African Journal of Microbiology Research Vol. 7(11), pp. 941-947, 12 March, 2013
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.904
ISSN 1996-0808 ©2013 Academic Journals

Full Length Research Paper

Decolorization of indigo dye and indigo dye-containing textile effluent by *Ganoderma weberianum*

Chang-en Tian[1]*, Ruicai Tian[2], Yuping Zhou[1], Qionghua Chen[1] and Huizhen Cheng[1]

[1]School of Life Sciences, Guangzhou University, Guangzhou 510006, China.
[2]School of Life Science and Engineering, North Nationality University, Yinchuan 750021, China.

Accepted 8 March, 2013

Many natural and synthetic dyes present in industrial effluents are resistant to degradation by conventional treatments. The decolorization of indigo dye and indigo dye-containing textile wastewater was carried out using the newly isolated ligninolytic fungus strain *Ganoderma weberianum* TZC1. This strain was shown to decolorize both indigo dye and its effluents efficiently; the optimal pH and temperature ranges for activity were pH 4.0-5.0 and 28-33°C for indigo dye, and pH 3.0 and 28-33°C for indigo dye effluents, respectively. A decolorization efficiency of 92% for indigo dye was achieved by the cultivation of *G. weberianum* TZC1 for 1 h at an initial dye concentration of 20 mg l^{-1}. The highest decolorization efficiency for the indigo dye effluent (A_{650}=0.52) was 25%, and it was achieved by the cultivation of the culture containing equal volumes of the dye effluent and mycelial pellets for 1 h. The correlation between decolorization rate and initial concentration of indigo dye can be interpreted by Michaelis-Menten model. The Michaelis constant (K_m) and the maximum specific decolorization rate (V_{max}) were 24.33 mg l^{-1} and 13.99 mg g $FW^{-1}h^{-1}$, respectively. In addition, analyses of lignin peroxidase, manganese peroxidase and laccase activities as well as native polyacrylamide gel electrophoresis of *G. weberianum* TZC1 crude enzyme, confirmed that laccase played a major role in indigo dye decolorization. Furthermore, *G. weberianum* was shown to be used efficiently and repeatedly in repeated-batch decolorization operations for both indigo dye and its effluents. Our results suggest that the strain *G. weberianum* TZC1 had promising applications in the treatment of indigo dye-containing wastewater.

Key words: Decolorization, *G. weberianum*, indigo dye, indigo dye-containing textile effluent, laccase.

INTRODUCTION

Textile effluents usually contain dyes that are discharged in large quantities into natural water bodies such as the sea, rivers and lakes (Mohan et al., 2001; Meehan et al., 2000). Most dyes used in the textile industry are toxic to plants, microbes and protozoa living in the water bodies and are recalcitrant to decolorization and degradation (Birhanli and Ozmen, 2005; Couto, 2009). Thus, even the presence of very small amounts of dyes in the effluents is highly undesirable (Kaushik and Malik, 2009; Mishra and Tripathy, 1993). Furthermore, the discharge of dye-containing effluents into waters without appropriate treatment will reduce light penetration and in turn limit and even kill aerobic aquatic organisms by creating anaerobic conditions (Banat et al., 1996). Therefore, color removal from dye-containing wastewaters by different methods has garnered scientific interest (Forgacs et al., 2004).

The methods for dye removal from wastewater broadly fall into three categories: physical, chemical and biological. The major disadvantages of physico-chemical methods have been the high cost, low efficiency, limited

*Corresponding author. E-mail: changentian@yahoo.com.cn. Tel: +86 20 39366995. Fax: +86 20 39366995.

versatility, interference by other wastewater constituents and handling of the waste generated (Kaushik and Malik, 2009). Microbial decolorization is a cost-effective process, which is receiving much attention for the treatment of dye-containing wastewater (Banat et al., 1996; Zee and Villaverde, 2005). Fungi are suitable for dye removal from textile effluent because they have the following advantages over single cell organisms: solubilization of the insoluble substrates and tolerance for high concentrations of the toxicants, as well as greater physical and enzymatic contact with the environment because of an increased cell-to-surface ratio (Couto 2009; Kaushik and Malik 2009; Yesilada et al., 2003).

Vat dyes are extensively used for dyeing cotton fabrics. Amongst the vat dyes, indigo dyes are commonly used for the manufacture of denim (Harazono and Nakamura, 2005). The world consumption of dyes for cellulosic fibers is about 60 000 ton year^{-1}, 5% of which is indigo dye (Paradise, 1999; Spadaro et al., 1994). The annual production of synthetic indigo dye was estimated at 22 000 tons in 2001 (Schrott, 2001). Therefore, very large amounts of indigo dye-containing wastewater, especially textile effluent, must be treated before being discharged into the environment. Indigo dye is water-insoluble and is considered a recalcitrant substance that causes environmental concern (Balan and Monteiro, 2001), and its effluents have been primarily treated by chemical coagulation and floculation-filtration methods, which generate large amounts of sludge that pose handling and disposal problems (Nyanhongo et al., 2002).

