Water Air Soil Pollut (2014) 225:1801
DOI 10.1007/s11270-013-1801-0

Decolorization and Detoxification of Wastewater Containing Industrial Dyes by *Bjerkandera adusta* KUC9065

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Received: 18 February 2013 / Accepted: 29 October 2013 / Published online: 30 November 2013

Abstract This study was performed to evaluate the ability of white-rot fungi to decolorize dye effluents. A total of 222 isolates of white-rot fungi were initially investigated to assess their ability to decolorize chemically different synthetic dyes in solid medium, resulting in selection of 25 isolates including four isolates of *Berkandera adusta*, five isolates of *Ceriporia lacerata*, three isolates of *Irpex lacteus*, one isolate of *Perenniporia fraxinea*, ten isolates of *Phanerochaete* spp., one isolate of *Phlebia radiata*, and one isolate of *Porostereum spadiceum*. Of the 25 isolates, *B. adusta* KUC9065, *C. lacerata* KUC8090, *P. calotricha* KUC8003, and *P. spadiceum* KUC8602 were finally selected on the basis of their ability to decolorize synthetic dyes in liquid medium, and were used to decolorize industrial effluents. *B. adusta* KUC9065 increased the transmittance of visible light by 71–92 %. Decolorization of wastewater by *B. adusta* KUC9065 was probably caused by the lignin-modifying enzymes produced by the fungus. In addition, the acute toxicity to *Daphnia magna* decreased from 2.5 to 2.1 and from 3.5 to 2.6 toxic units over 24 and 48 h, respectively.

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Keywords White-rot fungi · Industrial effluent · Decolorization · Detoxification

1 Introduction

Numerous industries including textile dyeing industry use various synthetic dyes which consist of chemically different basic structures such as diazonium, acridine, anthraquinone, triarylmethane, azo, quinone-immine, phthalocyanine, and xanthene (Rauf and Salman Ashraf 2012). It is reported that over 1.5 million tons of synthetic dyes were produced every year in the late 2000s (Jiang and Murmann 2011). In wastewater treatment, the decolorization of dyeing effluents is one of the most crucial steps, because the dyes in wastewater are a potential environmental pollutant. It is estimated that 10–500 mg/l dyestuff is included in textile effluents (O'Neill et al. 1999), although dyes are highly visible at low concentrations (>1 mg/l) (Banat et al. 1996). Without a decolorization step before discharge, the dyestuff may block out visible rays, resulting in a decrease in photosynthesis (Banat et al. 1996). In addition, a number of synthetic dyes are potentially poisonous; some are known to be carcinogenic. Currently, physicochemical methods are applied to dye wastewater treatment, with high dye-removal efficiencies (Yadav et al. 2013). However, alternatives need to be developed to overcome several drawbacks, including high operating

costs in large-scale applications, and secondary environmental contamination by the chemicals used.

Biodecolorization using white-rot fungi has recently been considered as a promising alternative to the physicochemical treatments (Choi et al. 2013; Novotný et al. 2009), although the optimal biodecolorization process to handle the residues and byproducts from the biodecolorization process still needs to be developed for practical application on real industrial effluents (Kaushik and Malik 2009). White-rot fungi secrete lignin-modifying enzymes (LMEs), including manganese peroxidase (MnP), laccase (Lac), and lignin peroxidase (LiP) that are involved in the oxidative breakdown of lignin. With low substrate specificity, LMEs have been widely applied to bioremediation of organopollutants such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and synthetic dyes (Diwaniyan et al. 2010; Guo et al. 2008; Keharia and Madamwar 2002; Lee et al. 2013). In particular, genera *Phanerochaete*, *Bjerkandera*, and *Irpex* are well-known isolates useful for the decolorization of synthetic dyes, which work by removing the chromophore groups of the dye (Choi et al. 2013; Heinfling et al. 1998; Lee et al. 2010; Novotný et al. 2001, 2009; Paszczynski et al. 1992).

To date, most of the research into biodecolorization using white-rot fungi has focused on the decolorization characteristic of synthetic dyes, such as the decolorization efficiency and the relationship between decolorization and LMEs. The decolorization characteristics of several synthetic dyes have been thoroughly investigated. In addition, few studies focused on metabolites of synthetic dyes by white-rot fungi which may have higher toxicity than original form of the synthetic dyes (Choi et al. 2013; Kaushik and Malik 2009). However, application of white-rot fungi to the decolorization of real industrial dye effluents has not been fully reported. Because industrial dye effluents contain salts, surfactants, metals, toxic organic chemicals, and biocides as well as synthetic dyes, decolorization of solutions that only contain the synthetic dye are different from that of real effluents. More recently, few studies have targeted the decolorization of real industrial dye effluents by well-known isolates of white-rot fungi *P. chrysosporium*, but only moderate decolorization efficiency was reported (Sangeeta et al. 2011). The objectives of this study were to screen prominent fungi for the decolorization of industrial dye effluents from a variety of white-rot fungi and to evaluate the decolorization characteristics of the selected isolates.

