

# Combined biological removal of methylene blue from aqueous solutions using rice straw and *Phanerochaete chrysosporium*

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Received: 26 November 2014 / Revised: 16 December 2014 / Accepted: 20 December 2014 / Published online: 5 March 2015  
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**Abstract** The aim of this study was to develop a low-cost and environmental friendly treatment method for the removal of cationic dye from wastewater. Rice straw powder combined with the white rot fungus *Phanerochaete chrysosporium* (*P. chrysosporium*) were used to remove methylene blue (MB) from wastewater at varied operational conditions. Results demonstrated that this combined biodegradation and adsorption method was effective for the removal of MB from aqueous solutions; a maximum removal efficiency of 88 % was achieved for an initial dye concentration of 400 mg/L. Of the total removed MB, 28.4 % was biodegraded by *P. chrysosporium*, and the rest was adsorbed on the biodegraded rice straw and the fungal cells. The amount of total adsorbed MB in the treatment group (400 mg/L) is almost three times of that in the control without *P. chrysosporium* group. This significant improvement in the adsorption capacity is mainly because *P. chrysosporium* increased the specific surface area of the straw and produced more functional groups on it. Besides, *P. chrysosporium* also affect the pH in a positive way for the adsorption.

**Keywords** Methylene blue · Adsorption · Biodegradation · Rice straw · *Phanerochaete chrysosporium*

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## Introduction

Every year, millions of tons of highly colored wastewater are discharged from different sources including plastic, textile, leather, cosmetics, paper-making, printing, and dye manufacturing industries (Feng et al. 2011; Liu et al. 2012). Methylene blue (MB) is the most commonly used material for dyeing cotton, wood, and silk. However, MB can cause eye burns which may be responsible for permanent injury to eyes of human and animals (Tan et al. 2008). Furthermore, MB can also cause heart rate increasing, vomiting, shock, Heinz body formation, cyanosis, jaundice, and tissue necrosis in humans (Vadivelan and Kumar 2005).

Accordingly, several treatment methods, including adsorption, electrolysis, chemical oxidation, microbiological or enzymatic decomposition, and membrane filtration, have been developed to remove MB from wastewater. As one of the most employed methods, adsorption technology is frequently applied because of its low investment, simplicity of design, and ease of operation characteristics (Gong et al. 2009; Wan et al. 2011; Zeng et al. 2013). Many biological materials, especially agricultural residues such as straw, have been applied as adsorbents to adsorb MB from wastewater in recent years. As a type of reproducible agricultural wastes, straw materials are especially abundant in nature and rich in available reactive groups existing in cellulose, hemicellulose, and lignin structures (Zhang et al. 2011). However, about 300 million tons of straw is deserted or incinerated in China every year, which is not only destruction of soil and atmospheric environment but also waste of resources (Xu et al. 2011). One promising way to utilize this precious bioresource is to produce straw-based adsorbents (Oei et al. 2009; Hofrichter et al. 2010; Xu et al. 2011). For example, in one recently published work, it is reported that the maximum adsorption capacity of tartaric acid-modified rice straw was calculated as 246.2 mg/g (Feng et al. 2012). But these adsorbents were

either inefficient in adsorption capacity (Zhou et al. 2011) or may cause more serious damage to the environment during the production of chemical modified adsorbents (Dawood and Sen 2012; Rivera et al. 2011).

Biological treatment is often a cost effective and environmentally friendly method. Extensive work has been done on the microbiological or enzymatic decomposition of MB in the wastewater; nevertheless, according to literature, microbial degradation is advisable only when the dye concentration is within 80 mg/L (Wesenberg et al. 2003; Jin et al. 2007; Singh and Chen 2008). Considering the above facts, we developed a combined biological MB removal system. The white rot fungus *Phanerochaete chrysosporium* (*P. chrysosporium*) combined with rice straw powder were employed to remove MB from aqueous solutions via biodegradation and adsorption. This system has the following advantages: (1) by far, white rot fungi are the most efficient microorganisms in breaking down synthetic dyes, and the representative species *P. chrysosporium* has been shown to degrade MB to carbon dioxide by secreting ligninolytic enzymes (Wesenberg et al. 2003); (2) rice straw can work as immobilization supports for the production of the ligninolytic enzymes (Sedighi et al. 2009); (3) *P. chrysosporium* may improve the adsorption ability of rice straw by increasing the specific surface area (Huang et al. 2008) and adding functional groups, for example  $-\text{COO}^-$  groups (Asgher et al. 2008).

The objective of this study was to assess the feasibility of employing the rice straw from agricultural waste and white rot fungus *P. chrysosporium* for the removal of MB from wastewater. The removal efficiencies of MB with different initial dye concentrations were investigated. The impact factors of removal efficiency, such as pH values and the activities of ligninolytic enzymes, were also evaluated.