Indigo dye and indigo dye-containing effluents could be decolorized by photocatalysis (Vautier et al., 2001), electrochemical reduction (Doğan and Türkdemir, 2005; Thomas et al., 2006), ligninolytic enzymes (Balan and Monteiro, 2001; Campos et al., 2001b; Nyanhongo et al., 2002; Abadulla et al., 2000), anaerobic mixed cultures (Manu and Chaudhari, 2003), and aerobic bioreactor (Khelifi et al., 2008; Couto et al., 2006). We previously found that a laccase generated by white rot fungus *G. weberianum* TZC1 was able to decolorize indigo dye (Chen et al., 2010). We therefore hypothesized that the mycelial pellets of *G. weberianum* TZC1 can directly decolorize indigo dye. Accordingly, we designed the present study to examine the usefulness of *G. weberianum* TZC1 in decolorizing indigo dye solutions and indigo dye-containing textile effluents. The mechanism underlying the decolorization of indigo dye was also explored.

MATERIALS AND METHODS

Chemicals

Indigo dye, ABTS (2, 20-azinobis(3-ethylbenzthiazoline-6-sulphonate)), Phenol Red (Phenolsulfonphthalein) and veratryl alcohol (3,4-Dimethoxybenzyl alcohol) were obtained from Sigma-Aldrich (Milwaukee, USA). All other reagents and chemicals were of analytical grade and were used without further purification. The indigo dye solution was prepared by mixing 200 mg of powdered indigo dye, 1 g of $Na_2S_2O_4$, and 2 g of 48 Bomè NaOH in a vessel containing 800 ml of water. The newly prepared solution is light yellow in color. After the prepared solution turned blue, the pH was adjusted to 5 with HCl. The final volume of the dye solution was 1000 ml.

Storage of *G. weberianum* TZC1

The *G. weberianum* TZC1 strain was isolated from fruiting bodies grown on tree stumps in the suburb of Guangzhou, P.R. China; the TCZ1 strain was identified as *G. weberianum* based on its morphology and sequence identity to the ITS regions and 5.8S rRNA gene found in GenBank (Chen et al., 2010). The strain was maintained on potato dextrose agar (PDA) medium slants. The slants were inoculated and incubated at 28°C for 7-8 d followed by storage at 4°C, and subcultured periodically.

Medium for mycelium-pellet culture

One liter of medium used for the mycelial pellet cultures was composed of 200 g of peeled potatoes, 20 g of dextrose, 3 g of KH_2PO_4, 1.5 g of $MgSO_4 \cdot 7H_2O$, 0.002 g of vitamin B1, 5 g of yeast extract, 70 ml of microelement solution and water. The microelement solution includes 0.5 g l^{-1} of $MnSO_4 \cdot H_2O$, 1 g l^{-1} of NaCl, 0.1 g l^{-1} of $FeSO_4 \cdot 7H_2O$, 0.1 g l^{-1} of $CoCl_2$, 0.1 g l^{-1} of $ZnSO_4 \cdot 7H_2O$, 0.1 g l^{-1} of $CuSO_4 \cdot 5H_2O$, 0.01 g l^{-1} of $KAl(SO_4)_2 \cdot 12H_2O$, 0.01 g l^{-1} of H_3BO_3 and 0.01 g l^{-1} of $Na_2MoO_4 \cdot 2H_2O$. The media were sterilized by autoclaving at 121°C for 20 min.

Decolorization experiments

For the decolorization experiments, 100 µl of spore suspension of *G. weberianum* TZC1 (10^8 spores ml^{-1}) from a slant were transferred aseptically into 500 ml Erlenmeyer flasks containing 100 ml of the autoclaved medium described above. The pH of the medium was adjusted according to the requirement of the experiment before sterilization, and the cultures were incubated for 4 d in an orbital shaker at 160 rpm and 28°C. Then, 100 ml of indigo dye or indigo dye-containing textile effluent (A_{650}=0.52) obtained from a denim factory in Guangzhou were added to the culture. The resulting mixture was further incubated under same conditions.

Effects of pH and temperature on dye and effluent decolorization

The effect of pH on decolorization was monitored at pH ranges of 3.0 to 7.0 for indigo dye and 2.0 to 6.0 for indigo dye-containing effluents. The pH of the medium was adjusted using 1.0 M NaOH or 1.0 M HCl. To determine the effect of temperature on decolorization, the culture was incubated at temperatures ranging from 13 to 38°C for indigo dye and from 23 to 43°C for indigo dye-containing effluents.

