# Decolorization of the dye Remazol Brilliant Violet by the white rot fungus *Stereum ostrea*

[1]Ramya. A, [2]Kanderi Dileep Kumar, [3]B. S. Shanthi Kumari, [4]S.N. Krushna Naik, [5]B. Rajasekhar Reddy

Department of Microbiology, Sri Krishnadevaraya University,
Anantapuramu, Andhra Pradesh – 515003, India.

***Abstract*: The release of textile azo dyes to the environment is an issue of health concern while the use of microorganisms has proved to be the best option for remediation.Thus in the present studyan attempt was made to examine the potential of*Stereum ostrea*,a white rot fungi for Decolorization of azo dye – Remazol Brilliant Violet (RBV). *S. ostrea* was grown in Koroljova liquid medium spiked with a concentration of RBV dye at 200ppm. RBV dye at 200ppm level was not toxic to *S. ostrea* as recovery of larger biomass from dye amended medium than from control. pH changes occurred in the culture medium within a range of 3.93 to 5.32 without dye upon growth of the fungal culture. Decolorization of RBV dye took place in culture broth during growth of *S. ostrea* in medium spiked with 200ppm level of dye. There was increase in Decolorization of dye from 14% within two days of incubation to 70% in 10 days of incubation.Impact of RBV dye on secretory capacity of *S. ostrea* was also assessed. Secretion of extracellular protein by *S. ostrea* was not affected by the presence of dye at 200ppm level. Soluble protein was secreted in to culture broth to the extent of 160 µg/ml in control as against 170 µg/ml within 6 days of incubation. The white rot fungus *S. ostrea* is known for secretion of ligninolytic enzymes – laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP). Therefore ligninolytic enzymes, secreted by *S .ostrea* in medium with/without dye were compared.Laccase appeared to be a dominant one in ligninolytic enzymes of *S. ostrea* as reflected by recovery of this enzyme in maximum titers. Yields of laccase, MnP and LiP obtained from medium ammended with 200 ppm dye were always higher than those of respective enzymes from control during course of growth of *S. ostrea.* At 10$^{th}$dayof incubation, secretion of three ligninolytic enzymes was enhanced 1.2 to 2.6 folds in medium with dye in comparison to control.**

***Keywords*: *Stereum ostrea*, Remazol Brilliant Violet, Decolorization, ligninolytic enzymes**

## I. Introduction

One of the major problems that humans are facing is the restoration of the contaminated environment. Textile dyes contribute as the most important environment-polluting agents (Maulinet al., 2013). By and large, these dyes contain aromatic and heterocyclic compoundswith accomplishing characteristics like color, intensity, solubility, fastness, and substantiveness (Zhang W and Wu C W, 2014) These dyes are very difficult to degrade and thus pose an environmental threat (Park et al., 2007). The presence these dyes in water bodies are not only highly toxic to aquatic life but also may cause various problems in human beings like, respiratory problems and gastrointestinal problems (Robinsonet al.,2002). Reports showed that approximately 100 tons of used dyes per annum are discharged into water streams worldwide (Yagub et al., 2012). The majority of these dyes and their transformed productsarehighlytoxicandmutagenictobioticcommunities (Benigni et al., 2000; Poonkuzhali et al., 2011; Sathishkumar et al., 2013).

Dyes can be of many different structural varieties like acidic, basic, disperse, azo, anthraquinone based and metal complex dyes. Among those azo dyes are the main chemical class of dyes with the greatest variety of colors, therefore they have been extensively used by the industry. Due to the presence of sulfonic groups and nitrogen-nitrogen double bond, azo dyes are categorized under highly recalcitrant compound and it is very difficult to degrade these compounds. Since these dyes are highly colored compounds they cause evidential environment threat by reducing the transparency of water bodies (AnuradhaKumari, 2015). Therefore, treatment of the dye-loaded effluent without causing secondary pollution is essential to protect the ecosystems receiving the effluent (Sathishkumar et al., 2014). In recent decades, several physicochemical and biological treatment techniques have been reported for the remediation of reactive azo dyes (Sathishkumar et al., 2012; Adnan et al., 2016; Saadon et al., 2016). Physicochemical treatment techniques include processes,such as coagulation, flocculation, adsorption, flotation, precipitation, oxidation and reduction, ozonation and membrane separation. However, they are very expensive and have drawbacks (Azmi et al., 1998; Robinson et al., 2001). Biological treatments have received a great deal of interest owing to their minimal impact on the ecosystem and their cost-effectiveness. Although bacterial treatment is economical and simple, there is a problem associated with the formation of toxic aromaticaminesduringthedegradationprocess(Vyridesetal., 2014).