## Materials and methods

### Strains and chemicals

The fungus *P. chrysosporium* strain BKMF-1767 (ATCC 24725) was purchased from China Center for type Culture Collection (Wuhan, China). *P. chrysosporium* was maintained on potato dextrose agar slants at 4 °C. Before the inoculation, the spores were gently scraped from the agar surface and blended in the sterile distilled water as spore suspension. The spore concentration was assessed by microscope with a blood cell counting chamber and adjusted to  $2.0 \times 10^6$  spores per mL (Huang et al. 2008). Rice straw used in this experiment was purchased from a farm in Yuelu District (Changsha, China). Rice straw was dried at 45 °C, and then, it was ground to pass through a 2 mm nylon screen. Methylene blue ( $\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S} \cdot 3\text{H}_2\text{O}$ , FW=373.9) was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

Based on the results obtained by other researchers (Ferreira-Leitão et al. 2003; Zhang et al. 2012a, b), experiments were started at different initial dye concentrations of 100, 200, and 400 mg/L. Stock solutions were prepared by dissolving MB in distilled water. These initial dye concentrations we selected were. All other chemicals used were of analytical grade.

### Experimental conditions

All experiments were performed in 1000 mL flasks with 1 g straw, 5 mL spore suspension (equal ultrapure water in the control group), and 400 mL of dye solution (100, 200, 400 mg/L) at 37 °C. The flasks were agitated in a shaking incubator at a constant shaking rate of 60 rpm. pH of the solutions was adjusted by using HCl or NaOH. All flasks including A (100), B (200), C (400) and the three control groups were sterilized in an autoclave for half an hour at 121 °C before spore suspension was added.

### Analysis of MB removal efficiency

The supernatant was measured for the determination of the concentration of residual MB. MB concentration was measured with a Shimadzu 2570 UV-visible spectrophotometer at a wavelength of 664 nm (Kim et al. 2014). Appropriate dilution was processed to ensure that the concentration of the solution was within the range of the standard curve. The total residual MB,  $Q$  (mg/g), was calculated according to the following equation:

$$Q_a = CV/m \quad (1)$$

where  $C$  (mg/L) is the concentration of MB in supernatant,  $V$  (L) is the volume of the dye solution (0.4 L), and  $m$  (g) is dry weight of the rice straw (1 g).

The amount of adsorption of the control group is the difference between the amounts of total added MB and the total residual MB. In the treatment group, hydrochloric acid solutions were used as eluent to desorb the adsorbed MB at the end of the experiment (Feng et al. 2012). The amount of adsorption,  $Q$  (mg/g), was calculated according to the following equation:

$$Q_a = C_d V_d / m_1 \quad (2)$$

where  $C_d$  (mg/L) is the concentration of MB in eluent,  $V_d$  (L) is the volume of the eluent, and  $m_1$  (g) is dry weight of the adsorbent (rice straw and fungal cells). The amount of biodegraded MB is the difference between the amounts of total removed MB and the total absorbed MB after the treatment.

### Extracellular enzyme activity assays

The supernatant fluid was filtered through 0.45 µm filter papers. Substrate filtrate was used for ligninolytic peroxidase activity analyses with a Shimadzu 2550 UV-visible spectrophotometer. Ligninolytic enzyme activity was the sum of LiP activity and MnP activity. LiP activity was measured as described by Tien and Kirk (1988), and one unit (U) of LiP activity was defined as the amount of the enzyme required to produce 1 mM veratryl aldehyde from the oxidation of veratryl alcohol per minute. Each reaction mixture (total volume 3 mL) contained 1.5 mL of 100 mM sodium tartrate (pH 3.0), 1 mL of 10 mM veratryl alcohol which was replaced by the same volume of sodium tartrate in the control mixture, 0.4 mL of enzyme extract, and 0.1 mL of 10 mM H<sub>2</sub>O<sub>2</sub>. The reaction was started with H<sub>2</sub>O<sub>2</sub>, and the formation of veratraldehyde was monitored at 310 nm. MnP activity was measured as described by Huang et al. (2008), and one unit (U) of MnP was defined as the amount of enzyme required for producing 1 mM Mn<sup>3+</sup> from the oxidation of Mn<sup>2+</sup> per minute. Each 3-mL reaction mixture contained 2.4 mL of 50 mM sodium succinate (pH 4.5), 0.1 mL of 15 mM MnSO<sub>4</sub> replaced by 0.1 mL of sodium succinate in the control mixture, 0.4 mL of crude enzyme solution, and 0.1 mL of 10 mM H<sub>2</sub>O<sub>2</sub>. The reaction was initiated at 37 °C by adding H<sub>2</sub>O<sub>2</sub>, and the rate of Mn<sup>3+</sup>-succinate complex formation was monitored by measuring the increase in absorbance at 240 nm.

### Analysis of fungal biomass

The fungal biomass carbon was measured by fumigation of the filtered sample with ethanol-free chloroform and extraction with 0.5 M K<sub>2</sub>SO<sub>4</sub>, adapted from a previously published method (Huang et al. 2010).

### Determination of pH in the medium

The pH of the supernatant fluid was measured by using a Mettler Toledo FE 20 pH meter.

### Structure analysis of samples

The untreated rice straw and biodegraded rice straw were characterized by analytical instruments. The specific surface area and pore size of samples were analyzed by using a BET-N2 surface analyzer (Beckman Coulter SA3100). The Fourier transform infrared (FTIR) spectra of rice straw before and after biodegradation were obtained with a Bruker IFS 66/S IR Spectrophotometer. One hundred milligram KBr and 1 mg of fully dried sample were mixed and prepared as tablets, and the KBr blank was calibrated every time before each analysis; the scanning range was 400–4000 cm<sup>-1</sup>. The samples were also characterized by scanning electron microscope

(SEM) scanning (QUANTA 200, FEI) at a magnification of 800.