Repeated-batch studies

Repeated-batch operations were performed to evaluate the decolorization efficiency of mycelial pellets at the different ages. We

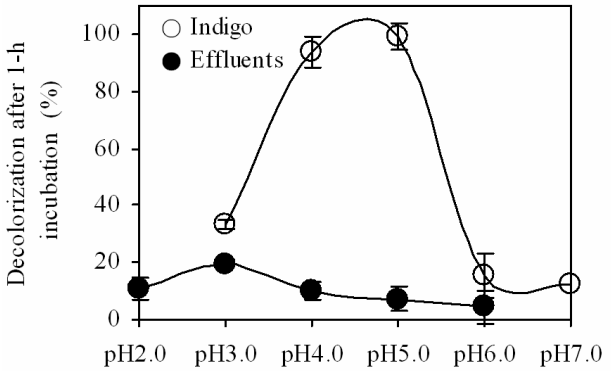

Figure 1. Decolorization of indigo dye and its effluents by *G. weberianum* TZC1 at 28°C, agitation at 160 rpm and different pH values. The values shown are the mean of triplicate cultivation experiments.

achieved this by harvesting fungal pellets after each cultivation for decolorization; we then inoculated the pellets into 50 ml of media plus 50 ml of effluents every 5 days, and the decolorization was detected after treatments of 1 or 4 h for both the indigo dye and indigo dye-containing effluent. All cultures were incubated at 33°C and were shaken at 160 rpm.

Decolorization assay

The decolorization of the dye and effluent was detected by UV-Vis spectrophotometer (Beckam Coulter DU® 800, USA) at the λ_{max} of indigo dye (650 nm) using the supernatant of the liquid culture medium. The supernatant was obtained by centrifugation at 10,000 rpm for 10 min in a refrigerated centrifuge (Beckam Coulter Microfuge® 1, USA) followed by decantation. The removal of color was reported as % decolorization (% = $100(A_{ini} – A_{obs}) / A_{ini}$), where A_{ini} and A_{obs} are the absorbance of the dye solution initially and at cultivation time (t), respectively. Each decolorization value is the mean of three replicated experiments. Abiotic controls (without the microorganism) were always included.

Native-polyacryl amide gel electrophresis (PAGE)

To determine whether the laccase is the major enzyme in the crude extract involved in dye decolorization, the crude enzyme was subjected to PAGE on a 12% native polyacrylamide gel. After separation of the proteins by electrophoresis, the gel was divided into 3 pieces; the first piece was soaked in 20 ml of sodium acetate buffer (50 mM, pH 4.5) containing 25 mg of ABTS to detect the laccase activity; the second piece was stained with indigo dye [20 mg l^{-1} in sodium acetate buffer (50 mM, pH 4.5)] to detect decolorization activity, and the third was stained with Coomassie brilliant blue R250 to identify the proteins. After 10 min to 4 h of incubation, the dye solution was discarded, and pictures of the gels were taken.

Protein detection

The protein content was determined by the method of Bradford using bovine serum albumin as a standard (Bradford, 1976).

Methods employed for the kinetics study

To analyze the kinetics of indigo dye decolorization by *G. weberianum* TZC1, a batch kinetic test in a flask was conducted with the culture obtained from Decolorization experiments described above containing different initial concentration of indigo dye. The pH was maintained at an optimal level of 4.0. The temperature was controlled at 33°C. The agitation was kept at 160 rpm. An entire experiment lasted 120 min. The decolorization rate in this system was calculated by use of the following equation:

$$V = (C_0 – C_1) \times Volume / FW$$

Where V is the specific decolorization rate (mg $gFW^{-1}h^{-1}$); C_0 and C_1 are the initial and final dye concentration (mg l^{-1}), respectively; Volume is the culture volume (l); FW is the fresh weight (g) of mycelium pellet used in a flask.

RESULTS AND DISCUSSION

Effect of pH on the decolorization of indigo dye and its effluents

To investigate the effect of pH on dye decolorization, the initial pH values of the media were adjusted to a pH range of 3.0 to 7.0 for indigo dye and 2.0 to 6.0 for indigo dye effluents, respectively. The decolorization rates were measured in culture filtrates after 2 and 4 h of incubation for both indigo dye and its effluents. The results revealed that the fungus was capable of decolorizing indigo dye over a pH range of 3.0 to 7.0, and the highest decolorization rate was observed between pH 4.0 and 5.0 (Figure 1). Meanwhile, *G. weberianum* TZC1 could also decolorize indigo dye-containing effluents, and the optimum pH for decolorization of effluents was pH 3.0 (Figure 1). The difference in the optimal pH for indigo dye and its effluents may result from the different substances contained in the different liquids. Thereafter, pH 4.0 and pH 3.0 were used for indigo dye and its effluents, respectively in our further decolorization experiments.

