Use of Cassava Residue for the Removal of Congo Red from Aqueous Solution by a Novel Process Incorporating Adsorption and *In Vivo* Decolorization

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To investigate the removal of Congo red (CR) from an aqueous solution using cassava residue, a novel process incorporating adsorption and *in vivo* decolorization was proposed. The conditions and characteristics of CR adsorption on cassava residue were investigated by batch adsorption experiments. Langmuir and pseudo-second order models were found to fit well with the data of equilibrium adsorption and kinetics adsorption, respectively. The adsorption was affected considerably by the adsorbent (cassava residue) dosage and CR concentration. The maximum adsorptive capacity was 59.2 mg/g ($m_{CR}/m_{cassava\ residue}$), calculated by the Langmuir model. Then, CR-loaded cassava residue was further decolorized *via* an *in vivo* process by *Trametes* sp. SYBC-L4. The laccase produced by *Trametes* sp. SYBC-L4 effectively decolorized CR, which was revealed by native polyacrylamide gel electrophoresis. The moisture content affected the performance of *in vivo* decolorization considerably. Decolorization of 81.6 ± 2.4% was achieved under the conditions of pH 5.5, temperature 30 °C, and moisture content of 60% after 16 days of cultivation. Moreover, analyses of Fourier transform infrared spectroscopy and scanning electron microscopy indicated that the carbonyl (C=O), hydroxyl (-OH), and amino (-NH) groups in cassava residue were the potential adsorption sites for interaction with CR and that the structure of cassava residue was modified in the process of *in vivo* decolorization.

Keywords: Cassava residue; Adsorption; Congo red; Decolorization; Laccase

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INTRODUCTION

There are more than 1 million tons of synthetic dyes produced each year (Singh and Arora 2011), and at least 10% of those dyes are released into the environment during the process of their production and application (Husain 2006). The discharged dyes create a bulk of colored wastewater and cause serious pollution problems (Husain 2006; Singh and Arora 2011). Among structural dyes, anionic dyes are the most problematic (Vimonses *et al*. 2009). Congo red (CR) is a benzidine-based anionic disazo dye that is widely used in textiles, printing, and paper industries, although CR is toxic to many organisms and is a suspected carcinogen and mutagen. CR-contaminated wastewater is hardly treated since the complex aromatic structure of CR endowed its physicochemical, thermal and optical stability (Vimonses *et al.* 2009; Afkhami and Moosavi 2010).

Among known techniques for dye removal from aqueous solutions, adsorption is an attractive technique due to its low operational cost, ease of design, and insensitivity to toxic substances (Afkhami and Moosavi 2010; Sharma *et al.* 2013). Moreover, many lignocellulosic biomasses have been extensively utilized as alternative adsorbents for the removal of dyes from aqueous solutions (Han *et al.* 2008; Tamez Uddin *et al.* 2009; Senthil Kumar *et al.* 2010; Dawood and Sen 2012; Gautam *et al.* 2013). These adsorbents are economically viable for dye removal because they are abundant (Lin and Juang 2009). Moreover, equilibrium, kinetics, thermodynamics, and the mechanism of dye adsorption using lignocellulosic biomass were explored for the design of a full-scale plant (Senthil Kumar *et al.* 2010; Sen *et al.* 2011; Dawood and Sen 2012; Gautam *et al.* 2013). Although the adsorption of dyes using lignocellulosic biomass could remove the color from dye-containing wastewater, the dye only accumulated on the biomass, rather than being degraded or mineralized. Therefore, the dye-loaded biomass cannot be directly discarded. Like many other valuable adsorbents, desorption dye for recovery of the adsorbent is not economically viable (Afkhami and Moosavi 2010; An *et al.* 2010). Subsequent processing of the dye-loaded biomass should be further investigated to eradicate dye pollution in the environment. However, few works have reported on further treatment of the dye-loaded biomass (Chakraborty *et al.* 2011).

White-rot fungi have been known to effectively degrade and decolorize synthetic dyes because of their extra-cellular ligninolytic enzymes such as laccase, manganese peroxidase, and lignin peroxidase (Singh and Arora 2011; Zeng *et al.* 2011). Moreover, white-rot fungal decolorization with solid-state culture (SSC) using lignocellulosic biomass as substrate, *i.e.*, *in vivo* decolorization, has received more attention since SSC mimics the natural environment of white-rot fungi (Boer *et al.* 2004; Tychanowicz *et al.* 2004). However, it is hard to balance the dye absorption and fungal growth when the substrate was chosen for fungal cultivation. The poor absorption of the dye to the substrate will affect the efficiency of dye removal, whereas a large amount of dye absorption will inhibit fungal growth and consequent ligninolytic enzymes production due to the toxicity of synthetic dyes (Ali 2010; Zeng *et al.* 2011). Thus, a process successfully incorporated into dye adsorption using lignocellulosic biomass and *in vivo* decolorization is rarely reported (Robinson *et al.* 2001).

Cassava is widely available and is an attractive feedstock for the production of fuel ethanol because it is cheap and does not compete with food for arable land (Zhang *et al.* 2010). Cassava residue is a lignocellulosic waste generated in the distilling step of cassava-based ethanol production. However, due to low nutrition and the recalcitrant lignocellulosic structure of the waste, it is unsuitable for producing distillers dried grains with solubles *via* the processes of evaporation and drying. Handling the waste is a problem that will become increasingly severe in the future with increased industrial production of ethanol from cassava. Therefore, effective and reasonable use of the waste is a significant issue (Zhang *et al.* 2011). In the present work, cassava residue was utilized as an adsorbent for the removal of CR from an aqueous solution and the adsorptive capacity of cassava residue was evaluated by batch adsorption experiments. Moreover, the isotherm, kinetics, thermodynamics, and mechanism of CR adsorption on cassava residue were explored from the data obtained in the batch adsorption experiments. Next, *in vivo* decolorization of CR-loaded cassava residue was performed with a white-rot fungus, *Trametes* sp. SYBC-L4. Lastly, a novel process that incorporated adsorption with cassava residue and *in vivo* decolorization using *Trametes* sp. SYBC-L4 was proposed for the economical and eco-friendly removal of CR from an aqueous

solution. Moreover, the process was characterized by using scanning electron microscopy and Fourier transform infrared spectroscopy, respectively.

