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Polyvinyl alcohol-immobilized *Phanerochaete chrysosporium* and its application in the bioremediation of composite-polluted wastewater

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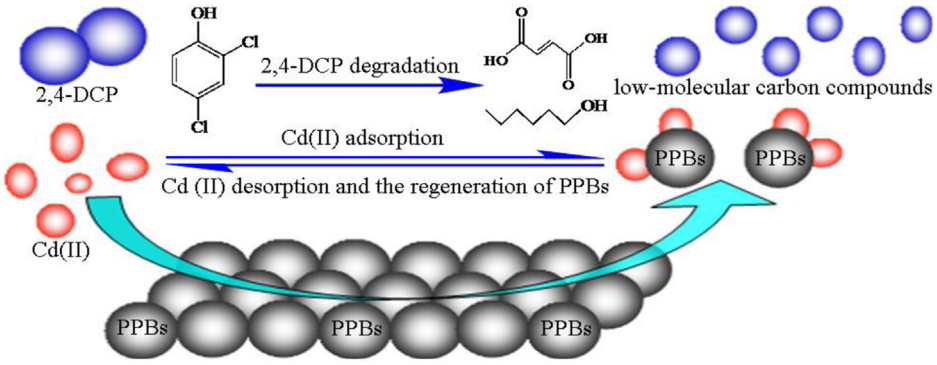
HIGHLIGHTS

- PVA-immobilized *P. chrysosporium* beads (PPBs) were fit for wastewater treatment.
- Removal rates of Cd(II) and 2,4-DCP at optimum conditions were up to 78% and 95.4%.
- 2,4-DCP removal rates were beyond 90% with varying initial 2,4-DCP concentrations.
- PVA was vital to Cd(II) removal besides the function groups in *P. chrysosporium*.
- Maximum recovery of the Cd(II)-laden PPBs after reuse three times was 98.9%.

GRAPHICAL ABSTRACT

Schematic diagram of polyvinyl alcohol-immobilized *Phanerochaete chrysosporium* beads (PPBs) for Cd(II) removal and 2,4-DCP degradation.



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ABSTRACT

A novel biosorbent, polyvinyl alcohol (PVA)-immobilized *Phanerochaete chrysosporium*, was applied to the bioremediation of composite-polluted wastewater, containing both cadmium and 2,4-dichlorophenol (2,4-DCP). The optimum removal efficiency achieved was 78% for Cd(II) and 95.4% for 2,4-DCP at initial concentrations of 20 mg/L Cd(II) and 40 mg/L 2,4-DCP. PPBs had significantly enhanced the resistance of *P. chrysosporium* to 2,4-DCP, leading to the degradation rates of 2,4-DCP beyond 90% with varying initial 2,4-DCP concentrations. This research demonstrated that 2,4-DCP and secreted proteins might be used as carbon and nitrogen sources by PVA-immobilized *P. chrysosporium* beads (PPBs) for Cd(II) removal. Fourier transform infrared spectroscopy analysis showed that hydroxyl and carboxyl groups on the surface of PPBs were dominant in Cd(II) binding. The mechanism underlying the degradation of 2,4-DCP into fumaric acid and 1-hexanol was investigated. The adsorption–desorption studies indicated that PPBs kept up to 98.9% of desorption efficiency over three cycles.

1. Introduction

Population growth, rapid industrialization, and urbanization have contributed to contamination of water with metal and organic pollutants. Contamination of water with a combination of heavy

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metal and organic pollutants has received increasing attention with rapid industrial development in recent years. Contamination by cadmium (Cd) is a major public concern due to its high toxicity to aquatic organisms and humans [1,2]. Trace amounts of Cd(II) in the environment could cause severe damage to the biological organization, from the molecular level to the community level [3]. According to U.S. Environmental Protection Agency (EPA) and World Health Organization (WHO), the permissible limit of cadmium in drinking water is 0.005 mg/L. 2,4-Dichlorophenol (2,4-DCP), widely used as a fungicide, pesticide, and wood preservative, can provoke disturbances in the structure of cellular bilayer phospholipids, causing strongly carcinogenic and mutagenic effects on organisms [4]. Listed as a priority pollutant by EPA, the limit for 2,4-DCP content has been set at 0.093 mg/L by the Grade I–III National Surface Water Environmental Quality Standard.

Biosorption is an efficient, cost-effective, flexible, and environmentally friendly technique [5] that has attracted more attention owing to its application in the treatment of wastewater containing composite pollutants of heavy metal and organic compounds. *Phanerochaete chrysosporium* (*P. chrysosporium*), the model strain of white rot fungi, is well known for its unique xenobiotics degradation and heavy metal removal with admirable biosorption capacity [6–10]. However, the application of *P. chrysosporium* is limited in wastewater treatment processes owing to its poor mechanical strength, low resistance, and long degradation time [11].