### Statistical analysis

Data are presented as the means of three replicates, and the standard deviations were used to analyze the experimental data. Statistical analyses were performed using the software package SPSS (version 18.0). One-way analysis of variance (ANOVA) was used for testing difference among the data, and differences were considered statistically significant at  $P < 0.05$ . All figures were derived from Origin 8.0.

## Results

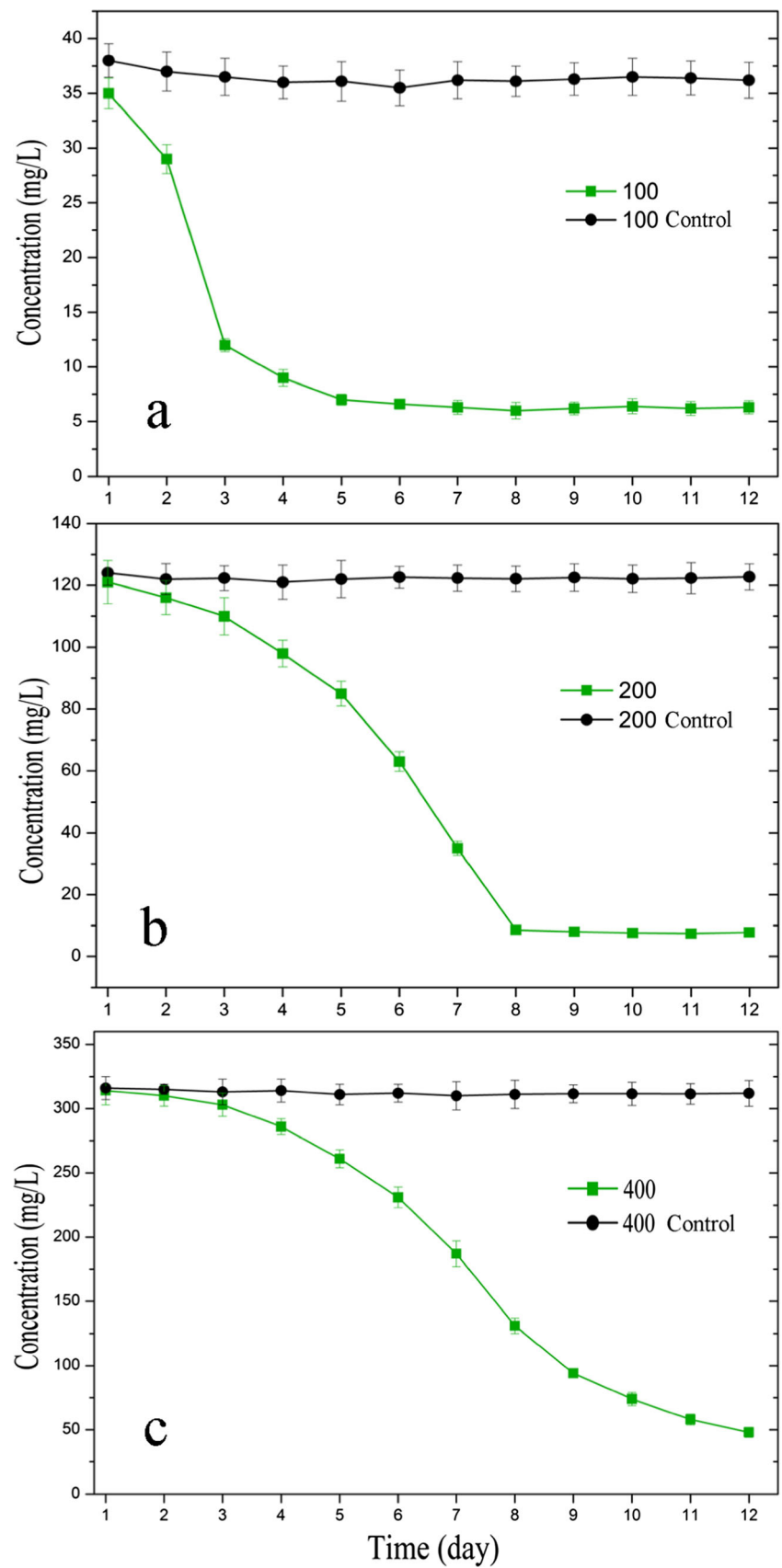
### The changes of MB concentration

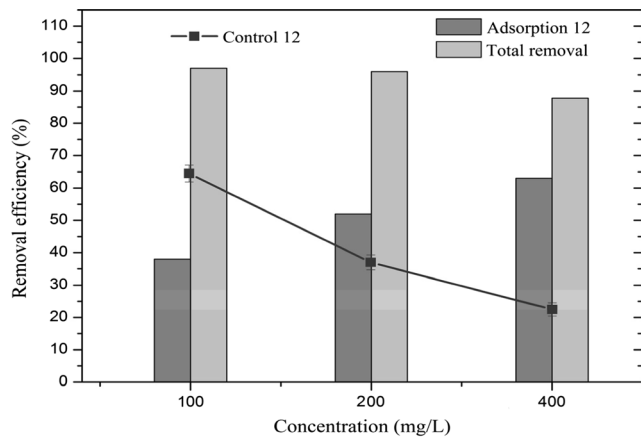
Figure 1 demonstrates the changes of MB concentration during the experiment. Almost the same reductions in MB concentration were found in the treatment group and the control group with the same initial MB concentration after 24 h treatment. No obvious variation in MB concentration was observed in the control groups from day 1 to day 12 (Fig. 1). At the end of the experiment, the removal efficiencies for the control groups with initial MB concentration of 100, 200, and 400 mg/L were 64, 37, and 22 %, respectively. Meanwhile, we clearly observed that most of soluble MB in the treatment groups was removed even at the initial MB concentration of 400 mg/L (Fig. 1). It was found that when the initial MB concentration was 100 mg/L, over 90 % of the total dissolved MB was removed in 4 days (Fig. 1a), and when the initial MB concentration increased to 400 mg/L, approximately 90 % removal was obtained on day 12 (Fig. 1c). After 12 day treatment, the amount of total removed MB in A (100), B (200), and C (400) were 37.6, 76.8, and 140.8 mg, respectively. At the end of the operation, the rice straw and fungal cells in the treatment groups were removed from the flasks and hydrochloric acid solution was used as eluent to desorb the adsorbed MB. In this study, the percentages of MB adsorbed by rice straw and fungal cells in A (100), B (200), and C (400) were 38, 52, and 63 %, respectively (Fig. 2). Figure 2 also shows that the total MB removal percentages in the three flasks were 95, 96, and 88 %, respectively. The results indicate that 57, 44, and 25 % of the original MB in A (100), B (100), and C (400), respectively, were removed due to the biodegradation.

### Dynamic changes of ligninolytic enzyme activities

The dynamic changes of the total production of the ligninolytic enzymes with different initial MB concentrations are shown in Fig. 3. The fluctuating curves of all treatments

