Decolorization of Three Acid Dyes by Enzymes from Fungal Strains

PARK, CHULHWAN[1,2], YURI LEE[1], TAK-HYUN KIM[1,3], BYUNGHWAN LEE[1], JINWON LEE[4], AND SANGYONG KIM[1]*

[1]*Industrial Ecology National Research Laboratory, Korea Institute of Industrial Technology (KITECH), Chonan 330-825, Korea*
[2]*Department of Chemical Engineering, University of Cambridge, Pembroke Street, Cambridge CB2 3RA, U.K.*
[3]*Department of Civil and Environmental Engineering, University of Wisconsin-Madison, 1415 Engineering Drive, Madison, WI 53705, U.S.A.*
[4]*Department of Chemical Engineering, Kwangwoon University, Seoul 139-710, Korea*

Abstract In recent years, there has been an intensive research on decolorization of dye and textile wastewater by various fungal strains. In this study, the decolorization ability of three commercial dyes, acid yellow 99, acid blue 350, and acid red 114, were investigated using 10 fungal strains. Among the fungal strains tested, *Trametes versicolor* KCTC 16781 completely decolorized all dyes in both solid and liquid experiments, and was also able to decolorize the mixture of those three dyes in liquid experiments. The secretion of the ligninolytic enzymes into the extracellular medium during decolorization by *T. versicolor* KCTC 16781 was also studied. No lignin peroxidase activity was detected, and manganese peroxidase and laccase activities were investigated.

Key words: Dye, decolorization, enzyme, laccase, manganese peroxidase, *Trametes versicolor*

Many synthetic dyes are extensively used in textile dyeing, paper printing, color photography, pharmaceutical, food, cosmetic, and other industries [1–3]. The structural diversity of dyes derives from the use of different chromophoric groups such as azo, anthraquinone, triarylmethane, and phthalocyanine, and different application technologies such as reactive, direct, disperse, and vat dyeing. Azo dyes constitute the largest class of dyes used commercially [4, 5]. There are more than 8,000 chemical products listed in the Color Index which are associated with the dyeing process, while over 100,000 commercially available dyes exist with over 700,000 metric tons of dyestuff annually produced [6, 7]. It is known that about 90% of reactive dyes entering activated sludge sewage treatment plants will pass through unchanged and be discharged into rivers [8]. Among industrial wastewaters, dye wastewater from textile and dyestuff industries is one of the most difficult to treat, because dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to be biodegraded [9]. As synthetic dyes are relatively resistant to biodegradation, the decolorization of dye in wastewater is based mainly on physical or chemical methods. Although these methods are effective, they have some drawbacks such as high cost, formation of hazardous byproducts, and intensive energy requirements. Therefore, the applications of microbial biodegradation as a better alternative are attracting much attention, and several microorganisms for degrading toxic compounds have been reported and their characteristics have been investigated [10–13].

Various fungal strains are known to degrade a wide variety of recalcitrant compounds, such as xenobiotics, lignin, and dyestuffs, with their extracellular enzymes. Many studies have also demonstrated that many fungal strains are capable of degrading various types of synthetic dyes such as azo, triphenyl methane, polymeric, phthalocyanine, and heterocyclic dyes [14, 15]. Some fungal strains produce three kinds of enzymes [laccase, lignin peroxidase (LiP), and manganese peroxidase (MnP)], whereas others produce only one or two of them [16]. In view of these considerations, we undertook to study the decolorization patterns of three dyes by 10 fungal strains through solid cultivation, and to assess the potential of *Trametes versicolor* KCTC 16781 and extracellular enzymes from this fungal strain to decolorize three acid dyes through liquid cultivation. To verify the possibility of application to wastewater, we also attempted to confirm the decolorization performance of mixture dye.