Effect of temperature on decolorization of indigo dye and its effluents

The effects of temperature on dye and effluents decolorization were evaluated by incubating the cultures at various temperatures. Figure 2 shows that the decolorization rates of both indigo dye and its effluents were higher at 28 and 33°C than those at other temperatures (that is 13, 18, 23, 38 and 43°C), which indicated that 28 and 33°C were the optimum temperature for the decolorization of both indigo dye and its effluents. The lower decolorization rate at temperatures higher than 33°C may be attributed to the deactivation of the decolorization enzymes. Based on the above results, the following decolorization experiments

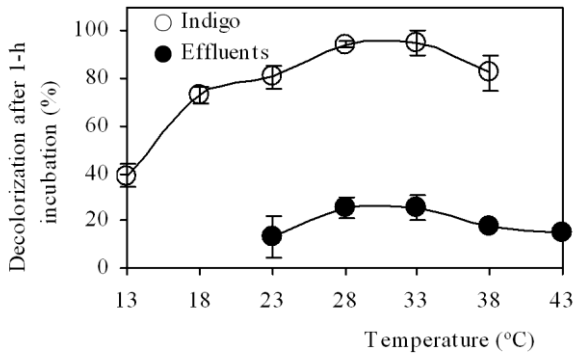

Figure 2. Effect of temperature on the decolorization of indigo dye and its effluents by *G. weberianum* TZC1 at different temperatures, under agitation at 160 rpm and at pH 4.0 for indigo dye, and at pH 3.0 for the indigo dye effluents. The values shown are the mean of triplicate cultivation experiments.

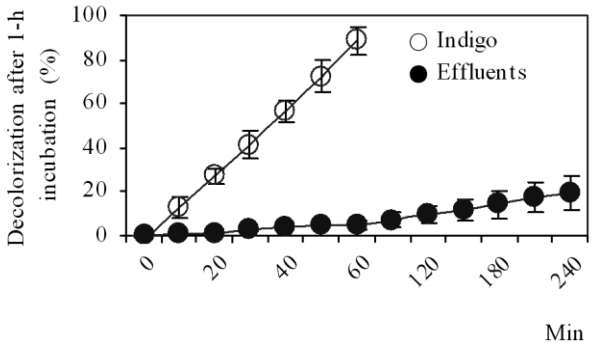

Figure 3. Decolorization of indigo dye and its effluents by *G. weberianum* TZC1 under optimal conditions of 33°C, agitation at 160 rpm and at pH 4.0 for indigo dye, and at pH 3.0 for the indigo dye effluents. The values shown are the mean of triplicate cultivation experiments.

using strain TZC1 were performed at 33°C.

Decolorization of indigo dye and its effluents under the optimal condition

Based on the above results, indigo dye and its effluents were treated at 33°C, under agitation at 160 rpm at pH 4.0 for indigo dye and pH 3.0 for its effluents to probe the decolorization capability of *G. weberianum* TZC1. As shown in Figure 3, the rate of decolorization reached 92 and 25% per hour for 20 mg l^{-1} indigo dye and for indigo dye effluents (A_{650}=0.52), respectively. The lower decolorization rate for indigo dye effluents may result from inhibiting substances contained in the wastewater

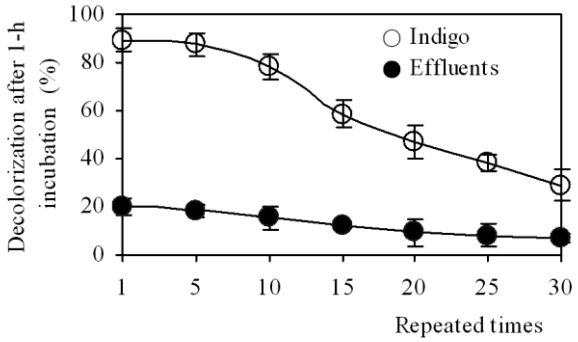

Figure 4. Color removal of indigo dye and its effluents in liquid batch cultivation with *G. weberianum* TZC1 at 33°C, under agitation at 160 rpm and at pH 4.0 for indigo dye, and at pH 3.0 for the indigo dye effluents. The values shown are the mean of triplicate cultivation experiments.