2 Materials and Methods

2.1 Fungal Isolate

A total of 222 white-rot fungi were obtained from the Korea University Culture (KUC) collection, Korea, for use in this study (Table 1). The fungi were transferred from stock cultures to malt extract glucose agar (MEGA) medium containing 5 g/l malt extract, 1 g/l glucose, and 20 g/l agar, and allowed to grow at 27 °C in the dark for 5–10 days prior to use in the decolorization of synthetic dyes and wastewater containing industrial dyes.

As a reference, the 222 isolates from 47 genera were isolated from several woody substrates such as fallen tree trunks, sawn wood, and wood products in Korea, and identified on the basis of the nucleotide sequence of the large subunit rDNA region. The partial large subunits of rDNA were amplified by polymerase chain reaction (PCR) using the LR0R-LR3 primer set (Vilgalys and Hester 1990) under the reaction condition descrived by Kim et al. (2004). Analysis of the amplified PCR products were performed at Macrogen (Seoul, Korea) and the sequences of the PCR products were compared with data sets from GenBank.

2.2 Decolorization of Synthetic Dyes

Three chemically different dyes, namely, Congo red (CR), Reactive Blue 4 (RB4), and Orange II (OII), which include diazo, anthraquinone, and azo groups as their basic structure, respectively, were chosen as synthetic dyes for testing.

In the first investigation, the abilities of the isolates to decolorize CR, RB4, and OII in solid medium were evaluated according to the method described by Novotný et al. (2004). Inoculums of each fungus (5-mm diameter) from the leading edge of the mycelium in the pre-cultured plate were inoculated into the center of a MEGA plate containing 100 mg/l CR, RB4, or OII. The media were then kept at 27 °C for 6 days, and the percentage of color removal was determined by comparing the diameter of the decolorized zone and the diameter of the petri dish (90 mm). Of the 222 white-rot fungi tested, 25 isolates, which removed >70 % of the original color of each dye, were further investigated for their ability to decolorize synthetic dyes in liquid medium.

To investigate the decolorization ability of the 25 isolates for synthetic dyes in the liquid phase, a

Table 1 White-rot fungi used in this study

Fungal genus	Number of isolates
Amphinema	1
Antrodiella	1
Bjerkandera	10
Ceriporia	8
Cerrena	3
Crepidotus	1
Cryptoporus	1
Daedaleopsis	1
Dendrocorticium	2
Dentipellis	1
Fomes	2
Fuscoporia	1
Heterobasidion	2
Hyphodontia	1
Hypholoma	3
Hypochinicium	12
Irpex	23
Lenzites	3
Megalocystidium	4
Meruliopsis	1
Microporus	2
Mucronella	2
Mycoaciella	2
Oligoporus	1
Pachykytospora	2
Panellus	2
Peniophora	4
Peniophorella	1
Perenniporia	2
Phanerochaete	39
Phlebia	13
Phlebiella	6
Phlebiopsis	3
Phyllotopsis	1
Pleurotus	1
Polyporus	1
Porostereum	2
Pseudochaete	1
Radulomyces	2
Rhizochaete	7
Schizophyllum	9
Schizopora	10
Skeletocutis	2
Stereum	5
Trametes	16
Trichaptum	2
Tyromyces	3
Total	222

miniaturized fermentation process was applied according to Lee et al. (2011). Briefly, for each sample, a fungal disk (5-mm diameter) was inoculated into a 50-ml conical tube containing 10 ml nutrient solution (5 g/l malt extract and 1 g/l glucose) and 100 mg/l of CR, RB4, OII, or a mixture of the three dyes. The tube was stopped with a sili stopper, and placed into a tube rack at an angle of 50°. Cultivation of the tube was performed at 27 °C for 7 days on a rotary shaker (150 rpm). After cultivation, samples were filtered with a 0.45-μm syringe filter, and the percentage of color removal of each sample was estimated using a spectrophotometer (Optizen 2120UV; Mecasys, Korea) by measuring the absorbance at 495, 595, 485, and 490 nm for CR, RB4, OII, and the mixture of the three dyes, respectively.

