatus*. Experiments were carried out by varying one parameter i.e. pH (2.5-6.5), temperature (30°C-60°C), enzyme activity (3.3U-20U), dye concentration (10mg/L-125mg/L) and time (0-480mins), while others constant to study its effects on decolorization of RBBR. From the results obtained, the optimum conditions for decolorization of RBBR by extracts of *C. versicolor* and *P. ostreatus* were pH 4.0, temperature of 30°C, enzyme activity 20U, dye concentrations of 100mg/L and 50mg/L for *C. versicolor* and *P. ostreatus* respectively at the end of 480 minutes. At the optimized conditions, decolorizations for *C. versicolor* and *P. ostreatus* were 80.42% and 70.42% respectively. Highest laccase activity (19.50U) was recorded in *C. versicolor* compared to *P. ostreatus* (1.41U).

Keywords: Decolorization, RBBR, *Coriolus versicolor*, *Pleurotus ostreatus*

Introduction

The textile industry is a substantial consumer of water and produces enormous volume of contaminated water during various processes of production (Verma *et al.*, 2012). This wastewater is highly polluted containing high COD, BOD, salts, color (residue of reactive dye) and organic matter. The presence of dyes as the most important contaminants in the effluents is making biodegradation complex (Tekoğlu, and Özdemir, 2010). The water load causes an imbalance to water ecosystem by limiting the amount of light penetration into water bodies thereby affecting aquatic life (Wang *et al.*, 2011), depleting dissolve oxygen in waters and possibility of some ions such as chromium to enter into food chain.

The hazardous nature of dyed wastewaters released by textile industries is widely accepted. They are highly toxic with others and carcinogenic (Chung and Stevens, 1993; Zollinger, 1987) as cited by Swamy, 1998). The need for an efficient, economic and environmentally benign technology to treat textile effluents is highly demanding. Various physico-chemical methods available for their treatment are expensive and environmentally unpleasant. However, biological methods or its combination could serve a solution due to its efficiency in removing color at appreciable cost (Robinson *et al.*, 2001; Padmavathy *et al.*, 2003). The versatile white rot fungi (basidiomycetes fungi) have developed specialized ligninolytic enzymes for reductive cleavage of azo bond which are generally recalcitrant to other biological degradation (Pandey *et al.*, 2007). Enzymes of white rot fungi break aromatic rings and decolorize dyes (Singh, 2006). Several researches acknowledged the efficiency of white rot fungi for biological treatments of dyes (Hardin *et al.*, 2003;

Papadopoulou *et al.*, 2013; Hadibarata *et al.*, 2013). The current study focused on decolorization potentials of *pleurotustosreatus* and *Coriolus versicolor* on reactive blue 19. Remazol brilliant blue royal was selected from a list of an important recalcitrant anthraquinone type of dye largely used by textile industries (due to its excellent color fastness especially in cotton fabrics) and released to the surroundings.

Materials and Methods

Collection of fungal cultures and chemicals

Pure cultures of *Pleurotus ostreatus* and *Coriolus versicolor* were collected from Biotechnology research laboratory, faculty of Environmental Engineering, Cyprus International University, Turkish republic of Northern Cyprus (TRNC). Cultures were maintained on potato dextrose Agar (PDA) slants and incubated at 30°C for 15days and stored at +4°C. RBBR textile dye and reagents used in the present study were obtained from Merck and Sigma Aldrich companies.

Preparation of stock dye solution

A Stock dye solution of Remazolbrilliant blue royal (RBBR) dye 1g/L of 0.1M sodium phosphate buffer pH 6.0 was prepared. Standard solutions of 10mg/L, 25mg/L, 50mg/L, 100mg/L and 200mg/L were prepared from the stock dye using the formula $M_1 V_1 = M_2 V_2$.

where M_1 = Concentration of stock dye solution V_1 = Volume of dye (RBBR) M_2 = Concentration of dye (RBBR) V_2 = Volume of standard solution (50ml). UV visible Spectrophotometer (2450 shimadzu model) was used to determine the maximum dye absorption wave length and absorptions of standard solutions as shown in Fig.1.