(Nyanhongo et al., 2002). To our knowledge, the decolorization efficiency for indigo dye is the highest one when compared to all other organisms reported to date. Also this strain can decolorize indigo dye and its effluents without a mediator in a very short time, suggesting that strain TZC1 is a promising fungus to depollute the indigo dye-containing effluents. To our knowledge, this is the first report on the decolorization of dye-containing effluents by the fungus *G. weberianum*.

Repeated-batch liquid cultivation

Although there are many reports concerning decolorization of indigo or indigo carmine, the repeated-batch methods were only employed in several ones. A packed-bed reactor with silica-gel-bound laccase beads can decolorize indigo carmine efficiently for 18 days (Rekuć et al., 2010). One milligram of spores displaying *Bacillus subtilis* laccase can decolorize 44.6 µg indigo carmine in 2 h (Cho et al., 2011). Indigo Carmine (70 mg/l) was almost totally decolourised in 3 days in a bioreactor with *Trametes hirsuta* immobilized by a stainless steel sponge (Rodríguez et al., 2004). Others involves in absorption of Indigo Carmine (Prado et al., 2004; Lakshmi et al., 2009).

The repeated-batch experiments for the removal of indigo dye and its effluents were carried out to investigate whether *G. weberianum* TZC1 can be used efficiently and repeatedly. As shown in Figure 4, the rate of decolorization decreased as the number of repeats increased. Initially, the rate of decolorization after incubation for 1 h was 92 and 20% for indigo dye and its effluents, respectively. After the fungus was used 30 times, the rate of decolorization after 1 h was recorded at 37 and 8% for indigo dye and its effluents, respectively.

These results suggest that the strain was indeed able to decolorize indigo dye and its effluents efficiently and that it can be used repeatedly.

The mechanism for decolorization of indigo dye

We found that decolorization could be mainly attributed to biodegradation but not to adsorption, because the pellets were not stained by indigo dye throughout incubation. During incubation, we first observed that the solution color was changed from deep blue to light blue to red and from red to a natural color, like that of the control without dye. This is consistent with the result of Campos and college (2001a), whereby the laccases from the fungi *Trametes hirsuta* (THL1 and THL2) and *Sclerotium rolfsii* (SRL1) were able to oxidize indigo dye yielding isatin (indole-2, 3-dione) and further decompose the latter to anthranilic acid (2-aminobenzoic acid) (Campos et al., 2001a). Isatin is a red substance, and anthranilic acid is colorless. The bacterium *Paenibacillus larvae* decomposed indigo carmine into anthranilic acid via isatin sulfonic acid (Ramya et al., 2008). The above results indicate the involvement of laccase in the decolorization of indigo dye by strain TZC1 and the same degradation pathway of indigo dye by strain TZC1 as the bacterium *P. larvae* (Ramya et al., 2008) and the laccases from the fungi (Campos et al., 2001a).

It is clear that lignin-modifying enzymes (LMEs) play significant roles in dye metabolism by white rot fungi (WRF) (McMullan et al., 2001). WRF are able to produce LMEs such as laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP) (Asgher et al., 2008). LMEs are directly involved in not only the degradation of lignin in their natural lignocellulosic substrates but also in the degradation of various xenobiotic compounds (Pointing, 2001), including dyes (Glenn and Gold, 1983). Some WRF produce all three LMEs, while others produce only one or two of them (Hatakka, 1994). We previously determined that strain TZC1 can generate a laccase, and the latter can in turn decolorize indigo dye *in vitro*. In an attempt to identify the role of ligninolytic enzymes, we first measured activities of those enzymes. A high level of laccase activity has been detected in the supernatants of pellet-cultured medium (Chen et al., 2010). However, neither MnP activity nor LiP activity were detected in the supernatants of *G. weberianum* TZC1 culture medium (data not shown). Other fungi such as *Phlebia tremellosa* (Kirby et al., 2000; Robinson et al., 2001), *Pleurotus sajorcaju* (Chagas and Durrant, 2001), and *Coriolus versicolor f. antarcticus* (Levin et al., 2004) were also demonstrated to decolorize and/or degrade dye mainly through laccase. Taken together, laccase may be the main biocatalyst in the decolorization and degradation of indigo dye.