2.3 Decolorization of Wastewater Containing Industrial Dyes

A textile effluent sample was collected from the wastewater treatment plant at the Keom-jun Dyeing Enterprise Cooperation (KDEC), Yang-ju, South Korea. Industrial wastewater generated from approximately 50 textile and dyeing facilities near the KDEC is treated at the plant. We collected the raw wastewater sample without any pretreatment, and the sample was kept at 4 °C throughout the period of research. General characteristics of the wastewater such as acidity, dissolved oxygen, salinity, conductivity, and total dissolved solids were measured using a multi-parameter water quality meter (YSI-556; Yellow Springs Instruments, Yellow Springs, OH, USA), and dissolved organic carbon (DOC) was analyzed using a Shimadzu TOC analyzer (model 5000A; Shimadzu, Tokyo, Japan). The amounts of metal elements in the wastewater were determined using inductively coupled plasma-optical emission spectrometer (Vista Pro, Varian Inc., USA). Table 2 shows the general characteristics of the wastewater used in this study.

Table 2 General characteristics of wastewater used in this study

Acidity (pH)	Dissolved oxygen (mg/l)	Salinity (‰)	Conductivity (mS/cm)	Total dissolved solids (g/l)	Dissolved organic carbon (mg/l)	Metal concentration (mg/l)						
						Cd	Cr	Cu	Pb	As	Zn	Fe
9.03	1.63	0.97	1.359	1.234	90.7	0.12	0.05	0.03	0.12	0.05	0.71	0.34

Four white-rot fungi, namely, *B. adusta* KUC9065, *Ceriporia lacerata* KUC8090, *P. calotricha* KUC8003, and *Porostereum spadiceum* KUC8602, which were selected on the basis of their ability to decolorize synthetic dyes (see later), were used for the decolorization of real wastewater. For decolorization of wastewater, each of ten fungal disks (5-mm diameter), which were removed from the pre-cultured medium, was inoculated into a flask containing 100 ml wastewater with the nutrients described by Tien and Kirt (1988). As a reference, the nutrients added were consisted of 10 g glucose, 2 g KH_2PO_4, 2.5 g $MgSO_4$ $7H_2O$, 0.1 g $CaCl_2$ $2H_2O$, 0.02 g ammonium tartrate, 10 ml thiamin–HCl (100 mg/l), 100 ml veratryl alcohol (4 mM), and 10 ml trace elements solution per 1 l of wastewater, and the trace elements were consisted of 3 g $MgSO_4$, 0.5 g $MnSO_4$ H_2O, 0.1 g NaCl, 0.1 g $FeSO_4$ $7H_2O$, 0.1 g $CoCl_2$ $6H_2O$, 0.1 g $ZnSO_4$ $7H_2O$, 0.1 g $CuSO_4$ $5H_2O$, 10 mg $AlK(SO_4)_2$ $12H_2O$, 10 mg H_3BO_3, 10 mg Na_2MoO_4 $2H_2O$, and 1.5 g nitrilotriacetate per 1 l of distilled water. Each flask was then incubated at 27 °C for 2 weeks with shaking at 150 rpm. After incubation, each sample was removed from the flask, and the percentage of color removal was estimated by measuring the absorbance from 380 to 750 nm.

A white-rot fungus, *B. adusta* KUC9065, which showed significant wastewater decolorization efficiency during the 2-week incubation period, was further investigated to compare the relationship between the decolorization of wastewater and the secretion of LMEs. During the 3-week incubation period of *B. adusta* KUC9065, samples were withdrawn every 3 days to determine wastewater decolorization effectiveness and LME activity. The fermentation broth containing nutrients described above with distilled water instead of wastewater was also prepared and incubated to confirm the effect of wastewater on LME activity. For each sample, a 2-ml aliquot was drawn using a pre-sterilized syringe and filtered by a 0.45-μm syringe filter to remove the mycelium. Filtered samples were used immediately for enzyme assay and stored at −20 °C before absorbance assay. All tests for decolorization of wastewater containing industrial dyes were performed in triplicate, and flasks without fungal inoculation were also served as an abiotic control. The flask containing the wastewater and nutrients which were steam-sterilized at 121 °C for 30 min were incubated at 27 °C for 3 weeks with shaking at 150 rpm.