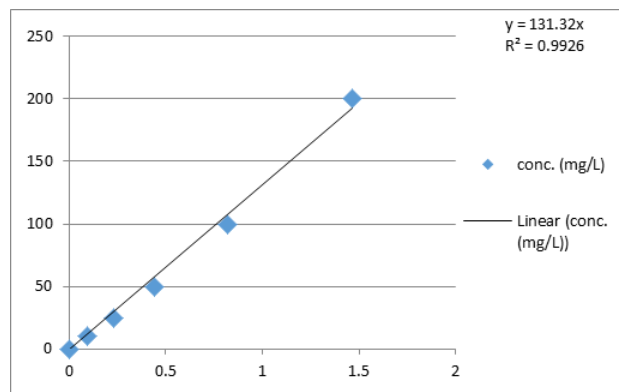


Fig. 1. Calibration Curve for RBBR textile dye.

Preparation of culture medium

Solid state fermentation medium was prepared based on the protocol described by Pensupa *et al.* (2013) with composition; wheat bran 90g (90% w/w) and soybean residue 10g (w/w). Medium was humidified with a 0.1M pH 5.5 sodium phosphate buffer at a ratio 70% (v/w) and autoclaved at 121.5 °C and 1.2 atm conditions for 30 min, and left to stand for 2days under room temperature. Medium was then inoculated with fungal stock cultures of *Coriolus versicolor* and *Pleurotus ostreatus* grown on PDA under sterile conditions. The Inoculated medium was incubated for 17 days at 30°C. Cultures were dehumidified in a memmert oven at 45°C for 24h. Dehumidified cultures were grinded in a Mixer/millier at 8000rpm for 2 mins and stored in are fridgerator at 4°C for further work.

Enzyme extraction

Enzyme extraction was carried according to the protocol of Ergun and Urek (2017). Following this protocol, one gramme of dehumidified culture medium for *Coriolus versicolor* and *Pleurotus ostreatus* were dissolved in 10ml each of 0.1M sodium phosphate buffer pH 6.0 for enzyme extraction. The prepared solutions were shaken in a Heridolph shaker for 15mins, centrifuged using Hettich Micro 22R at 6000rpm for 5mins and filtered using filter paper (Whatman No. 1). The filtrate was used as a source of enzyme for the present decolorization studies.

Optimization studies for decolorization of RBBR

The decolorization of RBBR textile dye was optimized by studying the effect of various physical parameters i.e, pH (2.5-6.5), temperature (30°C-60°C), initial dye conc. (10mg/L-125mg/L), enzyme activity(3.3U-20U) and time (0-480mins). This was done by varying one parameter while previously optimized parameters at optimum level as described by Asgher *et al.* (2013). Samples were drawn periodically at intervals of 0,5,10,30,60, and 120 minutes and analyzed using UV-vis spectrophotometer at maximum absorption wavelength to observe the change in absorbance. Percentage decolorization was calculated as follows: % Decolorization= $100(A_0 - A_t) / A_0$. Where, A_0 is the absorbance value of the initial dye concentration, and A_t is the absorbance value of the dye concentration in sample at time t.

Enzyme Assay

Laccase activity was assayed spectrophotometrically with ABTS as substrate (0.4 mM) in 0.05 mM Citrate /0.1 M phosphate buffer at pH 4.5 as described by Iqbal *et al.*

(2011). 0.1 mL of the incubated enzyme solution was added to 1.9 mL of the ABTS solution at room temperature. The change in absorbance at 420 nm ($\epsilon = 36,000 \text{ M}^{-1} \text{ cm}^{-1}$) was recorded automatically by the spectrophotometer for 120s and 180s for *Coriolus versicolor* and *Pleurotus ostreatus* respectively.

Results and Discussions

pH Effect

Varying pH (2.5-6.5) indecolorization of RBBR by both extracts of *Coriolus versicolor* and *Pleurotus ostreatus* (Fig 1 and 2) shows that the maximum decolorization occurs at pH 4. At this optimum, maximum decolorizations were 58% and 47% by *C. versicolor* and *P. ostreatus* respectively. The result obtained was in agreement with previous findings which showed optimum decolorization by white rot fungi in acidic condition (Mansur *et al.*, 2003; Palmieri *et al.*, 2005; Kaushik and Malik, 2009; Asgher *et al.*, 2009).