Fungi could be an excellent candidate for dye removal. Most of them use an extracellular enzymatic system that transforms aromatic substances, such as lignin, PAH or pesticides. Much attention is currently focused on fungal decolorisation processes. The most widely studied are white rot fungi. They produce non-specific enzymes, such as lignin peroxidase, manganese peroxidase and laccase, which degrade many aromatic compounds. Fungi are used as sorbents and/or enzyme producers involved in biodegradation/biotransformation (Knapp et al., 1995;Wesenberg et al., 2003; Przystas et al., 2009; Diwaniyam et al., 2010; Przystas et al., 2013; Hadibarata et al., 2013; Si et al., 2013). The main objective of our study is to assess the efficacy of Decolorization of dye RemazolBrilliant Violet by fungus *Stereum ostrea.*

## II. Materials and Methods

**Organism and culture conditions:**

The White rot fungus *Stereum Ostrea*, isolated from wood logs was by prof. M.A. Singaracharya, Department of Microbiology, Kakatiya University, Andhra Pradesh, India. *S. ostrea* was maintained on Koroljova-Skorobogat medium and preserved at 4°C(Koroljova-Skorobogat`ko et al.,1998). The maintenance medium was prepared according the following composition (g/L):3.0 peptone, 10.0 glucose, 0.6 $KH_2PO_4$ 0.001 $ZnSO_4$, 0.4 $K_2HPO_4$, 0.0005 $FeSO_4$, 0.05 $MnSO_4$,0.5 $MgSO_4$ and 20.0 agar (pH 5.00).

**Dye and dye solution preparation:**

The commercial dye Remazol Brilliant Violet (RBV) was chosen for Decolorization in the present study. RBV is an azo dye with absorption maxima at 570nm. The dye was dissolved in sterile distilled water to a concentration of 100 mg/10 ml and was stored in the dark. The dye from the stock was added to the medium before sterilization to achieve the required dye concentration.

**Testing of decolorisation of Remazol Brilliant Violet(RBV) by *Stereum ostrea*by**

**1. Plate method**

Koroljova agar mediumwas amended with dye RBV at 200ppm and poured into petriplates.

Plates were inoculated with a disc of 6-day old fungus *S. ostrea* grown on Koroljova agar medium. Plates were incubated at its optimum growth temperature of 30°C for 12 to 14 days. Observations were made for dye Decolorization.

**2. Liquid cultures**

30ml of Koroljova broth spiked with dye concentration of 200ppmis dispensed into 250ml conical flasks. *S. ostrea* culture grown on agar slants was inoculated into Koroljova broth in flasks.The flasks were incubated in a gyratory orbital shaker at 150rpm at a temperature of 30°C.Flasks were withdrawn at regular time intervals from the shaker. The contents of the flask filtered through Whatman No.1 filter paper to separate mycelial biomass and cell free extract.Decolorization of the dye was assayed by measuring the decrease in absorbance at the λmax 557nm over a period of 12 days at 2-day interval by using UV Spectrophotometer. Ligninolytic enzyme activities were also assayed with cell free extract.

**Analytical procedures:**

**Decolorisation assay:**

Decolorisationactivity was expressed in terms of percentage Decolorization and was determined by monitoring the decrease in absorbance at 570 nm against the medium.