Some of the advantages of immobilized whole cells over freely suspended cells include increased mechanical strength, efficient biosorbent regeneration, easier solid–liquid separation, and higher bacterial cell density [5,8,12]. The immobilized biomass can also be shielded from unfavorable environmental conditions, including high pollutant concentrations and predators [13]. PVA is preferred over other polymers for cell immobilization owing to its lower cost, higher mechanical strength, durability, non-toxicity, good biocompatibility, and biodegradable properties [14–16]. Moreover, PVA beads demonstrate high stability within a pH range of 1.0–13.0 [17]. PVA-immobilized cells have been proven to be efficient in the biodegradation of hazardous contaminants, including aromatic compounds [15,18], phenolic compounds [19–21], and crystal violet [22], as well as in the uptake of heavy metals by immobilized fungal biomass [23,24].

The objective of this study was to immobilize *P. chrysosporium* using PVA as the support material. Sodium alginate, zeolite powder, silicon dioxide, activated carbon, and Tween 80 were also used as additives to improve the mechanical properties of the crosslinking immobilized carrier. Using NaH_2PO_4 solution as a crosslinking agent, Cd(II) removal and 2,4-DCP degradation by PVA-immobilized *P. chrysosporium* beads (PPBs) were studied. The effects of biomass dosage, and initial concentrations on adsorption and degradation capacities in a batch system were investigated. The pH of solutions and the content of extracellular proteins of *P. chrysosporium* were also analyzed. The removal pathways and mechanisms of PPBs were explored by using scanning electron microscopy (SEM) equipped with the energy dispersive X-ray analysis (EDAX), Fourier transform infrared spectroscopy (FTIR), and gas chromatography–mass spectrometry (GC–MS). The current study demonstrated that PPBs had great removal efficiency and practical applicability in wastewater treatment with co-pollutants (Cd(II) and 2,4-DCP).

2. Materials and methods

2.1. Materials

P. chrysosporium (BKMF-1767) was maintained by subculturing on potato dextrose agar (PDA) slants at 4 °C, and then introduced into PDA plates for 7 days at 37 °C. Spore suspensions were obtained by dissolving the spores into sterile distilled water, and then adjusted to a concentration of 2.0×10^6 CFU/mL using a turbidimeter (WGZ-200, Shanghai, China).

2,4-DCP was procured from Tianjin Guangfu Fine Chemical Research Institute. All other reagents used were of analytical grade and were purchased from Shanghai First Reagent Co., China. 1.0 g/L Cd(II) was prepared as the experimental stock solution by dissolving $Cd(NO_3)_2{\cdot}4H_2O$ in ultrapure water (18.25 MΩ/cm). 500 mg/L of 2,4-DCP stock solution was prepared and stored at 4 °C. Different concentrations of Cd(II) and 2,4-DCP were prepared by diluting the stock solutions.

2.2. Preparation of the gel solution and the crosslinking agent

The gel solution was prepared by dissolving PVA (10%, w/v), Na–alginate (1%, w/v), silicon dioxide (2%, w/v) and zeolite powder (1%, w/v) into 0.9% NaCl solution in a reactor, which was immersed in a 80 °C water bath with slowly stirring. 1.0 M NaH_2PO_4 solution, as the crosslinking agent, was obtained by dissolving NaH_2PO_4 into saturated boric acid solution. The gel solution and the crosslinking agent were sterilized by autoclave at 111 °C for 30 min and then cooled to approximately 45 °C before using.

2.3. Preparation of PPBs

P. chrysosporium spore suspension of 20% (w/v) firstly mixed evenly with activated carbon (3%, w/v). Then, the spore suspension and sterile Tween 80 (1%) were added to the gel solution and mixed well. The mixture was trickled into a gently stirred 1.0 M NaH_2PO_4 solution using 5 mL syringe and cured for 2 h to form stable microspheres. After that, the prepared microspheres were rinsed and immersed in 0.9% NaCl solution for 12–24 h under sterile condition, and then transferred into the culture medium and incubated in an incubator (ZHWY, Shanghai, China) at 37 °C and 150 rpm. After 3 days of incubation, all samples (PPBs) were harvested for the succeeding experiments.

The pH of solutions was adjusted to 6.5 according to previous study with 0.1 M HNO_3 or NaOH at the beginning of experiments. All experiments were carried out in 500 mL conical flasks containing 200 mL aqueous solution at 37 °C with 150 rpm.

