1. Introduction

The increasing worldwide contamination of surface waters by heavy metals is an important environmental problem facing humanity because heavy metals are not biodegradable and can be accumulated in living tissues [1–4]. Cadmium, copper, zinc, and their compounds are widely existent in surface water. Their removal and recovery from wastewater with low heavy metal concentration and continuously secrete extracellular substances during adsorption. The removal rate and recyclability were enhanced by living biomass biosorption. In addition, live P. chrysosporium could degrade organic pollutants (chlorophenol, synthetic dyes, etc.) during its growth process [15,16]. Live P. chrysosporium mycelia could degrade organic pollutants (chlorophenol, synthetic dyes, etc.) during its growth process [15,16]. Live P. chrysosporium have a vast potential to treat inorganic and organic polluted wastewater.

In this study, the use of live P. chrysosporium mycelia as biosorbent for Cd(II), Cu(II), and Zn(II) biosorption from artificial wastewaters was investigated. The maximum biosorption capacity of the fungal biomass was determined under pH-controlled conditions in simulated heavy metal contaminated wastewater. Different kinetic models were
compared in terms of their ability to describe Cd(II), Cu(II), and Zn(II) binding by *P. chrysosporium*. The effects of environmental conditions including pH, temperature, contact time, and concentration were studied. Further information regarding the binding mechanism was obtained from changes in pH during incremental addition of Cd(II), Cu(II), and Zn(II) to *P. chrysosporium* suspensions. Functional groups involved in metal binding were characterized by Fourier transform infrared (FT-IR) spectroscopy. Scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDAX) were used to describe the change in *P. chrysosporium* mycelia after adsorption.

2. Materials and methods

2.1. Biomass and culture medium

The *P. chrysosporium* BKM-F1767 (ATCC 24725) used in this study was purchased from the China Center for Type Culture Collection (Wuhan). The fungus was cultivated in a Kirk liquid culture medium using the shake flask method [17]. The pH of the medium was adjusted to 4.5 before autoclaving, and 100 mL of the Kirk liquid culture medium was used in each 250 mL flask. Every 100 mL liquid culture contained 5 × 10^7 spores using a turbidimeter (WGZ-200, Shanghai, China). Once inoculated, the flasks were incubated in an incubator at 150 rev/min for 3 days at 37°C.

2.2. Methods of adsorption studies

Each adsorption study was conducted by shaking the flasks at 150 rev/min for a certain period using an air bath shaker. After 3 days of cultivation, the heavy metal ion solution was added into the flasks. At the setting intervals, 1 mL of solution was sampled and centrifuged at 10,000 rev/min for 10 min before analyzing the heavy metal concentration with atomic absorption spectrometry (AAnalyst 700, Perkin Elmer). The pellets were dried at −40°C in a freezer dryer (FD-1, Boyikang, Beijing, China). Batch biosorption experiments were repeated three times. The amount of adsorbed metal ions per unit of dry biomass was calculated using the following equation:

\[
Q = \frac{(C_0 - C) \cdot V}{M},
\]

where \(Q\) is the amount of metal ions adsorbed onto the biomass (mg/g), \(C_0\) is the initial metal concentration and \(C\) is the final metal concentration after biosorption (mg/L), \(V\) is the volume of the medium (L), and \(M\) is the amount of the dry biomass (g).

2.2.1. Effect of pH on adsorption

The stored solutions of Cd(II) (10 g/L), Cu(II) (10 g/L), and Zn(II) (10 g/L) were respectively prepared by dissolving cadmium nitrate, cupric sulfate, and zinc sulfate in deionized water. After 3 days of cultivation, the pH of each solution in the flask was adjusted to the desired value. Because heavy metal precipitation could occur at higher pH values, a series of pH values were prepared at 3.5, 4.5, 5.5, 6.5, 7.5, and 8.5 by adding 1 mol/L HCl or 1 mol/L NaOH solution. The heavy metal solution was then added into each flask to an initial heavy metal concentration of 20 mg/L.

2.2.2. Effect of temperature and contact time on adsorption

Biosorption of *P. chrysosporium* was performed in the initial heavy metal concentration of 20 and 50 mg/L at 25, 30, 35, 37, 40, and 45°C. The pH of the solutions was adjusted to the optimum value for the biosorption of heavy metal with 1 mol/L HCl or 1 mol/L NaOH solution. Samples were taken at intervals of 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h to analyze the residual heavy metal concentrations.

