

RESEARCH PAPER



Decolorization of mordant yellow 1 using *Aspergillus sp.* TS-A CGMCC 12964 by biosorption and biodegradation

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ABSTRACT

In this report, the decolorization features of extracellular enzymes and mycelia separately prepared from *Aspergillus sp.* TS-A CGMCC 12,964 (120 h) were investigated. The fermentation broth of TS-A degraded 98.6% of Mordant Yellow 1 (50 mg/L) at an initial pH 6 within 1 h with over 70% of the dye (50 mg/L) degraded by extracellular enzymes and 18.8% removed by live mycelia. The degradation products of the dye were analyzed by UV-Vis and FTIR spectra. The decolorization rates of extracellular enzymes and mycelia were examined under different contact periods, dye concentrations and pH values. The extracellular enzymes exhibited excellent degradation activity under weak acidic conditions. In addition, biosorption models of mycelia fitted well the Langmuir isotherm model and the pseudo-second-order kinetic equation. Although the decolorization process was achieved through the synergistic effects of mycelia and extracellular enzymes, decolorization was dominated by the biodegradation activity of the extracellular enzymes from TS-A.

ARTICLE HISTORY

Received 08 Dec 2017
Accepted 01 May 2018

KEYWORDS



Aspergillus; Azo dye; biosorption; biodegradation; mycelia; enzymes

Introduction

Azo dyes represent the majority of synthetic dyes in the textile industry and are used widely because of their wide spectrum of colors, facile attachment to fibers and low cost [1,2]. However, the discharge of dye wastewater into the environment is undesirable because the dyes possess intensive colors and are a serious health hazard [2,3]. Synthetic dyes and their breakdown products present in textile industry wastewaters have become a major pollution source with about 280,000 tons of textile dyes discharged in such industrial effluents every year [4]. Therefore, there is an urgent need to remove dyes from the effluents before discharging the wastewater into the natural environment.

Compared with physical and chemical methods, biological methods are widely used to remediate dyes present in effluents because of their high efficiency, low cost and secondary pollution [2]. Decolorization by microorganisms has focused on using bacteria, fungi, algae and yeasts, which absorb and/or degrade a wide range of azo dyes [5–7]. Traditional technologies have used bacteria to remove dyes because of their strong adaptability and high

activity. However, azo dyes are generally resistant to degradation under aerobic conditions using bacteria; thus, degradation by bacteria requires alternating between anaerobic and aerobic conditions [2,8,9]. Moreover, light intensity and the concentration of CO₂ are limiting factors for azo dye decolorization by algae [7]. Substantial information has accumulated over the last few years that describes the activity of ligninolytic fungi, especially white rot fungi, which has been shown to degrade multiple types of synthetic dyes [4,6]. Interestingly, fungi possess ligninolytic enzymes that degrade complex dyes, including laccase (Lac), manganese peroxidase (MnP) and lignin peroxidase (LiP) [10]. Among these ligninolytic enzymes, Lac is a copper-containing enzyme that oxidizes various benzene and non-benzene compounds using molecular oxygen as the electron acceptor [10,11]. For example, Lac produced by *Trichoderma lixii* F21 is involved in the conversion of polar dye ARS and non-polar dye QGSS to nontoxic intermediate micro-molecules [12]. MnP oxidizes Mn²⁺ into Mn³⁺ in the presence of H₂O₂ and the stable Mn³⁺ formed functions as an oxidizer of pollutants [13]. MnP immobilized by chitosan achieved a 97.31% degradation rate

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of dye effluents, and reduced Chemical Oxygen Demand (COD), Total Organic Carbon (TOC) and Biochemical Oxygen Demand (BOD) at degradation values of 82.40, 78.30 and 91.7%, respectively [14]. LiP degraded most recalcitrant aromatic compounds when veratryl alcohol (VA) acted as a redox mediator [15]. The white rot fungus *Phanerochaete chrysosporium* showed complete decolorization of Direct Red-80 because of its highly active LiP [16]. Apart from white-rot fungi, other fungi such as *Aspergillus niger*, *Scheffersomyces spartinae* and *Galactomyces geotrichum* also degrade and/or adsorb a diverse range of dyes [17–21]. Methyl orange was degraded by the blue laccase from *Aspergillus ochraceus* NCIM-1146 and its intermediates such as p-N,N'-dimethylamine phenyldiazine and p-hydroxybenzene sulfonic acid were confirmed by FTIR (Fourier transform infrared spectroscopy), HPLC and GC-MS [19]. Immobilized *Aspergillus flavus* was able to decolorize and degrade Drimarene Blue dye into nontoxic metabolites [22].

Besides the use of enzymes to degrade dyes, biosorbents from the biomass of bacteria, fungi and algae have also been used to remove dyes from wastewater [23,24]. The highest decolorization efficiency was achieved by *Aspergillus fumigatus* mycelia at pH 3.0, biomass 3.0 g/L within 30 min, where $-\text{COOH}^-$ and $-\text{NH}_3^{3+}$ functional groups on the mycelium surface were responsible for dye adsorption [17]. In general, dyes could be removed efficiently by *Aspergillus* using the biodegradation properties of enzymes and the biosorption capacity of mycelia [10,18,22]. We postulate that biosorption may dominate the decolorization system; however, the dye is only transferred onto the mycelium surface and remains a threat to the environment. Only nontoxic endproducts of dye degradation are considered to be acceptable and non-harmful to the environment. However, there is a paucity of data showing a correlation between the decolorization role by *Aspergillus* and the removal of dyes and a consequent improvement in wastewater quality.

In this work, *Aspergillus sp.* TS-A CGMCC 12964 (TS-A) was found to remove dye color effectively. Extracellular enzymes and mycelia from TS-A were applied separately to decolorize Mordant Yellow 1 (MY1) in the simulated dye wastewater. Biodegradation products of Mordant Yellow 1 with the extracellular enzymes were analyzed by UV-Vis

spectrophotometry and FTIR analysis. Decolorization activity of TS-A was examined under different conditions, including contact period, dye concentration and pH.

Materials and methods

Chemicals

MY1 was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). ABTS (2, 2'-azion-bis (3-ethyl-benzothiazoline)-6-sulphonic acid) and VA were purchased from Bio Basic Inc. (Canada). All of chemicals and reagents used in this study were of analytical grade.