*Corresponding author
Phone: 82-41-589-8356; Fax: 82-41-589-8340;
E-mail: sykim@kitech.re.kr

MATERIALS AND METHODS

Fungal Strains

Ten fungal strains used for the decolorization ability were *Chrysonilia crassa* KCTC 6124, *T. versicolor* ATCC 200801, *T. versicolor* KCTC 16781, *T. versicolor* ATCC 12679, *Phanerochaete chrysosporium* KCCM 60256, *P. chrysosporium* KCTC 6147, *Aspergillus niger* KCCM 60317, *Pleurotus ostreatus* ATCC 34675, *P. ostreatus* ATCC 9427, and *Sclerotium rolfsii* ATCC 200224. These strains were grown on potato dextrose agar (PDA) plates at 28°C for 7 days and were maintained at 4°C. Acid yellow 99, acid blue 350, and acid red 114 are commonly used in the textile industry. Mixture dye consisted of those three dyes, and its concentration was 100 mg/l.

Media Compositions and Culture Conditions

The fungal strains were inoculated on PDA and incubated until extensive mycelium growth occurred. They were divided into 1 cm^2 pieces, and were placed on the center of agar plates containing yeast malt peptone glucose (YMPG) with 100 mg/l dyes. YMPG consists of the following components (g/l of distilled water): glucose, 10; malt extract, 10; peptone, 2; asparagine, 1; KH$_2$PO$_4$, 2; MgSO$_4$·7H$_2$O, 1; and thiamine·HCl, 0.001 [17]. They were statically incubated at 28°C for 15 days. Inoculums for the decolorization experiments were prepared by growing the fungal strains in potato dextrose broth (PDB) in the controlled incubator shaker under the condition of 28°C and 100 rpm. After 4 days of cultivation in the small pellet form, these were homogenized by using the Ace Homogenizer (Nissei, Tokyo, Japan) at 1,500 rpm for 30 sec. The 8 ml homogenized samples were used to inoculate each flask, which contained 80 ml of Kirks Basal Salts medium without tween solution and veratryl alcohol (g/l of distilled water): glucose, 5; ammonium tartrate, 0.22; KH$_2$PO$_4$, 0.2; MgSO$_4$·7H$_2$O, 0.05; CaCl$_2$, 0.01; thiamine·HCl, 0.001, and trace elements, 10 ml [18]. Then, dyes were added to each flask and pH was adjusted to 4.5 with 2,2'-dimethyl succinic acid. Trace elements solution had the following compositions (g/l of distilled water): CuSO$_4$·7H$_2$O, 0.08; H$_2$MoO$_4$, 0.05; MnSO$_4$·4H$_2$O, 0.07; ZnSO$_4$·7H$_2$O, 0.043; and Fe$_2$(SO$_4$)$_3$, 0.05. Shaken culture flasks of the fungal strains were grown at 28°C with continuous agitation of 100 rpm.

Analytical Methods

Samples were withdrawn from the culture at appropriate time intervals, centrifuged (10,000 rpm, 15 min), and the clear supernatant was analyzed for pH, glucose concentration, decolorization, and ligninolytic enzyme production. Dye decolorization was measured photometrically at maximum wavelength of each dye using UV/Visible spectrophotometer (Bio-Tek Instruments, Milano, Italy), and was calculated from the decrease in absorbance of the maximum peak for each dye. The wavelengths showing maximum absorbance for acid yellow 99, acid blue 350, acid red 114, and mixture dye were 452, 616, 510, and 460 nm, respectively. Glucose concentration was determined by using enzyme kits (YD Diagnostics, Seoul, Korea).

