Research Article

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Adsorption of copper ions on *Magnolia officinalis* residues after solid-phase fermentation with *Phanerochaete chrysosporium*

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Abstract: The disposal of residues while manufacturing Chinese medicine has always been an issue that concerns pharmaceutical factories. *Phanerochaete chrysosporium* was inoculated into the residues of *Magnolia officinalis* for solid-phase fermentation to enzymatically hydrolyze the lignin in the residues and thus to improve the efficiency of removal of the copper ions from residues for the utilization of residues from Chinese medicine. With the increase in activities of lignin-degrading enzymes, especially during the fermentation days 6 to 9, the removal rate of copper ions using *M. officinalis* residues increased dramatically. The rate of removal reached the maximum on the 14th day and was 3.15 times higher than the initial value. The rate of adsorption of copper ions on the fermentation-modified *M. officinalis* residues followed the pseudo-second-order kinetics. The adsorption isotherms were consistent with the Freundlich models. The adsorption enthalpy was positive, indicating that it was endothermic and elevation in temperature was favorable to this adsorption process. The adsorption free energy was negative, implying the spontaneity of the process. The copper ions adsorbed could be effectively recovered using 0.2 M hydrochloric acid solution. After five successive cycles of adsorption-regeneration, the fermentation-modified *M. officinalis* residues exhibited a stable adsorption capacity and greater reusability. The *M. officinalis* residues fermented with *P. chrysosporium* are low-cost and environmentally

friendly copper ions adsorbent, and this preparation technique realizes the optimum utilization of Chinese medicine residues.

Keywords: *Magnolia officinalis* residues; fermentation modification; adsorbent; copper ion; adsorption model.

1 Introduction

Clean water resources are requisite for the ecological environment. Due to the booming industries for metal smelting, electroplating, printed circuit board, and others, the amount of wastewater discharge containing copper ions has been increased substantially, resulting in the pollution of a large amount of soil and surface water and jeopardizing the environment [1]. Copper ions are widely dispersed, difficult to degrade, and are eventually captured by animals and plants through bioaccumulation, or get into human bodies through the food chain. The excess copper ions cause stomach discomfort and ulcers, liver and brain damage, etc. [2]. The allowable upper limit of copper content in drinking water is 1.3 mg/L [3]; therefore, removing excess copper from drinking water is an urgent environmental issue globally.

The traditional copper-removing methods include coagulation, precipitation, membrane separation, extraction, evaporation [4], which require equipments, high cost, high energy, and toxic chemicals. While these approaches are mainly applied to treat concentrated copper-containing wastewater, adsorption is a common and easy method to treat dilute wastewater [5]. Recently, many studies have shown that microorganism biomass can be used as adsorbent to remove micro-pollutants from wastewater [6,7,8]. The microorganism biomass are economic materials because they are easy to grow using inexpensive and abundant carbon sources. Furthermore, additional functional groups such as amino, carboxyl,

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hydroxyl and sulfate can improve the adsorption capacity as adsorption capacity depending on surface properties and functional groups [9,10,11]. However, the strength of microbial biomass is usually poor, and they are easy to be compressed and deformed, which affecting their practical application. Moreover, the cost of chemically modified adsorbents is usually high, considering the large volumes of dilute copper-containing wastewater. It is necessary to develop cheaper and eco-friendly alternative adsorbents.

China is a leading producer and user of Chinese herbal medicines. After the extraction of effective medical ingredients from herbs, a large number of residues are treated as a waste [12], which not only increase the production costs but also have a negative impact on the environment. These residues are rich in lignocellulose and various functional groups, which are easy to interact with heavy metals, so they could potentially function as adsorbents. However, the adsorption capacity of natural Chinese medicine residues is relatively low. Hence, they are modified using acid, alkali, oxidant and other reagents to improve the adsorption capacity. Although these methods favorably enhance the capacity, a large amount of effluent containing acid, alkali, oxidant and other chemicals are discharged, resulting in secondary contamination [13].