Microbial source and culture mediums

Aspergillus sp. TS-A CGMCC12964 (TS-A) was isolated from activated sludge of a textile factory in Shihezi, Xinjiang, China, and stored at the China General Microbiological Culture Collection Center (CGMCC 12964). Czapek's medium used in this study contained 2 g/L NaNO_3 , 1 g/L K_2HPO_4 , 0.5 g/L KCl, 0.5 g/L MgSO_4 , 0.01 g/L FeSO_4 and 30 g/L sucrose (pH 6). All culture media were autoclaved at 120°C for 20 min before inoculation.

Preparation of extracellular enzymes and mycelia

TS-A was inoculated in 100 mL culture medium in a 250 mL flask, and shaken at 160 rpm and 30°C for 120 h. The fermentation broth of TS-A (120 h) was centrifuged at 10,000 r/min for 10 min at 4°C, and the live mycelia were washed and stored at 4°C. The supernatant was filtered with a 0.22- μm membrane and used as the source of extracellular enzymes. Sterilized and powder mycelia were prepared from the same batch of TS-A live mycelia (120 h). Sterilized mycelia were obtained by sterilizing the collected mycelia at 120°C for 20 min. Powder mycelia were prepared from pretreated mycelia, which were dried at 80°C for 24 h, pulverized in a mortar and pestle, and then sieved into uniform particle sizes using an 80-mesh screen.

Decolorization activity of the extracellular enzymes and mycelia

The extracellular enzymes, live mycelia, sterilized mycelia and powder mycelia were used separately to remove MY1 (50 mg/L) in 100 mL decolorization reactions. The decolorization rate of the fermentation broth of TS-A (120 h) was used as the control. All decolorization experiments of MY1 were performed at pH 6, 30°C and 160 rpm for 1 h. The degradation liquid was centrifuged at 10,000 r/min for 10 min. Supernatants were monitored by UV-Vis spectrophotometry (Spectrumlab S22PC, China). The decolorization rate was calculated using the following equation:

$$\text{Decolorization}(\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where A_0 and A_1 represent the initial and final absorbance of the dye at 354 nm, respectively [25]. All the decolorization experiments were performed in triplicate and the average values were used in calculations.

Enzymes activities and biomass assay

Activities of three lignin oxidases (MnP, LiP and Lac) were detected from 24 to 120 h during the fermentation broth of TS-A by a S22pc spectrophotometer. The determination of enzyme activities was referenced to Pan et al [25]. One unit (U) of enzyme activity was defined as the amount of enzyme required to oxidize 1 μM substrate in 1 min. In addition, the wet weight of the TS-A biomass and pH values of the fermentation broth were measured in the decolorization process of TS-A.

Uv-Vis and FTIR spectral analysis of enzyme degradation activity

MY1 was degraded by the extracellular enzymes for 1 h in the same decolorization system. The degradation liquid was centrifuged at 10,000 r/min for 10 min. The supernatant was scanned over the range of 200–500 nm with a UV6100S UV-Vis scanning spectrophotometer (Shanghai Mapada Instruments Co., Ltd., China). The supernatant was also mixed with KBr at a ratio of 1:1000 and ground in an agate mortar. The prepared mixtures were analyzed over

the wavenumber range of 4000–600 cm^{-1} by FTIR (Magna-IR 750, Thermo Nicolet) where pure KBr powder was used as the background.

Effects on decolorization activity of extracellular enzymes and mycelia

The effect of different conditions on decolorization by the extracellular enzymes was investigated. This included characterizing the effects of Mn^{2+} (0.05, 0.1, 0.15, 0.2, 0.5, 1, 1.5 and 2.5 mM), H_2O_2 (0.05, 0.1, 0.15, 0.2, 0.5, 1 and 1.5 mM), time (1, 3, 5, 8, 10, 20, 30 and 60 min), dye concentrations (30, 50, 70, 90 and 110 mg/L) and pH values (3, 4, 5, 6, 7 and 8). The effects of the mycelium on decolorization were investigated without Mn^{2+} and H_2O_2 present.

Kinetics and isotherm model analysis of mycelia biosorption

The Langmuir and Freundlich isotherms were used to describe the adsorption onto the surface of the mycelium. The linear formulas of the Langmuir and Freundlich isotherms are [26]:

$$\frac{1}{q_e} = \frac{1}{q_m K_L C_e} + \frac{1}{q_m} \quad (2)$$

$$\ln q_e = \frac{1}{n} \ln C_e + \ln K_F \quad (3)$$

where C_e is the equilibrium dye concentration in solution (mg/L), q_e is the equilibrium adsorbed dye concentration on the biomass (mg/g), K_L represents the Langmuir constant rate and was evaluated by the affinity of dye for mycelia (L/mg) and q_m is the complete monolayer maximum capacity of the biomass (mg/g). K_F and n represent the biosorption capacity and the affinity of dye for mycelia, respectively.

Different kinetic models were estimated. The linear formulas of pseudo-first-order and pseudo-second-order kinetics are expressed as [27]:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (4)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (5)$$

where q_e (mg/g) and q_t (mg/g) are the amounts of mycelia adsorbed dye at equilibrium and at time t . K_1 (min^{-1}) is the pseudo-first-order biosorption rate, K_2 ($\text{g/mg}\cdot\text{min}^{-1}$) is the equilibrium rate constant of the

pseudo-second-order reaction. The biosorption effect of different MY1 concentrations on mycelia was carried out, where different isotherm models were fitted to the experiment data.

SEM and BET analysis of mycelia

Mycelia were examined by scanning electron microscopy (SEM; JEOL, JSM-6490LV, Japan) under an acceleration voltage of 12 kV. Prior to analysis, the mycelia were dried at 383 K for 24 h and stored in a desiccator. The surface area and porous properties of mycelia were determined with BET surface area analyzer by nitrogen adsorption-desorption at 78 K (SI/MP, Quantachrome, USA).