Laccase (EC. 1.10.3.2) activity was determined by oxidation of 4.47 mM syringaldazine in Mellvaine buffer (0.1 M citric acid and 0.2 M sodium phosphate) (pH 4.6). The reaction mixture (3 ml) consisted of 2.5 ml of Mellvaine buffer (pH 4.6) containing 500 µl of culture filtrate. The reaction was initiated by adding 10 µl of syringaldazine (4.47 mM) solution. The increase of absorbance at 525 nm was measured at 40°C. One unit of laccase activity was defined as the amount of enzyme to increase 1.0 absorbance at 525 nm per min [19]. MnP (EC 1.11.1.13) activity was determined by oxidation of 2,2'-azinobis(3-ethyl-6-benzothiazoline sulfonic acid) (ABTS) in 100 mM sodium lactate buffer (pH 4.5) [20]. One milliliter sample mixture consisted of 500 µl of 50 mM sodium lactate buffer containing 0.2 mM MnSO$_4$ and 80 µg/ml ABTS, and 500 µl of 100 µM H$_2$O$_2$. The reaction at room temperature was initiated by the addition of 10 µl culture filtrate. One unit of MnP activity was defined as the amount of enzyme to increase 1.0 absorbance at 415 nm per min. LiP (EC 1.11.1.14) activity was assayed, based on the oxidation by azure B [21, 22]. The reaction mixture contained 1 ml of 125 mM sodium tartrate buffer (pH 3.0), 500 µl of 0.16 mM azure B, 500 µl of the culture filtrate, and 500 µl of 2 mM H$_2$O$_2$. The reaction was initiated at 24°C by adding H$_2$O$_2$, one unit of the enzyme activity was defined as the amount of enzyme to decrease 0.1 unit absorbance at 651 nm per min per ml of the culture filtrate.

RESULTS AND DISCUSSION

Selection of Fungal Strains through Solid and Liquid Cultivation

Decolorization of three acid dyes was investigated by 10 fungal strains selected through solid cultivation. Three acid dyes were two azo dyes (acid yellow 99 and acid red 114) and one anthraquinone dye (acid blue 350). Since our similar results were related with other six dyes [23], the decolorization abilities of 10 fungal strains were investigated during 15 days through solid cultivation (Table 1). Except for *A. niger* KCCM 60317, the other fungal strains completely decolorized one of three dyes. *T. versicolor* ATCC 200801, *T. versicolor* KCTC 16781, and *P. chrysosporium* KCCM 60256 were especially able to effectively decolorize all three dyes in 10 days. Decolorization began with formation of
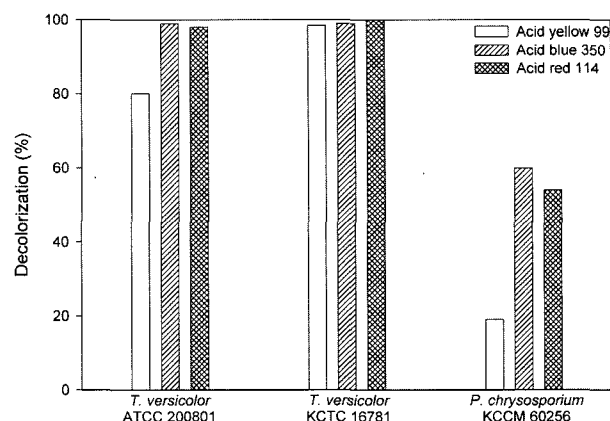
Table 1. Dye decolorization by 10 fungal strains in solid cultivation.

Fungal strains	Acid yellow 99	Acid blue 350	Acid red 114
C. crassa KCTC 6124	-	-	+
T. versicolor ATCC 200801	+	+	+
T. versicolor KCTC 16781	+	+	+
T. versicolor ATCC 12679	+	+	-
P. chrysosporium KCCM 60256	+	+	+
P. chrysosporium KCTC 6147	-	+	-
A. niger KCCM 60317	-	-	-
P. ostreatus ATCC 34675	+	-	-
P. ostreatus ATCC 9427	-	+	-
S. rolfsii ATCC 200224	-	+	-

(+) represents complete decolorization, while (-) represents partial decolorization.

Fig. 2. Decolorization of three acid dyes by three fungal strains after 48 h.

clear zones around the colonies, and complete decolorization was then achieved, showing total disappearance of color without visible biosorption to the biomass. This result indicates that decolorization is due to extracellular enzymes.