Decolorisation activity (%) was calculated according to the formula:

$$\textbf{Decolorization activity (\%)} = \frac{(\textbf{Initialabsorbance}) - (\textbf{Observedabsorbance})}{\textbf{Initialabsorbance}} \times 100$$

**Assay of ligninolytic enzymes:**

- Laccase activity was assayed using 10 mMguaiacol in 100 mM acetate buffer containing 10% (v/v) acetone. The changes in absorbance of the reaction mixtures containing guaiacol were monitored at 470 nm (Das et al., 1997). The enzyme activity was expressed in IU where one unit of laccase corresponded to the amount of enzyme that oxidized one micromole of guaiacol per minute.
- Lignin peroxidase (LiP) activity was determined by the oxidation of veratryl alcohol at 310 nm (Tien and Kirk, 1988). The enzyme activity was expressed in IU where one unit of LiP corresponded to the amount of enzyme that oxidized one micromole of veratryl alcohol per minute.
- Manganese peroxidase (MnP) activity was determined by the oxidation of 1mM guaiacol in presence of $H_2O_2$ in 10mM citrate phosphate buffer at 465 nm (Bonnen et al., 1994). The enzyme activity was expressed in IU where one unit of MnP was defined as the amount of enzyme that oxidized one micromole guaiacol of per minute.

**Protein estimation**

Protein concentrations were determined by the Folin-Lowry method with bovine serum albumin as the standard (Lowry et al., 1951).

## III. Results:

*S. ostrea* was initially grown in solid Koroljova medium amended with RBV dye at a concentration of 200 ppm. The plate surface appearance, such as fungal growth and clear halo formation was observed visually daily. Decolorization of the agar, without colouration of the fungal mat was observed which indicates the enzymatic degradation, which is further confirmed by enzymatic assay.

**Figure 1: Screening for Decolorization of the dye RBV at 200 ppm by *S. ostrea* on solid media**


*S. ostrea* was later grown in Koroljova liquid medium spiked with RBV dye at a concentration of 200ppm. RBV dye at 200ppm level was not toxic to *S. ostrea* as recovery of larger biomass from dye amended medium than from control (Table 1). pH changes occurred in the culture medium within a range of 3.93 – 5.32 without dye upon growth of the fungal culture

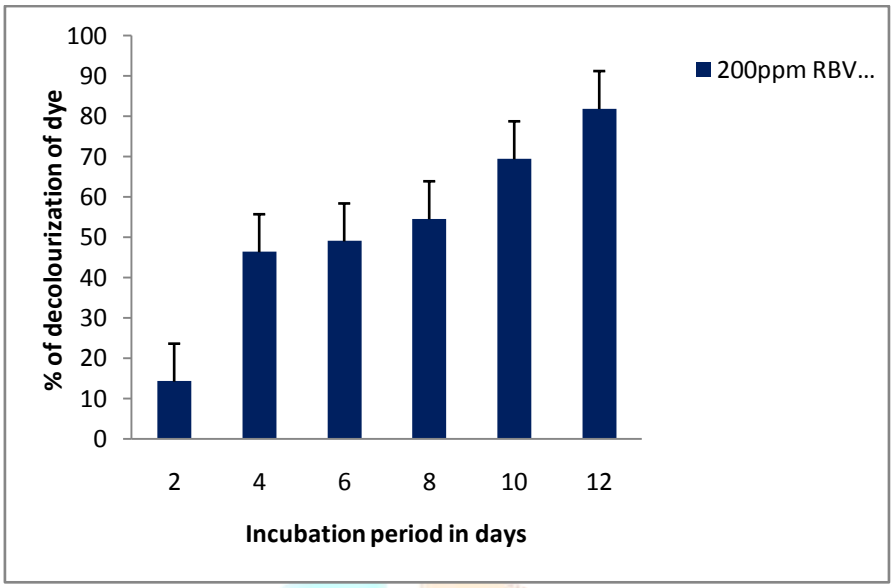
**Table 1. Growth of fungal culture (*Stereum ostrea*) and pH changes in the culture medium in the presence of RBV dye at 200ppm concentration**

| Incubation period in days | Biomass* (g/30ml) | | pHof the culture broth | |
|---|---|---|---|---|
| | control | 200ppm of dye | Control | 200ppm of dye |
| 2 | 0.04 | 0.045 | 5.15 | 5.32 |
| 4 | 0.052 | 0.06 | 4.81 | 4.97 |
| 6 | 0.07 | 0.087 | 4.21 | 4.45 |
| 8 | 0.085 | 0.091 | 4.05 | 4.22 |
| 10 | 0.09 | 0.108 | 3.93 | 3.97 |
| 12 | 0.11 | 0.121 | 3.72 | 4.82 |

*Biomass recovered from the entire medium (30ml) in the presence of the dye in the flask
Control: Devoid of the dye RBV
Values are the means of duplicate experimental set ups

Decolorization of RBV dye took place in culture broth during growth of *S. ostrea* in medium spiked with 200ppm level of dye (Figure 2). There was increase in Decolorization of dye from 14% within two days of incubation to 70% in 10 days of incubation.