Magnolia officinalis residue is the waste of pharmaceutical factory. It's cheap and easy to get. There is no report about *Magnolia officinalis* residue as an adsorbent. Compared with microbial biomass, *Magnolia officinalis* residue is not easy to be compressed and deformed, which is more conducive to practical application. Further, it is necessary to modify *Magnolia officinalis* residue by appropriate methods to improve the adsorption capacity. In this work, it is treated by solid-phase fermentation. White rot fungi *Phanerochaete chrysosporium* (*P. chrysosporium*) can grow on a lignocellulosic substrate, and they can make use of lignocellulosic materials as carbon source, secrete lignin-degrading enzymes [14], which are capable of partially hydrolyzing the lignin structure. The objectives of this study were to determine the suitable fermentation conditions for modification of *Magnolia officinalis* residue by *P. chrysosporium*, and prepare biosorbent capable of adsorbing copper ions with higher adsorption capacity in solution. Compared with chemical modification, this treatment process is simple and mild, no toxic and expensive chemical reagents are used, and the secondary pollution to the environment is less.

2 Materials and methods

2.1 Reagents and strains

Magnolia officinalis residues were obtained after the extraction of magnolol from *Magnolia officinalis*. The residues were thoroughly washed with deionized water, and were then dried at 80°C for 48 h in an oven. The dry solid was shredded with a grinder, and the 60-mesh particles were collected for fermentation. *Phanerochaete chrysosporium* CICC 40299 was purchased from the Industrial Microbial Species Conservation Center (Beijing, China). Potato liquid medium was prepared as follows: 200 g of potato, 20 g of glucose, 3 g of KH_2PO_4 , 1.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g of vitamin B₁, and 1000 g of water. The solid medium was prepared by adding 2% agar powder to the above liquid medium. Solid-phase fermentation medium was prepared as follows: 0.06 g of glucose, 0.10 g of KH_2PO_4 , and 0.10 g of NH_4Cl was added to 45 mL of 10% wheat bran extract. An original solution of copper ions was prepared as follows: anhydrous copper sulfate powder was dried in an oven at 80°C for 4 hours, and then 1.2559 g of copper sulfate was dissolved in distilled water to form a 500-mL 1000 mg/L solution. In the experiment, the original solution was diluted with distilled water to derive a target concentration. All reagents used had analytical or biochemical purity.

2.2 Solid-phase fermentation of *Magnolia officinalis* residues with *Phanerochaete chrysosporium*

The spores of *Phanerochaete chrysosporium* were sprayed on the solid potato medium for activation. Simultaneously, 200 μL of spore suspension were inoculated into 100 mL of liquid potato medium for proliferation. The media were cultured in a thermostatic shaker at 30°C and 180 r/min for vigorous growth.

A 15 g portion of *Magnolia officinalis* residues were placed into a 500-mL conical bottle, and was wetted with 45 mL of the solid-phase fermentation medium. The residues were sterilized at 115°C for 30 min, and were cooled to room temperature. After inoculated with 3 mL of *Phanerochaete chrysosporium* suspension, the residues were placed into a thermostatic incubator at 30°C for solid-phase fermentation. During the fermentation, water was added regularly to maintain the humidity of the residues, and the activity of lignin peroxidase was detected daily. After two weeks of fermentation, the solid-

phase fermentation products were washed 4–5 times with deionized water, and were then dried in an oven at 80°C until the weight was constant. Thus, the fermented *Magnolia officinalis* residues were obtained.

2.3 Adsorbent Characterization

The samples were stored at room temperature for 2 weeks before characterization analysis. Water content of samples were determined by weight loss at 110°C long enough to keep the weight constant. The specific surface area of the material was determined by nitrogen adsorption method (Nova 4200e adsorption analyzer, Conta Instruments Company, USA) and calculated by Brunauer-Emmet-Teller (BET) method based on adsorption isotherm. Material surface micro-morphology was obtained by field emission scanning electron microscopy (Hitachi S4800, Japan). A Fourier transform infrared (FT-IR) spectroscopy (SP400, PE Company, USA) was used to determine the presence of functional groups in the adsorbents at room temperature using KBr as background over the range of 4 000 to 400 cm^{-1} .