Results

Dye decolorization analysis

Filamentous fungi normally display biodegradation and biosorption activities because they contain a rich source of lignin modifying enzymes and a large specific surface area. Figure 1(a) shows the biodegradation and biosorption performance of the decolorization process by TS-A. The decolorization rate reached 98.6% when MY1 was incubated with the fermentation broth of TS-A, in which over 70% was achieved by extracellular enzyme degradation of MY1 and 18.8% of MY1 was removed by live mycelia. The results showed that the decolorization rate of powder mycelia (44.2%) was higher than that of sterilized mycelia (30.6%). The powder mycelia were chosen for further adsorption experiments. Figure 1(b) describes the enzyme activities and the biomass of the TS-A fermentation broth over a period of 120 h. The activity of manganese peroxidase, which increased from 2.4 to 14.6 U/L, was more obvious than that of laccase and lignin peroxidase. The biomass of TS-A reached 4 g (wet cell)/L after 120 h. The pH of the fermentation broth decreased from 6 to 3 during the 120 h incubation period.

Uv-Vis and FTIR analysis of the changes to the MY1 structure

MY1 and its breakdown products were examined by UV-Vis and FTIR analysis (Figure 2). The UV-Vis spectra showed that the characteristic peak representing MY1 at 354 nm disappeared following incubation

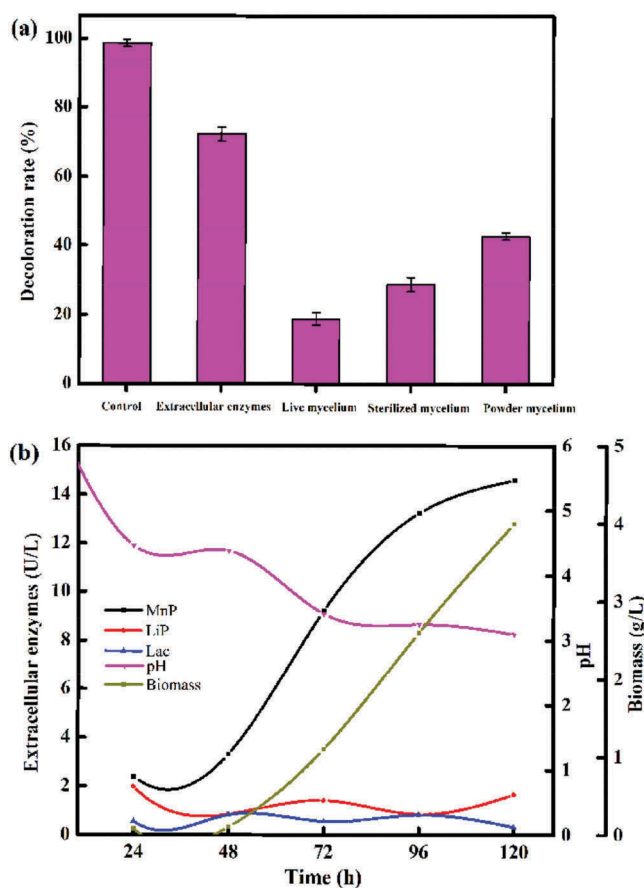


Figure 1. Decolorization activity and growth responses of *Aspergillus sp.* TS-A. (a) Decolorization rates of mycelia and extracellular enzymes and (b) changes of extracellular enzymes activities, biomass and pH with time. The control is the fermentation broth of TS-A.

with TS-A (Figure 2(a)). FTIR spectra of MY1 and its breakdown products showed three groups of specific peaks over the range of 4000–600 cm^{-1} (Figure 2(b)). The first group of peaks of MY1 was observed over the broad range of 3700 to 3000 cm^{-1} , which showed peaks at 3440 cm^{-1} for the asymmetric stretching vibration of –OH, and peaks at 3282 cm^{-1} and 3102 cm^{-1} that represented hydrogen stretching in the aromatic ring. The second group ranged from 2000 to 1000 cm^{-1} with peaks at 1532 cm^{-1} for N–CH₃ bending vibrations, 1352 cm^{-1} for N = N stretching vibrations, 1352 to 1165 cm^{-1} for primary aromatic amines with C–N vibrations and 1078 cm^{-1} for the C–OH stretching vibrations. The third group of peaks ranged from 900–600 cm^{-1} with peaks at 881 cm^{-1} , 811 cm^{-1} and 735 cm^{-1} for the benzene ring vibration, and at 699 cm^{-1} for C–O stretching vibrations in the benzene ring. After biodegradation with the extracellular enzymes, the peaks in the first

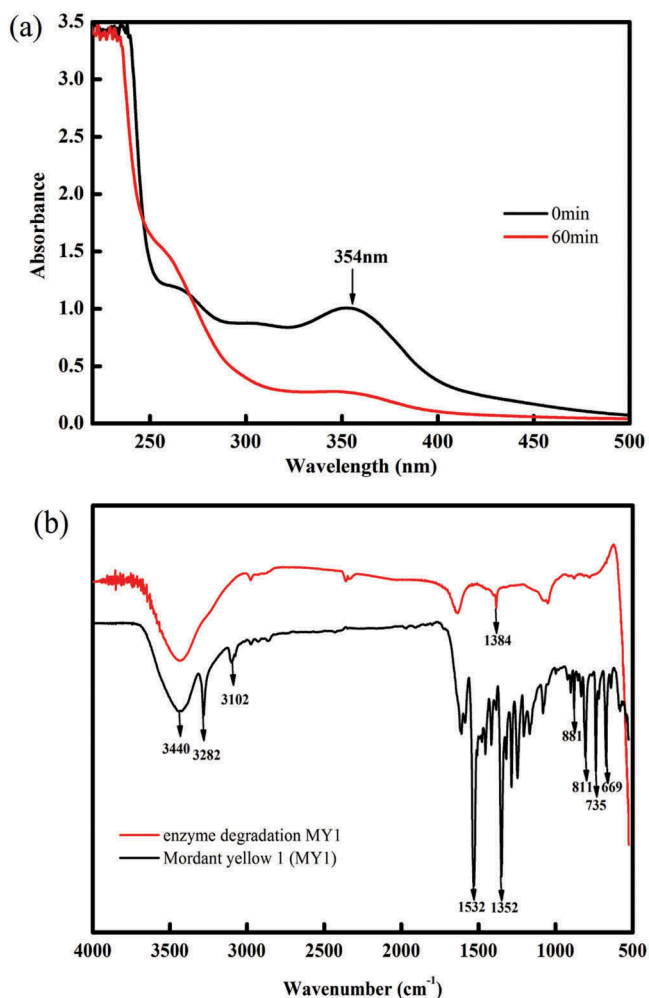


Figure 2. Analysis of the MY1 structure and its intermediate products. (a) UV-Vis spectrum and (b) FTIR spectra.

group at 3282 cm^{-1} and 3102 cm^{-1} , and in the second group at 1532 cm^{-1} and 1352 cm^{-1} were significantly reduced in intensity. The peaks in the third group at 811 cm^{-1} , 735 cm^{-1} and 669 cm^{-1} were undetectable. The results showed that the azo dye MY1 could be degraded efficiently by extracellular enzymes prepared from TS-A.