2.2.3. Effect of initial concentration on adsorption

Another main factor that influences the uptake of heavy metals onto *P. chrysosporium* is the initial ion concentration. The effect of initial concentration on the adsorption capacity of *P. chrysosporium* was investigated at concentrations of 10, 20, 30, 40, 50, and 60 mg/L with 6 h of contact time at 37°C.

2.3. FT-IR experiments

The surface functional groups of *P. chrysosporium* were characterized using FT-IR analysis which was often used to evaluate the functional groups related to binding of heavy metal. Freeze-dried original *P. chrysosporium* and Cd(II)-loaded *P. chrysosporium* were mixed with KBr and compressed into thin films for FT-IR analysis (WQF-410, Beijing, China). The spectral range was 400–4000 cm\(^{-1}\).

2.4. SEM and EDAX analysis

SEM and EDAX analysis were used to characterize the changes in *P. chrysosporium* mycelia before and after the adsorption and the compositions of the crystal in the heavy metal loaded *P. chrysosporium*. Freeze-dried *P. chrysosporium* and heavy metal loaded *P. chrysosporium* were used for SEM (FEI QUANTA-200, FEI Company, the Netherlands). The heavy metal loaded *P. chrysosporium* was also used for EDAX analysis (FEI QUANTA-200, FEI Company, the Netherlands).

2.5. Kinetic experiments

To determine the equilibration time and the rate of heavy metal binding by *P. chrysosporium*, kinetics experiments are required. In this study, the experimental data were modeled by two kinetics models, the pseudo first-order
reaction [18] and the pseudo second-order reaction [19]. The pseudo first-order reaction assumes that the change in the number of surface sorption sites is proportional to the number of remaining free surface sites, and the pseudo second-order model assumes that the rate is proportional to the square of the number of remaining unoccupied surface sites [20].

The pseudo first-order model is given by

\[
\log(Q_e - Q_t) = \log Q_e - \frac{k_1}{2.303} t,
\]

and the pseudo second-order kinetic model is given by

\[
\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{1}{Q_e} t,
\]

where \(Q_e\) and \(Q_t\) are the adsorbed amounts (mg/g) per unit adsorbent at equilibrium and any time \(t\) (min), respectively; \(k_1\) is the pseudo first-order rate constant for the adsorption process (min\(^{-1}\)); \(k_2\) is the rate constant of pseudo second-order adsorption (g \cdot mg\(^{-1}\) \cdot min\(^{-1}\)).

3. Results and discussion

3.1. Effect of pH

The pH is an important factor in the adsorption process, which affects the activity of the functional groups of biomass [21]. The variations in pH greatly affected the uptake of heavy metal from the aqueous solution, as shown in Figure 1. When the pH was lower than 5.5, the uptake sharply increased with the increasing pH. However, when the pH was higher than 6.5, the uptake decreased. The maximum metal uptake was obtained at pH 5.5–6.5 with 23.89, 15.21, and 13.18 mg/g for Cd(II), Cu(II), and Zn(II), respectively. Several researchers have reported the effects of pH on heavy metal uptake using different kinds of P. chrysosporium. For example, the optimum pH for Cd(II) uptake was 6.0 for nonliving P. chrysosporium [10] and for loofah sponge-immobilized P. chrysosporium [5]. At lower pH, protonation of the cell wall components reduced the attraction between the biomass and the Cd(II). With increase of pH, more negatively charged groups were formed which facilitate heavy metal uptake.

3.2. Effect of temperature and contact time

To determine the effect of temperature and contact time, biosorption of heavy metals at temperatures ranging from 25 to 45°C were observed under the conditions of 50 mg/L of Cd(II), 20 mg/L of Cu(II), or 20 mg/L of Zn(II). The temperature significantly affected the uptake of Cd(II) by P. chrysosporium as shown in Figure 2. At 37°C, a noticeable increase of heavy metal uptake was observed. The Cd(II), Cu(II), and Zn(II) adsorption was 59.77, 15.21, and 13.18 mg/g, respectively. The reason for this phenomenon is that 37°C is the optimal temperature for the growth of P. chrysosporium. For example, the optimum pH for Cd(II) uptake was 6.0 for nonliving P. chrysosporium [10] and for loofah sponge-immobilized P. chrysosporium [5]. At lower pH, protonation of the cell wall components reduced the attraction between the biomass and the Cd(II). With increase of pH, more negatively charged groups were formed which facilitate heavy metal uptake.
weight of wet biomass in solution was 50 g/L. The increase of the initial concentration resulted in an increase in metal uptake by *P. chrysosporium*. However, when the metal concentration was too high, the uptake of ions decreased. The metal removal capacities of Cd(II), Cu(II), and Zn(II) achieved a maximum when the metal concentration was 47.2, 75.4, and 65.0 mg/L, respectively. This trend indicated that at lower metal concentrations, the binding sites were enough for adsorbing heavy metal. When the initial concentration increased, the percentage of metal removal is expected to be reduced even though the amount of metal uptake increases. That is to say, the increased initial concentration led to a lower ratio of the amount metal uptake to the initial amount of metal in the solution at the start of the experiment. However, at higher ion concentrations, the binding efficiency decreased, which could be attributed to the competition between the heavy metal ions for the sites available for the sorption process.