**Figure 2. Decolorization of the dye RBV by *Stereum ostrea* at 200ppm of dye level**




*Percent Decolorization of dye – RBV in the culture filtrate expressed in terms of initially added dye
Control: Devoid of the dye RBV
Values are the means of duplicate experimental set ups

Impact of RBV dye on secretory capacity of *S. ostrea* was assessed (Table 2). Secretion of extracellular protein by *S. ostrea* was not affected by the presence of dye at 200ppm level. Soluble protein was secreted in to culture broth to the extent of 160 µg/ml in control as against 170 µg/ml within 6 days of incubation.

**Table 2. Secretion of extracellular protein by the fungal culture *Stereum ostrea* in presence of the dye RBV at a concentration of 200ppm**

| Incubation period in days | Extracellular protein content (µg/ml) | |
|---|---|---|
| | Control | 200ppm of dye |
| 2 | 138.18 | 144.75 |
| 4 | 142.46 | 151.9 |
| 6 | 160.28 | 170.22 |
| 8 | 178.67 | 183.86 |
| 10 | 190.45 | 196.21 |
| 12 | 199.05 | 206.63 |

Control : Devoid of the dye RBV
Values are the means of duplicate experimental set ups

The white rot fungus *S. ostrea* is known for secretion of ligninolytic enzymes – laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP). Therefore ligninolytic enzymes, secreted by *S .ostrea* in medium with/without dye were compared (Table 3).All these enzymes were secreted by *S. ostrea* upon growth in Koroljova medium devoid of dye. Laccase appeared to be a dominant one in ligninolytic enzymes of *S. ostrea* as reflected by recovery of this enzyme in maximum titers. Yields of laccase, MnP and LiP obtained from medium amended with 200 ppm dye were always higher than those of respective enzymes from control during course of growth of *S. ostrea.* At $10^{th}$ of incubation, secretion of three ligninolytic enzymes was enhanced 1.2 – 2.6 folds in medium with dye in comparison to control.

**Table 3. Secretion of Ligninolytic enzymes by *Stereum ostrea* upon growth in medium with RBV dye at 200ppm concentration**

| Incubation period in days | Titers expressed in U*/ml | | | | | |
|---|---|---|---|---|---|---|
| | Laccase | | MnP | | LiP | |
| | Control | 200ppm of dye | Control | 200ppm of dye | Control | 200ppm of dye |
| 2 | 1.68 | 3.26 | 0.40 | 0.55 | Nd | 1.28 |
| 4 | 19.82 | 28.09 | 6.72 | 9.23 | 0.85 | 6.21 |
| 6 | 39.31 | 50.59 | 9.71 | 15.86 | 2.18 | 9.3 |
| 8 | 57.31 | 63.01 | 17.25 | 27.38 | 4.07 | 16.21 |
| 10 | 80.92 | 98.11 | 22.36 | 41.23 | 8.24 | 21.32 |
| 12 | 98.64 | 137.26 | 31.22 | 56.75 | 1.36 | 5.24 |

*One unit of laccase&MnP corresponds to the amount of enzyme that oxidized one micromole of guaiacol per minute.
*One unit of LiP correspond to the amount of enzyme that oxidized one micromole of veratryl alcohol per minute.
nd-not detected
Control : Devoid of the dye RBV
Values are the means of duplicate experimental set