Effects of Mn^{2+} and H_2O_2 on MY1 degradation by the extracellular enzymes

MnPs catalyze the H_2O_2 -dependent oxidation of Mn^{2+} to Mn^{3+} , which can oxidize various pollutants [14,28,29]. LiPs were able to oxidize aromatic compounds by generating free radicals in the presence H_2O_2 [30]. Therefore, Mn^{2+} and H_2O_2 were required for the extracellular degradation system. Figure 3(a) shows that the degradation rate of

extracellular enzymes increased rapidly at low concentrations of Mn^{2+} (i.e., 0.1 to 1 mM). The activity of the extracellular enzymes was inhibited when Mn^{2+} was over the optimum concentration (1 mM). The maximum degradation rate by the extracellular enzymes was 78% with increasing concentration of H_2O_2 (Figure 3(b)). However, the decolorization rate gradually decreased under increased concentrations of H_2O_2 .

Effects of time, dye concentration and pH on decolorization

Figure 4 shows the effects of contact time, MY1 dye concentration and pH on decolorization by the powder mycelia and extracellular enzymes from TS-A. The degradation rate of MY1 by the extracellular enzymes reached 70% within 10 min and then gradually stabilized (Figure 4(a)). The decolorization rate by powder mycelia reached a relatively constant value

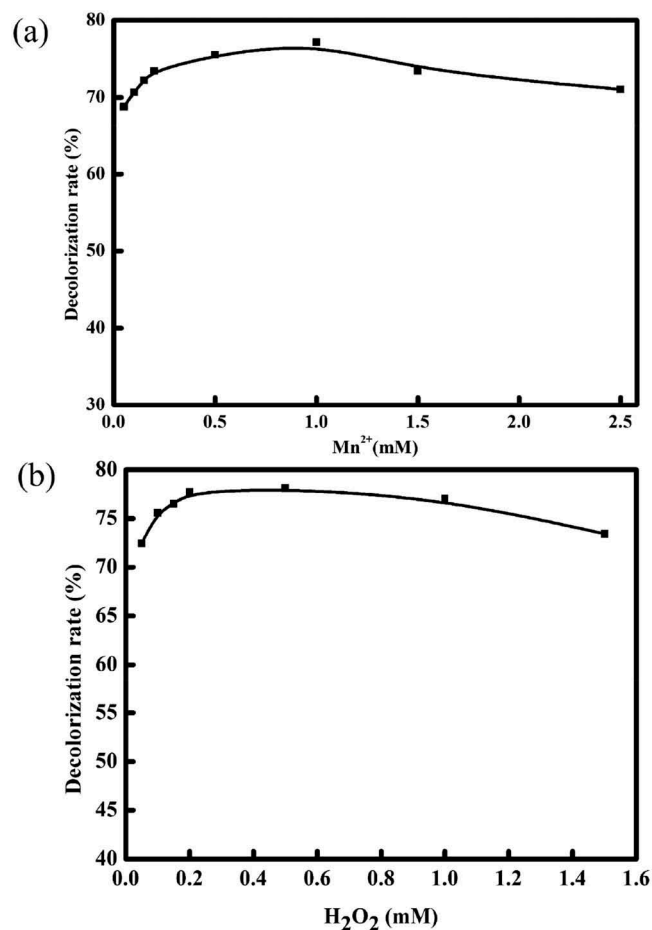


Figure 3. Effects of (a) Mn^{2+} and (b) H_2O_2 on the degradation of MY1 with extracellular enzymes.

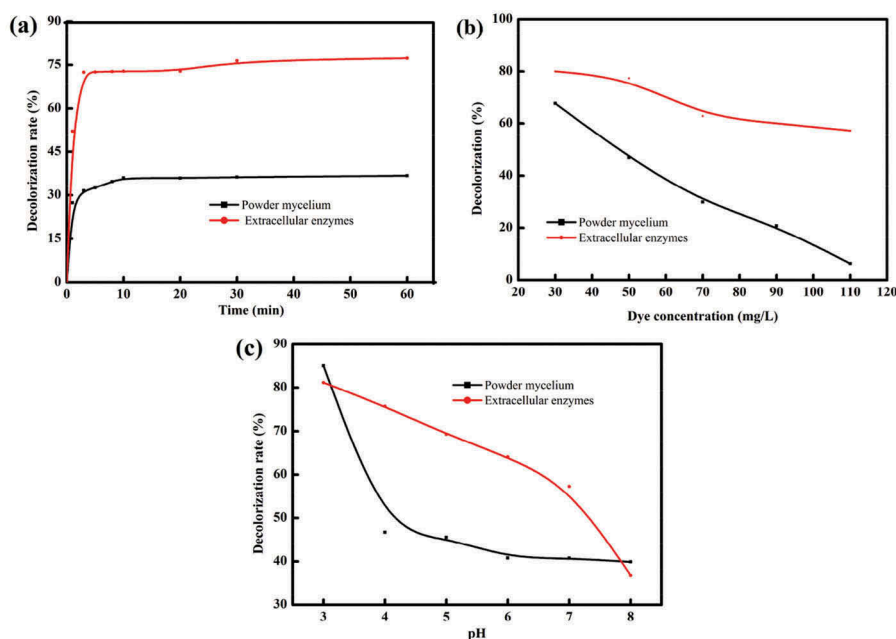


Figure 4. Effects of different environments on the decolorization rate of MY1. (a) Contact time, (b) dye concentration and (c) pH.