**Fig. 1** The changes of methylene blue concentration in the control groups (without *P. chrysosporium*) and in treatment groups. Initial methylene blue concentration: **a** 100, **b** 200, **c** 400 mg/L. The bars represent the standard deviations of the means ( $n=3$ )



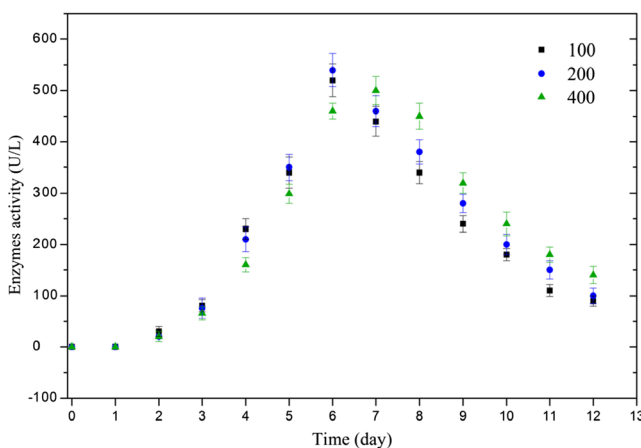


**Fig. 2** Removal efficiency of methylene blue in the experiment. Control 12: after 12 days, the removal efficiencies of methylene blue in the control groups (100, 200, and 400 mg/L, respectively, without *P. chrysosporium*); adsorption 12: after 12 days, the percentage of adsorbed methylene blue from the total added methylene blue in the treatment groups (100, 200, and 400 mg/L, respectively, with *P. chrysosporium*); total removal: after 12 days, the removal efficiencies of methylene blue in the treatment groups. The bars represent the standard deviations of the means ( $n=3$ )

are similar, and ligninolytic enzyme activity increased rapidly during the initial stage followed by the fast decline along with the time shift. In the initial stage (day 0 to day 3) of the experiment, the low activities of enzymes were observed. The highest ligninolytic enzyme activity ( $538 \text{ U L}^{-1}$ ) was found in B (200) on day 6. It was shown that ligninolytic enzyme activities of all the three groups reached the peak on day 6, except in C (400) that did not reach a maximum value of  $502 \text{ U L}^{-1}$  until day 7.

Structure analysis of rice straw and biodegraded rice straw

The surface physical parameters of the samples, including the specific surface area and pore structure, are summarized in Table 1. The surface properties of the biodegraded rice straw



**Fig. 3** Dynamic changes of enzyme activities in treatment groups (100, 200, and 400 mg/L, respectively, with *P. chrysosporium*). The bars represent the standard deviations of the means ( $n=3$ )

were significantly increased compared with crude rice straw, implying that additional pores were developed during the biodegradation. To more directly examine the changes of surface morphology, SEM was used to analyze the surface morphology of crude rice straw and biodegraded rice straw. As shown in Fig. 4a, the surface of untreated rice straw was relatively smooth, flat, and continuous. After biodegradation, the external surface area was clearly increased by forming more holes and cracks on the surface (Fig. 4b) which was believed to affect adsorption capacity in a positive way.