2.4 Ligninolytic Enzyme Assay

The activities of three types of LME in the samples, MnP, Lac, and LiP, were determined according to the methods described by Wariishi et al. (1992); Johannes and Majcherczyk (2000), and Tien and Kirt (1988), respectively. MnP activity was determined by oxidation of 2,6-dimethoxyphenol (2,6-DMP). A 100-μl aliquot of the sample, manganese sulfate solution, and 2,6-DMP solution were added to 600 μl of malonate buffer solution, and 100 μl of hydrogen peroxide solution was added (total volume 1000 μl) to initiate the oxidation reaction. Laccase activity was determined by oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS). A 100- μl aliquot of ABTS solution was added to 800 μl of 0.1 M sodium acetate buffer (pH 4.5), and 100 μl of the sample was added to initiate oxidation reaction. LiP activity was determined by oxidation of veratryl alcohol. A 100-μl aliquot of 10 mM veratryl alcohol solution and 100 μl of the sample were added to 700 μl of 0.25 M sodium acetate buffer (pH 2.5), and 100 μl of hydrogen peroxide solution was added to initiate the oxidation reaction. The oxidation of 2,6-DMP by MnP, ABTS by laccase, and veratryl alcohol by LiP were followed spectroscopically at 469, 420, and 310 nm, respectively, by using a spectrophotometer for 3 min at room temperature. The enzyme activity was calculated by the Beer–Lambert law, and one unit was defined as producing 1 μmol/min of product under the assay conditions.

2.5 Acute Toxicity Test

A detoxification assay was conducted according to the Organization for Economic Co-operation and Development (OECD) standard procedures with neonates of *Daphnia magna* (less than 24 h old) (OECD 2004). Sample solutions were filtered with a 0.45-μm syringe filter from the wastewater before and after decolorization. Five dilutions (50 %, 25 %, 12.5 %, 6.25 %, and 3.125 %) of these samples and one control with four replicates were prepared, with 10 ml of test solution and five individuals placed in each vessel. The test was conducted at 20±2 °C for 48 h with 16 h of light and 8 h of dark photoperiods. During the test, organisms were not fed. The immobilization (defined as no response to gentle agitation) of the test species was used to calculate the EC_{50} values by the Trimmed Spearman–Karber method. EC_{50} values were transformed into toxic units ($TU = 100/EC_{50}$) for the comparison of toxicities before and after decolorization. One-way analysis of variance (ANOVA) was then performed to compare the toxicities before and after decolorization and P values less than 0.1 were regarded as statistically significant.

3 Results and Discussion

3.1 Decolorization of Synthetic Dyes

The synthetic dye-decolorization abilities of the white-rot fungi are summarized in Table 3. Among 222 white-rot fungi, a total of 25 isolates, including four isolates of *B. adusta*, five isolates of *Ceriporia lacerata*, three isolates of *I. lacteus*, one isolate of *Perenniporia fraxinea*, ten isolates of *Phanerochaete* spp., one isolate of *Phlebia radiata*, and one isolate of *Porostereum spadiceum*, removed >70 % of the original color of the dyes in the solid medium. Of the three synthetic dyes, RB4 and OII were more easily decolorized than CR in solid medium. *C. lacerata* KUC8814 alone decolorized >90 % of all the dyes in solid medium.

The percentage of color removal of the synthetic dyes in liquid medium is shown in Table 3. All strains of *C. lacerata* showed outstanding ability to decolorize individual dyes, and their isolates caused >90 % color removal when applied to the mixture of three dyes. *B. adusta*, a well-known species with useful synthetic dye biodecolorization capabilities (Heinfling et al. 1998), also significantly decolorized synthetic dyes in the present study. In the liquid medium, the *B. adusta* strains exhibited >90 % color removal when applied to CR, RB4, and OII, with an exception of strain KUC8204. Of the *B. adusta* strains, KUC8065 alone achieved >90 % percentage of color removal when applied to the three-dye mixture. The intra-specific variation of *B. adusta* for decolorization abilities was considered as general phenomenon which is frequently observed during fungal degradation of organopollutants, as noted by others (Choi et al. 2013; Lee et al. 2011). Following *C. lacerata* and *B. adusta*, *P. calotricha* KUC8003 and *P. spadiceum* KUC8602 had the next highest decolorizing rates when applied to the three-dye mixture (>80 %). Therefore, four isolates, namely, *B. adusta* KUC9065, *C. lacerata* KUC8090, *P. calotricha* KUC8003, and *P. spadiceum* KUC8602, were finally chosen for the decolorization of real wastewater.