(36.8%), indicating that the dye was no longer absorbed. The degradation rate of MY1 by extracellular enzymes decreased to 57.2% at a dye concentration of 110 mg/L (Figure 4(b)). The degradation rate of MY1 with extracellular enzymes reached 82% when the pH of the decolorization reaction was adjusted to 3 (Figure 4(c)), whereas the degradation rate decreased to 36.8% at a pH 8. This observation indicated that the extracellular enzymes showed the highest enzymatic activity under weak acidic conditions. The dye removal rate by powder mycelia was 85.1% at pH 3 and when the pH was increased above 3 the decolorization rate decreased rapidly.

Analysis of biosorption isotherm and kinetic models

In this report, two common models were used to describe the biosorption equilibrium by powder mycelia (Figure 5). The parameter n was > 1 , which indicated a favorable biosorption process when using the Freundlich isotherms model (Table 1). Comparing the R^2 values between the Langmuir and Freundlich isotherms models, the Langmuir isotherm model fitted the data better than the Freundlich isotherms model to describe the biosorption behavior of powder mycelia.

The pseudo-second-order kinetic model was found to be suitable for describing the behavior of removing MY1 by powder mycelia (Figure 5(c)), and the data of biosorption capacity (q_{exp}) were also close to the calculated values ($q_{e,cal}$) at the set temperature (Table 2). This modeling also demonstrated that the results were well fitted ($R^2 = 0.9999$) to the pseudo-second-order kinetic model.

SEM images and BET analysis

SEM images of the surface of the powder mycelia are shown in Figure 6(a). Numerous wrinkles could be observed on the surface of the mycelium, which contribute to the adsorption ability of powder mycelia. Figure 6(b) shows that many particles were found on the powder mycelium surface and these particles may represent non-dissolved dye. The BET data analysis showed that powder mycelia had a high specific surface area of 335.5 m²/g and an average pore diameter of 2.2 nm (Table 3).

Discussion

The major mechanism for decolorization of dye by fungi is biosorption, biodegradation or a combination of both [10]. The biosorption ability of mycelia has a

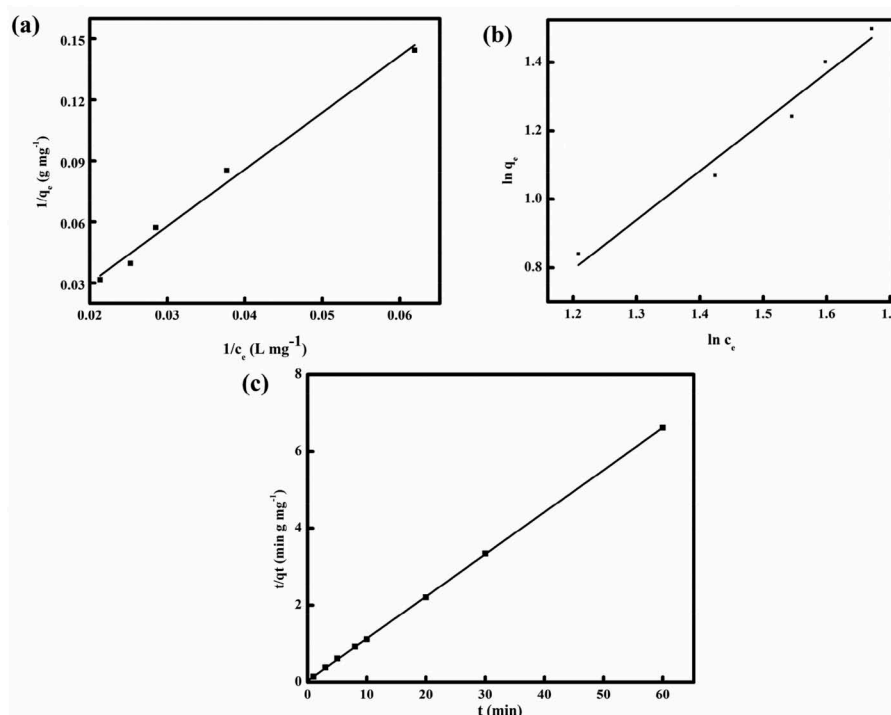


Figure 5. Different models for biosorption of MY1 onto pretreated mycelia: (a) Langmuir isotherm, (b) Freundlich isotherm and (c) pseudo-second-order kinetics.

Table 1. Isotherm constants for the biosorption of MY1 onto powder mycelia.

T (°C)	Parameter	Langmuir model	Parameter	Freundlich model
30	q_{max} ($\text{mg}\cdot\text{g}^{-1}$)	38.7597	k_F	2.5211
	k_L	0.00925	n	1.4337
	R^2	0.9867	R^2	0.9631

Table 2. Parameters for the two kinetic models for biosorption.

T (°C)	Kinetic models	q_{exp} ($\text{mg}\cdot\text{g}^{-1}$)	q_{ecal} ($\text{mg}\cdot\text{g}^{-1}$)	k	R^2
30	Pseudo-first order	9.1927	476,869,998.1	0.1110	0.0915
	Pseudo-second order		9.1912	0.2372	0.9999

strong correlation with the surface area, functional groups on the cell surface and solution pH [10,31]. Function groups such as carboxyl and amino moieties were found to be the main binding sites on the mycelium surface [32]. In this work, the decolorization rate of sterilized mycelia was higher than that of live mycelia, which showed that the intracellular enzymes contributed less to the degradation of MY1. Autoclaving of live mycelia increased the dye adsorption capacity. This observation is consistent with

previous studies that showed that autoclaving ruptures fungal structures and exposes potential binding sites for dyes [32,33]. The decolorization rate of powder mycelia was higher than that of sterilized mycelia because of an increase of the mycelium surface area. The surface area and available binding sites are critical to the adsorption ability of powder mycelia. Increasing of the adsorption time did not improve the adsorption efficiency of powder mycelia because the available binding sites were saturated. Khalaf et al. reported that the adsorption ability of mycelia also depends on the equilibrium between dye molecules binding to the powder mycelium surface and those remaining in the powder mycelium pores [24]. The pH may influence the surface of the mycelium consisting of biopolymers with many functional groups [34]. Chitin, proteins, polysaccharide and other compounds on the mycelium surface act as donors of $-\text{COOH}^-$ and $-\text{NH}^{3+}$ groups, which could bind the dye anion via electrostatic interactions [10,24]. The removal rate of MY1 with powder mycelia was 85.1% at pH 3. Similar results revealed that the biomass of *L. sajor-caju* exhibited maximum dye uptake at pH 2 [35]. Therefore, pH strongly affects the surface dye binding capacity of mycelia.