## IV. Discussion

Solid-plate experiments, evaluating the dye Decolorization potential of white-rot fungi, equally good results with different *Pleurotus* species and isolates were found e.g. with *P. lindquistii* (Levin L., 2005), *P. florida* (Sathiya et al., 2007), *P. ostreatus* (~93% Decolorization of Reactive dye 222, on the 6th day of incubation) (Kiran et al., 2012).The biodegradation of three azo dyes (Congo red, Orange II and Tropaeolin O) was done by the fungus *Phaenerocheatechrysosporium* (Cripps et al., 1990). Kitwechkun and Khanongnuch (36) studied the decolourization of azo dye (Orange II) by immobilized white rot fungus *Coriolusversicolor* (Kitwechkun and Khanongnuch, 2004).A group of researchers evaluated the possibility of a fungal wastewater treatment for a mixture of bio accessible reactive azo dyes using biodegradation assays(Martins et al., 2003). Whiterot fungus *Phlebiatremellosa* was found capable of decolorizing an array of synthetic textile dyes (Kirby et al., 2000).

The first dye decolourization by white rot fungi *Phanerochaetechrysosporium* was reported (Tien and Kirk, 1983). Other white rot such as *Hirschioporuslarincinus, Inonotushispidus, Phlebiatremellosa* and *Coriolusversicolor* can be used to decolorise dye effluent (Banat et al., 1996). Later on 29 other white rot fungi capable of dye decolourization were surveyed (Wesenberg et al. 2003).Decolorisation of dyes by using lignin modifying enzymes were studied extensively using laccase from *Trametesversicolor* (Khammuanget al., 2009), *Trametes hirsute, Trametesmodesta, Sclerotiumroysii* (Nyanhongo et al., 2002), *Laccariafraterna, Pleurotusostreatus* (Balarajuet al., 2007), *Lentinuspolychrous* (Khammuang and Rakrudee**,** 2009); LiP from *Phanerochaetechrysosporium* and MnP from *Phanerochaetechrysosporium, Bjerkanderaadusta, Pleurotuseryngii*(Heinfling et al., 1998).

**Conclusions:**

The results presented in the present communication show that the white rot fungus *S. ostrea* has the potential to remove an azo dye RBV from aqueous solutions and the results of this work, conclude that *S. ostrea* has the potentiality in Decolorization of dye Remazol Brilliant Violet, stimulation of secretion of ligninolytic enzymes at lower concentrations of dye, toxicity of dye at higher concentration to growth and ligninolyticenzyme production of *S. ostrea*.

**References**

[1] Adnan, LA. Hadibarata, T. Sathishkumar, P. MohdYusoff, AR. 2016. Biodegradation pathway of Acid Red 27 by whiterot fungus *Armillaria* sp. F022 and phytotoxicity evaluation. CSAWAC 44:239–246
[2] Anuradha, K. 2015. Biodegradation of dyes by Basidiomycetes fungi using HPLC and UV –Visible Spectrophotometer. Research and Reviews: Journal of Chemistry
[3] Azmi, W. Sani, RK. Banerjee, UC. 1998. Biodegradation of triphenylmethane dyes. Enzyme MicrobTechnol 22:185-191.
[4] Balaraju, K. Gnanadoss, JJ. Arokiyaraj, S. Agastian, P. Kaviyarasan, V. 2007. Production of cellulase and laccase by *Laccariafraterna* and *Pleurotusostreatus* under submerged and solid state fermentation. The ICFAI Journal of Biotechnology 2007; 1: 23-34.
[5] Banat, IM. Nigam, P. Singh, D. Marchant R. 1996. Microbial decolourization of textile-dyecontaining effluents: a review. Bioresour. Technol 1996; 58: 217-227.
[6] Benigni, R. Giuliani, A. Franke, R. Gruska, A. 2000. Quantitative structure–activity relationships of mutagenic and carcinogenic aromatic amines. Chem Rev 100:3697–3714