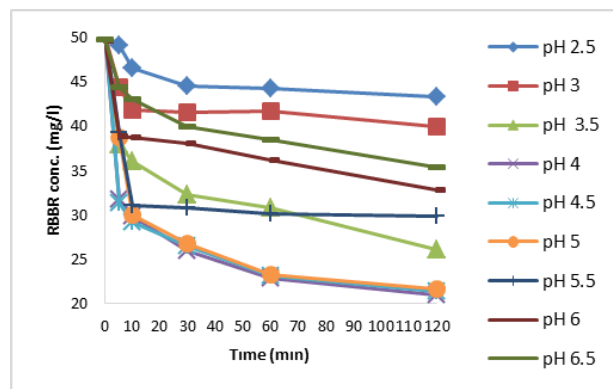


Fig. 2. pH curve for Decolorization of RBBR by *Coriolus versicolor* extracts (after 120 minutes).

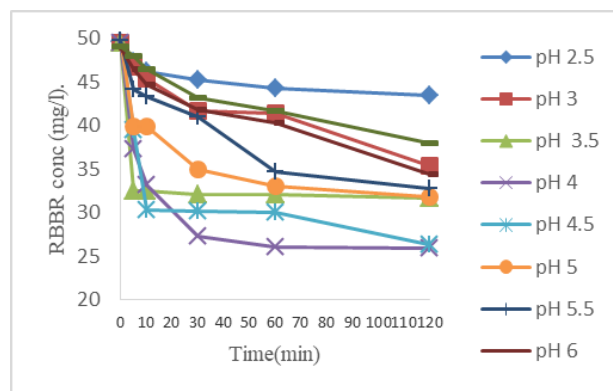


Fig. 3. pH curves for decolorization of RBBR by *Pleurotus ostreatus* extract (after 120 minutes).

Temperature Effect

The decolorization patterns for RBBR showed that maximum decolorizations of 69% & 39% were achieved by *Coriolus versicolor* and *Pleurotus ostreatus* respectively at 30 °C among other tested temperatures (30 °C, 40 °C, 45 °C, 50 °C and 60 °C) after 120 minutes (Fig 4 and 5). Further increase in temperature results in decreased the percentage decolorizations in both *Coriolus versicolor* and *Pleurotus ostreatus*. This study was in consistent to the study by Asgher *et al.* (2009); Naghdi *et al.* (2013) and Venkatachalam and Venkatachalam (2008), whom observed that decolorization of dye stuffs by white rot fungi work best at moderate

temperatures. The decrease in decolorization at higher temperatures could be due to denaturation of enzymes resulting from the breakdown of the weak ionic and hydrogen bonding that stabilizes the 3-dimensional structure of the enzyme active site.

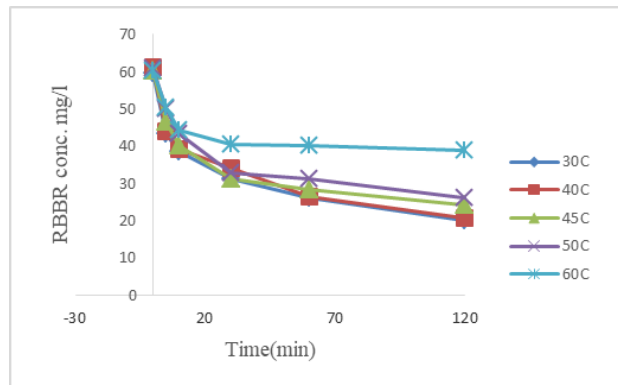


Fig. 4. Temperature curve for Decolorization of RBBR by *Coriolus versicolor* extract (after 120 minutes)

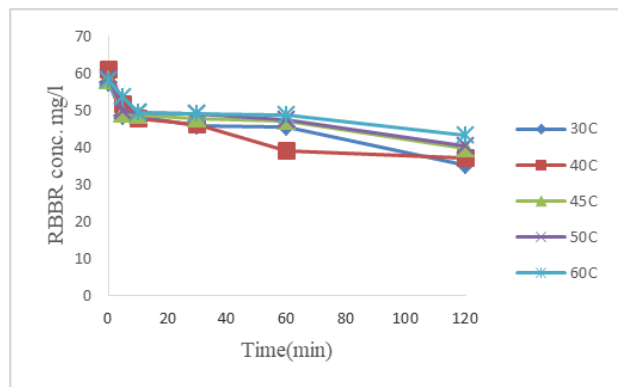


Fig. 5. Temperature curve for Decolorization of RBBR by *Pleurotus ostreatus* extract (after 120 minutes)