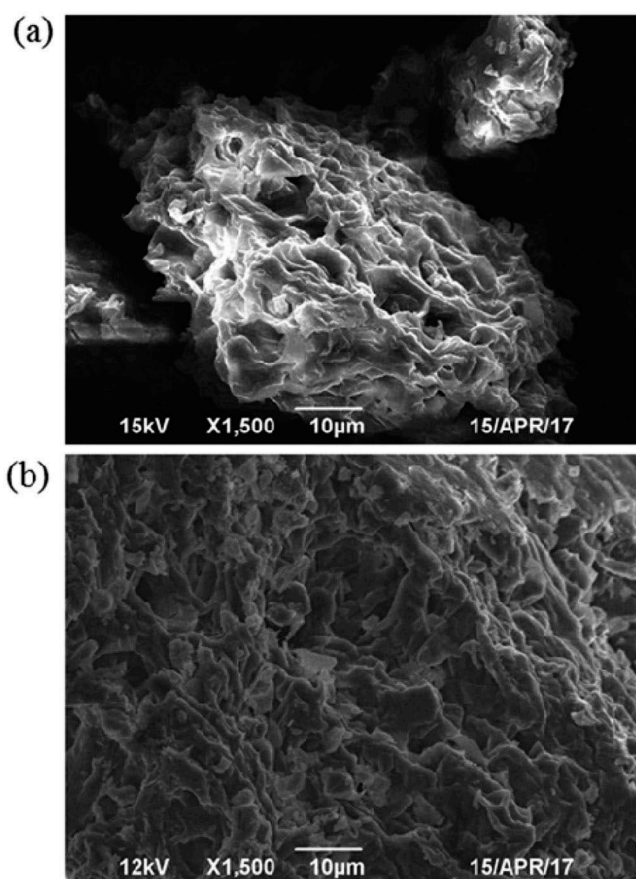


Figure 6. SEM images of powder mycelia. (a) Before adsorption and (b) after adsorption.

Table 3. BET analyses of surface parameter for powder mycelia.

Parameters	BET surface area (m ² /g)	Total Pore Volume (cm ³ /g)	Average Pore Size (nm)
Value	335.5	0.3	2.2

In this report, the Langmuir and Freundlich isotherms revealed that both models were applicable to describe the decolorization process. Comparison of the R^2 values indicated that the monolayer adsorption of MY1 played a dominant role in dye removal. Similar results were obtained in a previous report of *Aspergillus fumigatus* biosorption [17]. The pseudo-second-order kinetic model indicated that the decolorization of MY1 was determined mainly by the chemical adsorption process. The mechanism of adsorption may be described as a type of chemisorption that involves sharing or ion exchange of electrons between the surface of the powder mycelium and the dye. It was reported previously that biosorption of a reactive acid dye with *Enteromorpha flexuosa* and *Gracilaria* also followed pseudo-second-order

kinetics [33]. The biomass surface likely facilitates surface complexation through electrostatic attraction and ion exchange because of the presence of functional groups on the cell surface such as carboxyl, amino and phosphate moieties, and the lipid fraction [24,31].

In previous reports, the biomass from bacteria, fungi and algae had been selected as biosorbents for dye removal [7,10]. Although the biosorption capacity of biomasses can be regenerated and used for several biosorption cycles without considerable loss of biosorption ability, dye transfer onto mycelium surface is toxic and does not dissipate. Nonetheless, biosorption of toxic dyes by fungi biomasses can be removed through degradation because of the presence of active laccase, manganese peroxidase and lignin peroxidase enzymes [10,23].

In this study, biodegradation products of MY1 were monitored by UV-Vis and FTIR. Results illustrated clearly the breakdown of chromogenic groups in MY1. The change in MnP activity was more apparent than that of Lac and LiP in the fermentation broth of TS-A. A similar report revealed that laccase and manganese peroxidase were involved in the degradation process of Malachite Green by ligninolytic fungus *Aspergillus flavus* [22]. In the presence of H₂O₂, MnP could oxidize Mn²⁺ to Mn³⁺, which was stabilized by forming Mn³⁺ chelates as diffusing oxidizers [28,29]. LiP increased degradation capacity in the presence of H₂O₂ [10]. The low concentration of Mn²⁺ and H₂O₂ could enhance degradation of the extracellular enzyme degradation system. The activities of MnP and LiP can be inhibited when Mn²⁺ and H₂O₂ exceed optimum concentrations. The catalytic efficiency of MnP was dependent on the high enzyme-substrate ratio [29].

Besides substrate concentration, pH is also an important parameter that defines the decolorization activity of extracellular enzymes and powder mycelia. In this study, extracellular enzymes showed higher potential decolorization activity under weak acidic conditions. The pH-dependent activity of enzymes likely explains the importance of the pH in defining the efficiency of dye degradation. Similarly, Wen et al. reported that the degradation rate was highest when the pH was 2.96–4.8 [36]. The decrease in pH of the fermentation broth could arise from the production of acidic metabolites during the

growth of TS-A. Three kinds of acidic metabolic products were examined from a *T. lixii* F21 culture extract of ARS-treated medium [12]. In general, fungi were found to grow under weak acidic conditions, which was beneficial to the degradation of dyes. In the present study, although the decolorization activity of powder mycelia exceeded that of the extracellular enzymes at pH 3, the decolorization rate of the extracellular enzymes was higher than that of powder mycelia under weak acidic conditions.