EXPERIMENTAL

Raw Materials

Cassava residue was provided by Yong Xiang Ethanol Co. Ltd., Wu Jiang, China. The cassava residue was washed and dried at 105 °C until a constant weight was obtained. The dried residue was milled to a fine powder and passed through an 80-mesh screen. The powdered residue was stored in an airtight plastic container and used for experiments. The components of cassava residue were as follows: total nitrogen (TN), 1.53 ± 0.08%; lignin, 20.19 ± 0.22%; cellulose, 31.46 ± 0.63%; hemicellulose, 10.49 ± 0.20%. Congo red (C.I. 22120, MF: $C_{32}H_{22}N_6Na_2O_6S_2$, MW: 696.68, λ_{max}: 495 nm) was obtained from Shanghai Yuming Industrial Co. Ltd., Yuanhang Reagent Factory, China. The stock solution of CR (1000 mg/L) was prepared in sodium phosphate buffer (50 mM). All working solutions were prepared by diluting the stock solution with sodium phosphate buffer (50 mM) to the desired concentration. All reagents used were of analytical grade.

Batch Adsorption of CR on Cassava Residue

The adsorptive capacity was determined by batch adsorption experiments of a known amount of cassava residue mixed with 50 mL of CR solution of a known concentration in a series of 250-mL Erlenmeyer flasks. The mixtures were shaken in an orbital shaker incubator at 150 rpm for 240 min under a constant temperature. At a predetermined time, the flasks were withdrawn from the shaker, and the residual dye concentration in the mixture was analysed by centrifuging the mixture (4500×*g*, 10 min) and measuring the optical density of the supernatant at the wavelength of maximum absorbance with a UV/VIS spectrometer (Model UV2100, Unic, Shanghai). The experiments were conducted by varying the pH value (5.5 to 8.5), adsorbent (cassava residue) dosages (0.05 to 0.4 g), CR concentration (50 to 400 mg/L), and temperature (30 to 50 °C) to investigate the effects of the conditions on adsorptive capacity. The data were utilized to investigate the kinetics, isotherm, thermodynamics, and mechanism of adsorption. The capacity of dye adsorbed per unit adsorbent (mg dye per g adsorbent) was calculated according to Eq. 1 (An *et al.* 2010; Senthil Kumar *et al.* 2010),

$$q_t = \frac{(C_0 - C_t)}{m} \cdot V \quad (1)$$

where q_t (mg/g) is the capacity of CR adsorbed per unit of adsorbent at time *t*, C_0 (mg/L) is initial CR concentration in the flasks, C_t (mg/L) is CR concentration in the mixture at time *t*, *V* (L) is the volume of solution, and *m* (g) is the mass of cassava residue.

Adsorption Isotherm, Kinetics, Thermodynamics and Mechanism

Adsorption isotherm

To simulate the adsorption isotherm of CR on cassava residue, two commonly used equilibrium isotherm models, the Langmuir model and Freundlich model, were applied to describe the adsorption isotherm. The Langmuir model (Eq. 2) (Sen *et al.*

2011; OuYang *et al*. 2014) and Freundlich model (Eq. 3) (Sen *et al.* 2011; OuYang *et al*. 2014) can be expressed as follows,

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

$$\mathrm{In}(q_e) = \frac{1}{n}\mathrm{In}(C_e) + \mathrm{In}(K_f) \quad (3)$$

where C_e (mg/L) is the equilibrium concentration of CR in solution, q_e (mg/g) is the capacity of CR adsorbed per unit of adsorbent at equilibrium time, q_m (mg/g) is the maximum adsorptive capacity calculated by the model, K_a is the Langmuir constant, and K_f and n are Freundlich constants.

Adsorption kinetics

To investigate the adsorption kinetics of CR on cassava residue, the adsorption process was described in terms of a pseudo-second order model (Ho and McKay 2000). The model (Eq. 4) was expressed as follows,

$$\frac{t}{q_t} = \frac{t}{q_{e,cal}} + (\frac{1}{k_2 q_{e,cal}^2}) \quad (4)$$

where $q_{e,cal}$ (mg/g) is the capacity of CR adsorbed per unit of adsorbent at equilibrium time calculated by the model, q_t (mg/g) is the capacity of CR adsorbed per unit of adsorbent at time t, and k_2 is the model constant.

Adsorption thermodynamics

Adsorption thermodynamics of CR on cassava residue was investigated by using the following equations (Eq. 5 and Eq. 6) (Dawood and Sen 2012),

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (5)$$

$$\log(\frac{q_e}{C_e}) = \frac{\Delta S^\circ}{2.303R} - \frac{\Delta H^\circ}{2.303RT} \quad (6)$$

where q_e (mg/g) is the capacity of CR adsorbed per unit of adsorbent at equilibrium time, C_e (mg/L) is the equilibrium concentration of CR in solution, T is temperature in K, R is the gas constant (8.314 J/mol·K), and $\Delta G°$, $\Delta H°$, and $\Delta S°$ are Gibb's free energy, enthalpy change, and entropy change, respectively.

Adsorption mechanism

To investigate the mechanism of adsorption, the film diffusion model (Eq. 7) (Qiu *et al.* 2009) and intra-particle diffusion model (Eq. 8) (Weber and Morris 1963; Vimonses *et al.* 2009) were employed,

$$\mathrm{In}(1 - \frac{q_t}{q_e}) = -K_b t \quad (7)$$

$$q_t = K_{id} \acute{} t^{0.5} + I \quad (8)$$

where q_e (mg/g) is the capacity of CR adsorbed per unit of adsorbent at equilibrium time, q_t (mg/g) is the capacity of CR adsorbed per unit of adsorbent at time t, $t^{0.5}$ is square root of the time t, K_b is the constant of the film diffusion model, and K_{id} and I are the constants of the intra-particle diffusion model.