[7] Bonnen, A.M. Anton, L.H. and Orth, A.B. 1994. Lignin-degrading enzymes of the commercial button mushroom, *Agaricusbisporus*. Applied and Environmental Microbiology, vol. 60, no. 3, pp. 960–965.
[8] Cripps, C. Bumpus, J.A. Aust, S.D. 1990. Biodegradation of azo and heterocyclic dyes by Phanerochaetechrysosporium. Appl. Environ. Microb., 56 (4): 1114- 1118
[9] Das, N. Sengupta, S. Mukherjee, M. 1997. Importance of laccase in vegetative growth of *Pleurotusorida*. Applied and Environmental Microbiology, vol. 63, no. 10, pp. 4120–4122.
[10] Diwaniyan, S. Kharb, D. Raghukumar, C. 2010. Decolorization of synthetic dyes and textile effluents by basidiomycetous fungi. Water Air Soil Pollut 210:409-419.
[11] Hadibarata, T. Adnan, LA. MohdYusoff, AR. 2013. Microbial Decolorization of an Azo Dye Reactive Black 5 Using White-Rot Fungus *Pleurotuseryngii* F032. Water Air Soil Pollut 224:1595
[12] Heinfling, A. Martinez, MJ. Martinez, AT. Bergbauer, M. Szewzyk, U. 1998. Transformation of industrial dyes by manganese peroxidases from *Bjerkanderaadusta* and *Pleurotuseryngii* in a manganese-independent reaction. Appl Environ Microbiol ; 64(8): 2788– 2793.
[13] Khammuang, S. and Rakrudee, S. 2009. Laccase activity from fresh fruiting bodies of *Ganoderma sp*. MK05: Purification and remazol brilliant blue R decolourization. J BiolSci; 9: 8387.
[14] Khammuang, S. and Sarnthima, R. 2009. Mediator assisted rhodamine B decolourization by *Tramatesversicolor*laccase. Pak J Biol Sci;12: 616-623.
[15] Kiran, S. Ali, S. Asgher, M. Anwar, F. 2012. Comparative study on decolorization of reactive dye 222 by white rot fungi *Pleurotusostreatus* IBL-02 and *Phanerochaetechrysosporium* IBL-03. African Journal of Biotechnology Vol.6, pp.3639-3650.
[16] Kirby, N. Marchant, R. McMullan, G. 2000. Decolorisation of synthetic textile dyes by *Phlebiatremellosa*. FEMS Microbiology Letters, (188): 93-96.
[17] Kitwechkun, W. Khanongnuch, C. 2004. Decolourization of azo dye (OrangeII) by immobilized white-rot fungus *Coriolusversicolor* RC3. Proceeding of The 15th Annual Meeting of the Thai Society for Biotechnology.
[18] Knapp, JS. Newby, PS. Reece, LP. 1995. Decolorization of wood rotting basidiomycete fungi. Enzyme Microb Tech 17:664668.
[19] Koroljova-Skorobogat'ko, O.V. Stepanova, E.V. Gavrilova, V.P. 1998. Purification and characterization of the constitutive form of laccase from the basidiomyceteCoriolushirsutus and effect of inducers on laccase synthesis. Biotechnology and Applied Biochemistry, vol. 28, no. 1, pp. 47–54.
[20] Levin, L. Forchiassin, F. Viale, A. 2005. Ligninolytic enzyme production and dye decolorization by *Trametestrogii*: application of the PlackettBurman experimental design to evaluate nutritional requirements. Process Biochemistry, Vol.40, pp. 1381-1387.
[21] Lowry, O.H. Rosebrough, N.J. Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, vol. 193, no. 1, pp. 265–275.
[22] Martins, M.A.M. Lima, N. Silvestre, A.J. Queiroz, M.J. 2003. Comparative studies of fungal degradation of single or mixed bioaccessible reactive azo dyes. Chemosphere, (52): 967-973.
[23] Maulin, P. Kavita, A. Sunu, S. Darji, A. M. 2013. Isolation, Identification and Screening of Dye Decolorizing Bacteria.American Journal of Microbiological Research, Vol. 1, No. 4, 62-70
[24] Nyanhongo, GS. Gomes, J. Gubitz, GM. Zvauya, R. Read, J. Steiner, W. 2002. Decolourization of textile dyes by laccases from a newly isolated strain of *Trametesmodesta*. Water Res; 36(6): 1449–1456.
[25] Park, C., Lee, M., Lee, B., Kim, S. W., Chase, H. A., Lee, J., & Kim, S. (2007). Biodegradation and biosorption for decolorization of synthetic dyes by *Funaliatrogii*. Biochemical Engineering Journal, 36(1), 59-65. DOI: 10.1016/j.bej.2006.06.007
[26] Poonkuzhali, K. Sathishkumar, P. Boopathy, R. Palvannan, T. 2011. Aqueous state laccasethermostabilization using carbohydrate polymers: effect on toxicity assessment of azo dye. CarbohydrPolym 85:341–348
[27] Przystas, W. Zablocka-Godlewska, E. Grabinska-Sota, E. 2009. Screening of dyes decolorizing microorganisms strains. Pol J Environ Stud 18:69-73.
[28] Przystas, W. Zablocka-Godlewska, E. Grabinska-Sota, E. 2013. Effectiveness of dyes removal by mixed fungal cultures and toxicity of their metabolites. Water Air Soil Pollut 224:1534-1543.
[29] Robinson, T. Chandran, B. Nigam, P. 2002. Removal of dyes from a synthetic textile dye effluent by biosorption on apple pomace and wheat straw, Water Research. 36(11) 2824 – 2830.
[30] Robinson, T. McMullan, G. Marchant, R. 2001. Remediation of dyes in textile effluents: a critical review on current treatment technologies with a proposed alternative. Bioresour. Technol. 77:247-255.
[31] Saadon, SA. Sathishkumar, P. Yusoff, ARM. Wirzal, MDH. Rahmalan, MT. Nur, H. 2016. Photocatalytic activity and reusability of ZnO layer synthesised by electrolysis, hydrogen peroxide and heat treatment. Environ Technol 37:1875–1882
[32] Sathishkumar, P. Arulkumar, M. Palvannan, T. 2012. Utilization of agro industrial waste Jatrophacurcaspods as an activated carbon for the adsorption of reactive dye remazol brilliant blue R(RBBR).J Clean Prod 22:67–75
[33] Sathishkumar, P. Balan, K. Palvannan, T. Kamala-Kannan, S. Oh, BT. Rodríguez-Couto, S. 2013. Efficiency of *Pleurotusflorida*laccase on decolorization and detoxification of the reactive dye Remazol Brilliant Blue R (RBBR) under optimized conditions. CSAWAC 41:665–672
[34] Sathishkumar, P. Kamala-Kannan, S. Cho, M. Kim, JS. Hadibarata, T. Salim, MR. Oh, BT. 2014. Laccase immobilization on cellulose nanofiber: the catalytic efficiency and recyclic application for simulated dye effluent treatment. J MolCatal B Enzym 100:111–120