As mentioned above, the decolorization process was achieved through the synergistic effects of mycelia and extracellular enzymes with the activity of the extracellular enzymes displaying the predominant role. The possible decolorization process of MY1 by TS-A is depicted in Figure 7. When the spore hypha of TS-A are inoculated in medium, active enzymes of MnP, Lac and LiP are secreted into the extracellular environment. The degradation rate of MY1 by extracellular enzymes is enhanced because of the gradual decrease in pH during the growth of TS-A. Live mycelia may behave in a particular manner that involves self-immobilization to form mycelial pellets, which have considerable mechanical strength and a large specific surface area [37]. The amino acid and polysaccharose on the surface of live mycelia provide functional groups as the main azo dye binding sites because they contain carboxyl, amino and hydroxyl groups [10,17,27]. The mycelial pellets may play a role as a bioactive carrier and gather dye molecules from dye wastewater. According to the equilibrium time of enzyme degradation and mycelium adsorption, we hypothesize that mycelia could reduce dye inhibition when extracellular enzymes degrade the high concentration of dyes during the initial incubation period. Furthermore, UV-Vis and FTIR spectra showed that dye molecules were degraded into small molecules, which might enter cells and even enter a metabolic flux until completely mineralized.

Decolorization features of TS-A could be transformed between biodegradation and biosorption under different conditions. The decolorization efficiency of *Aspergillus* could be controlled with the adjustment of pH. Extracellular enzymes performed suitable degradation activity against MY1. Thus, generation of lignin oxidase by gene recombination could be carried out in the future to investigate the specific mechanism of MY1 degradation. The activity sludge could be strengthened by adding *Aspergillus sp.* TS-A

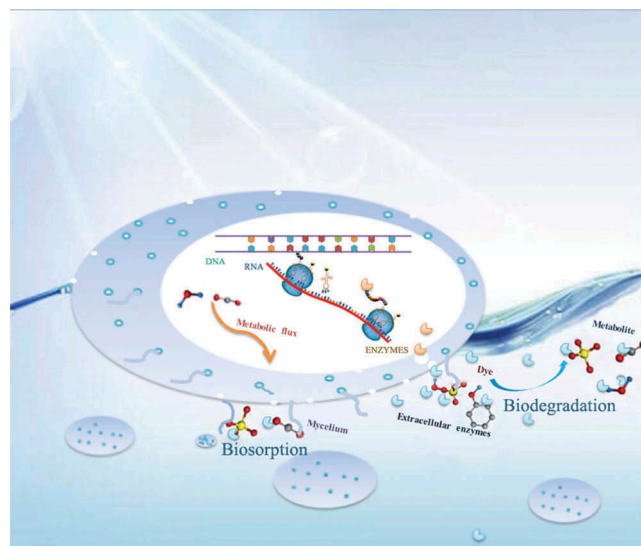


Figure 7. Possible decolorization process of MY1 by *Aspergillus sp.* TS-A.

because of its excellent biosorption and biodegradation activity. *Aspergillus sp.* TS-A could also be potentially used in biofilm reactors because this species can adhere to surfaces, and thus be an effective system for degradation of azo dyes in wastewater.

Conclusions

This work investigated the decolorization of the azo dye MY1 by extracellular enzymes and mycelia separately prepared from *Aspergillus sp.* TS-A CGMCC12964 (120 h). The synergistic effects of the extracellular enzymes and mycelia of TS-A successfully decolorized MY1 under acidic conditions. The breakdown of chromogenic groups in MY1 was examined. The biosorption behavior of TS-A was found to be a monolayer adsorption process, accompanied with particular heterogeneous adsorption. Biosorption was determined to involve primarily a chemical adsorption process. The decolorization activity of mycelia and the extracellular enzymes was clearly affected by pH. Biodegradation by the extracellular enzymes in a weak acidic environment was found to be the main decolorization process of the TS-A fermentation broth.

Acknowledgments

The work was financially supported by the National Natural Science Foundation of China (No. 21466032) and Scientific

Research Foundation for Changjiang Scholars of Shihezi University (No. CJXZ201501). We would like to thank the editor and anonymous reviewers for their valuable comments in this work.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Natural Science Foundation of China [No. 21466032]; Scientific Research Foundation for Changjiang Scholars of Shihezi University [No. CJXZ201501].