In vivo Decolorization with *Trametes* sp. SYBC-L4

Trametes sp. SYBC-L4 (NCBI Genbank accession no. HQ891288) was kindly provided by Professor Liao from Jiangnan University. *In vivo* decolorization with the fungus was performed to treat CR-loaded cassava residue. The fungus was cultured on PDA plates for 10 days at 30 °C, and fungal mycelial plugs (diameter 10 mm) were used as inoculum. In the process of *in vivo* decolorization, the four mycelial plugs were transferred to 250-mL Erlenmeyer flasks containing 10.0 g cassava residue, 592.0 mg CR, and distilled water at different pH, which was adjusted by 1.0 M NaOH or HCl solution. Cultivation was done at a constant temperature and humidity of 70%. CR was given a final concentration of 59.2 mg/g ($m_{CR}/m_{cassava\ residue}$) based on the results of the adsorption experiments above. The effects of temperature (25 to 35 °C), moisture content (40% to 80%), and pH (5.5 to 8.5) on the decolorization of CR, activity of ligninolytic enzymes, and weight loss of CR-loaded cassava residue were investigated, respectively. At a predetermined time, the residual dye on cultures was extracted using a mixture of methanol, acetone, and water (1:1:1) (1/200, *W/V*), shaken at 150 rpm for 3 h, and then centrifuged at 4500×*g* for 10 min (Tychanowicz *et al.* 2004). The collected supernatant was analyzed for dye decolorization. Dye disappearance was determined by monitoring the optical density at the maximum wavelength of absorbance. In the control culture, the fungus (abiotic control) was omitted, and the total dye extracted with the organic mixture in the abiotic control was considered 100% (Tychanowicz *et al.* 2004). Residue on the culture was dried at 105 °C until a constant weight was obtained. The dried residue was then weighed and the weight loss was calculated by the following equation: $(m_0\text{-}m)/m_0 \times 100\%$, where, m_0 (g) is the initial weight of CR-loaded cassava residue and m (g) is the weight of the residue after incubation.

Ligninolytic Enzymes Extraction, Determination, and Native-PAGE Analysis

Ligninolytic enzymes extraction and determination

To determine the activity of ligninolytic enzymes derived from *Trametes* sp. SYBC-L4, one gram of the culture was taken from each flask and mixed with 50 mM sodium tartrate buffer (1/10, *W/V*) at pH 4.5. The mixture was placed in a shaker at 150 rpm for 1 h and then centrifuged at 4500×*g* for 10 min. The supernatant was analyzed for the activity of ligninolytic enzymes. The activity was expressed as unit per gram of dry culture by measuring the optical density. The dry weight of the culture was determined by weighing the culture that had been dried at 105 °C until a constant weight was obtained. The activity of laccase was determined with 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) as the substrate. Oxidation of ABTS was monitored by the increase in A_{420} (ε=36 $mM^{-1}\cdot cm^{-1}$). One unit of laccase activity was

defined as the amount of enzyme required to oxidize 1 μmol of ABTS per min at 30 °C (Zeng *et al.* 2011; Schubert *et al.* 2012). The activity of manganese peroxidase (MnP) was measured by quantifying the formation of Mn (III)-malonate complex at 270 nm after incubating reaction mixtures containing 1 mM $MnSO_4$ and 0.1 mM H_2O_2 at 30 °C at certain time intervals (Wariishi *et al.* 1992). To measure the enzyme activity of lignin peroxidase (LiP), the oxidation of veratryl alcohol to veratraldehyde was monitored at 310 nm (Tien and Kirk 1984).

Native-PAGE analysis

Native polyacrylamide gel electrophoresis (native-PAGE) was carried out to visualize the protein band and CR decolorization band of laccase activity (Zeng *et al.* 2011; Si *et al.* 2013). A 10% polyacrylamide gel and 1.5 M Tris buffer (pH 8.8) in a DYCZ-24DN (LiuYi, China) electrophoresis chamber were used at a constant voltage of 120 V. Afterwards, the gel was incubated in 100 mL 50 mM sodium tartrate buffer (pH 4.5) containing 0.5 mM ABTS and 0.5 mM CR for 1 h to reveal the protein band and CR decolorization band, respectively.

Scanning Electron Microscopy

Cassava residue, CR-loaded cassava residue, and CR-loaded cassava residue after *in vivo* decolorization were characterized by scanning electron microscopy (SEM) (FEI Quanta 200, Holland).

Fourier Transform Infrared Spectroscopy

Cassava residue, CR-loaded cassava residue, and CR-loaded cassava residue after *in vivo* decolorization were characterized by Fourier transform infrared spectroscopy (FTIR) scanning at a range of 4000 to 400 cm^{-1} (Nicolet Nexus 470, Thermo Electro Co., USA).