To investigate the functional groups responsible for adsorption of MB, the FTIR spectra of the two samples were studied (Fig. 4c). As depicted in Fig. 4c, the infrared spectra show numerous adsorption peaks in the scanning range of  $4000\text{--}400 \text{ cm}^{-1}$ . Very strong absorption at  $3453 \text{ cm}^{-1}$  was attributed to  $\text{--OH}$  stretching, and the adsorption peaks at  $2930$  and  $1360 \text{ cm}^{-1}$  were attributed to  $\text{C--H}$  stretching vibration from the  $\text{CH}_2$  groups (Kim et al. 2014). The peaks at  $1654 \text{ cm}^{-1}$  were related to the  $\text{--COO}^-$  groups from carboxylic acid in rice straw (Zhang et al. 2011). Absorption bands observed around  $1600$  and  $1500 \text{ cm}^{-1}$  verified the presence of aromatic rings present in lignin (Kim et al. 2014). Peaks between  $1200$  and  $400 \text{ cm}^{-1}$  represent the deformation of cellulose, lignin, and residual hemicellulose bands (Zhang et al. 2012a, b). According to the previous studies, the adsorption ability of rice straw was mainly depended on  $\text{--OH}$  and  $\text{--COO}^-$  groups (Feng et al. 2012; Zhang et al. 2011). Comparing the spectra of two samples (Fig. 4c), significant changes can be observed. The absorption at  $3453 \text{ cm}^{-1}$  of biodegraded rice straw is higher than crude straw, which indicates that more  $\text{--OH}$  was generated after the biodegradation. The peaks at  $1654 \text{ cm}^{-1}$  enhanced show higher amount of  $\text{--COO}^-$  groups formed after biodegradation. The peaks around  $1600$  and  $1500 \text{ cm}^{-1}$  weakened after biodegradation suggest that *P. chrysosporium* destroyed the aromatic skeletal carbon of lignin (Dorado et al. 1999). Results showed that more functional groups beneficial to adsorption were developed on rice straw after the biodegradation.

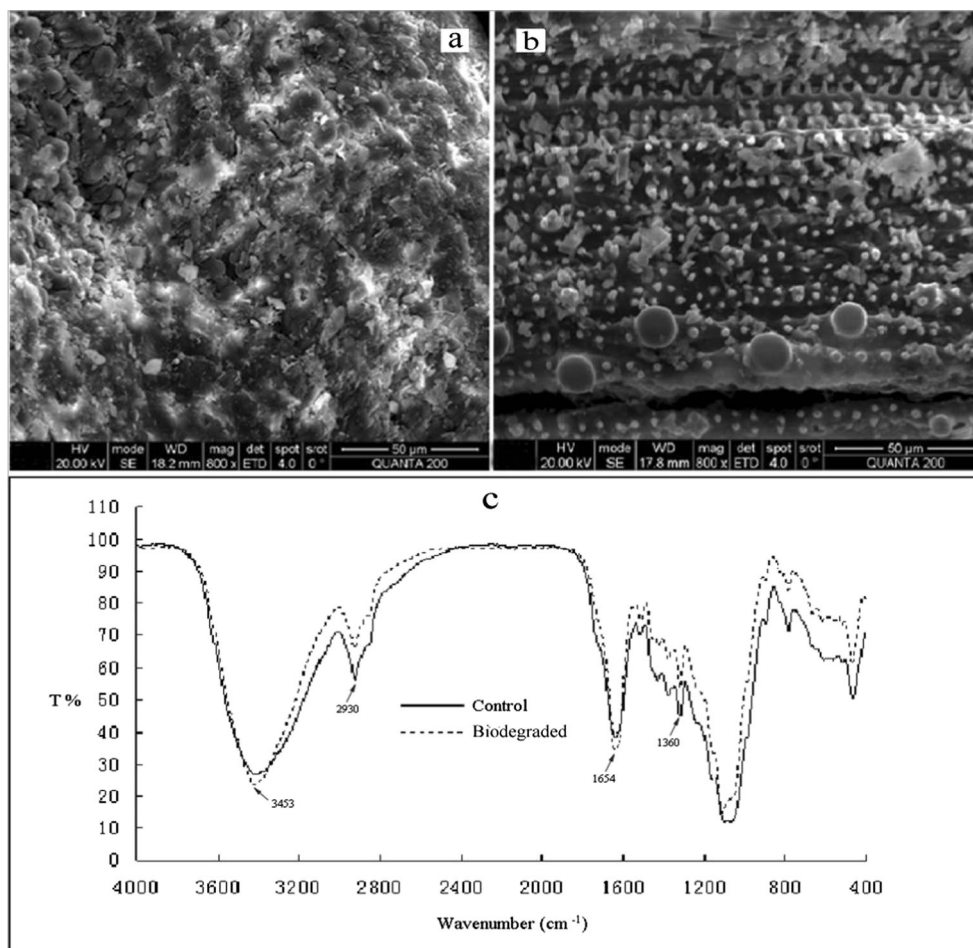
Changes of pH in the medium

As shown in Fig. 6, the initial pH of all the groups is about 5.0. In the process of the experiment, pH of the control group (the other two control groups were not shown in the figure because there was no obvious difference between them) showed little