References

- [1] Saratale RG, Gd S, Js C, et al. Bacterial decolorization and degradation of azo dyes: a review. *J Taiwan Inst Chem Eng.* **2011**;42:138–157.
- [2] Popli S, Patel UD. Destruction of azo dyes by anaerobic–aerobic sequential biological treatment: a review. *Int J Environ Sci Technol.* **2015**;12:405–420.
- [3] Santos ABD, Cervantes FJ, Lier JBV. Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology. *Bioresour Technol.* **2007**;98:2369–2385.
- [4] Maas R, Chaudhari S. Adsorption and biological decolorization of azo dye reactive red 2 in semicontinuous anaerobic reactors. *Process Biochem.* **2005**;40:699–705.
- [5] Ren S, Guo J, Zeng G, et al. Decolorization of triphenylmethane, azo and anthraquinone dyes by a newly isolated *Aeromonas hydrophila* strain. *Appl Microbiol Biotechnol.* **2006**;72:1316–1321.
- [6] Eichlerová I, Homolka L, Benada O, et al. Decolorization of Orange G and Remazol Brilliant Blue R by the white rot fungus *Dichomitus squalens*: toxicological evaluation and morphological study. *Chemosphere.* **2007**;69:795–802.
- [7] Park JB, Rj C, An S. Wastewater treatment high rate algal ponds for biofuel production. *Bioresour Technol.* **2011**;102:35–42.
- [8] Lin J, Zhang X, Li Z, et al. Biodegradation of reactive blue 13 in a two-stage anaerobic/aerobic fluidized beds system with a *Pseudomonas sp.* isolate. *Bioresour Technol.* **2010**;101:34–40.
- [9] Pearce CI, Lloyd JR, Guthrie JT. The removal of colour from textile wastewater using whole bacterial cells: a review. *Dyes Pigm.* **2003**;58:179–196.
- [10] Sen SK, Raut S, Bandyopadhyay P, et al. Fungal decoloration and degradation of azo dyes: a review. *Fungal Biol Rev.* **2016**;30:112–133.
- [11] Jiang M, Ten Z, Ding S. Decolorization of synthetic dyes by crude and purified laccases from *Coprinus comatus* grown under different cultures: the role of major isoenzyme in dyes decolorization. *Appl Biochem Biotechnol.* **2013**;169:660–672.
- [12] Adnan LA, Sathishkumar P, Yusoff ARM, et al. Rapid bioremediation of Alizarin Red S and Quinizarine Green SS dyes using *Trichoderma lixii* F21 mediated by biosorption and enzymatic processes. *Bioprocess Biosyst Eng.* **2017**;40:85–97.
- [13] Kuan IC, Tien M. Stimulation of Mn peroxidase activity: a possible role for oxalate in lignin biodegradation. *Proc Natl Acad Sci.* **1993**;90:1242–1246.
- [14] Bilal M, Asgher M, Iqbal M, et al. Chitosan beads immobilized manganese peroxidase catalytic potential for detoxification and decolorization of textile effluent. *Int J Biol Macromol.* **2016**;89:181–189.
- [15] Asgher M, Bhatti HN, Ashraf M, et al. Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. *Biodegradation.* **2008**;19:771–783.
- [16] Singh S, Pakshirajan K, Daverey A. Enhanced decolorization of Direct Red-80 dye by the white rot fungus *Phanerochaete chrysosporium* employing sequential design of experiments. *Biodegradation.* **2010**;21:501–511.
- [17] Chaudhry MT, Zohaib M, Rauf N, et al. Biosorption characteristics of *Aspergillus fumigatus* for the decolorization of triphenylmethane dye acid violet 49. *Appl Microbiol Biotechnol.* **2014**;98:3133–3141.
- [18] Ali N, Hameed A, Ahmed S, et al. Decolorization of structurally different textile dyes by *Aspergillus niger* SA1. *World J Microbiol Biotechnol.* **2008**;24:1067–1072.
- [19] Telke AA, Kadam AA, Jagtap SS, et al. Biochemical characterization and potential for textile dye degradation of blue laccase from *Aspergillus ochraceus* NCIM-1146. *Biotechnol Bioprocess Eng.* **2010**;15:696–703.
- [20] Liang T, He M, Li S, et al. Aerobic decolorization, degradation and detoxification of azo dyes by a newly isolated salt-tolerant yeast *Scheffersomyces spartinae* TLHS-SF1. *Bioresour Technol.* **2015**;203:287–294.
- [21] Waghmode TR, Kurade MB, Govindwar SP. Time dependent degradation of mixture of structurally different azo and non azo dyes by using *Galactomyces geotrichum* MTCC 1360. *Int Biodeterior Biodegrad.* **2011**;65:479–486.
- [22] Andleeb S, Atiq N, Robson GD, et al. An investigation of anthraquinone dye biodegradation by immobilized *Aspergillus flavus* in fluidized bed bioreactor. *Environ Sci Pollut Res.* **2012**;19:1728–1737.
- [23] Srinivasan A, Viraraghavan T. Decolorization of dye wastewaters by biosorbents: A review. *J Environ Manage.* **2010**;91:1915–1929.
- [24] Khalaf MA. Biosorption of reactive dye from textile wastewater by non-viable biomass of *Aspergillus niger* and *Spirogyra sp.* *Bioresour Technol.* **2008**;99:6631–6634.
- [25] Pan H, Xu X, Wen Z, et al. Decolorization pathways of anthraquinone dye Disperse Blue 2BLN by *Aspergillus sp.* XJ-2 CGMCC12963. *Bioengineered.* **2017**;8:630–641.
- [26] Nguyen TA, Fu CC, Juang RS. Biosorption and biodegradation of a sulfur dye in high-strength dyeing

- wastewater by *Acidithiobacillus thiooxidans*. *J Environ Manage.* **2016**;182:265–271.
- [27] Simonin JP. On the comparison of pseudo-first order and pseudo-second order rate laws in the modeling of adsorption kinetics. *Chem Eng J.* **2016**;300:254–263.
- [28] Urzúa U, L L F, Lobos S, et al. Oxidation reactions catalyzed by manganese peroxidase isoenzymes from *Ceriporiopsis subvermispota*. *Febs Letters.* **1995**;371:132–136.
- [29] Chen W, Zheng L, Jia R, et al. Cloning and expression of a new manganese peroxidase from *Irpex lacteus* F17 and its application in decolorization of reactive black 5. *Process Biochem.* **2015**;50:1748–1759.
- [30] Bilal M, Asgher M, Parra-Saldivar R, et al. Immobilized ligninolytic enzymes: an innovative and environmental responsive technology to tackle dye-based industrial pollutants – A review. *Sci Total Environ.* **2017**;576:646–659.
- [31] Sivasamy A, Nethaji S, Nisha LL. Equilibrium, kinetic and thermodynamic studies on the biosorption of reactive acid dye on *Enteromorpha flexuosa* and *Gracilaria corticata*. *Environ Sci Pollut Res.* **2012**;19:1687–1695.
- [32] Fu YZ, Viraraghavan T. Removal of Congo Red from aqueous solution by the fungus *Aspergillus niger*. *Water Qual Res J Can.* **2002**;35:95–111.
- [33] Arica MY, Bayramoğlu G. Biosorption of Reactive Red-120 dye from aqueous solution by native and modified fungus biomass preparations of *Lentinus sajor-caju*. *J Hazard Mater.* **2007**;149:499–507.
- [34] Maurya NS, Mittal AK, Cornel P, et al. Biosorption of dyes using dead macro fungi: effect of dye structure, ionic strength and pH. *Bioresour Technol.* **2006**;97:512–521.
- [35] O'Mahony T, Guibal E, Tobin JM. Reactive dye biosorption by *Rhizopus arrhizus* biomass. *Enzym Microb Technol.* **2002**;31:456–463.
- [36] Wen X, Jia Y, Li J. Enzymatic degradation of tetracycline and oxytetracycline by crude manganese peroxidase prepared from *Phanerochaete chrysosporium*. *J Hazard Mater.* **2010**;177:924–928.
- [37] Lu T, Zhang Q, Yao S. Efficient decolorization of dye-containing wastewater using mycelial pellets formed of marine-derived *Aspergillus niger*. *Chin J Chem Eng.* **2017**;25:330–337.