2.4. Effect of biomass dosage

The various dosages of PPBs (0.9, 2.5, 4.1, 8.0, 11.9, and 15.8 g/L) and 4.1 g/L of PVA beads without *P. chrysosporium* were added to the solutions with 20 mg/L Cd(II) and 40 mg/L 2,4-DCP to determine their influence on the adsorption and degradation performance.

2.5. Effect of initial Cd(II) concentration

In order to determine the effect of initial Cd(II) concentration on Cd(II) removal and 2,4-DCP degradation, a series of Cd(II) solutions (0, 5, 10, 20, 40, 80, 120, 160, and 200 mg/L) were prepared and the initial 2,4-DCP concentration in each of the flask was maintained at 40 mg/L.

2.6. Effect of initial 2,4-DCP concentration

Various concentrations of 2,4-DCP and an initial Cd(II) concentration of 20 mg/L were prepared. The initial 2,4-DCP concentration was adjusted to 0, 5, 10, 20, 80, 100, 120, and 160 mg/L to investigate the optimum initial 2,4-DCP concentration and the effect of initial 2,4-DCP concentration on the removal of Cd(II) and 2,4-DCP.

2.7. Desorption and reusability

Desorption of Cd(II) adsorbed on PPBs was performed by shaking with 10 mM HCl solution at 200 rpm for 2 h. Then, the PPBs were washed several times with 0.9% NaCl solution and reintroduced into the solutions containing 20 mg/L Cd(II) and 40 mg/L 2,4-DCP to test the reusability of PPBs with three adsorption–desorption cycles. Desorption efficiency was calculated using the following equation:

$$\text{Desorption efficiency}\,(\%) = \frac{\text{Amount of Cd(II) desorbed}}{\text{Amount of Cd(II) adsorbed}} \times 100\% \quad (1)$$

2.8. Analytical methods and calculations

The supernatant was decanted from the solution in flasks at pre-decided intervals, and centrifuged in a centrifuger (TGL20-M, Hukang, China) at 10,000 rpm, 4 °C, for 10 min. Then the residual Cd(II) and 2,4-DCP concentrations in the supernatant were analyzed.

The concentration of Cd(II) was determined by using a flame atomic absorption spectrometer (FAAS, PerkinElmer AA700, USA). The 2,4-DCP concentration in solution was analyzed quantitatively by high performance liquid chromatography (HPLC, Agilent 1100) on a column of Zorbax Eclipse XDB-C18 (150 × 4.6 mm, 5 μm), using the mobile phase consisting acetonitrile and water (80:20, v/v) with a flow rate of 1.0 mL/min and detected at 287 nm under the column temperature of 35 °C. Samples were filtered through a 0.45 μm filter membrane and 20 μL filtrate was injected into column. Protein content in the extracellular medium was measured using Bradford method at 595 nm by a UV–vis spectrophotometer (UV754N, Shanghai, China).

The removal percentage and amount of removed Cd(II) or 2,4-DCP were calculated from the following equations:

$$\text{Removal rate}(\%) = \frac{(C_i - C_f)}{C_i} \times 100\% \quad (2)$$

$$q = \frac{(C_i - C_f)V}{W} \quad (3)$$

where q (mg/g) is the amount of removed Cd(II) or 2,4-DCP by the unit mass of PPBs, C_i and C_f are the initial and final residual concentrations (mg/L) of Cd(II) or 2,4-DCP, V is the volume of the aqueous solution (L), and W is the weight (g) of PPBs. All the experiments were performed in triplicate and the results obtained were the mean values from these independent experiments. All statistical tests were performed utilizing SPSS software and Origin v8.0 software.

2.9. Mechanism exploration

SEM (FEI Quanta-200, FEI Company, Holland) photomicrographs were taken to determine the surface morphology of PPBs before and after reacting with composite-polluted wastewater, operated at 20 kV after coating the samples with gold by sputtering to improve the image quality. FTIR (WQF-410, Beijing, China) spectra of KBr powder-pressed pellets were recorded ranging from 4000 cm^{-1} to 400 cm^{-1}. The PPBs were harvested through filtering after experiments and then dried at –60 °C in a freezer dryer (FD-1, Boyikang, Beijing, China). GCMS-QP2010 Ultra (Shimadzu, Japan) equipped with a RTX-5 capillary column (30 m × 0.25 mm × 0.25 μm) was employed to identify the intermediates of 2,4-DCP after degradation by PPBs according to the method reported by Chen et al. [25,26].