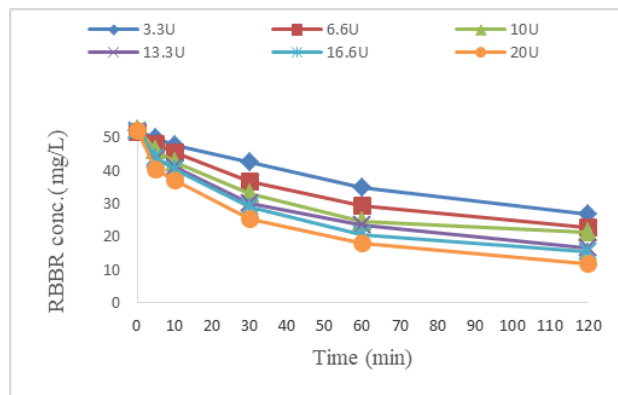


Fig. 6. Enzyme curve for decolorization of RBBR by *Coriolus versicolor* extract (after 120 minutes).

Enzyme effect

Enzyme activity of 20U was optimum for decolorization of RBBR by both *Coriolus versicolor* and *Pleurotus ostreatus* compared to other enzyme activities tested (3.3U, 6.6U, 10U, 13.3 and 16.6) (Fig 6 & 7). Up to 78% and 43% color removal were achieved by *Coriolus versicolor* and *Pleurotus ostreatus* respectively at the optimum enzyme activity (20U) after 120 minutes. This is in harmony to the findings of Yesilada *et al.* (2014), who obtained maximum

decolorization at highest enzyme activity. Similarly, Husain (2006) and Murugesan *et al.* (2006) obtained similar results.

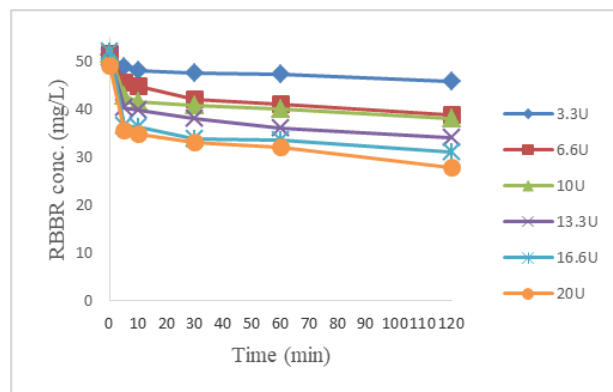


Fig. 7. Enzyme curve for Decolorization of RBBR by *Pleurotus ostreatus* extract (after 120 minutes).

Dye effect

This was carried out with initial concentration of dye ranging from 10-125mg/L. From the results obtained, color removal progressively decreased with increase in concentration of dye (Fig 8 and 9). Maximum decolorizations (94% and 83% in *Coriolus versicolor* and *Pleurotus ostreatus* respectively) were obtained at 10mg/L. However, it has been observed that higher concentration of dye yielded increase the rate of dye removal.

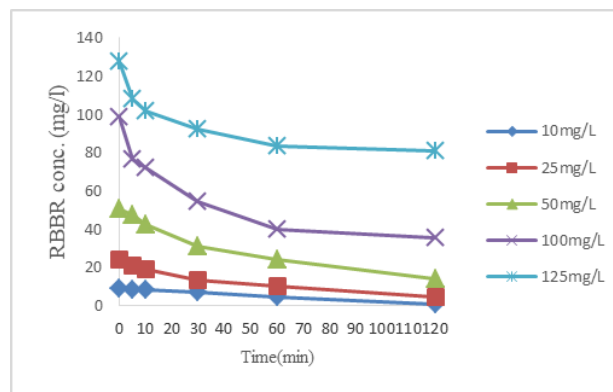


Fig. 8. Initial Dye curves for decolorization of RBBR by *Coriolus versicolor* extract (after 120 minutes).

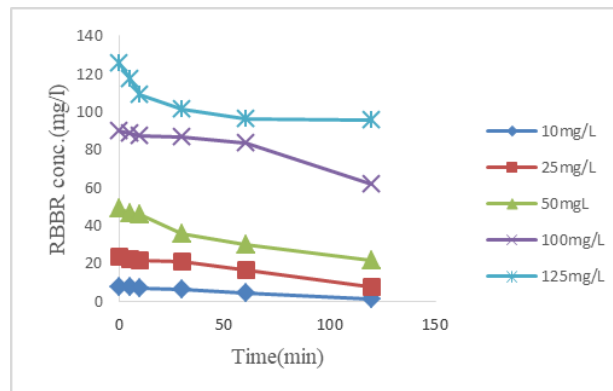


Fig. 9. Dye curve for decolorization of RBBR by *Pleurotus ostreatus* extract (after 120 minutes).