Three isolates of *I. lacteus*, one isolate of *P. fraxinea*, nine isolates of *Phanerochaete* spp. (*P. aculeata*, *P. sordida*, and *P. velutina*), and one isolate of *P. radiata* poorly decolorized synthetic dyes in the liquid medium, although they showed reasonable decolorization abilities in the solid medium (Table 3). Theses isolates were excluded from the screening process for the decolorization of real wastewater because decolorization ability in liquid medium is a high priority in terms of practical application.

3.2 Decolorization of Wastewater Containing Industrial Dyes

Table 4 shows the absorbance of visible light wavelengths (380–750 nm) by wastewater after a 2-week decolorization by four selected white-rot fungi, *B. adusta* KUC9065, *C. lacerata* KUC8090, *P. calotricha* KUC8003, and *P. spadiceum* KUC8602. The absorbance by wastewater of all wavelengths was significantly decreased by *B. adusta* KUC9065 during the 2-week incubation. Although *C. lacerata* KUC8090, *P. calotricha* KUC8003, and *P. spadiceum* slightly decolorized the wastewater, these decolorizing abilities were relatively low compared to their notable ability to decolorize synthetic dyes both in solid and liquid media. It is considered that the low decolorizing abilities of the above three isolates arise from the influence of substances other than dyes in the dye effluents, such as salts, surfactants, metals, toxic organic chemicals, and biocides. Therefore, we suggest that decolorizing ability

Table 3 Decolorization of synthetic dyes by white-rot fungi in solid and liquid media

Fungal isolate	Decolorization ability in solid medium[a]			Color removal in liquid medium (%)			
	CR	RB4	OII	CR	RB4	OII	Mixed dyes
Bjerkandera adusta KUC8204	++	++++	++++	75	50	–[b]	4
Bjerkandera adusta KUC8808	++	+++++	+++++	91	97	92	76
Bjerkandera adusta KUC9065	+++	++++	++++	92	98	97	94
Bjerkandera adusta KUC9107	++	++++	++++	92	96	93	83
Ceriporia lacerata KUC8090	++++	+++++	+++++	90	95	99	94
Ceriporia lacerata KUC8138	++++	+++++	+++++	89	93	99	93
Ceriporia lacerata KUC8139	++++	++++	++++	89	93	99	91
Ceriporia lacerata KUC8614	+++	++++	++++	85	85	98	93
Ceriporia lacerata KUC8814	+++++	+++++	+++++	88	92	98	94
Irpex lacteus KUC8508	++++	++++	++++	78	86	14	27
Irpex lacteus KUC9013	++++	+++	+++++	64	58	–	5
Irpex lacteus KUC9014	++++	++++	+++	73	85	31	23
Perenniporia fraxinea KUC8728	++++	++++	+++++	49	26	–	28
Phanerochaete aculeata KUC9032	++	+++++	++++	33	97	46	13
Phanerochaete calotricha KUC8003	++	++++	+++++	64	91	62	87
Phanerochaete sordida KUC8032	++++	+++++	+++++	44	37	24	18
Phanerochaete sordida KUC8405	++	+++++	+++++	38	18	–	17
Phanerochaete sordida KUC9201	++++	+++++	+++++	37	–	–	19
Phanerochaete velutina KUC8301	++	+++++	+++++	39	–	–	26
Phanerochaete velutina KUC8727	++	+++++	+++++	40	6	–	18
Phanerochaete velutina KUC8801	++	+++++	+++++	48	–	–	24
Phanerochaete velutina KUC8815	++	+++++	+++++	44	2	–	15
Phanerochaete sp. KUC8370	++++	+++++	+++++	47	90	6	1
Phlebia radiata KUC8406	++	+++++	+++++	56	42	1	37
Porostereum spadiceum KUC8602	++	+++++	+++++	88	98	99	80

[a] Dye decolorization ability in solid medium was determined by the percentage of color removal on MEA medium amended with CR, RB4, or OII, and was expressed as follows: +++++, 100≥% color removal>90; ++++, 90≥% color removal>70; +++, 70≥% color removal>50; ++, 50≥% color removal>30; +, 30≥% color removal>0; − no removal

[b] Not decolorized

should be evaluated in real effluents in order to screen candidates for the biodecolorization of wastewater containing industrial dyes, although the basic structures of synthetic dyes, such as diazo, anthraquinone, and azo compounds, were similar or identical to commercial dyes.