3. Results and discussion

3.1. Effect of biomass dosage

The effect of biomass dosage on Cd(II) removal and 2,4-DCP degradation was investigated by varying the biomass dosage of PPBs from 0.9 to 15.8 g/L in 20 mg/L Cd(II) and 40 mg/L 2,4-DCP solutions. The Cd(II) removal efficiency by using PPBs increased with, but was not proportional to, an increase in the biomass dosage (Fig. 1a). Cd(II) removal rates were 49.9%, 66.2%, 78.0%, 78.9%, 83.4%, and 92.4%, for 0.9, 2.5, 4.1, 8.0, 11.9, and 15.8 g/L PPBs, respectively. This was higher than that by using PVA beads without *P. chrysosporium* (44.2% for 4.1 g/L). Similar results were obtained for 2,4-DCP degradation (Fig. 1b).

Although the removal efficiency increased from 49.9% to 92.4% when the PPBs biomass dosage was increased from 0.9 to 15.8 g/L, the Cd(II) uptake capacity decreased considerably from 10.66 to 1.18 mg/g (Fig. 1c). Similar phenomena for Cd(II) uptake were also reported in previous studies [27–29]. The increase of removal efficiency was due to an excess in the available binding sites or surface area with the increase in biomass dosage, where virtually all the Cd(II) ions were sorbed. However, some binding sites were saturated during the process with more biomass dosages, leading to a decrease in uptake capacity. Furthermore, both the curves in Fig. 1c displayed a similar trend, i.e., an initial rapid decrease followed by the final stability, with an increase in biomass dosage. When the biomass dosage increased up to 4.1 g/L, the uptake capacities of Cd(II) and 2,4-DCP approached their equilibrium values. This might be attributed to the fact that a PPBs biomass dosage of 4.1 g/L attained the sorbent/sorbate equilibrium of the system in the removal of Cd(II) and 2,4-DCP.

3.2. Effect of initial Cd(II) concentration

The effect of initial Cd(II) concentration (varying between 5 and 200 mg/L), keeping the initial 2,4-DCP concentration constant at 40 mg/L, on Cd(II) removal and 2,4-DCP degradation was presented in Fig. 2. As shown in Fig. 2a, an optimum uptake rate of 78.0% was observed at a Cd(II) concentration of 20 mg/L. Increase in the initial Cd(II) concentration resulted in a decrease in Cd(II) removal efficiency from 78.0% (at 20 mg/L) to 62.4% (at 200 mg/L). This could be attributed to a higher ratio of metal ions to sorption sites or the less competition of Cd(II) ions for a fixed number of available binding sites at a low initial Cd(II) concentration [23]. However, high removal rates were also obtained at high Cd(II) concentrations

Table 1
Estimated parameters with Langmuir model for Cd(II) removal by various biosorbents.

Biosorbent type	q_{max}	b	Reference
Sodium alginate-immobilized *Pycnoporus sanguineus*	3.32	1.34	[33]
Alginate/PVA-immobilized *Scenedesmus quadricauda*	81.09	0.046	[34]
PVA-immobilized *Aspergillus niger*	60.24	0.058	[24]
PVA/Sodium alginate-immobilized *Lentinus edodes*	6.45	2.62	[35]
loofa sponge-immobilized *P. chrysosporium*	85.98	0.064	[36]
PPBs	60.06	0.013	This study
PVA beads without *P. chrysosporium*	2.71	0.290	This study

q_{max} is maximum Cd(II) uptake (mg/g) and b is the equilibrium constant (L/mg).