RESULTS AND DISCUSSION

Effect of Conditions on Adsorptive Capacity of Cassava residue

The effect of conditions on the adsorption of CR was investigated in batch adsorption experiments, and the results are presented in Fig. 1. A pH range of 5.5 to 8.5 and a temperature range of 30 to 50 °C were investigated, since conditions beyond these ranges are too extreme to be easily and economically applied on an industrial scale. The pH would be expected to significantly affect the adsorptive capacity of adsorbent if electrostatic interaction, including attraction or repulsion, was the predominant interaction between the dye and adsorbent (Dawood and Sen 2012). As shown in Fig. 1a, the adsorptive capacity was approximately 40 mg/g in the pH range of 5.5 to 8.5. The pH did not markedly affect the adsorptive capacity. This result suggested that the predominant interaction between cassava residue and CR molecules was not electrostatic. The effect of temperature on the adsorptive capacity of adsorbent is an indicator of whether adsorption is an exothermic or endothermic process (Sen *et al.* 2011; Dawood and Sen 2012). Figure 1b shows that the adsorptive capacity did not vary markedly with the increase of temperature from 30 °C to 50 °C, indicating that energy acquired or discharged by the adsorption was negligible. The adsorbent (cassava residue) dosage, however, had a considerable effect on the adsorptive capacity. Figure 1c shows that an

increase of cassava residue dosage from 0.05 g to 0.4 g resulted in a decrease in the adsorptive capacity from 52.35 ± 1.05 mg/g to 11.90 ± 0.22 mg/g at 240 min of adsorption. This is due to the concentration gradient between solute concentration in the solution and the solute concentration at the surface of adsorbent (Senthil Kumar *et al.* 2010; Sen *et al.* 2011). CR concentration considerably affected the CR adsorptive capacity of cassava residue (Fig. 1d); the adsorptive capacity increased from 23.03 ± 0.58 mg/g to 57.80 ± 1.11 mg/g at 240 min of adsorption with increasing concentration of CR (50 mg/L to 400 mg/L). A higher dye concentration provided a greater driving force because of the concentration gradient between the aqueous and the solid phase (Vimonses *et al.* 2009). Therefore, an increase in CR concentration led to a considerable increase in the adsorptive capacity.

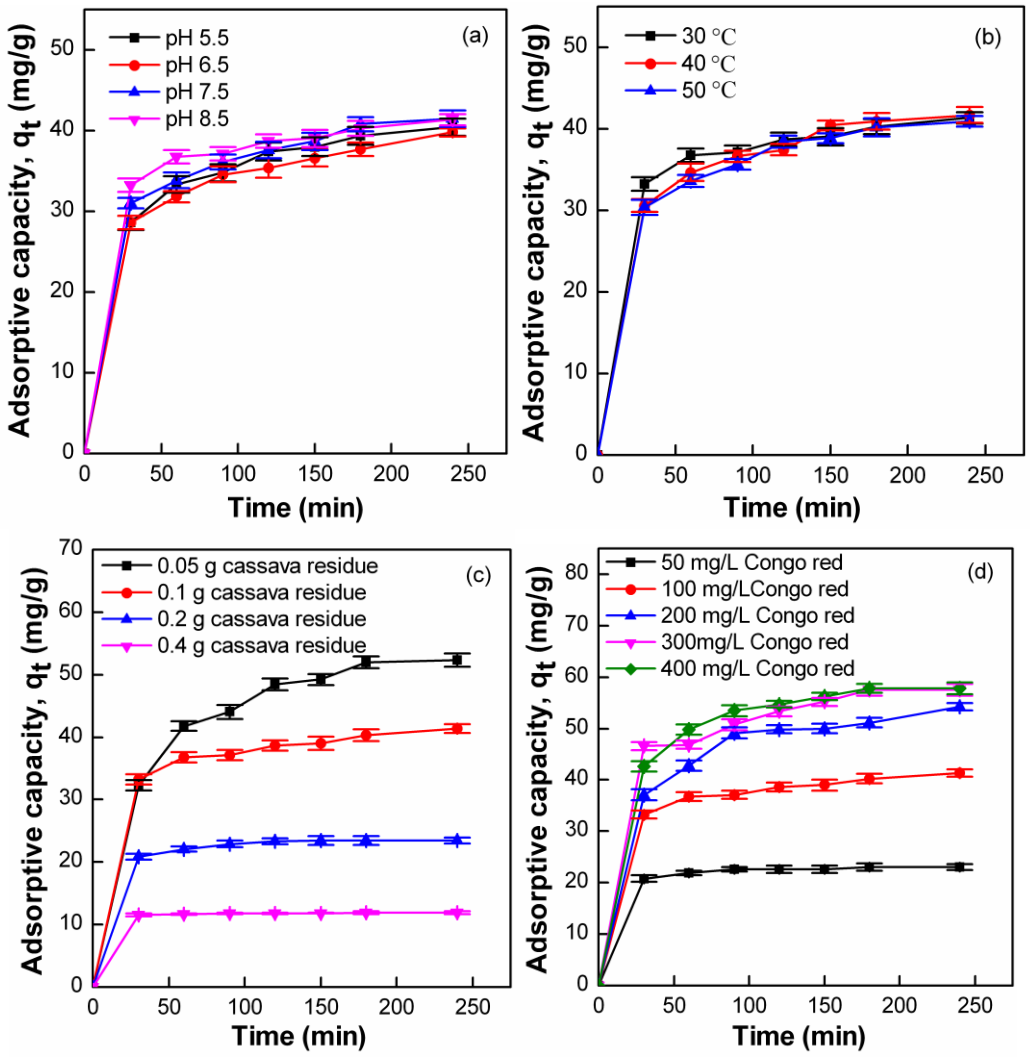

Fig. 1. Effect of: (a) pH at cassava residue dosages of 0.1 g, temperature of 30 °C, and Congo red concentration of 100 mg/L; (b) temperature at cassava residue dosages of 0.1 g, pH 8.5, and Congo red concentration of 100 mg/L; (c) cassava residue dosage at temperature of 30 °C, pH 8.5, and Congo red concentration of 100 mg/L; (d) Congo red concentration at temperature of 30 °C, pH 8.5, and cassava residue dosages of 0.1 g on the Congo red adsorptive capacity of cassava residue

Thermodynamics, Kinetics, Isotherm, and Mechanism of CR Adsorption on Cassava Residue

Table 1. Pseudo-Second Order Kinetic Parameters of Congo Red Adsorption on Cassava Residue