Dye concentrations up to 100mg/L and 50mg/L for *C. versicolor* and *P. ostreatus* respectively resulted in maximum color removal at the previously optimized conditions. Further

increase in concentration from the optimum (100mg/L & 50 mg/L for *Coriolus versicolor* and *P. ostreatus* respectively) decreased the removal of color, which could be due to saturation of enzyme active site. The decolorization of dye at higher concentrations could be due to an acidic condition which support dye removal by most white rot fungi as reported in literatures (Mansur *et al.*, 2003; Palmieri *et al.*, 2005; Kaushik and Malik, 2009; Asgher *et al.*, 2009).

Time Effect

Varying time from 0-480minutes in decolorization of RBBR by *C. versicolor* and *P. ostreatus* at previously optimized conditions showed that color removal rapidly increases as time increases, up to a limit when no significant decolorization was observed (Fig 10 and 11). At the end of 480 minutes, maximum decolorizations were 80.42% and 70.42 % for *C. versicolor* and *P. ostreatus* respectively. However, increase in time may increase the cost of treatment due to high energy input. This finding was in consistent to the work of Hadibarata *et al.* (2013) and Asgher *et al.* (2009), who obtained similar, results in a time dependent manner.

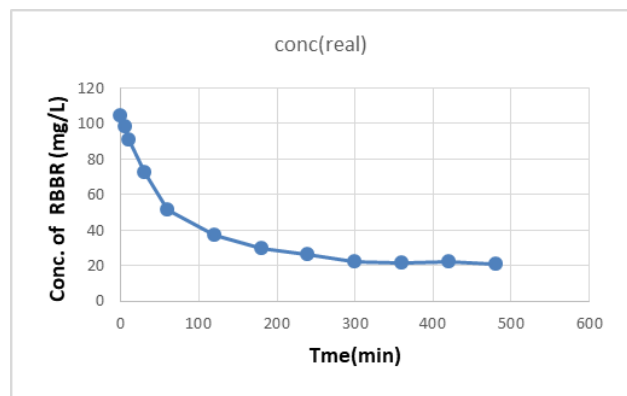


Fig. 10. Time effect in Decolorization of RBBR by extract of *Coriolus versicolor* (after 480 minutes)

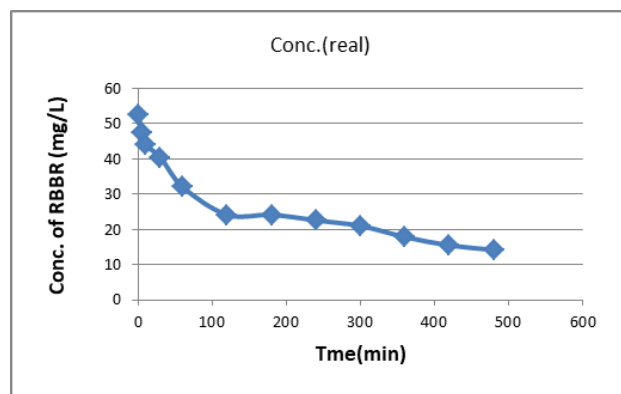


Fig. 11. Time effect in Decolorization of RBBR by extract of *Pleurotus ostreatus* (after 480 minutes)

Laccase Assay

High laccase activity was recorded in *C. versicolor* compared to *P.ostreatus* (Fig 12 & 13). Optimum laccase activities were 20.46U and 1.55U in *C. versicolor* and *P. ostreatus* respectively at the end of 30 and 60 minutes respectively. Further increase in time reduced the enzyme activity from the optimum. The lost in activity could be due to the effect of prolong agitation/shaking which may denature enzyme proteins.