### 3.4. Batch kinetics

Adsorption kinetics is not only necessary for predicting the removal rate of a pollutant from aqueous solutions, but also necessary for providing valuable insights into the mechanism of sorption reactions [20]. The parameters for the two kinetics models and correlation coefficients of Cd(II) under different temperatures were calculated as listed in Table 1. Pseudo second-order kinetic parameters for the adsorption of Cd(II), Cu(II), and Zn(II) at various initial heavy metal concentrations by *P. chrysosporium* are listed in Table 2.

As shown in Table 1, the value of $r_2$ of the pseudo second-order kinetic model for Cd(II) is very high, followed by the pseudo first-order equation (Table 1). For the pseudo second-order model, the rate constant and the adsorbed amounts at equilibrium increased with the increase of temperature. These represented sorption processes predominantly followed by the pseudo second-order sorption mechanism, where the rate was proportional to the square of the number of remaining sites. If a divalent ion binds to two monovalent negatively charged surface sites, the rate should be proportional to the square of the free surface sites, which was the assumption of this model [18,20,22].

### Table 1. Kinetic parameters for the adsorption of Cd(II) at various temperatures by *P. chrysosporium*.

<table>
<thead>
<tr>
<th>$T$ (°C)</th>
<th>$k_1$ (Qe (mg/g))</th>
<th>$r_1$</th>
<th>$k_2$ (Qe (mg/g))</th>
<th>$r_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.0025</td>
<td>28.87</td>
<td>0.953</td>
<td>27.93</td>
</tr>
<tr>
<td>35</td>
<td>0.0071</td>
<td>43.23</td>
<td>0.817</td>
<td>47.39</td>
</tr>
<tr>
<td>40</td>
<td>0.0078</td>
<td>47.52</td>
<td>0.873</td>
<td>49.02</td>
</tr>
<tr>
<td>45</td>
<td>0.0083</td>
<td>46.20</td>
<td>0.992</td>
<td>47.62</td>
</tr>
</tbody>
</table>

*P. chrysosporium*. At this temperature, large quantities of proteins and amino acids are secreted by *P. chrysosporium*, which are available for binding of heavy metal.

The uptake equilibrium of Cd(II), Cu(II), and Zn(II) was achieved after 6 h and no obvious changes were observed for longer reaction times, as shown in Figure 3. The results suggested that a two-step mechanism occurs. The first portion represented a rapid adsorption during the first 3 h. The equilibrium time required for maximum uptake of heavy metal was 6 h at all setting temperatures.

#### 3.3. Effect of initial heavy metal concentration

The results of the removal of Cu(II), Cu(II), and Zn(II) in different initial concentrations are shown in Figure 4. The uptake equilibrium of Cd(II), Cu(II), and Zn(II) was achieved after 6 h at all setting temperatures.

### Table 2. Pseudo second-order kinetic parameters for the adsorption of Cd(II), Cu(II), and Zn(II) at various initial metal-ion concentrations by *P. chrysosporium*.