Transmittance of all visible light wavelengths by wastewater continuously increased with incubation time in the presence of *B. adusta* KUC9065, which emerged as the most effective isolate for decolorization of wastewater (Fig. 1). After a 3-week decolorization process, the absorbance at violet, blue, cyan, green, yellow, orange, and red wavelengths was decreased by 71.1 %, 82.4 %, 85.7 %, 89.8 %, 91.8 %, 92.1 %, and 90.2 %, respectively (Table 5). The change in the color of the wastewater before and after the decolorization was obvious to the naked eye (Fig. 2). It is apparent that this remarkable decolorization of wastewater by of *B. adusta* KUC9065 before discharge might increase light penetration in natural bodies of water, thereby improving photosynthesis. However, further studies are necessary to reduce the operating time for practical application of biodecolorization. As a reference, the absorbance of wastewater without fungal inoculation also slightly decreased (17.5–34.4 %) during 3-week agitation (Table 5).

Table 4 Percentage of color removal of wastewater after 2-week decolorization by four selected white-rot fungi

Fungal isolate	Color removal[a] (%)						
	Violet (380–450 nm)	Blue (450–475 nm)	Cyan (476–495 nm)	Green (495–570 nm)	Yellow (570–590 nm)	Orange (590–620 nm)	Red (620–750 nm)
Bjerkandera adusta KUC9065	63.5±6.4 A[b]	75.4±6.5 A	78.9±5.5 A	84.9±2.8 A	89.6±0.7 A	90.3±0.1 A	86.2±1.8 A
Ceriporia lacerata KUC8090	26.9±4.5 B	32.2±2.7 D	31.4±1.3 D	35.4±0.4 C	38.4±0.9 D	33.5±0.8 D	−5.0±2.0 D
Phanerochaete calotricha KUC8003	39.7±1.6 B	45.7±1.8 B	44.5±2.2 B	45.7±1.8 B	46.2±1.3 C	41.6±1.1 C	7.2±0.8 C
Porostereum spadiceum KUC8602	28.4±7.8 B	38.1±0.5 C	40.2±8.1 C	48.5±4.8 B	55.0±5.0 B	54.5±4.2 B	40.2±2.8 B

[a] Values represent the average of three replicates with standard deviations

[b] Numbers followed by the same letter in each column are not significantly different (α=0.05) according to Duncan's method

3.3 Ligninolytic Enzyme Activity

Figure 3 shows Lac and MnP activities during the 3-week decolorization process of wastewater by *B. adusta* KUC9065. In the wastewater, Lac and MnP activities reached their peaks at 6 days (4.2 U/ml) and 12 days (48.3 U/ml), respectively, while in the liquid medium without wastewater, Lac and MnP activities were remarkably lower than those in the wastewater. Lip activity was not detected in either wastewater or liquid medium in this study. LMEs involved in the oxidative breakdown of phenolic compounds are considered key substances in the degradation of organopollutants by white-rot fungi. Secretion of LMEs by white-rot fungi is stimulated by the addition of organopollutants, including dyes, as noted by Kasinath et al. (2003). It is anticipated that Lac and MnP secretion, which is stimulated by wastewater, might play a major role in the decolorization of wastewater by *B. adusta* KUC8065. Another explanation for the decolorization of wastewater by *B. adusta* KUC8065 is the ionic sorption of dye compounds by fungal hyphae (Kaushik and Malik 2009; Salas-Veizaga et al. 2013), which will be studied further.

3.4 Detoxification of Wastewater

Acute toxicity of wastewater before and after the decolorization process, which is represented by TU values, is shown in Fig. 4. The TU values resulting from