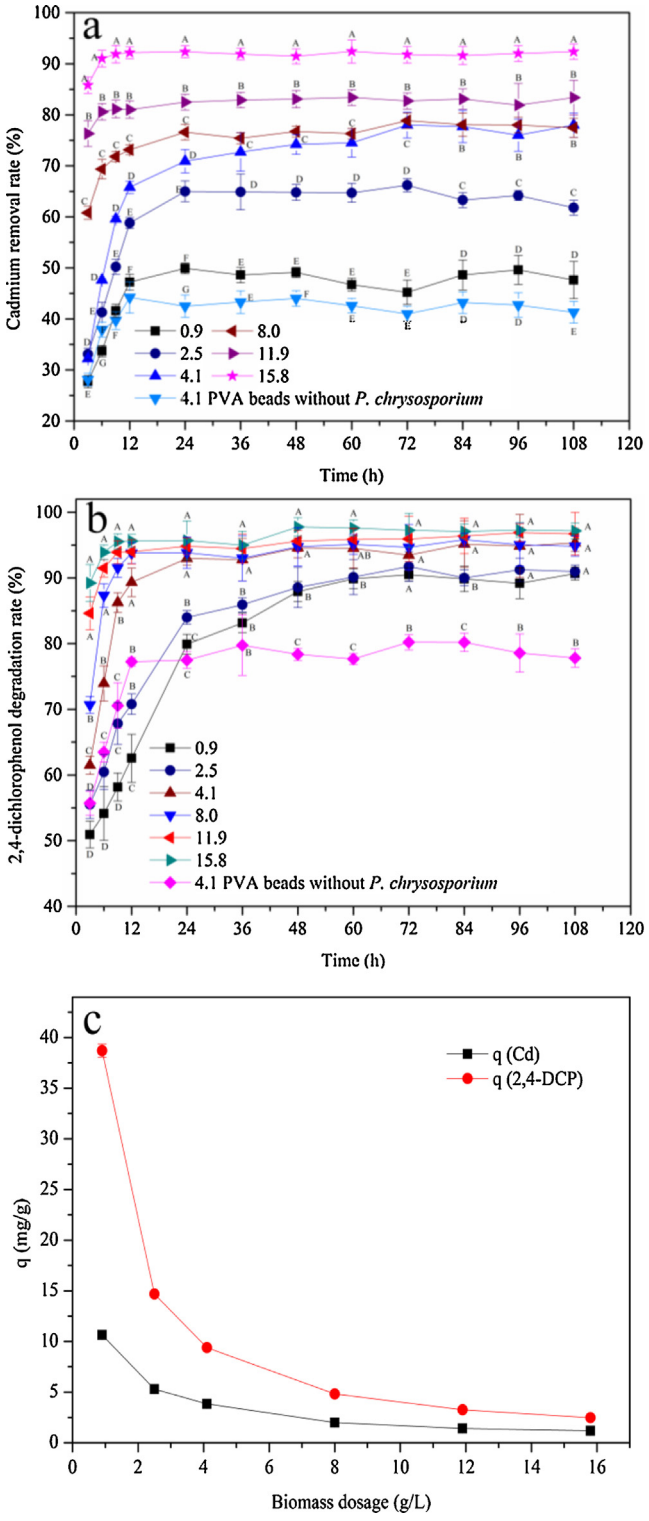

Fig. 1. Effect of biomass dosage on (a) Cd(II) removal and (b) 2,4-DCP degradation by using PPBs and PVA beads without *P. chrysosporium*, at an initial Cd(II) concentration of 20 mg/L, an initial 2,4-DCP concentration of 40 mg/L, and an initial pH 6.5, with PPBs biomass dosages of 0.9, 2.5, 4.1, 8.0, 11.9, and 15.8 g/L; (c) were evaluated using the plateau values of the (a) adsorption and (b) degradation capacities.

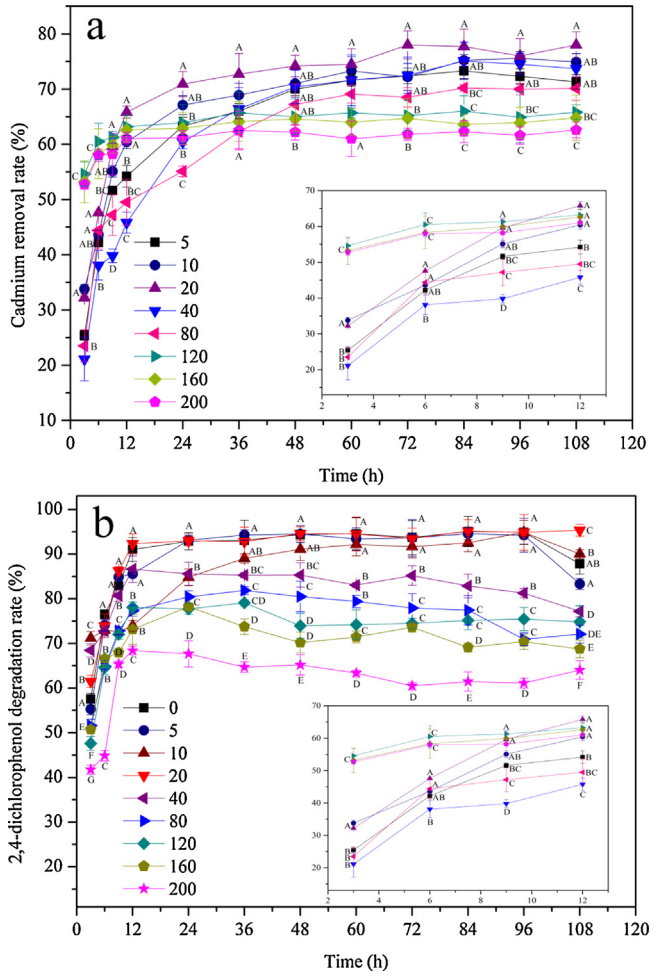

Fig. 2. Effect of initial Cd(II) concentration on (a) Cd(II) removal and (b) 2,4-DCP degradation, using 4.1 g/L of PPBs at an initial pH 6.5 and an initial 2,4-DCP concentration of 40 mg/L; the initial Cd(II) concentration in the medium was adjusted to 0, 5, 10, 20, 40, 80, 120, 160, and 200 mg/L.