2.4 Detection of extracellular enzyme activity

During the fermentation, 0.5 g of the solid-phase fermentation culture was sampled every day. 25 mL of distilled water was used to extract the crude enzymes in the culture. The extract was filtered and the extracellular enzyme activity of the filtrate was determined by spectrophotometry. The activity of lignin peroxidase (LiP) was measured with the method documented in the literature [15]: An LiP activity unit (U) was defined as the amount of enzyme required to oxidize 1 μmol of veratrol into veratral per minute. In detail, 2.9 mL of a reaction system, including 1.5 mL of a 0.1 mol/L tartaric acid buffer (pH = 3.0), 1.0 mL of 10 mmol/L veratrol (1.0 mL of buffer in the control group), and 0.4 mL of a crude enzyme solution, was prepared in the first place. Then, at 37°C, 0.1 mL of 10 mmol/L H_2O_2 was added to the system to commence reactions. The veratral formed was determined by measuring the increasing rate of absorbance value at 310 nm. On the other hand, the activity of manganese peroxidase (MnP) was determined following the approach proposed by Huang et al. [16]. A unit of MnP activity (U) was defined as the amount of enzyme required to oxidize 1 μmol of Mn^{2+} into Mn^{3+} per minute. In detail, 2.9 mL of a reaction system, including 2.4 mL of a 50 mM sodium

succinate (pH = 4.5), 0.1 mL of 15 mM MnSO_4 (0.1 mL of sodium succinate in the control group), and 0.4 mL of a crude enzyme solution, was prepared. Then, 0.1 mL of 10 mmol/L H_2O_2 was added to the reaction system to start reactions at 37°C. The complex of Mn^{3+} and succinic acid formed was determined by measuring the increasing rate of absorbance value at 240 nm.

2.5 Determination of copper ion concentration by spectrophotometry with sodium diethyldithiocarbamate (DDTC-Na)

0.0270 g of DDTC-Na was dissolved in 1 L of distilled water to derive a 27 mg/L DDTC-Na solution stored in a brown bottle. 0, 0.4, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mL of a 10 mg/L copper ion solution was separately mixed with the DDTC-Na solution of which the volume was 1.5 times that of the corresponding copper solution. ~30 mL of distilled water was added, and the pH was adjusted to be 9.0 with a 1% ammonia solution. The volume of the mixture was increased to 50 mL with distilled water. The absorbance of these copper ion solutions at 452 nm was determined [17]. The calibration curve of absorbance values against copper ion concentrations was thus obtained. The copper ion concentration of a solution would be thus determined by measuring its absorbance at 452 nm to match the point on the calibration curve. The concentration should lie in the linear range of the curve, or else the solution tested should be diluted properly.

2.6 Comparison of Cu^{2+} adsorption capacities of the fermented and unfermented residues

A 0.1 g portion of the fermented and unfermented *Magnolia officinalis* residues were separately added to 100 mL of 100 mg/L copper ion solutions. The mixtures were placed in a shaker at 120 r/min for adsorption at 30°C for 6 hours. Then, 1 mL of filtrate was separately withdrawn and their absorbance values were determined by the above-mentioned DDTC-Na method. The copper ion concentrations of both filtrate samples were thus calculated on the basis of the calibration curve. The copper ion removal rates and the adsorption capacity values were calculated as follows [18]:

$$\text{Removal rate(\%)} = \left(\frac{C_0 - C_t}{C_0} \right) \times 100\% \quad (1)$$

$$q_t = \frac{(C_0 - C_t)V}{m} \quad (2)$$

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (3)$$

wherein C_0 represents the initial content of copper ions (mg/L); C_t corresponds to the content of copper ions at time t (mg/L) and q_t is the adsorption capacity at time t (mg/g); C_e represents the equilibrium Cu^{2+} content in the solution at the end of adsorption (mg/L) and q_e is the equilibrium adsorption capacity (mg/g). V represents the volume (L) of the adsorption system and m represents the mass of the adsorbent (g). Statistical variance analysis was carried out to examine the difference of copper ion adsorption removal rate between fermented and unfermented *Magnolia officinalis* residues.

2.7 Adsorption kinetics experiment

Typically, 0.2 g of an adsorbent was added to 100 mL of a solution with an initial Cu^{2+} content of 100 mg/L and pH of 5.0. The mixture was shaken in a water bath at 30°C. The mixture was separately sampled after 10, 20, 30, 60, 90, and 120 min, and the samples were centrifuged to derive clean solutions. The Cu^{2+} contents of these solutions were determined to calculate the adsorption capacity values at different times.