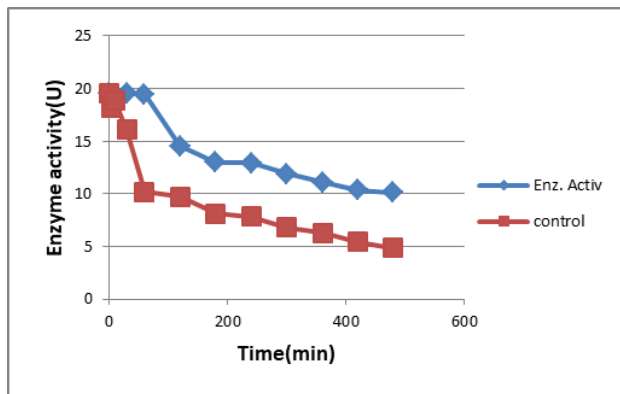


Fig. 12. Laccase activity of *C. versicolor* at optimized conditions

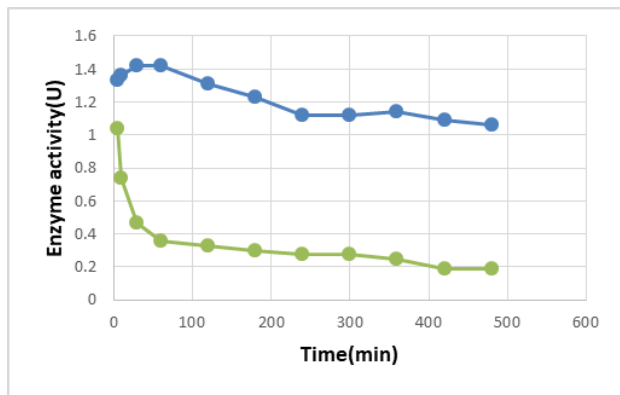


Fig. 13. Laccase activity of *P. ostreatus* at optimized conditions

Conclusions

In the current study, *P. ostreatus* and *C. versicolor* were used for decolorization of RBBR textile dye. *C. versicolor* was found to be more effective than *P. ostreatus* in decolorizing the tested dye, with up to 80% color removal at the end of 480 minutes, while *P. ostreatus* 70% at the same time. The optimum conditions for these results were pH 4, temperature 30 °C, enzyme activity 20U, dye concentrations of 50mg/L and 100mg/L for *P.ostreatus* and *C. versicolor* respectively. Laccase assay revealed higher activity of this enzyme in *C. versicolor* (20.46U) compared to *P. ostreatus* (1.55U), which could be the reason for higher decolorization in *C. versicolor*.

Recommendations

The application of this enzyme technology should be expanded to other textile dyes. However, advance study has to be done to investigate the toxicity of dye metabolites that might be produced due to degradation of dye, and potential fate of used biomass for an environmentally benign technology. Similarly, further study has to be done for application of this technology to large industrial scale to preserve our environments.

References

Asgher, M., Yasmeen, Q., Muhammad, H. and Iqbal, N. (2013). Enhanced decolorization of Solar brilliant red 80 textile dye by an indigenous white rot fungus *Schizophyllum commune* IBL-06. *Saudi Journal of Biological Sciences*, 20, 347-352.