(65.6%, 64.5%, and 62.4% at initial Cd(II) concentrations of 120, 160, and 200 mg/L, respectively). It might be closely associated with the immobilized materials we used. The materials, such as zeolite, silicon dioxide, and activated carbon, had good adsorption properties for heavy metals. It also suggested that Cd(II) removal were indeed greatly improved under the protection of immobilized materials. Moreover, the amounts of potential Cd(II)-binding sites were probably enhanced on account of the unfolding of other cell-surface polymers and denaturation of proteins [30].

It should be noted that higher Cd(II) uptake capacity was observed at higher Cd(II) concentrations, which might be the reason that higher Cd(II) concentrations led to an increase in the driving force to overcome all mass transfer resistance of metal ions between PPBs and the aqueous phase. It resulted in a higher probability of collision between PPBs and Cd(II). Thus, enhanced interaction between PPBs and Cd(II) during adsorption processes led to easier penetration of heavy metal ions to sorption sites and higher Cd(II) uptake [31,32].

Furthermore, according to Langmuir model, the maximum Cd(II) uptake capacity (q_{max}) of PPBs reached 60.06 mg/g (Fig. S1a, $R^2 = 0.931$), which was greatly enhanced compared to several biosorbents listed in Table 1. However, the maximum uptake ability of PVA beads without *P. chrysosporium* was only 2.71 mg/g (Fig. S1b, $R^2 = 0.982$).

As indicated by Fig. 2b, the initial Cd(II) concentration had a significant effect on 2,4-DCP degradation. When the initial Cd(II) concentration was set to below 20 mg/L, 2,4-DCP degradation rates were beyond 90%. The removal rate of 2,4-DCP followed a similar trend to that of Cd(II); however, it displayed great degradation rate (95.4%) for an initial Cd(II) concentration of 20 mg/L. However,

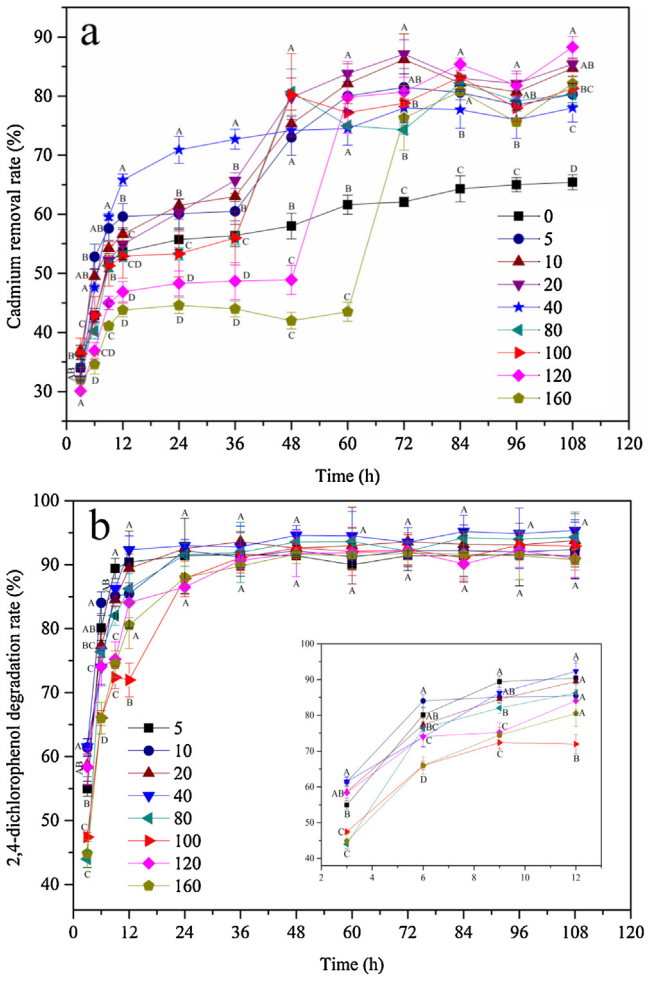

Fig. 3. Effect of initial 2,4-DCP concentration on (a) Cd(II) removal and (b) 2,4-DCP degradation, using 4.1 g/L of PPBs at an initial pH 6.5 and an initial Cd(II) concentration of 20 mg/L; the initial 2,4-DCP concentration in the medium was adjusted to 0, 5, 10, 20, 40, 80, 100, 120, and 160 mg/L.