[35] Sathiya, M.P. Periyar, S.S. Sasikalaveni, A. Murugesan, K. Kalaichelvan, P.T. 2007. Decolorization of textile dyes and their effluents using white rot fungi, African Journal of Biotechnology, Vol.6, No.4, pp. 424429.
[36] Si, J. Cui, B-K. Dai, Y-Ch. 2013. Decolorization of chemically different dyes by white-rot fungi in submerged cultures. Ann Microbiol 63:1099-1108.
[37] Tien, M. and Kirk, T.K. 1988. "Wood," in Methods in Enzymology: Biomass, Part B : Lignin, Pectin, and Chitin, A. Willis and T. Scott, Eds., vol. 161, pp. 238–249, Academic Press, San Diego, Calif, USA,
[38] Tien, M. Kirk, TK. 1983. Lignin-degrading enzyme from the hymenomycete*Phanerochaetechrysosporium*burds. Science; 221(4611): 661–663.
[39] Vyrides, I. Bonakdarpour, B. Stuckey, DC. 2014. Salinity effects on biodegradation of reactive black 5 for one stage and two stages sequential anaerobic aerobic biological processes employing different anaerobic sludge. Int. Biodeterior. Biodegrad 95:294–300
[40] Wesenberg, D. Kyriakides, I. Agathos, SN. 2003. White-rot fungi and their enzymes for the treatment of industrial dye effluents. BiotechnolAdv; 22(1-2): 161–187.
[41] Yagub, MT. Sen, TK. Ang, HM. 2012. Equilibrium, kinetics, and thermodynamics of methylene blue adsorption by pine tree leaves. Water Air Soil Pollut 223:5267–5282
[42] Zhang, W. and Wu, C. W. 2014. Dyeing of multiple types of fabrics with a single reactive azo disperse dye. Chem Papers, vol. 68, pp.330–335.