**Table 1** Surface properties of untreated rice straw and biodegraded rice straw

	Surface area ( $\text{m}^2/\text{g}$ )	Pore volume ( $\text{cm}^3/\text{g}$ )
Untreated rice straw	3.256	0.0026
Biodegraded rice straw	11.541	0.0219

**Fig. 4** Morphology of crude rice straw (a) and the biodegraded rice straw (b). The FTIR spectra of rice straw and the biodegraded rice straw (c)



fluctuation, while pH of the treatment groups varied over time. In the early stages of the experiment, pH of samples from flasks A (100), B (200), and C (400) decreased rapidly to about 4.3. After 5 days, the pH values of all the three samples increased significantly and later tended to stabilize (Fig. 6). After 8 days, pH turned to be 5.3, 5.6, and 5.9 for the reactors A (100), B (200), and C (400), respectively. The results showed that the final pH values increased with the increase of initial concentration of MB. On the whole, the pH values in C (400) were higher than those in A (100) and B (200) (Fig. 6).

## Discussion

### The changes of MB concentration

In the control without *P. chrysosporium* groups, sharp reductions in the dye concentration were found in 24 h (Fig. 1), after which no obvious changes were observed. This came about because the adsorption has reached the equilibrium, since previous studies have indicated that the adsorption behavior of

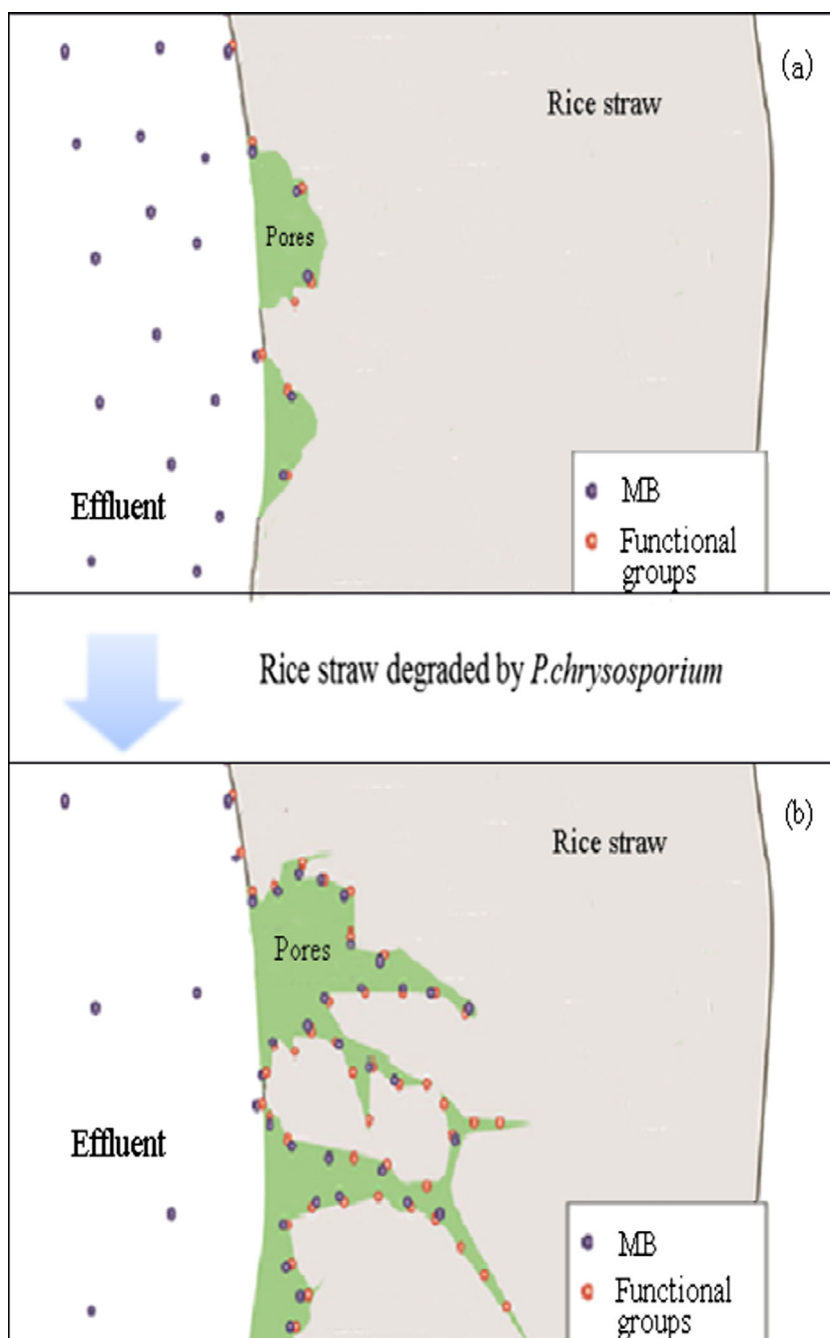
MB onto rice straw was Langmuir-type adsorption, which reaches the equilibrium in 4 h (Zhang et al. 2011). Our data indicated that with the increasing of MB doses, the adsorption capacity increased. A proposed reason is that dye concentration provides an important driving force to overcome the mass transfer resistance between the solid phase (rice straw) and the liquid phase (water) (Feng et al. 2012).