2.8 Adsorption thermodynamics experiment

A 0.2 g portion of an adsorbent was separately added to 100 mL of solutions with pH of 5.0 and Cu^{2+} contents of 20, 40, 60, 80, 100 and 120 mg/L. These systems were shaken in a water bath at 30°C for 120 min. Transparent solutions were obtained through centrifugation. The residual Cu^{2+} contents at adsorption equilibrium states were determined for the calculation of equilibrium adsorption capacity q_e (mg/g).

2.9 Desorption

The copper ions adsorbed could be recovered with 0.2 M hydrochloric acid solution at 25°C and 150 r/min shaking for 2 hours for desorption, the regenerated adsorbent was then used in the next adsorption experiment. The reutilization performance of the fermented *Magnolia officinalis* residues was studied by a 5-round adsorption and desorption regeneration experiment. All experiments

Figure 1: Purity detection of the *Phanerochaete chrysosporium* strain.

were repeated 3 times, and the results consisted of mean values and deviations.

2.10 Statistical analysis

All the experiments were performed in triplicate and the results obtained were statistically analyzed and presented as mean $\pm$ S.E. (standard error). The S.E. values have been displayed as Y-error bars in figures.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Solid-phase fermentation of *Magnolia officinalis* residues with *P. chrysosporium*

P. chrysosporium is a classic strain for lignin degradation [19]. The spore suspension was dispersed on the potato agar plate and cultured at 30°C for 5 days. Afterwards, white mycelia with the same shape covered the plate, suggesting that the purity of the strain was qualified. Then, enrichment culture of the strain was further carried out.

The spore suspension of *P. chrysosporium* was inoculated into the potato liquid medium. After incubated at 30°C and 120 r/min for 7 days in a shaker, the medium was covered with yellow mycelia (no mycelial spheres), indicating that enrichment culture was completed and the liquid could be used for solid-state fermentation inoculation.

The contents of various components, including bran extract, glucose, KH_2PO_4 , and NH_4Cl , in the solid-phase fermentation substrate had significant effects on mycelial growth and enzymes production. The composition of solid-phase substrate was optimized and listed as follows: 15 g of *Magnolia officinalis* residues were mixed

Figure 2: Enrichment culture of *Phanerochaete chrysosporium*.

Figure 3: On day 9 during the fermentation of *Phanerochaete chrysosporium* on the *Magnolia officinalis* residues.

with 45 mL of 10% bran extract, 0.08 g of glucose, 0.10 g of KH_2PO_4 and 0.21 g of NH_4Cl . After the inoculation of 3 mL of culture medium of *P. chrysosporium* into the solid-phase substrate, the mixture was incubated in an incubator at 30°C. During the fermentation, sterile water was added to maintain a wet state of the solid-phase substrate. At the initial stage, the mycelia grew slowly; From day 5 to 9, the mycelia grew fast, and the surface and interior of the residues were covered with white mycelia (Figure 3). Afterwards, the mycelia stopped growing even if the incubation continued. Finally, the fermentation terminated on day 14.

Figure 4: Activities of LiP and MnP during the solid-state fermentation.

3.2 Changes of lignin peroxidase activity during the fermentation

During the solid-phase fermentation, *P. chrysosporium* grow on solid materials, they usually make use of lignocellulosic materials as carbon source, and their hyphae can grow on particle surfaces and penetrate into the interparticle spaces and, therefore, degrade the lignocellulosic materials by the secretion of lignocellulosic enzyme [14]. LiP and MnP enzymes were produced during the solid-phase fermentation of *Magnolia officinalis* residues with *P. chrysosporium*. The relationship of enzymes activity against fermentation days is depicted in Figure 4.

The enzymes activity was detected in the second day of fermentation. On day 7, the enzyme activities of LiP and MnP in the medium reached the maximum, and the maximum LiP enzyme activity in the *Magnolia officinalis* residues substrate was 35.37 U/g, MnP enzyme activity was 20.51 U/g. The enzyme activity gradually decreased after day 7.

3.3 Structural Characterization

The water content, specific surface area and surface morphology of *Magnolia officinalis* adsorbents before and after fermentation were analyzed to understand the structural changes caused by solid-state fermentation. From Figure 5, it can be seen that the water content increased from 4.06% to 4.97%, and the specific surface area increased from 0.394 m^2/g to 1.013 m^2/g , which increased to 1.22 times and 2.57 times respectively. T-test

Figure 5: Comparison of water content (a), specific surface area (b) and surface morphology of *Magnolia officinalis* adsorbent before (c) and after fermentation (d). (The statistical significance difference ($p < 0.001$) is marked).