Next, *T. versicolor* ATCC 200801, *T. versicolor* KCTC 16781, and *P. chrysosporium* KCCM 60256 were selected to study decolorization in liquid cultivation. Three fungal strains showed different decolorization activities, and the results of three dyes after 48 h are shown in Fig. 2. *T. versicolor* ATCC 200801 efficiently decolorized over 95% of two dyes, acid blue 350 and acid red 114. *P. chrysosporium* KCCM 60256 could decolorize only a little

Fig. 1. Chemical structures of three acid dyes. (A) Acid yellow 99. (B) Acid blue 350. (C) Acid red 114.

amount of the dyes. The fungal strains exhibited different decolorization abilities for the dyes, depending on their specificity toward particular dyes. About 20% of acid yellow 99 was decolorized by *P. chrysosporium* KCCM 60256, and about 60% and 55% of acid blue 350 and acid red 114 were decolorized, respectively. Among the three fungal strains, *T. versicolor* KCTC 16781 showed the highest decolorization activity. Biodegradation of textile dyes by *P. chrysosporium* has recently received increasing attention [24], however, *T. versicolor* KCTC 16781, under conditions used in this liquid culture transformation study, showed higher capability for dye degradation than *P. chrysosporium* KCCM 60256. Different fungal strains appear to have different decolorization tendency. In addition, even small structural differences of dyes are expected to markedly affect the decolorization level of dyes. This is one of the reasons why excellent microorganisms are required for various cases. The results showed that acid blue 350 was the most rapidly decolorized, while acid yellow 99 was difficult to be decolorized. Acid red 114 and acid yellow 99 are azo-based dyes, which constitute the largest class of synthetic dyes used in this industry. In order to obtain better results, the systematic study on the relationship between dye structure and fungal decolorization is necessary; however, this kind of study until now has been rather scarce [25, 26].

Decolorization and Extracellular Enzymes by *T. versicolor*

The most active fungal strain, *T. versicolor* KCTC 16781, was selected and then investigated for decolorization and enzyme production. As seen in Table 2, different trends of enzyme production, glucose consumption, and decolorization capacities were observed, depending on the dye structures. About 30% of acid yellow 99 was decolorized after 24 h, and decolorization of acid yellow 99 was the lowest value among the three dyes. But, as the enzyme activity increased sharply in the early cultivation period, decolorization
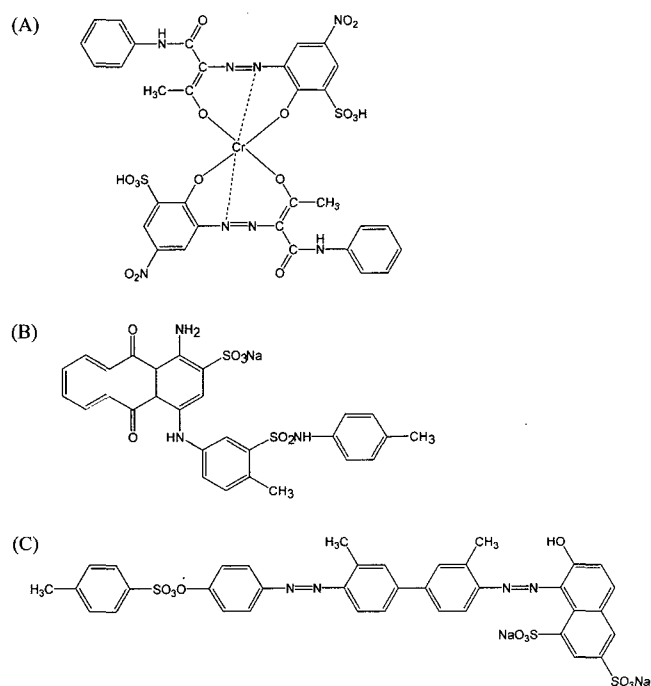
Table 2. Decolorization, enzyme activity, and glucose concentration for three acid dyes by *T. versicolor* KCTC 16781.