As shown in (Fig. 1b, c), in spite that less than half of the total dissolved MB in B (200) and C (400) were removed after 24 h, most of them was removed after 12 day treatment. In particularly, removal efficiency in C (400) was significantly enhanced; the removal efficiency on day 12 was nearly four times of that on day 1 (Fig. 1c). We speculate that that is mainly because *P. chrysosporium* degraded part of MB and also improved the adsorption ability of rice straw.

### Ligninolytic enzyme activities and the biodegradation

It has well been known that the white rot fungi are the most efficient single class of microorganisms in discoloration. This property is mainly based on its capacity to produce extracellular ligninolytic enzymes which are able to degrade a broad array of environmental pollutants (Asgher et al. 2008). In

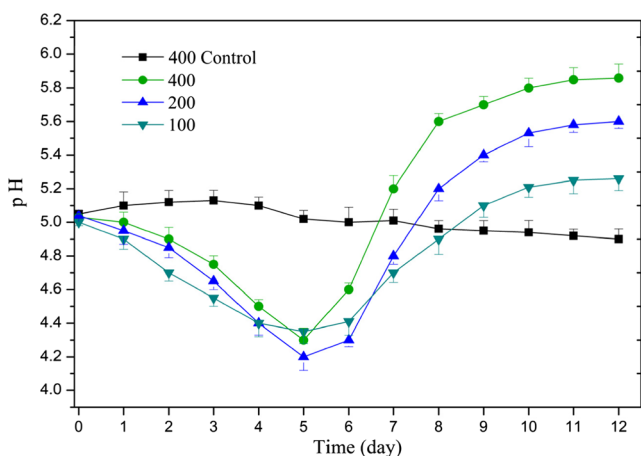
**Fig. 5** The schematic diagram of the adsorption process and mechanism. **a** Rice straw and **b** biodegraded rice straw. Functional groups presented in this schematic diagram mainly include carboxyl groups and hydroxyl groups



order to acquire a precise understanding of the biodegradation process, the dynamic changes of ligninolytic enzyme activities were studied. Higher enzyme activities were detected in the middle stage (day 5 to day 9) of the experiment (Fig. 3). This is due to the fact that the ligninolytic enzymes are produced by *P. chrysosporium* during their secondary metabolism since limited nutrient and energy were provided to the fungus (Wesenberg et al. 2003). In the initial stage of the experiment, the higher MB concentration (400 mg/L) corresponding lower ligninolytic enzyme activities is probably because less dissolved oxygen exists in high concentration MB solutions,

while the production of ligninolytic enzymes is generally optimal at high oxygen tension in liquid culture (Wesenberg et al. 2003).

In this study, 57 % (22.8 mg), 44 % (35.2 mg), and 25 % (40.0 mg) of the original MB in A (100), B (100), and C (400), respectively, were removed due to the biodegradation. As shown in Fig. 2, MB concentrations declined sharply from day 4 to day 9; this is in accord with the high ligninolytic enzyme activities detected in this period, Fig. 3. The highest biodegraded MB amount was found in C (400). One possible explanation is that higher dye concentration facilitated the



**Fig. 6** The changes of pH in the control group (400 mg/L, without *P. chrysosporium*) and in treatment groups (100, 200, and 400 mg/L, respectively, with *P. chrysosporium*). The bars represent the standard deviations of the means ( $n=3$ )

reaction between ligninolytic enzymes and dye molecules. Furthermore, the higher ligninolytic enzyme activities in C (400) were observed at the last period of experiment.

#### Effect of *P. chrysosporium* on adsorption

*P. chrysosporium* can improve the adsorption ability of rice straw by changing the surface morphology of the straw and producing more functional groups on it and also adsorb MB by the mycelium. To determine the effect of *P. chrysosporium* on the adsorption, we examined the change of the amount of adsorbed MB. As shown in Fig. 2, unlike the other two groups, A (100) present a low adsorption quantity compared to the control with *P. chrysosporium* group. This is mainly because part of adsorbed MB was degraded by *P. chrysosporium*. The amounts of adsorption in the other two groups were found increased with the increasing concentration of MB; the amount of total adsorbed MB in C (400) is almost three times of that in the control group (Fig. 2). To more directly examine the effect of *P. chrysosporium* cells on the adsorption, the content of fungal biomass in the flask was analyzed. Results show that the content of fungal biomass in C (400) is 76.3 mg/g dry straw. According to the literature (Iqbal and Saeed 2007; Singh and Chen 2008; Kumar et al. 2009), the biosorption capacities of *P. chrysosporium* is within 40–110 mg/g. Taken together, it is obvious that the amount of MB absorbed on *P. chrysosporium* was less than 10 mg. As shown in Fig. 2, the amount of total adsorbed MB in C (400) was 102.4 g. It suggests that rice straw plays the major role in the adsorption, and the adsorption capacity was improved by *P. chrysosporium* significantly.