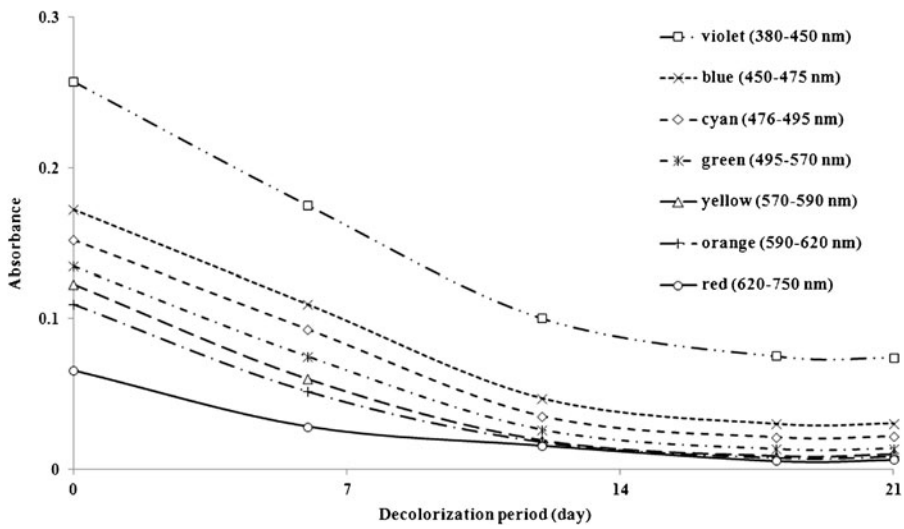

Fig. 1 Transition of absorbance at visible light wavelengths of the wastewater samples during 3-week decolorization by *B. adusta* KUC9065

Table 5 Percentage of color removal of wastewater after 3-week decolorization by *B. adusta* KUC9065

Wavelength	Color removal[a] (%)
Violet (380–450 nm)	71.1±3.4 (18.3)[b]
Blue (450–475 nm)	82.4±6.8 (17.5)
Cyan (476–495 nm)	85.7±7.7 (20.0)
Green (495–570 nm)	89.8±7.3 (27.3)
Yellow (570–590 nm)	91.8±7.5 (36.3)
Orange (590–620 nm)	92.1±8.2 (37.4)
Red (620–750 nm)	90.2±9.3 (33.4)

[a] Values represent the average of three replicates with standard deviations

[b] Abiotic control

biodecolorization by *B. adusta* KUC9065 were significantly reduced from 3.5 to 2.6 on the basis of the 48-h acute toxicity test, while there is no significant difference of TU values from the 24-h acute toxicity test. The reduction in toxicity during the treatment of the dyeing effluent has to be accomplished along with the increase in light penetration, because an increase in toxicity arising from metabolites during the fungal biodecolorization process has been reported frequently (Choi et al. 2013; Kaushik and Malik 2009; Olukanni et al. 2010). Choi et al. (2013) revealed that three toxic compounds were newly formed during the decolorization process of CR and OII by *I. lacteus*. Kaushik and Malik (2009) summarized previous reports relevant to fungal dye decolorization and demonstrated that handling the toxic metabolites released from decolorization

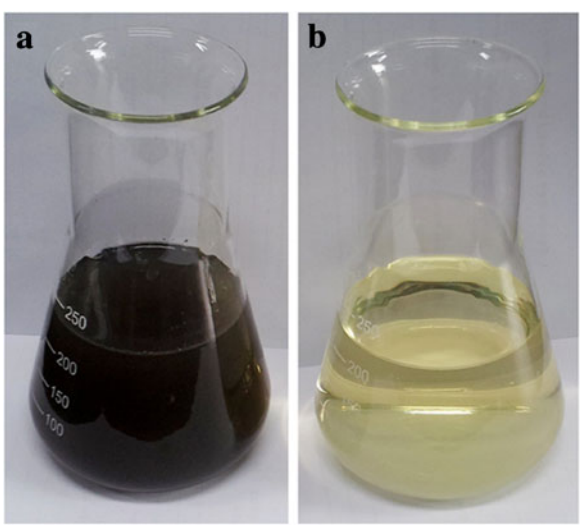

Fig. 2 Photographic comparison of wastewater before (**a**) and after (**b**) 3-week decolorization by *B. adusta* KUC9065

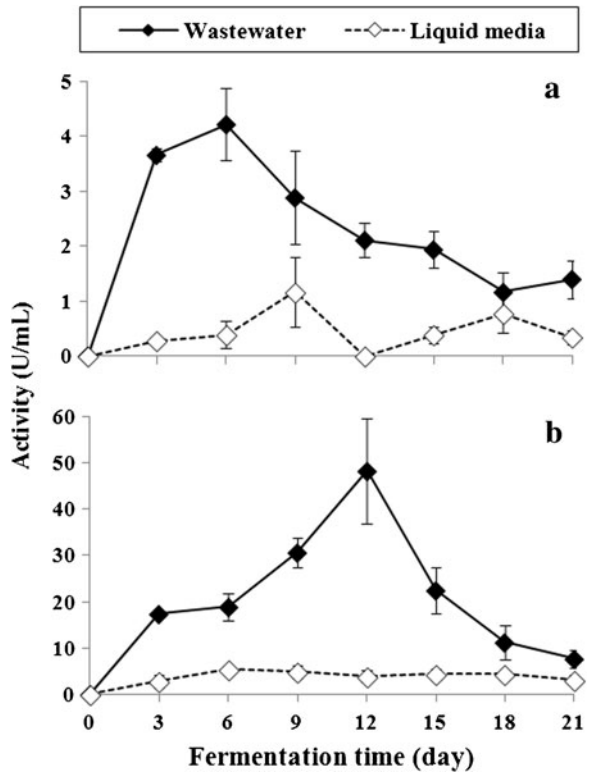

Fig. 3 Laccase (**a**) and manganese peroxidase (**b**) activities during 3-week decolorization of wastewater by *B. adusta* KUC9065. Error bars represent standard deviation (n=3)