SDS-PAGE of the purified laccase from *G. weberianum* TZC1 exhibited a protein of ~ 45 kDa (Chen et al., 2010). To determine whether the laccase is the major player in decolorizing indigo dye, the supernatants of the *G. weberianum* TZC1 culture medium were subjected to native-PAGE. After the separation of proteins by electrophoresis, the gel was divided into 3 pieces; the first piece was soaked in ABTS-containing solution to detect the laccase activity; the second was stained with indigo dye to detect decolorization activity, and the third was stained with Coomassie brilliant blue R250 to identify the proteins. As shown in Figure 5, only one decolorized zone was present in lane A as a band, and a single laccase activity band appeared in lane B. Moreover, the laccase activity band and the decolorized band were indeed at the same position of the native gel, suggesting that the decolorization was caused by laccase. Except for a protein band located at the same position as the laccase and decolorization band, almost no other obvious protein bands were present on the native-PAGE (Figure 5). This is in accordance with the observation that no LiP and MnP activities were detected in the supernatants

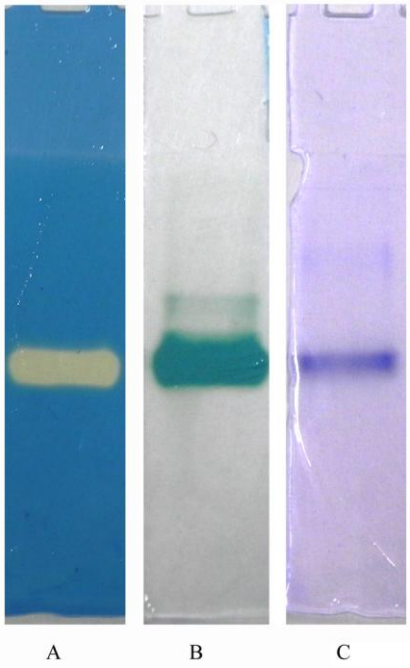

Figure 5. Native PAGE and zymogram of the supernatants from pellet-cultured medium. Lane A, a gel strip showing a clear white band of decolorized zone observed against a blue background after incubation with indigo dye; lane B, a gel strip showing a clear blue-green band for laccase activity after incubation with ABTS; lane C, a gel strip showing the protein bands stained with Coomassie brilliant blue R250.

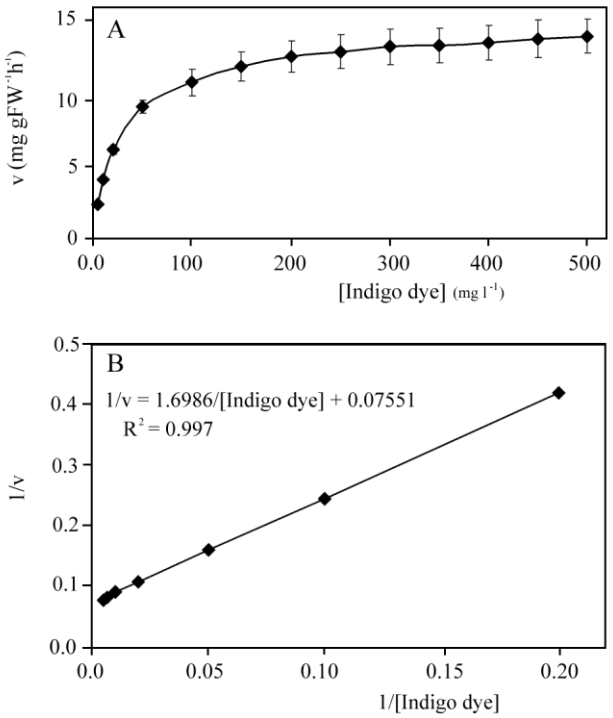

Figure 6. The kinetics of indigo dye decolorization by *G. weberianum* TZC1 under optimal conditions of 33°C, agitation at 160 rpm and at pH 4.0. (A) Dependence of specific decolorization rate on the initial concentration of indigo dye; (B) Lineweaver–Burk plot of indigo dye biodegradation.

from pellet-cultured medium.

To summarize, the extracellular laccase of *G. weberianum* TZC1 indeed plays a major role in indigo dye decolorization and degradation.

The kinetics of indigo dye decolorization by *G. weberianum* TZC1

Figure 6A shows the dependence of dye concentration on biological decolorization rate. When the initial dye concentration increased from 5 to 500 mg l^{-1}, the decolorization rate also increased. The kinetic trends were interpreted according to conventional Michaelis–Menten model. The decolorization rate in our system was calculated by use of the following equation derived from Lineweaver–Burk plot:

$$1/v_{dye} = (Km/V_{dye\ max}) \times 1/C_{dye} + 1/V_{dye\ max}$$

Where v_{dye} is the specific decolorization rate; C_{dye} is the dye concentration; V_{max} and K_m are the maximum specific decolorization rate and dissociation constant, respectively. V_{max} and K_m were calculated from the slope and intercept; the relationship is shown in Figure 6B. V_{max} and K_m are 13.99 mg gFW^{-1} h^{-1} and 24.33 mg l^{-1}, respectively. To our knowledge, no data concerning the kinetics of indigo dye decolorization by microorganisms has been reported so far. The K_m and V_{max} in indigo dye decolorization by *G. weberianum* TZC1 are lower and higher than other dyes decolorization by other microorganisms, respectively, such as malachite green by *Peseudomonas lutea* (Daneshvar et al., 2007) and *Pandoraea pulmonicola* YC32 (Chen et al., 2009), indicating a higher affinity between pellets and indigo dye and a higher efficiency in indigo dye decolorization by *G. weberianum* TZC1 than others.