To figure out the mechanism underlying the improvement in adsorption capacity, the morphology and responsible functional groups of rice straw and biodegraded rice straw were investigated. As described in Table 1 and Fig. 4, the surface

properties of the biodegraded rice straw were significantly increased compared with crude rice straw. The external surface area was clearly increased by forming more holes and cracks on the surface due to the fact that lignin was selectively degraded by extracellular ligninolytic enzymes (Huang et al. 2008). According to previous studies (Kim et al. 2014; Zhang et al. 2011), the removal capacity of rice straw increased with the increase external surface area; thus, the adsorption of MB would be facilitated after the biodegradation.

Several reports have suggested that the functional groups on the surface of rice were the dominant factor of the adsorption (Feng et al. 2012). According to FTIR spectra in Fig. 4c,  $-\text{COO}^-$  groups and  $-\text{OH}$  groups on the surface of rice straw increased prominently after biodegradation. The mechanism underlying this improvement could be as follows: (i) Intermediates such as phenols and carboxylic acid containing carboxyl or other electronegative oxygen-containing functional groups were produced on rice straw surface during lignocellulose degradation by *P. chrysosporium*; (ii) *P. chrysosporium*'s metabolites like organic acids are beneficial to form these functional groups, for example, oxalic acid can produce more  $-\text{COO}^-$  on the surface of rice straw and improve the adsorption capacity for the dye (Feng et al. 2013). Besides, more holes and cracks were formed on the surface after the biodegradation, which would also promote the adsorption. Based on the above analysis, the schematic diagram of the adsorption process and mechanism is demonstrated in Fig. 5a (rice straw) and b (biodegraded rice straw). Functional groups presented in this schematic diagram mainly include  $-\text{COO}^-$  groups and  $-\text{OH}$  groups. Taken together, the improvement in the adsorption capacity is mainly because *P. chrysosporium* increased the specific surface area of the straw and produced more functional groups on it (Fig. 5b).

#### Changes of pH in the medium

As was known, MB is a type of cationic dyes and has good affinity with anionic matters like  $-\text{COO}^-$  group on the surface of rice straw. At lower pH, carboxyl groups on rice straw will fully protonized and show low affinity with MB (Han et al. 2010). Hence, pH is one of the most important factors that influence adsorption capacity. In this study, after *P. chrysosporium* was inoculated, it was observed that pH of samples decreased rapidly (Fig. 6). Similar results were found when studying the biodegradation of rice straw by *P. chrysosporium* in other systems (Huang et al. 2008; Xu et al. 2012); this is likely attributed to the accumulation of acidic metabolites like oxalate (Cheng et al. 2014; Huang et al. 2010). pH increased in later stages is due to little accumulation of organic acid and ammonia matters produced during organic matter catabolism by *P. chrysosporium*, for example,  $\text{NH}_4^+$  (Hattori et al. 1999). The pH values of C (400) were higher than the pH values of A (100) and B (200) (Fig. 6); we



speculate that higher concentration of MB in C (400) facilitated the solubilization of ammonia or the volatilization of organic acid. Previous research showed that MB uptakes by straw were kept at a quite low level at low pH (pH<4.0), and with the increase of pH further, the adsorption capacities of MB increased evidently; however, when the pH exceeded 6.0, the improvement of adsorption capacities was not obvious (Zhang et al. 2011). In our experiment, with the inoculation of *P. chrysosporium*, the pH in C (400) increased from 5.03 to 5.86, which indicates that a better condition was formed for the adsorption.

#### Disposal of the dye-loaded rice straw

It is necessary to deal with the dye-loaded biosorbents properly, for there is a good amount of MB adsorbed on them and they may release to the environment again if not disposed properly. If these dye-loaded biosorbents released to the environment without properly treated, it may cause serious secondary pollution. Conventionally, large amount of solvent is applied as eluent to wash and recover adsorbents for recycle use, and the generated waste eluent usually requires further treatment to avoid the secondary pollution (Feng et al. 2013). Here, we propose a low-cost and environment-friendly treatment method: solid-state fermentation of dye-loaded rice straw with *P. chrysosporium*. On one hand, MB absorbed on the rice straw can be degraded by *P. chrysosporium* (Iqbal and Saeed 2007); on the other hand, after MB was removed, the biodegraded rice straw could be reused as biosorbents. However, further research is needed to verify the feasibility of this method.

Based on the experimental results, rice straw combined with *P. chrysosporium* has shown great effect for the removal of MB from aqueous solutions. This combined biological system showed a maximum removal capacity of 140.4 mg/g dry straw, which was about four times that of crude rice straw. Of the total removed MB, 28.4 % was biodegraded by *P. chrysosporium*, and the rest was mainly adsorbed on the biodegraded rice straw. The significant improvement in adsorption capacity was due to the increased specific surface area and functional groups on the surface of rice straw. The present study indicates that rice straw inoculated with *P. chrysosporium* is an efficient, low-cost, and environmental friendly treatment method for removing MB from wastewater.

**Acknowledgments** This study was financially supported by the National Natural Science Foundation of China (51039001, 51378190, 50808073, 51278176, 51108178), the Environmental Protection Technology Research Program of Hunan (2007185), the Fundamental Research Funds for the Central Universities, the Hunan University Fund for Multidisciplinary Developing (531107040762), the Program for Changjiang Scholars and Innovative Research Team in University (IRT-13R17), and the project of the Hunan Provincial Innovation Foundation for Postgraduate (CX2014B141).

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