| Parameters	Decolorization at time (%)			Maximum activity at time (U/ml)		Glucose conc. at time (g/l)	
	24 h	48 h	72 h	Laccase	MnP	48 h	96 h
Acid yellow 99	29.6	98.4	100	1.36 at 48 h	3.49 at 48 h	3.25	1.21
Acid blue 350	97.9	99.0	100	0.25 at 48 h	1.19 at 36 h	1.97	1.34
Acid red 114	70.7	100	100	1.64 at 48 h	3.41 at 60 h	2.69	0.00

activity also rose dramatically over 90%. The maximum enzyme activity was observed after 48 h of cultivation during which time glucose decreased gradually. The decrease of enzyme activity after 48 h can be due to limitation of nutrients, especially glucose, which are required for growth of the fungal strain studied. The maximum enzyme activities of laccase and MnP were 1.36 U/ml and 3.49 U/ml, respectively. Broad peaks of laccase activity were typically seen from 24 h until 60 h. In the case of acid blue 350, it was degraded dramatically in the early cultivation period. Although the enzyme activity was relatively low, compared with other dyes, over 97% of acid blue 350 was decolorized within 24 h. MnP also increased gradually and reached the maximum value at 36 h, while laccase showed low activity during the cultivation period. The activity of MnP reached a maximum value after dramatic decrease of glucose concentration. About 70% of acid red 114 was also degraded rapidly within 24 h, and complete decolorization was achieved within 48 h. MnP started to be produced at 24 h and achieved the highest peak of 3.41 U/ml at 60 h and the highest laccase activity was 1.64 U/ml at 48 h. This trend for MnP activity was similar to that seen for laccase, however, the amount of this enzyme was about 2-fold higher than that of laccase. Glucose was also consumed as the fungal strain grew. Neither lignin nor any pollutants degraded by these fungal strains can be utilized as growth substrates, and different carbon source such as glucose is required for the metabolic activity. Swamy and Ramsay [27] reported that the rate of decolorization was constant at higher than 0.13 g/l glucose concentration, but the rate of decolorization decreased as glucose concentration decreased below 0.13 g/l.

Chemical characteristics of different dyestuffs are highly variable, and they are selected according to the material to be dyed. Therefore, the composition of dyeing effluent varies with textiles produced. The generation of synthetic wastewaters of defined composition is a prerequisite for research, however, there is little information available on simulating the color of textile effluents [28]. Frequently, one dye has been used in simulated wastewaters, however, it is occasionally useful to study wastewaters that are more representative of real effluents in terms of color. Therefore, the mixture dye, which simulated color in a real dye wastewater, was adopted for liquid cultivation in the present study, and it involved three different dyes (acid

yellow 99, acid blue 350, and acid red 114) with peaks at different spectrum wavelengths. As seen in Fig. 3, over 90% of the mixture dye was decolorized within 48 h, and its decolorization trend was similar to those of single dyes. It is clear, therefore, that *T. versicolor* KCTC 16781 is able to degrade not only single dye, but also mixture dye. Laccase and MnP activities occurred first in culture supernatants, coinciding with the dye decolorization, with maximum of 1.19 U/ml at 36 h and 2.39 U/ml at 48 h, respectively.