Further studies should be carried out to confirm whether *G. weberianum* TZC1 is more useful in other dyes decolorization. These important parameters (Vmax and Km) can be used for comparison if other biological systems are to be utilized in the removal of indigo dye.

ACKNOWLEDGEMENTS

This work was supported in part by the Science and Technology Planning Project of Guangdong Province, China (2007B080701008) and the Science and Technology Planning Project of Guangzhou Municipality, China (10C52010807) to C-E Tian.

REFERENCES

Abadulla E, Tzanov T, Costa S, Robra KH, Cavaco-Paulo A, Gübitz GM (2000). Decolourisation and detoxification of textile dyes with laccase from *Trametes hirsute*. Appl. Environ. Microbiol. 66:3357-3362.

Asgher M, Nawaz H, Ashraf BM, Legge RL (2008). Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. Biodegradation 19:771-783.

Balan DSL, Monteiro RTR (2001). Decolorization of textile indigo dye by ligninolytic fungi. J. Biotechnol. 89:141-145.

Banat IM, Nigam P, Singh D, Marchant R (1996). Microbial decolorization of textile-dye-containing effluents: a review. Bioresour. Technol. 58:217-227.

Birhanli A, Ozmen M (2005). Evaluation of the toxicity teratogenity of six commercial textile dyes using the frog embryo teratogenesis assay-Xenopus. Drug Chem. Toxicol. 28:51-65.

Bradford MM (1976). A rapid and sensitive method for detecting microgram amounts of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254

Campos R, Cavaco-Paulo A, Robra KH (2001a). Indigo degradation with laccases from *Polyporus* sp. and *Sclerotium rolfsii*. Tex. Res. J. 71:420-424.

Campos R, Kandelbauer A, Robra KH, Cavaco-Paulo A, Gübitz GM (2001b). Indigo degradation with purified laccases from *Trametes hirsuta* and *Sclerotium rolfsii*. J. Biotechnol. 89:131-139.

Chagas EP, Durrant LR (2001). Decolorization of azo dyes by *Phanerochaete chrysosporium* and *Pleurotus sajorcaju.* Enzyme Microb. Technol. 29:473-477.

Chen CY, Kuo JT, Cheng CY, Huang YT, Ho IH, Chung YC (2009). Biological decolorization of dye solution containing malachite green by *Pandoraea pulmonicola* YC32 using a batch and continuous system. J. Hazard Mater. 172(2-3):1439-1445.