the 2,4-DCP degradation rate was inhibited at Cd(II) concentrations of over 20 mg/L, which was in accordance with the results of our previous studies [25,26]. It could be due to the inhibition of the degradation effect of *P. chrysosporium* by Cd, which was known to be most toxic for biomass. Rapid accumulation of high concentrations of toxic Cd(II) in *P. chrysosporium* cells might lead to restrained growth, reduction in biological activity, and changes in morphology and physiology. The activities of extracellular enzymes and fungal colonization were disturbed by Cd during the degradation process of 2,4-DCP [25]. In present study, it was assumed that the secretion of extracellular proteins involved in the process was closely associated with the Cd(II) concentration in the external environment.

3.3. Effect of initial 2,4-DCP concentration

The effect of initial 2,4-DCP concentration on Cd(II) removal and 2,4-DCP degradation was demonstrated over a 2,4-DCP concentration range of 0–160 mg/L, using a standard Cd(II) concentration of 20 mg/L to sustain a high level of Cd(II) adsorption capacity (Fig. 3a). It was observed that the initial 2,4-DCP concentration had a specific impact on Cd(II) removal. The maximum Cd(II) removal rates were determined to be 65.4%, 81.5%, 86.2%, 87.1%, 78.0%, 82.2%, 83.2%, 85.4%, and 82.1%, with the increase in initial 2,4-DCP concentrations (0, 5, 10, 20, 40, 80, 100, 120, and 160 mg/L, respectively).