Parameters	$q_{e,cal}$ (mg/g)	k_2	R^2	$q_{e,exp}$ (mg/g)
pH				
5.5	43.29	0.001	0.999	40.42
6.5	42.02	0.001	0.998	39.77
7.5	44.05	0.001	0.998	41.49
8.5	42.74	0.002	0.999	41.35
Cassava residue dosages (g)				
0.05	58.14	0.001	0.999	52.35
0.1	42.74	0.002	0.999	41.35
0.2	23.98	0.01	0.999	23.48
0.4	11.95	0.07	0.999	11.90
Congo red concentration (mg/L)				
50	23.41	0.011	0.999	23.03
100	42.74	0.002	0.999	41.35
200	57.47	0.001	0.998	54.25
300	60.98	0.001	0.998	57.50
400	61.35	0.001	0.999	57.80
Temperature (°C)				
30	42.74	0.002	0.999	41.35
40	44.44	0.001	0.998	41.71
50	43.67	0.001	0.999	40.95

Based on the experimental batch adsorption data, the thermodynamics, kinetics, isotherm, and mechanism of CR adsorption on cassava residue were investigated. The adsorption thermodynamics was investigated with Van't Hoff equation (Dawood and Sen 2012), but the experimental data did not agree with the equation. The coefficient of determination for the equation was low (R^2=0.2612) and hence the plot of $log(q_e/C_e)$ against $1/T$ at the temperature ranging from 30 °C to 50 °C did not show linearity. This is due to the finding that an increase of temperature (from 30 °C to 50 °C) did not markedly affect the adsorptive capacity.

The adsorption kinetics of CR on cassava residue was investigated with the pseudo-second-order adsorption model, since the applicability of the model has been noted in descriptions of the adsorption kinetics for many dyes (Vimonses *et al.* 2009; Senthil Kumar *et al.* 2010; Gautam *et al.* 2013). In the present study, the model was also fitted with data of the batch adsorption experiments at various conditions, for which all corresponding kinetic parameters from the model are listed in Table 1. The coefficient of determination (R^2) for the model had high values (> 0.99), and hence the plots of t/q_t against t at different conditions showed excellent linearity. It was suggested that the adsorption of CR on cassava residue was a multi-step process involving adsorption on the external surface and diffusion into the inner part of adsorbent.

The adsorption isotherm provides the maximum adsorptive capacity of adsorbent and describes interactions between the adsorbent surface phase and adsorbent, including multilayer adsorption and monolayer adsorption. Two equilibrium isotherm models are commonly employed in this regard: Langmuir and Freundlich models (Senthil Kumar *et al.* 2010; Gautam *et al.* 2013; OuYang *et al.* 2014). The equilibrium experimental data

were obtained from the batch adsorption experiment under the following conditions: temperature of 30 °C, pH 8.5, cassava residue dosage of 0.1 g, adsorption time of 240 min, and CR concentration ranging from 50 mg/L to 400 mg/L. As shown in Table 2, the experimental data exhibited a better fit to the Langmuir model since the value of the coefficient of determination ($R_F^2 < 0.95$) for the Freundlich model was much less than the coefficient of determination ($R_L^2 > 0.99$) for the Langmuir model. The better fit to the Langmuir model indicated that CR adsorption on the cassava residue surface was monolayer adsorption, *i.e.*, an active site was once occupied by an adsorbate molecule. Table 2 also shows that the maximum adsorptive capacity (q_m) obtained from the Langmuir model was 59.2 mg/g. The CR adsorptive capacity of cassava residue was higher than that of many other adsorbents that were reported in a previous study (Dawood and Sen 2012), indicating that cassava residue is a promising adsorbent for CR removal from an aqueous solution.

Table 2. Adsorption Isotherm Models of Congo Red on Cassava Residue

Isotherm models	Parameters	Values
Langmuir	q_m	59.2
	K_a	0.14
	R_L^2	0.999
Freundlich	n	4.77
	K_f	19.48
	R_F^2	0.912

For a solid/liquid adsorption process, the steps of adsorption are typically as follows: film diffusion, adsorption onto active sites of adsorbent, and intra-particle diffusion. The controlling step of adsorption is either intra-particle diffusion or film diffusion. However, the controlling step may be distributed between intra-particle diffusion and film diffusion (Qiu *et al.* 2009; Sen *et al.* 2011; Dawood and Sen 2012). From Table 3, it was found that the experimental data under different conditions were neither fitted to the film diffusion model nor fitted to the intra-particle diffusion model. The correlation coefficient values for the film diffusion model and intra-particle diffusion model were both low (< 0.95). These observations were consistent with those previously reported by other researchers for the adsorption of different dyes using pine cone biomass (Sen *et al.* 2011; Dawood and Sen 2012). The results suggested that the controlling step of CR adsorption on cassava residue was distributed between the intra-particle diffusion and film diffusion.

In vivo Decolorization of CR-loaded Cassava Residue with *Trametes* sp. SYBC-L4

White-rot fungi can effectively degrade and decolorize synthetic dyes mainly due to their extra-cellular ligninolytic enzymes, such as laccase, manganese peroxidase, and lignin peroxidase (Singh and Arora 2011; Zeng *et al.* 2011; Selvam *et al.* 2012). The production of ligninolytic enzymes was dependent on strains, cultivation methods, medium components, *etc.* (Levin *et al.* 2005; Winquist *et al.* 2008; Wan and Li 2012).