<table>
<thead>
<tr>
<th>Initial metal-ion concentration (mg/L)</th>
<th>Cd(II)</th>
<th>Cu(II)</th>
<th>Zn(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_2$</td>
<td>$Q_e$</td>
<td>$r_2$</td>
</tr>
<tr>
<td>9.85</td>
<td>0.105</td>
<td>12.21</td>
<td>0.9996</td>
</tr>
<tr>
<td>19.2</td>
<td>0.0093</td>
<td>25.19</td>
<td>0.998</td>
</tr>
<tr>
<td>28.45</td>
<td>0.0754</td>
<td>36.23</td>
<td>0.9994</td>
</tr>
<tr>
<td>40.4</td>
<td>0.044</td>
<td>54.05</td>
<td>0.9993</td>
</tr>
<tr>
<td>47.2</td>
<td>0.025</td>
<td>66.22</td>
<td>0.9996</td>
</tr>
</tbody>
</table>

Figure 4. Effects of initial metal-ion concentration on the Cd(II), Cu(II), and Zn(II) adsorption on *P. chrysosporium* in the single-ion situation (pH 6.5; temperature: 37 °C; agitation rate: 150 rev/min; 6 h).

### Figure 4.

The results of the removal of Cu(II), Cu(II), and Zn(II) in different initial concentrations are shown in Figure 4.
3.5. Biosorbent characterization

FT-IR analysis was used to examine the characteristics of the functional groups and their contribution to metal binding. The cell wall composition of *P. chrysosporium* biomass contained a large number of complex organic components, such as proteins and carbohydrate [23,24]. The FT-IR spectra of native and Cd(II)-treated *P. chrysosporium* biomass are shown in Figure 5. Based on the information in the literature [25–28], the characteristic absorption bands of OH and NH were identified between 3200 to 3600 cm\(^{-1}\), and the carboxylic and phenolic stretching bands appeared at 2925 cm\(^{-1}\). The strong peak at 1657 cm\(^{-1}\) corresponds to C=O stretching in the carboxyl or in amide I and amide II groups. The peak at 1149 cm\(^{-1}\) attributed to C–N stretching of the native sample shifted to 1151 cm\(^{-1}\) of the cadmium-treated sample, and the peak at 1381 cm\(^{-1}\) was the vibration of the carboxylate and carboxylic acid groups. The peak appearing at 696 cm\(^{-1}\) represents C–S bending. Analysis of the FTIR spectra showed that the presence of hydroxy, carboxylic and amino functional groups was important to interact with cadmium ions.

3.6. SEM and energy analysis

To obtain further insight into the characteristics of cadmium particles, analysis of the fungal mycelium sample bonded with cadmium was carried out using SEM and EDAX techniques. The images of the original and heavy metal loaded fungi are shown in Figure 6. From Figure 6(a), it can be seen that the mycelia were smooth and clean. Furthermore, the mycelia formed mesh structures, which led to the adsorption of cadmium. However, the micrograph of the ion loaded fungi looks different from that before adsorption. As shown in Figure 6(b)–(d), some micro-particles were distributed on the surface of the mycelia. This result indicates...
that the biosorption process takes place on the surface. The EDAX analysis spectrum of the biomaterial is shown in Figure 7. The spectrum showed strong signals of Cd aside from C, O, N, S, and P that are likely to be due to the fungal biomass background.

According to Figures 6 and 7, it could be speculated that: heavy metals in the solution were adsorbed onto the surface of the mycelia through interactions with functional groups such as carboxyl, sulfur, or peptide bonds in the proteins or amino acid, as well as the nitrogen oxide and hydroxyl found on the mycelia. This phenomenon was similar to those reported by Vigneshwaran et al. [11] and Baldrian [12]. This phenomenon and the FT-IR results indicated that the carbonyl group in the amino acid and the peptide bond of the proteins contributed to metal binding.

4. Conclusions

*P. chrysosporium* was a very effective biosorbent for binding of heavy metal in solutions. The uptake of Cd(II), Cu(II), and Zn(II) ions into the biomass was mainly dependent on pH values, temperature, contact time, and initial ion concentration. The optimum biosorption conditions were at pH 5.5–6.5 at 37°C with 6 h of equilibrium time. In addition, initial concentrations of Cd(II), Cu(II), and Zn(II) should not exceed 50, 80, and 60 mg/g, respectively. The adsorption kinetics of these heavy metals was in accordance with the pseudo second-order model, which suggested that the adsorption rate should be proportional to the square of the free surface sites. The EDAX analysis spectrum and FT-IR spectroscopic study indicated that the carbonyl group in the amino acid and the peptide, as well as the carboxyl and hydroxyl groups contributed to binding of the metal.

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![Figure 7. EDAX analysis of (a) original *P. chrysosporium*, (b) Cd(II)-loaded *P. chrysosporium*, (c) Cu(II)-loaded *P. chrysosporium*, and (d) Zn(II)-loaded *P. chrysosporium*.](image-url)
References