Figure 6: The calibration curve for copper ion determination.

showed that both the water content and specific surface area increase obviously ($P < 0.001$). Scanning electron microscopy (SEM) images showed that after fermentation, the surface of adsorbent is more uneven, there are more layered structure surfaces and holes, so the specific surface area is greatly increased.

3.4 Detection of copper ion concentration

In the ammonia solution ($pH = 9$), the copper ions reacted with sodium diethyldithiocarbamate (DDTC-Na) to form yellow complexes. The yellow color could last for about 1 h. The absorbance values of copper ion solutions with different contents at 452 nm were determined with a spectrophotometer. The absorbance values had a linear relationship with copper ion contents, i.e. calibration curve, as shown in Figure 6. Thus, the content of copper ions can be quantified based on the calibration curve.

The calibration curve accorded with the linear equation $A_{452\text{ nm}} = 0.00412x + 0.00107$ ($R^2 = 0.99881$) in the copper ion range of 0–40 mg/L. The content of copper ions in a sample was calculated as follows: the concentration in the 50 mL of solution (see the experimental parts) was quantified according to the calibration curve and absorbance value. Then, the content of the original sample (mg/L) was obtained by correcting the volume.

3.5 Effect of fermentation time of *Magnolia officinalis* residues on the copper ion removal rate

After washing and drying, 0.2 g of *Magnolia officinalis* residues with different fermentation duration were separately added to a 100 mL of adsorption system with the initial copper ion content of 100 mg/L. The adsorption took place at 30°C for 6 h, and the system was stirred at 120 r/min. The removal rates of copper ions are shown in Figure 7. In the initial stage of fermentation, the mycelia grew slowly and produced a small amount of enzymes, and thereby the removal rate increased very slowly. From the day 6 to 9, the mycelia grew fast and produced a large amount of enzymes, and thereby the removal rate significantly increased. From the day 10 to 14, the mycelia stopped growing and produced less and less enzymes, and thereby the removal rate increased very slowly. In the day 14, the removal rate reached a maximum, 3.15 times that of the initial. The reason may be that the lignin peroxidase and manganese peroxidase[20] secreted by the *P. chrysosporium* could partially degrade the lignin components in the residues, thus exposing more active sites for the adsorption of more copper ions.

3.6 Comparison of copper ion removal rates of fermented and unfermented *Magnolia officinalis* residues

In the initial copper ion content range of 50–300 mg/L, the copper ions removal rates using fermented and unfermented *Magnolia officinalis* residues were measured and the results are shown in Figure 8, and the corresponding variance analysis is shown in Table 1. Generally speaking, the removal rates using fermented *Magnolia officinalis* residues were evidently higher than those using the unfermented ($p < 0.05$). Especially, at the same concentration, statistical analysis shows extremely remarkable differences ($p < 0.001$). It is demonstrated that fermentation is an effective method to improve the adsorption capacity. It may be explained that when *P. chrysosporium* grow on a lignocellulosic substrate, the lignin-degrading enzymes they secreted can partially degrade the lignin structure of the *Magnolia officinalis* residues to expose more active sites for the increase in the adsorption capacity. When the initial content of copper ions was lower than 150 mg/L, all the removal rates using fermented residues were above 95%, and the concentrations of copper ions after adsorption met the safe drinking water standards. When the initial content

Figure 7: Effect of fermentation time on the copper ions removal rate (The initial content of copper ions was 100 mg/L. 0.2 g of the adsorbent was used).

Figure 8: Effect of initial copper ion contents on the removal rates of copper ions using the fermented and unfermented *Magnolia officinalis* residues (0.2 g of the adsorbent was used, and the adsorption occurred at 30°C with stirring at 120 r/min. The statistical significance difference ($p < 0.001$) is marked).

of copper ions was in the range of 200–300 mg/L, the removal rates using fermented residues were obviously decreased from 87.8% to 58.6%. Therefore, it is necessary to increase the dosage of *Magnolia officinalis* residues in the treatment of concentrated wastewater with the copper ion content of above 200 mg/L.

3.7 Adsorption kinetics

The relationships between Cu²⁺ adsorption capacities of the fermented residues and time at different temperatures

Table 1: variance analysis of the removal rate of copper ion by the fermented and unfermented *Magnolia officinalis* residues.