Table 3. Parameters of Intra-particle Diffusion and Film Diffusion Models for Congo Red Adsorption on Cassava Residue

Parameters	K_{id}	I	$R_{intra\text{-}particle}^2$	K_b	R_{film}^2
pH					
5.5	2.46	8.49	0.834	0.021	0.914
6.5	2.37	8.44	0.827	0.018	0.807
7.5	2.48	9.21	0.816	0.021	0.885
8.5	2.41	10.80	0.752	0.022	0.742
Cassava residue dosages (g)					
0.05	3.33	8.88	0.885	0.023	0.920
0.1	2.41	10.80	0.752	0.022	0.742
0.2	1.36	7.23	0.683	0.039	0.927
0.4	0.66	4.13	0.612	0.039	0.433
Congo red concentration (mg/L)					
50	1.32	7.27	0.667	0.035	0.803
100	2.41	10.80	0.752	0.022	0.742
200	3.29	11.05	0.837	0.019	0.775
300	3.42	13.95	0.785	0.023	0.813
400	3.54	13.48	0.803	0.026	0.920
Temperature (°C)					
30	2.41	10.80	0.752	0.022	0.742
40	2.53	9.18	0.821	0.023	0.924
50	2.48	9.08	0.819	0.022	0.924

During the process of *in vivo* decolorization of CR-loaded cassava residue with *Trametes* sp. SYBC-L4, laccase was the only ligninolytic enzyme involved in the decolorization. There were no other ligninolytic enzymes detected in the culture (data not shown). The observation was consistent with the findings in fungi *Ganoderma lucidum* and *Trametes trogii*, which secreted only laccase that contributed to dye decolorization (Zeng *et al.* 2011). The involvement of laccase derived from *Trametes* sp. SYBC-L4 in decolorization of CR was revealed by native-PAGE. As shown in Fig. 2, the laccase

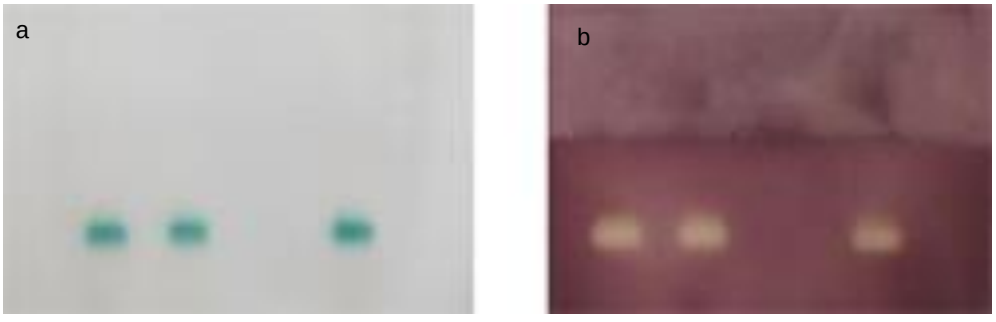

Fig. 2. Native-PAGE results of laccase staining with (a) 0.5 mM ABTS; (b) 0.5 mM Congo red: triplicated samples were performed in native-PAGE

activity band was detected after staining with ABTS. Additionally, a clear white band (decolorized zone) was observed against a red background after staining with CR.

The effects of cultivation conditions, including moisture content, temperature, and pH, on the performance of *in vivo* decolorization were further investigated. Time courses of laccase activity, decolorization, and weight loss are shown in Fig. 3.