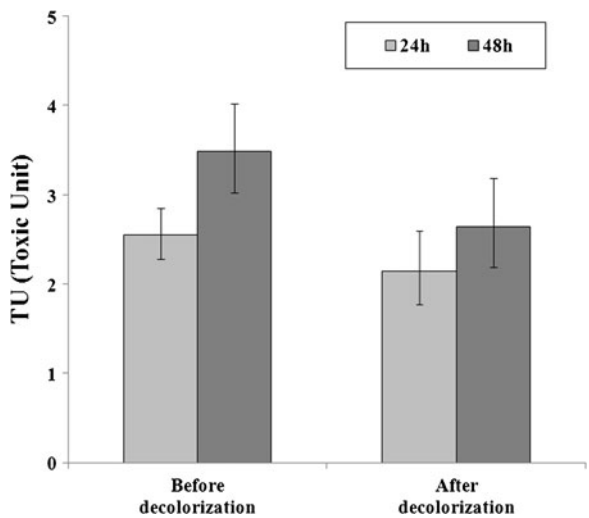

Fig. 4 Acute toxicity of wastewater before and after 3-week decolorization by *B. adusta* KUC9065. Error bars represent 95 % confidential intervals

process is necessary for the safe and eco-friendly application of biodecolorization. Taken together, it is suggested that the metabolites and their toxicities should be monitored continuously during biodecolorization process. In addition, phase transformations of metals by organic compounds might contribute to the change in toxicity of wastewater, because tiny quantities of heavy metals might affect acute toxicity to *D. magna* (Yi et al. 2009). As a reference, the concentration of DOC was increased from 90.7 mg/l to 418.9 mg/l by the addition of carbon sources (e.g., glucose) for the biodecolorization process in this study. Therefore, future investigations should be conducted to elucidate phase transformations of metals by organic compounds and their effect on the change of acute toxicity. Furthermore, additional toxicity reduction technology needs to be developed considering that a TU value of <1 is the threshold value for decisions regarding the discharge of wastewater into natural water bodies, although the toxicity of wastewater was somewhat decreased by biodecolorization in this work.

4 Conclusions

In the present study, 25 isolates including four isolates of *B. adusta*, five isolates of *C. lacerata*, three isolates of *I. lacteus*, one isolate of *P. fraxinea*, ten isolates of *Phanerochaete* spp., one isolate of *P. radiata*, and one isolate of *P. spadiceum* showed ability to decolorize chemically different synthetic dyes (i.e., CR, RB4 and OII) in solid medium among the 222 isolates from 47 genera of white-rot fungi. Of the 25 isolates, *B. adusta* KUC9065, *C. lacerata* KUC8090, *P. calotricha* KUC8003, and *P. spadiceum* KUC8602 were finally identified as outstanding fungi to decolorize synthetic dyes in liquid medium. However, only the white-rot fungus *B. adusta* KUC9065 caused extensive decolorization of industrial effluents, while the other strains showed low decolorization efficiency. During the 3-week biodecolorization process, *B. adusta* KUC9065 reduced the absorbance of visible light of the wastewater collected from the treatment plant at the Keom-jun Dyeing Enterprise Cooperation, Yang-ju, South Korea, by 71–92 % for different wavelengths. It is considered that Lac and MnP, which are produced by the fungus, might be involved in the decolorization process of wastewater. Furthermore, the acute toxicity of the wastewater was decreased by the decolorization process. Based on these results, it is suggested that *B. adusta* KUC9065 might be a notable candidate for wastewater biodecolorization. Further studies are necessary to reduce the processing time and to monitor the metabolites for practical biodecolorization by using *B. adusta* KUC9065.

Acknowledgments This work was supported by a Forest Science & Technology Project (Project No. S121012L110100), provided by the Korea Forest Service of the Korean Government, and by a Korea University Grant.

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