Source of Difference	SS	df	MS	F	P-value	F crit
copper ion content	5750.545	5	1150.109	0.2784017	0.914603	3.325835
fermented and unfermented	51288.3	2	25644.15	6.2075638	0.017672	4.102821
Error	41311.14	10	4131.114			
Total	98349.99	17				

are shown in Figure 9. Over time, the adsorption capacities increased. Due to the large initial mass transfer driving force and large number of vacancies on the surface of the adsorbent, the adsorption rates were high in 10–90 minutes. Along with the adsorption process, the concentrations of Cu^{2+} in the solutions decreased, and the diffusion resistance into the interior of the adsorbent was increased, resulting in a lower adsorption rate. The higher the adsorption temperature was, the greater the adsorption capacity was. An elevated temperature seemed to be favorable to the adsorption. After 120 min, the adsorption was in an equilibrium state. Hence, we chose 120 min as the adsorption time for an equilibrium state in the following sections.

The following linearized pseudo-first- and pseudo-second-order kinetic models [18] were used to fit the experimental data, separately.

$$\lg(q_e - q_t) = \lg(q_e) - \frac{k_1 t}{2.303} \quad (4)$$

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

where q_e and q_t represent the copper ions adsorbed on the adsorbent at equilibrium and at time t , whereas k_1 (min^{-1}) and k_2 (g/mg/min) are the rate constants for pseudo-first- and pseudo-second-order kinetic models, respectively.

The fitting results of both models are described in Table 2. The pseudo-second-order kinetic model had a better fitting performance because of the higher R^2 values, indicating that the kinetic behavior of Cu^{2+} adsorption process of fermented *Magnolia officinalis* residues with chemical adsorption process being the rate controlling step.

The activation energy for the adsorption system is an important parameter to determine the adsorption type. Generally, it is very small for physical adsorption, while it is larger for chemical adsorption. The adsorption rate constant obtained by fitting the pseudo-second-order

Figure 9: Relationships between Cu^{2+} adsorption capacities of the fermented residues and time at different temperatures.

kinetic model at different temperature can be introduced into the linearized Arrhenius equation [21]:

$$\ln k_2 = \ln A - \frac{E_a}{RT} \quad (6)$$

where k_2 is the pseudo-second-order kinetic rate constant, A is Arrhenius factor, E_a is the activation energy of adsorption (J/mol), R is the universal gas constant (8.314 J/mol/K), T shows the absolute temperature (K).

To find E_a , the adsorption runs were conducted at five different temperatures (20, 25, 30, 35 and 40°C), and the relationship between $\ln k_2$ and $1/T$ was shown in Figure 10. The values of E_a was determined from the slope of the plot of $\ln k_2$ versus $1/T$ (Figure 9), and was quantified to be 13.373 kJ/mol . According to the literature [1,21], the activation energy E_a is between 8 and 16 kJ/mol corresponding to chemical adsorption. Therefore, the Cu^{2+} adsorption on the fermented *Magnolia officinalis* residues is not only including physical adsorption, but may also including ion exchange, electrostatic and complexation.

Table 2: Fitting results of kinetic models for Cu^{2+} adsorption on the fermented *Magnolia officinalis* residues.

T/K	Pseudo-first order kinetic model			Pseudo-second order kinetic model		
	$q_e/\text{mg}\cdot\text{g}^{-1}$	k_1/min^{-1}	R^2	$k_2/\text{g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$	$q_e/\text{mg}\cdot\text{g}^{-1}$	R^2
293	57.890	3.662×10^{-2}	0.9341	4.42×10^{-4}	62.893	0.9830
298	56.403	3.685×10^{-2}	0.9899	4.99×10^{-4}	61.728	0.9858
303	56.637	3.823×10^{-2}	0.9354	5.47×10^{-4}	60.976	0.9884
308	57.188	3.961×10^{-2}	0.9331	5.91×10^{-4}	60.241	0.9899
313	57.227	4.076×10^{-2}	0.9373	6.29×10^{-4}	59.880	0.9921

Table 3: Fitting results of Cu^{2+} adsorption isotherms on the fermented *Magnolia officinalis* residues.