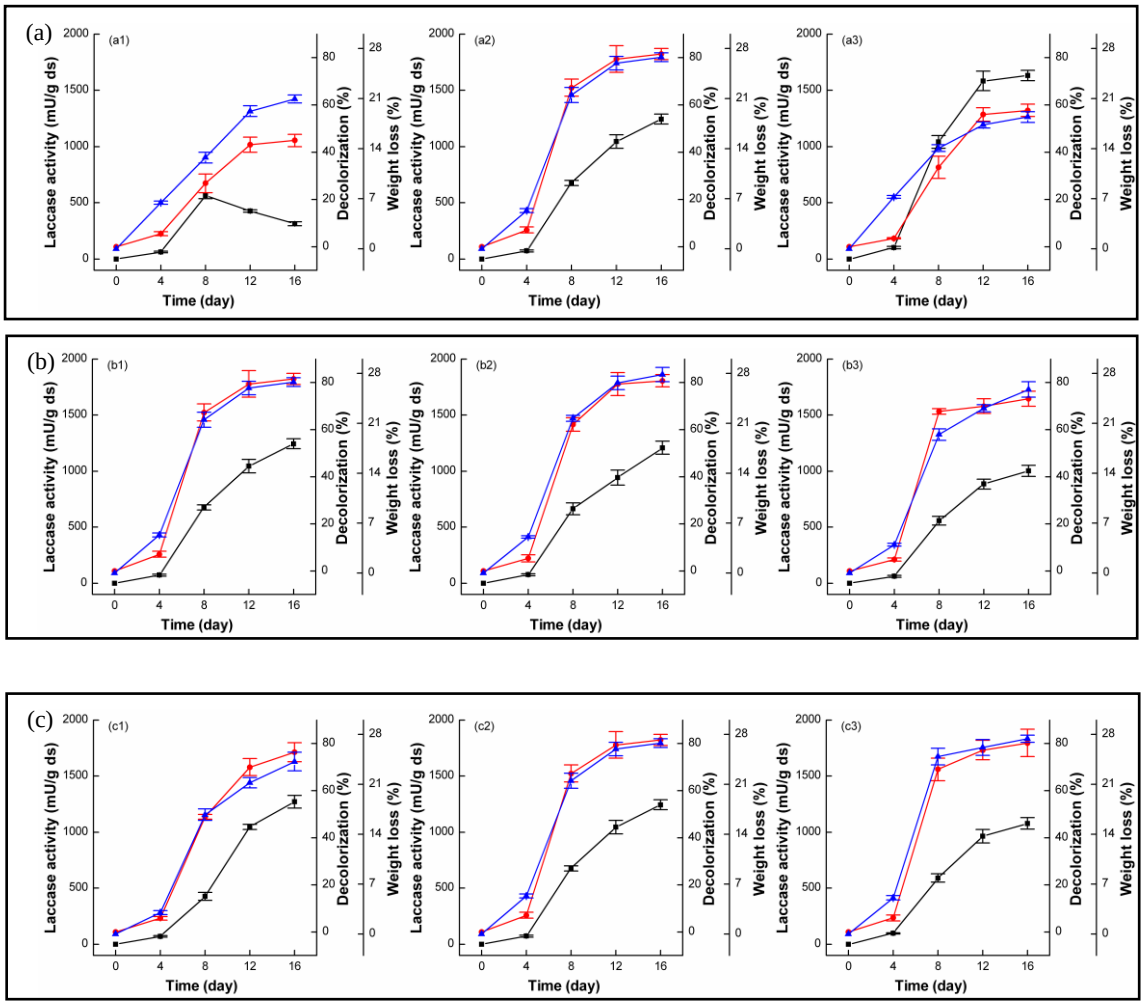

Fig. 3. Time courses of decolorization, laccase activity, and weight loss in the process of *in vivo* decolorization using *Trametes* sp. SYBC-L4: (a) effect of moisture content at pH 5.5 and temperature of 30 °C on the performance of *in vivo* decolorization (a1, a2, and a3 indicate moisture contents of 40%, 60%, and 80%, respectively); (b) effect of pH at moisture content of 60% and temperature of 30 °C on the performance of *in vivo* decolorization (b1, b2, and b3 indicate pH values of 5.5, 7.0, and 8.5, respectively); (c) effect of temperature at moisture content of 60% and pH 5.5 on the performance of *in vivo* decolorization (c1, c2, and c3 indicate temperatures of 25 °C, 30 °C, and 35 °C, respectively) (■ indicates laccase activity, ● indicates decolorization, ▲ indicates weight loss)

It was found that CR decolorization and weight loss increased with the extension of cultivation time. Decolorization was correlated with weight loss rather than laccase activity. This is attributed to the effect of fungal mycelium growth and extension on the decolorization (Papadopoulou *et al.* 2013). Moisture content considerably affected the performance of *in vivo* decolorization because water is a solvent for mass transfer and

oxygen diffusion in the process of *in vivo* decolorization. Low moisture content hampered the performance without providing sufficient water for fungal growth. On the other hand, high moisture content limited oxygen transfer, often inhibiting the performance of decolorization, as it is an oxidative process (Zadražil and Brunnert 1981; Wan and Li 2012). In the present work, the optimal moisture content was 60% in terms of decolorization (Fig. 3a). Fungi usually grow and metabolize in a mildly acidic and mild temperature environment (Zadražil and Brunnert 1981; Wan and Li 2012). The effects of pH (Fig. 3b) and temperature (Fig. 3c) on the performance of *in vivo* decolorization were less considerable than moisture content. After sixteen days of cultivation, decolorization was approximately 80% in the pH range of 5.5 to 7.0 and the temperature range of 30 to 35 °C. The maximal decolorization was 81.6 ± 2.4% under the conditions of pH 5.5, temperature 30 °C, and moisture content of 60%.

Characterization of Cassava Residue in a Novel Process for CR Decolorization

The results above indicated that cassava residue had not only large adsorptive capacity in CR but was also a solid substrate for the *in vivo* decolorization of CR with *Trametes* sp. SYBC-L4. Therefore, a novel process including adsorption and *in vivo* decolorization for CR removal from an aqueous solution was proposed in this study. CR was firstly adsorbed on cassava residue, and then CR-loaded cassava residue was decolorized by the fungi *via* an *in vivo* process. Scanning electron microscopy (SEM) images of cassava residue, CR-loaded cassava residue, and CR-loaded cassava residue after *in vivo* decolorization are shown in Fig. 4. The availability of pores and rough surface was clearly displayed in the SEM image of cassava residue (Fig. 4a), the morphological structure of cassava residue was not considerably different with that of CR-loaded cassava residue (Fig. 4b), indicating that the interaction between cassava residue and CR molecules did not markedly affect the morphological structure of cassava residue. Moreover, the morphological structure of CR-loaded cassava residue after *in vivo* decolorization (Fig. 4c) was loosed comparing with that of CR-loaded cassava residue. This is due to the biological action of *Trametes* sp. SYBC-L4 in the process of *in vivo* decolorization.

The FTIR spectra of cassava residue, CR-loaded cassava residue, and CR-loaded cassava residue after *in vivo* decolorization were further investigated and are shown in Fig. 5. FTIR spectroscopic analysis of cassava residue (Fig. 5a) indicated broad bands at 3415 cm^{-1}, representing hydroxyl (-OH) and amino (-NH) groups (Tamez Uddin *et al.* 2009; Zhou *et al.* 2011). The bands observed at 2920 cm^{-1} and 2845 cm^{-1} were assigned to stretching of -CH bond of methyl and methylene groups (Tamez Uddin *et al.* 2009). The band observed at 1731 cm^{-1} was assigned to carbonyl group (C=O) of unionized carboxylate stretching of carboxylic acid (Minamisawa *et al.* 2004; Tamez Uddin *et al.* 2009). The band observed at 1629 cm^{-1} was assigned to C=O group of acylamide (Zhou *et al.* 2011). The band observed at 1506 cm^{-1} was assigned to secondary amine group (Malkoc and Nuhoglu 2006). The bands observed at 1426 cm^{-1} and 1370 cm^{-1} were assigned to $-CH_3$ bending (Malkoc and Nuhoglu 2006; Senthil Kumar *et al.* 2010). The band observed at 1165 cm^{-1} was assigned to C-O stretching of ether group (Gautam *et al.* 2013). The bands at 500 to 800 cm^{-1} could be assigned to ester vibrations and monosubstituted aromatic rings, due to the lignin fraction of cassava residue biomass (Vieira *et al.* 2009; Gautam *et al.* 2013).