Since *T. versicolor* KCTC 16781 was able to degrade all three acid dyes, dye decolorization has long been considered to be associated with the production of ligninolytic enzymes. In addition, laccase is known as a multicopper enzyme, which catalyzes the oxidation of phenolic compounds [29], and MnP oxidizes Mn(II) to Mn(III), which is responsible for the oxidation of many phenolic compounds [30]. Various studies have shown that the major mechanisms of decolorization are biodegradation, because they can produce the lignin modifying enzymes, such as laccase, MnP, and LiP, to mineralize synthetic lignin or dyes [31, 32]. However, relative contributions of LiP, MnP, and laccase to the decolorization of dyes may be different for each fungal strain. For the fungal strain *P. chrysosporium*, LiP was found to be responsible for the decolorization of azo, thriphenyl methane, heterocyclic, and polymeric dyes, and MnP was not required to start the degradation of these dyes [33]. Young and Yu [34] studied the decolorization of eight synthetic dyes, including azo, anthraquinone, metal complex, and indigo dyes, by *T. versicolor*, and reported that
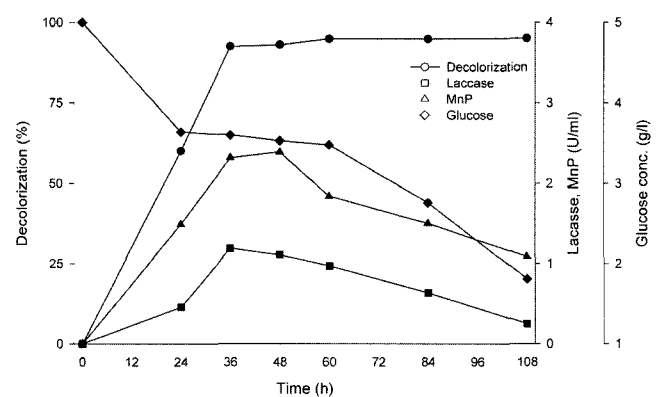
Fig. 3. Decolorization, enzyme activity, and glucose concentration for mixture dye by *T. versicolor* KCTC 16781.

MnP did not decolorize these dyes, while ligninase-catalyzed oxidation removed over 80% of the dyes. However, Zhang et al. [35] observed that MnP played an important role in the decolorization of cotton bleaching effluent by an unidentified white-rot fungus, while there was no LiP activity involved in this decolorization. Because T. versicolor releases laccase as its major extracellular enzyme, laccase activity appears to be the major mechanism in decolorizing anthraquinone, azo, and indigo dyes. In the present work, laccase and MnP from T. versicolor KCTC 16781 were found to be important enzymes for dye decolorization, and decolorization was not a single step reaction, but rather more than one enzyme were involved. Besides the decolorization of selected single dyes, which was shown for example in this study, there are several other factors to qualify ligninolytic fungal strains, such as T. versicolor KCTC 16781, as promising candidates for the development of novel treatment methods of dye-containing wastes and wastewaters. Their unspecific ligninolytic enzyme system in white-rot fungal strain, such as T. versicolor KCTC 16781, was able to decolorize various structural classes of dyes.

Three acid dyes were biocatalytically decolorized by extracellular enzymes from 10 fungal strains in solid and liquid cultivation. We confirmed that the decolorization was correlated with ligninolytic enzyme levels, and that T. versicolor KCTC 16781 was capable of decolorizing a range of industrially important textile dyes. The difference of decolorization rates according to dye types was due to various structures of the dyes. Decolorization by T. versicolor KCTC 16781 involved complex mechanism of enzyme reaction and dyes with different structures, and the decolorization occurred at different intrinsic enzymatic rates. In conclusion, fungal decolorization appears to be a promising alternative to replace or supplement currently available treatment processes. Nevertheless, more research is needed to develop practical applications.

Acknowledgments

REFERENCES

1. Kim, S., C. Park, T.-H. Kim, J. Lee, and S.-W. Kim. 2003. COD reduction and decolorization of textile effluent using a combined process. J. Biosci. Bioeng. 95: 102– 105.

2. Zheng, Z., R. E. Levin, J. L. Pinkham, and K. Shetty. 1999. Decolorization of polymeric dyes by a novel Penicillium isolate. Process Biochem. 34: 31– 37.