- Asgher, M., Azim, N., and Bhatti, H. N. (2009). Decolorization of practical textile industry effluents by white rot fungus *Coriolus versicolor* IBL-04. *Biochemical Engineering Journal*, 47, 61–65.
- Chung, K.T., and Stevens, S. E. (1993). Degradation of Azo dyes by environmental microorganisms and helminthes. *Environmental Toxicology and Chemistry*, 12(11), 2121-2132.
- Ergun, S.O., and Urek, R.O. (2017) Production of ligninolytic enzymes by solid state fermentation using *Pleurotus ostreatus*. *Annals of Agrarian Science*, 15, 273-277.
- Hadibarata, T., Adnan, I.A., Yusoff, A.R.M., Yuniarto, A., Rubiyatno, Zubir, M.M.F.A., Khudhair, A.B., Teh, Z.C. and Abu Naser, M.A. (2013). Microbial Decolorization of an Azo Dye Reactive Black 5 Using White-Rot Fungus *Pleurotuseryngii* F032. *Water Air Soil Pollutant*, 224, 1595.
- Hardin, I.R., Zhao, X., and Akin, D. E. (2003). Biotreatment approach to decolorizing textile waste effluents. *Georgia Water Resources Conference*, Georgia.
- Husain, Q. (2006). Potential Applications of the oxidoreductive enzymes in the decolorization and detoxification of textile and other synthetic dyes from polluted water. *Critical Reviews in Biotechnology*, 26(4), 201-221.
- Iqbal, H.M.N., Asgher, M., and Bhatti, H.N. (2011). Optimization of physical and nutritional factors for synthesis of lignin degrading enzymes by a novel strain of *Trametes versicolor*. *BioResources*, 6(2), 1273–1287.
- Kaushik, P., and Malik, A. (2009). Fungal dye decolourization: Recent advances and future potential. *Environment International*, 35, 127–141.
- Mansur, M., Arias, M.E., Patino, J. C., Flardh, M., and Gonzalez, A. E. (2003). The white- rot fungus *Pleurotus ostreatus* secretes laccase isozymes with different substrate specificities. *Mycologia*, 95, 1013-1020.
- Murugesan, K., Arulmani, M., Nam, I., Kim, Y., Chang, Y., and Kalaichelvan, P.T. (2006). Purification and characterization of laccase by a white rot fungus *Pleurotussajor-caju* under submerged culture condition and its potential in decolorization of azo dyes. *Applied Microbiology Biotechnology*, 75(5), 939-46.
- Naghdi, R., Karimi, A.N., Latibari, A.J., Hamzeh, Y., Mirshokraie, S.A., and Nadali, E. (2013). Biological removal of chloro-organic compounds from bagasse soda pulp bleaching effluent by *Coriolus versicolor*. *Global NEST Journal*, 15(1), 29-36.
- Padmavathy, S., Sandhya, S., Swaminathan, K., Subrahmanyam, Y.V., Chakrabarti, T., and Kaul, S.N. (2003). Aerobic decolorization of reactive azo dyes in presence of various Co substrates. *Chemical and Biochemical Engineering Quarterly*, 17, 147-151.
- Palmieri, G., Cennamo, G., and Sannia, G. (2005). Remazol Brilliant Blue R Decolourisation by the fungus *Pleurotus ostreatus* and its oxidative enzymatic system. *Enzyme and Microbial Technology*, 36(1), 17-24.
- Pandey, A., Singh, P., and Iyengar, L. (2007). Bacterial decolorization and degradation of Azo dyes. *International Biodeterioration & Biodegradation*, 59(2), 73–84.
- Papadopoulou, K., Kalagona, I. M., Phillippoussis, A., and Rigas, F. (2013). Optimization of fungal decolorization of azo and anthraquinone dyes via Box-Behnken design. *International Biodeterioration and Biodegradation*, 77, 31-38.
- Pensupa, N., Jin, M., Kokolski, M., Archer, D.B. and Du, D. (2013). A solid state fungal fermentation based strategy for the hydrolysis of wheat straw. *Bioresource Technology*, 149, 261-267.
- Robinson, T., McMullan, G., Marchant, R., and Nigam, P. (2001). Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative. *Bioresource Technology*, 77(3) 247-255.
- Singh, H. (2006) Mycoremediation: Fungal bioremediation. Wiley, Hoboken
- Swamy, J. (1998). The biodecoloration of textile Dyes by the white rot fungi *Trametes versicolor*. Master's thesis, Queen's University. Ontario, Canada.
- Tekoğlu, O., and Özdemir, C. (2010). Wastewater of Textile Industry and Its Treatment Processes. BALWOIS 2010 - Ohrid, Republic of Macedonia – 25.
- Venkatachalam, S., and Venkatachalam, C. (2008). Decolorization of Cibacron Yellow S-3 R Using *Coriolus versicolor* (MTCC 138). *Modern applied Science*, 2(5), 3-8
- Verma, A.K., Dash, R.R., and Bhunia, P. (2012). A review on chemical coagulation /floculation technologies for removal of color from textile waste waters. *Journal of Environmental Management*, 93(1), 54-168.
- Wang, Z., Xue, M., Huang, K. and Liu, Z. (2011). Textile Dyeing Wastewater Treatment. In: Advances in treating textile effluents. Peter Hauser (Eds.), Intech Open. UK. Doi: 10.5772/22670
- Yesilada, O., Birhanli, E., Ercan, S., and Ozmen, N. (2014). Reactive dye Decolorization activity of crude laccase enzyme from repeated –batch culture of *Funalia trogii*. *Turkish Journal of Biology*, 38, 103-110.
- Zollinger, H. (1987). Colour Chemistry-Synthesis, Properties of Organic Dyes and Pigments. VCH Publishers, New York, 92-100.