T/K	Langmuir isotherm			Freundlich isotherm		
	$Q_{\max}/\text{mg}\cdot\text{g}^{-1}$	KL	R2	KF	1/n	R2
293	131.578	0.1479	0.8446	16.406	0.8098	0.9600
303	185.185	0.1123	0.4348	18.088	0.8974	0.9286
313	277.778	0.0774	0.1908	12.930	0.9626	0.9230

3.8 Adsorption isotherms

The Cu^{2+} adsorption equilibrium data of fermented *Magnolia officinalis* residues are shown in Figure 11. With the increase of temperature, the adsorption capacity increased. The Langmuir and Freundlich models were independently fitted to the equilibrium data.

The Langmuir equation is the following:

$$\frac{c_e}{q_e} = \frac{c_e}{Q_{\max}} + \frac{1}{Q_{\max}K_L} \quad (7)$$

where $Q_{\max}$ is the maximum adsorption capacity (mg/g), and K_L is the Langmuir constant (L/mg).

The Freundlich equation is the following:

$$\lg q_e = \lg K_F + \frac{1}{n} \lg C_e \quad (8)$$

where K_F and n are the Freundlich adsorption isotherm constants, K_F represents the measure of adsorption capacity and $1/n$ represents the intensity of adsorption.

In the Langmuir model, the adsorption process is assumed to occur on a homogeneous surface by monolayer adsorption. However, the Freundlich model is described by an empirical model that considers the multilayer with reversible adsorption on a non-uniform distribution of the active site. The fitting results are shown in Table 3. The Cu^{2+} adsorption on the fermented *Magnolia officinalis* residues was in better agreement with the Freundlich equation, suggesting the adsorption capacity depends

Figure 10: The relationship between $\ln k_2$ and $1/T$. **Figure 11:** Cu^{2+} adsorption isotherms on the fermented *Magnolia officinalis* residues.

on the functional groups on the surface. The result is consistent with the kinetic analysis and activation energy analysis, as well as with Figure 7 results. Furthermore, with the increase of temperature, the adsorption capacity increased, and the increase of temperature was favorable to the adsorption.

Table 4: Fitting results of the thermodynamic equations for Cu²⁺ adsorption modeling.

Initial content / mg·L ⁻¹	ΔH / kJ·mol ⁻¹	ΔS / J·(mol·K) ⁻¹	ΔG / kJ·mol ⁻¹		
			293K	303K	313K
20	1.942	29.457	-6.689	-6.984	-7.278
40	5.837	42.844	-6.717	-7.145	-7.574
60	10.673	60.571	-7.074	-7.680	-8.286
80	14.208	71.160	-6.642	-7.354	-8.065
100	18.519	84.753	-6.313	-7.161	-8.008
120	14.071	67.299	-5.647	-6.320	-6.993

3.9 Adsorption thermodynamics

The thermodynamic equations for modeling are presented as follows[18].

$$K_d = \frac{q_e}{C_e} \quad (9)$$

$$\ln K_d = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (10)$$

$$\Delta G = \Delta H - T\Delta S \quad (11)$$

where ΔH is the enthalpy change, ΔG is Gibbs free energy, ΔS is the entropy change. The values of ΔH and ΔS were determined from the slope and intercept of the van't Hoff plot of $\ln K_d$ versus $1/T$.

These equations were fitted to the experimental data, and the results are given in Table 4.

The results in Table 4 show that the enthalpy change (ΔH) was positive, indicating that the reaction was endothermic, and the adsorption capacity increased with the increase of temperature. The Gibbs free energy (ΔG) was negative, indicating that the Cu²⁺ adsorption on the fermented *Magnolia officinalis* residues was a spontaneous process. The entropy change ΔS was positive, indicating that the chaos degree at the solid-liquid interface increased in the course of adsorption.

3.10 Fourier transform infrared spectroscopy

The Fourier transform infrared spectroscopy (FT-IR) spectra of the fermented *Magnolia officinalis* residues before and after the Cu²⁺ adsorption are shown in Figure 12. The following bands were changed significantly after the adsorption process: the intense and broad band at

Figure 12: FT-IR spectra of the fermented *Magnolia officinalis* residues before and after the adsorption of Cu²⁺.