3. Donmez, G. 2002. Bioaccumulation of the reactive textile dyes by Candida tropicalis growing in molasses medium. Enzyme Microb. Technol. 30: 363– 366.

4. Heinfling, A., M. J. Martinez, A. T. Martinez, M. Bergbauer, and U. Szewzyk. 1998. Transformation of industrial dyes by manganese peroxidases from Bjerkandera adusta and Pleurotus eryngii in a manganese-independent reaction. Appl. Environ. Microbiol. 64: 2788– 2793.

5. Lee, T. H., H. Aoki, Y. Sugano, and M. Shoda. 2000. Effect of molasses on the production and activity of dye-decolorizing peroxidase from Geotrichum candidum Dec1. J. Biosci. Bioeng. 89: 545– 549.

6. Banat, I. M., P. Nigam, D. Singh, and R. Marchant. 1996. Microbial decolorization of textile-dye-containing effluents: A review. Bioresource Technol. 58: 217– 227.

7. Robinson, T., B. Chandran, and P. Nigam. 2001. Studies on the production of enzymes by white rot fungi for the decolourisation of textile dyes. Enzyme Microb. Technol. 29: 575– 579.

8. Abadulla, E., K.-H. Robra, G. M. Gubitz, L. M. Silva, and A. Cavaco-Paulo. 2000. Enzymatic decolorization of textile dyeing effluents. J. Textile Res. 70: 409– 414.

9. Fu, Y. and T. Viraraghavan. 2001. Fungal decolorization of dye wastewaters: A review. Bioresource Technol. 79: 251– 262.

10. Lee, S.-K. and S. B. Lee. 2002. Substrate utilization patterns during BTEX biodegradation by an o-xylene-degrading bacterium Ralstonia sp. PHS1. J. Microbiol. Biotechnol. 12: 909– 915.

11. Jeon, J.-H., Y.-J. Han, T.-G. Kang, E.-S. Kim, S.-K. Hong, and B.-C. Jeong. 2002. Purification and characterization of 2,4-dichlorophenol oxidizing peroxidase from Streptomyces sp. AD001. J. Microbiol. Biotechnol. 12: 972– 978.

12. Yoon, K. 2003. Construction and characterization of multiple heavy metal-resistant phenol-degrading Pseudomonads strains. J. Microbiol. Biotechnol. 13: 1001– 1007.

13. Park, C., T.-H. Kim, J. Lee, S. Kim, and S.-W. Kim. 2002. Enhanced biodegradation of 2,4,6-trinitrotoluene (TNT) with various supplemental energy sources. J. Microbiol. Biotechnol. 12: 695– 698.

14. Heinfling, A., M. Bergbauer, and U. Szewzyk. 1997. Biodegradation of azo and phthalocyanine dyes by Trametes versicolor and Bjerkandera adusta. Appl. Microbiol. Biotechnol. 48: 261– 266.

15. Chung, N. Y., G. Y. Kang, G. H. Kim, I.-S. Lee, and W.-G. Bang. 2001. Effect of nutrient nitrogen on the degradation of pentachlorophenol by white rot fungus, Phanerochaete chrysosporium. J. Microbiol. Biotechnol. 11: 704– 708.

16. Ha, H.-C., Y. Honda, T. Watanabe, and M. Kuwahara. 2001. Production of manganese peroxidase by pellet culture of the lignin-degrading basidiomycete, Pleurotus ostreatus. Appl. Microbiol. Biotechnol. 55: 704– 711.

17. Jang, M. Y., W. R. Ryul, and M. H. Cho. 2002. Laccase production from repeated batch cultures using free mycelia of Trametes sp. Enzyme Microb. Technol. 30: 741– 746.

18. Kapdan, I. K., F. Kargi, G. MacMllan, and R. Marchant. 2000. Effect of environmental conditions on biological decolorization of textile dyestuff by *C. versicolor*. *Enzyme Microb. Technol.* **26:** 381–387.