Chen Q, Zhou Y, Jiang G, Li G, Cheng H, Tian C (2010). Laccase production by *Ganoderma weberianum* fermentation and its decolorization effect on Indigo dye. Food Ferment. Ind. 36(3):25-30.
Cho EA, Seo J, Lee DW, Pan JG (2011). Decolorization of indigo carmine by laccase displayed on *Bacillus subtilis* spores. Enzyme Microb. Technol. 49(1):100-104.
Cing S, Asma DH, Apohan E, Yesilada O (2003). Decolorization of textile dyeing wastewater by *Phanerochaete chrysosporium*. Folia Microbiol. 48:639-642.
Couto SR (2009). Dye removal by immobilised fungi. Biotechnol. Adv. 27:227-235.
Couto SR, Rosales E, Sanromán MA (2006). Decolourization of synthetic dyes by *Trametes hirsute* in expanded-bed reactors. Chemosphere 62:1558-1563.
Daneshvar N, Ayazloo M, Khataee AR, Pourhassan M (2007). Biological decolorization of dye solution containing Malachite Green by microalgae *Cosmarium* sp. Bioresour. Technol. 98(6):1176-1182.
Doğan D, Türkdemir H (2005). Electrochemical oxidation of textile dye indigo. J. Chem. Technol. Biotechnol. 80:916-923.
Forgacs E, Cserháti T, Oros G (2004). Removal of synthetic dyes from wastewaters: a review. Environ. Int. 30:953-971.
Glenn JK, Gold MH (1983). Decolorization of several polymeric dyes by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 45:1741-1747.
Harazono K, Nakamura K (2005). Decolourization of mixtures of different reactive textile dyes by the white-rot basidomycete *Phanerochaete sordaria* and inhibitory effect of polyvinyl alcohol. Chemosphere 59:63-68.
Hatakka A (1994). Lignin-modifying enzymes from selected white-rot fungi: production and role in lignin degradation. FEMS Microbiol. Rev. 13:125-135.
Kaushik P, Malik A (2009). Fungal dye decolourization: Recent advances and future potential. Environ. Int. 35:127-141.
Khelifi E, Gannoun H, Touham Y, Bouallagui H, Hamdi M (2008). Aerobic decolourization of the indigo dye-containing textile wastewater using continuous combined bioreactors. J. Hazard. Mater. 152:683-689.
Kirby N, Marchant R, McMullan G (2000). Decolourisation of synthetic textile dyes by *Phlebia tremellosa*. FEMS Microbiol. Lett. 188:93-96.
Lakshmi UR, Srivastava VC, Mall ID, Lataye DH (2009). Rice husk ash as an effective adsorbent: evaluation of adsorptive characteristics for Indigo Carmine dye. J. Environ. Manag. 90(2):710-720.
Levin L, Papinutt L, Forchiassin F (2004). Evaluation of Argentinean white rot fungi for their ability to produce lignin-modifying enzymes and decolorize industrial dyes. Bioresour. Technol. 94:169-176.
Manu B, Chaudhari S (2003). Decolorization of indigo and azo dyes in semicontinuous reactors with long hydraulic retention time. Process. Biochem. 38:1213-1221.
McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam P, Banat IM, Marchant R, Smyth WF (2001). Microbial decolourisation and degradation of textile dyes. Appl. Microbiol. Biotechnol. 56:81-87.
Meehan C, Banat IM, McMullan G, Nigam P, Amyth F, Marchant R (2000). Decolorization of remazol black B using a thermotolerant yeast, *Kluyveromyces marxianus* IMB3. Environ. Int. 26:75-79.
Mishra G, Tripathy M (1993). A critical review of the treatments for decolourization of textile effulent. Colourage 40:35-38.
Mohan N, Balasubramanian N, Subramanian V (2001). Electrochemical treatment of simulated textile effluent. Chem. Eng. Technol. 24:749-753.
Nyanhongo GS, Gomes J, Gubitz GM, Zvauya R, Read J, Steiner W (2002). Decolorization of textile dyes by laccases from a newly isolated strain of *Trametes modesta*. Water Res. 36:1449-1456.
Paradise PR (1999). Trademark counterfeiting, product piracy, and the billion dollar threat to the U.S. economy. Greenwood Publishing Group, Westport, USA. pp. 73-76.
Pointing SB (2001). Feasibility of bioremediation by white-rot fungi. Appl. Microbiol. Biotechnol. 57:20-33.
Prado AG, Torres JD, Faria EA, Dias SC (2004). Comparative adsorption studies of indigo carmine dye on chitin and chitosan. J. Colloid Interface Sci. 277(1):43-47.
Ramya M, Anusha B, Kalavathy S (2008). Decolorization and biodegradation of Indigo carmine by a textile soil isolate *Paenibacillus larvae*. Biodegradation 19:283-291.
Rekuć A, Bryjak J, Szymańska K, Jarzebski AB (2010). Very stable silica-gel-bound laccase biocatalysts for the selective oxidation in continuous systems. Bioresour. Technol. 101(7):2076-2083.
Robinson T, Chandran B, Nigam P (2001). Studies on the production of enzymes by white-rot fungi for the decolorisation of textile dyes. Enzyme Microb. Technol. 29:575-579.
Rodríguez CS, Sanromán MA, Hofer D, Gübitz GM (2004). Stainless steel sponge: a novel carrier for the immobilisation of the white-rot fungus *Trametes hirsuta* for decolourization of textile dyes. Bioresour. Technol. 95(1):67-72.
Schrott W (2001). Denim wieder im blickpunt der textilindustrie. Melliand Textilber 82:331-337.
Spadaro JT, Isabelle L, Renganathan V (1994). Hydroxy radical mediate degradation of azo dyes: evidence for benzene generation. Environ. Sci. Technol. 28:1389-1393.
Thomas B, Aurora T, Wolfgang S (2006). Electrochemical decolourization of dispersed indigo on boron-doped diamond anodes. Diamond Relat. Mater. 15:1513-1519.
Vautier M, Guillard C, Herrmann JM (2001). Photocatalytic degradation of dyes in water: case study of indigo and of indigo carmine. J. Catal. 201:46-59.
Yesilada O, Asma D, Cing S (2003). Decolorization of textile dyes by fungal pellets. Process. Biochem. 38:933-938.
Zee FP, Villaverde S (2005). Combined anaerobic-aerobic treatment of azo dyes - a short review of bioreactor studies. Water Res. 39:1425-1440.