After adsorption of CR, the peaks of -OH and -NH groups and that of C=O group of unionized carboxylate stretching of carboxylic acid shifted from 3415 cm^{-1} and 1731 cm^{-1} to 3423 cm^{-1} and 1737 cm^{-1}, respectively (Fig. 5b). It was suggested that hydroxyl (-OH), amino (-NH) and carbonyl (C=O) groups were the potential adsorption sites for interaction with CR molecules, and the predominant interaction might be hydrogen bonding. Similar conclusions have been reported in previous research on dye adsorption using other lignocellulosic biomasses (Tamez Uddin *et al*. 2009; Zhou *et al*. 2011). After *in vivo* decolorization with *Trametes* sp. SYBC-L4, the peaks of C=O of unionized carboxylate stretching of carboxylic acid (1731 cm^{-1}), secondary amine group (1506 cm^{-1}), -CH_3 bending (1426 cm^{-1}), and that of C-O stretching of ether group (1165 cm^{-1}) were all weakened (Fig. 5c), indicating structural modification of cassava residue since the residue is a substrate for the *in vivo* decolorization of CR.

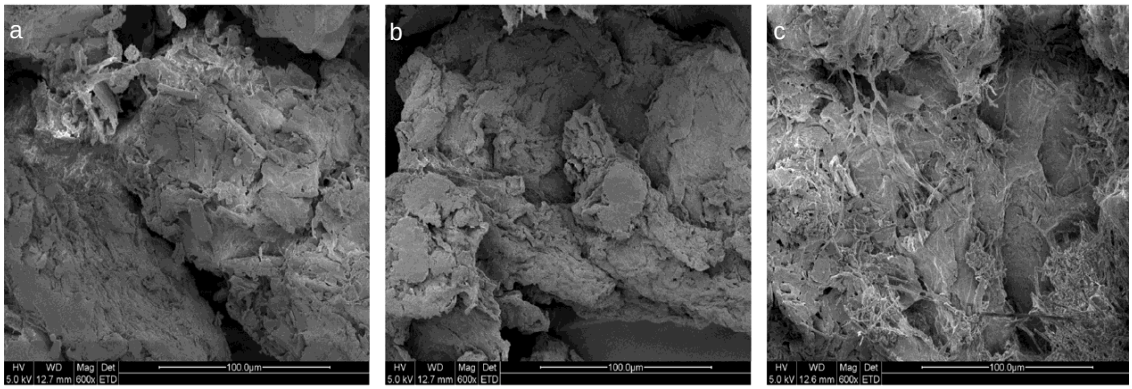

Fig. 4. SEM images (magnification×600) of (a) cassava residue, (b) Congo red-loaded cassava residue, and (c) Congo red-loaded cassava residue after *in vivo* decolorization

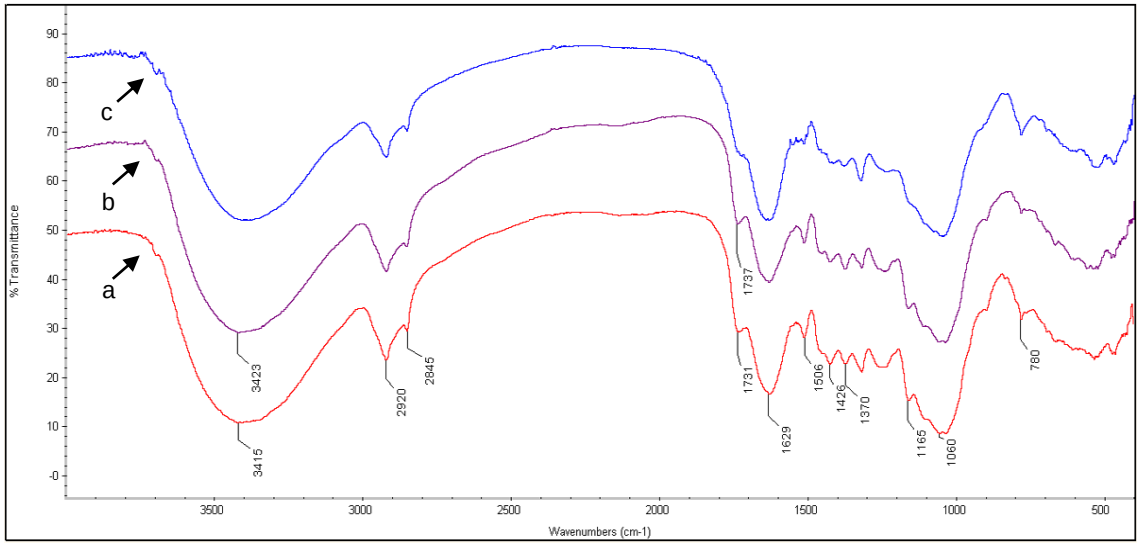

Fig. 5. FTIR spectra of (a) cassava residue, (b) Congo red-loaded cassava residue, and (c) Congo red-loaded cassava residue after *in vivo* decolorization

CONCLUSIONS

1. Cassava residue, a lignocellulosic waste, had not only large adsorptive capacity in Congo red (CR), but was also a solid substrate for the *in vivo* decolorization of CR with *Trametes* sp. SYBC-L4. The maximum adsorptive capacity was 59.2 mg/g ($m_{CR}/m_{cassava\ residue}$) and the maximum decolorization achieved was 81.6 ± 2.4% after sixteen days of cultivation.
2. Carbonyl (C=O), hydroxyl (-OH) and amino (-NH) groups in cassava residue were potential adsorption sites for interaction with CR molecules. The adsorption was considerably affected by adsorbent (cassava residue) dosage and CR concentration. Models of Langmuir and pseudo-second-order were found to fit well with the data of equilibrium adsorption and kinetics adsorption, respectively.
3. In the process of *in vivo* decolorization, CR decolorization correlated with weight loss rather than laccase activity. The effect of moisture content on the performance of *in vivo* decolorization was more considerable than that of pH and temperature. In terms of decolorization, the optimal cultivation conditions were pH 5.5, temperature 30 °C, and moisture content 60%.

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