19. Criquet, S., S. Tagger, G. Vogt, G. Iacazio, and J. L. Petit. 1999. Laccase activity of forest litter. *Soil Biol. Biochem.* **31:** 1239–1244.

20. Hofrichter, M., K. Vares, K. Scheibner, S. Galkin, J. Sipila, and A. Hatakka. 1999. Mineralization and solubilizaton of synthetic lignin by manganese peroxidases from *Nematoloma frowardii and Phkebia radiata*. *J. Biotechnol.* **67:** 217–228.

21. Archibald, F. S. 1992. A new assay for lignin-type peroxidase employing the dye azure B. *Appl. Environ. Microbiol.* **58:** 3110–3116.

22. Arora, D. S. and P. K. Gill. 2001. Comparison of two assay procedures for lignin peroxidase. *Enzyme Microb. Technol.* **28:** 602–605.

23. Park, C., Y. Lee, T. Kim, M. Lee, B. Lee, J. Lee, and S. Kim. 2003. Enzymatic decolorization of various dyes by *Trametes versicolor* KCTC 16781. *Korean J. Biotechnol. Bioeng.* **18:** 398–403.

24. Assadi, M. M., K. Rostami, M. Shahvali, and M. Azin. 2001. Decolorization of textile wastewater by *Phanerochaete chrysosporium*. *Desalination* **141:** 331–336.

25. Chagas, E. P. and L. R. Durrant. 2001. Decolorization of azo dyes by *Phanerochaete chrysosporium* and *Pleurotus sajorcaju*. *Enzyme Microb. Technol.* **29:** 473–477.

26. Knapp, J. S., P. S. Newby, and L. P. Reece. 1995. Decolorization of dyes by wood-rotting basidiomycete fungi. *Enzyme Microb. Technol.* **17:** 664–668.

27. Swamy, J. and J. A. Ramsay. 1999. Effects of glucose and NH_4^+ concentrations on sequential dye decolorization by *Trametes versicolor*. *Enzyme Microb. Technol.* **25:** 278–284.

28. Meehan, C., I. M. Banat, G. McMullan, P. Nigam, F. Smyth, and R. Marchant. 2000. Decolorization of Remazol Black-B using a thermotolerant yeast, *Kluyveromyces marxianus* IMB3. *Environ. Int.* **26:** 75–79.

29. Soares, G. M. B., M. T. P. Amorim, A. M. Oliveira-Campos, R. Hardina, and M. Costa-Ferreira. 2002. Specificity of phenolic diazo dyes in relation to transformation by laccase. *Enzyme Microb. Technol.* **30:** 607–612.

30. Sayadi, S. and R. Ellouz. 1995. Roles of lignin peroxidase and manganese peroxidase from *Phanerochaete chrysosporium* in the decolorization of olive mill wastewaters. *Appl. Environ. Microbiol.* **61:** 1098–1103.

31. Abadulla, E., T. Tzanov, S. Costa, K. Robra, A. Cavaco-Paulo, and G. M. Gubitz. 2000. Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsute*. *Appl. Environ. Microbiol.* **66:** 3357–3362.

32. Borchert, M. and J. A. Libra. 2001. Decolorization of reactive dyes by the white rot fungus *Trametes versicolor* in sequencing batch reactors. *Biotechnol. Bioeng.* **75:** 313–321.

33. Ollikka, P., K. Alhonmaki, V. M. Leppanen, T. Glumoff, T. Raijola, and I. Suominen. 1993. Decolorization of azo, triphenyl methane, heterocyclic and polymeric dyes by lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* **59:** 4010–4016.

34. Young, L. and J. Yu. 1997. Ligninase-catalyzed decolorization of synthetic dyes. *Water Res.* **31:** 1187–1193.

35. Zhang, F.-M., J. S. Knapp, and K. N. Tapley. 1999. Decolourisation of cotton bleaching effluent with wood rotting fungus. *Water Res.* **33:** 919–928.