3314 cm⁻¹ corresponding to the stretching vibration of O-H in -COOH of *Magnolia officinalis* cellulose, the band at 2922 cm⁻¹ corresponding to the asymmetric stretching vibration of C-H in -CH₃ of cellulose, the band at 1732 cm⁻¹ corresponding to the ester or carbonyl groups in hemicellulose, the band at 1605 cm⁻¹ corresponding to the stretching vibration of C=O in phosphates, the band at 1418 cm⁻¹ corresponding to the stretching vibration of C-O in carboxyl groups, the band at 1228 cm⁻¹ corresponding to the stretching vibration of C-O in lignin phenolic hydroxyl groups, and the band at 1015 cm⁻¹ corresponding to the stretching vibration of C-O-C in cellulose. These relative functional groups on *Magnolia officinalis* residues interacted with Cu²⁺ during the course of adsorption [18]. The result is consistent with the kinetic analysis, activation energy analysis and Freundlich models, suggesting the fermentation of *Magnolia officinalis* residue by *P. Chrysosporium* was advantageous to exposing functional groups and improving adsorption capacity.

3.11 Recycling of adsorbents

The reusability of any new type of adsorbent largely determined its practicality and cost effectiveness. The recycling performance of the fermented *Magnolia officinalis* residues is shown in Figure 13, and the corresponding variance analysis is shown in Table 5. In the 1-2 rounds of adsorption and desorption, the adsorption capacity decreased from 49.15 to 37.85 mg/g, indicating that there are some irreversible adsorption processes in the first cycle. However, in the subsequent rounds, there

Table 5: variance analysis of the recycling performance of adsorbent.

Source of Difference	SS	df	MS	F	P-value	F crit
Between groups	255.3401	4	63.83503	0.190953	0.939396	3.055568
In group	5014.465	15	334.2977			
Total	5269.805	19

Figure 13: The recycling performance of adsorbent (Desorption and regeneration of the fermented *Magnolia officinalis* residues. 0.2 g of the adsorbent was used, and the adsorption occurred at 25°C with stirring at 150 r/min).

were no significant decline ($p > 0.5$) as shown in Table 5. The adsorption capacity was maintained at approximately 36 mg/g.

4 Conclusions

Phanerochaete chrysosporium was inoculated into the residues of *Magnolia officinalis* for solid-phase fermentation. The mycelia grew vigorously and secreted lignin peroxidase and manganese peroxidase during the fermentation process. Over fermentation time, the activities of both enzymes increased gradually, and reached the highest values in the fermentation day 7. Afterwards, the activities decreased gradually and decreased to the initial levels in the fermentation day 14. On the other hand, over fermentation time, the removal rate of copper ions with *Magnolia officinalis* residues increased. Especially, from the day 6 to 9, the removal rate increased dramatically due to the increase of activities of lignin-degrading enzymes. Afterwards, although the enzymatic activities decreased gradually, the removal rate still increased gradually, and

reached the highest value in the fermentation day 14. The maximum removal rate was 3.15 times that of the initial value. In the range of initial copper ions content of 50–300 mg/L, in the fermentation day 14, the removal rate using the fermented *Magnolia officinalis* residues was significantly higher than that using the unfermented residues.

The adsorption of copper ions on the fermented *Magnolia officinalis* residues agreed with a pseudo-second-order kinetic model, suggesting that the kinetic behavior of Cu^{2+} adsorption process of fermented *Magnolia officinalis* residues with chemical adsorption process being the rate controlling step. Also, the values of E_a was quantified to be 13.373 kJ/mol, corresponding to chemical adsorption. The equilibrium data were in consistent with the Freundlich isotherms, which indicating the adsorption process occur on a non-uniform distribution of the active site. FT-IR spectra reveals some functional groups on *Magnolia officinalis* residues interacted with Cu^{2+} during the course of adsorption. All these results suggest the fermentation of *Magnolia officinalis* residue by *P. Chrysosporium* was advantageous to exposing functional groups and improving adsorption capacity. The negative Gibbs free energy shows this is a spontaneous process, and the adsorption enthalpy change was positive, indicating that the adsorption was endothermic and the increase of temperature was favorable to adsorption.

In summary, the preparation approach of copper ions adsorbents through the fermentation of *Magnolia officinalis* residues with *Phanerochaete chrysosporium* is efficient and eco-friendly, with low costs. The treatment process is simple and mild, no toxic and expensive chemical reagents are used, and the secondary pollution to the environment is less.

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