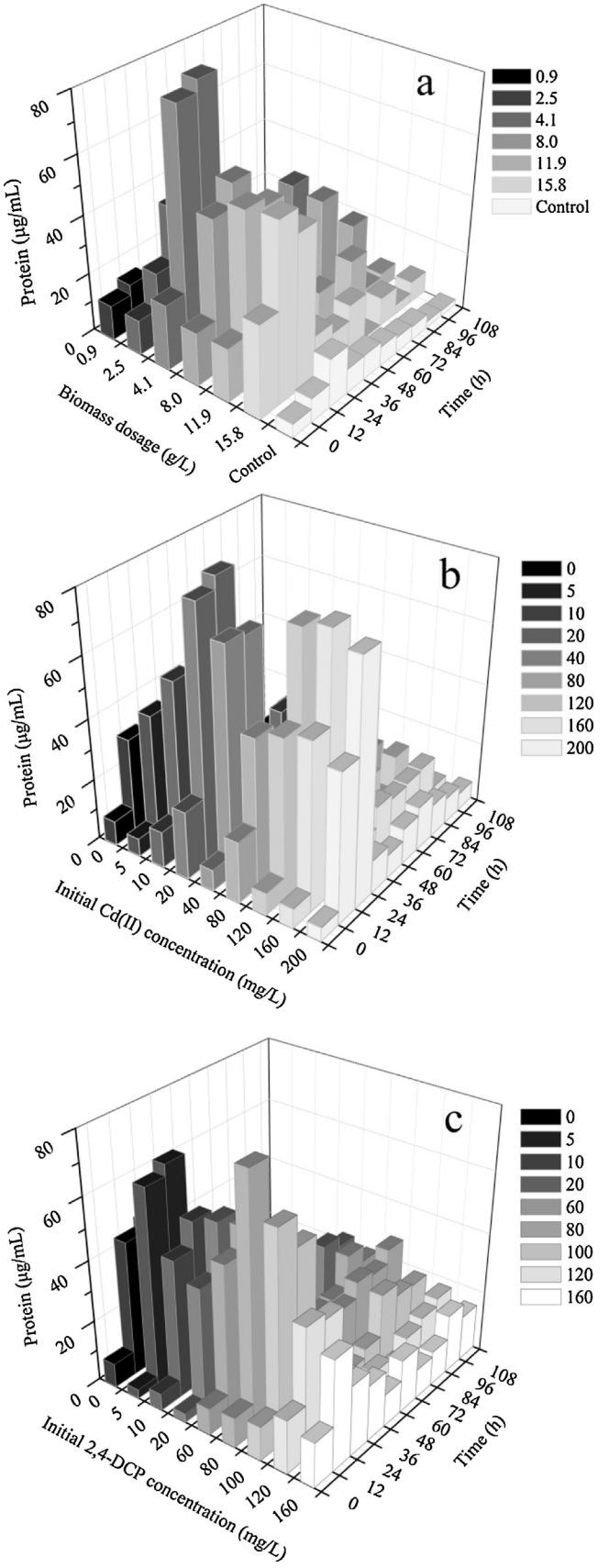

Fig. 4. Changes in protein concentration (μg/L) during the reaction process under different conditions. (a) The biomass dosages were set as 0.9, 2.5, 4.1, 8.0, 11.9, and 15.8 g/L. (b) When the initial 2,4-DCP concentration was 40 mg/L, the initial Cd(II) concentration in the medium was adjusted to 0, 5, 10, 20, 40, 80, 120, 160, and 200 mg/L. PPBs dosage was 4.1 g/L. (c) When the initial Cd(II) concentration was 20 mg/L, the initial 2,4-DCP concentration in the medium was adjusted to 0, 5, 10, 20, 60, 80, 100, 120, and 160 mg/L. PPBs dosage was 4.1 g/L.

Table 2
Changes of pH value with different initial Cd(II) concentrations during the reaction process.

Time (h)	Initial Cd(II) concentration (mg/L)									
	0	5	10	20	40	80	120	160	200	Control
0	6.51 ± 0.02e	6.51 ± 0.01h	6.49 ± 0.01e	6.49 ± 0.01e	6.50 ± 0.00d	6.50 ± 0.01b	6.50 ± 0.01a	6.50 ± 0.01c	6.49 ± 0.01a	6.50 ± 0.01f
12	7.23 ± 0.10d	7.18 ± 0.01g	6.94 ± 0.01d	6.68 ± 0.02d	6.56 ± 0.03cd	6.41 ± 0.01b	6.36 ± 0.01a	6.44 ± 0.00bc	6.32 ± 0.04a	7.31 ± 0.11d
24	5.35 ± 0.00c	7.40 ± 0.09ef	7.17 ± 0.02cd	6.89 ± 0.03bc	6.59 ± 0.04b–d	6.44 ± 0.03b	6.36 ± 0.04a	6.24 ± 0.07ab	6.35 ± 0.01a	5.96 ± 0.05h
36	5.45 ± 0.04c	7.52 ± 0.04e	7.18 ± 0.08cd	6.93 ± 0.06b	6.59 ± 0.05b–d	6.39 ± 0.05b	6.34 ± 0.04a	6.17 ± 0.08a	6.32 ± 0.02a	6.25 ± 0.03g
48	5.51 ± 0.06c	7.25 ± 0.09fg	7.18 ± 0.11cd	6.93 ± 0.01bc	6.68 ± 0.04b–d	6.40 ± 0.05b	6.34 ± 0.04a	6.13 ± 0.07a	6.32 ± 0.04a	6.50 ± 0.05f
60	5.78 ± 0.07b	6.95 ± 0.01d	7.22 ± 0.04c	6.90 ± 0.05bc	6.66 ± 0.12a–c	6.45 ± 0.06b	6.37 ± 0.02a	6.10 ± 0.08a	6.30 ± 0.03a	6.80 ± 0.09e
72	5.88 ± 0.06b	6.00 ± 0.08c	7.16 ± 0.10cd	6.86 ± 0.03bc	6.65 ± 0.08ab	6.45 ± 0.14b	6.37 ± 0.12a	6.09 ± 0.13a	6.29 ± 0.10a	7.45 ± 0.06d
84	6.05 ± 0.06a	5.57 ± 0.01b	6.53 ± 0.13e	6.83 ± 0.02c	6.63 ± 0.08bc	6.43 ± 0.16b	6.35 ± 0.13a	6.07 ± 0.13a	6.28 ± 0.08a	7.75 ± 0.02c
96	5.76 ± 0.08b	5.69 ± 0.13b	7.44 ± 0.14b	6.81 ± 0.01c	6.63 ± 0.06b–d	6.47 ± 0.12b	6.37 ± 0.11a	6.08 ± 0.08a	6.29 ± 0.07a	8.19 ± 0.01b
108	6.11 ± 0.04a	5.81 ± 0.10a	7.83 ± 0.08a	7.59 ± 0.12a	6.82 ± 0.07a	7.36 ± 0.08a	6.40 ± 0.10a	6.10 ± 0.11a	6.29 ± 0.08a	8.38 ± 0.06a

Different lowercase letters in the same column indicate significant differences ($P < 0.05$) among different contact time.

As shown in Fig. 3a, the experiment results supported the hypothesis that there were two steps in the adsorption process: bioaccumulation and chemical adsorption. The data suggested that bioaccumulation played a major role in Cd(II) removal during the first 36 h. Over this period, the Cd(II) removal rate increased from 56.4% to 72.7% when the 2,4-DCP concentration increased from 0 to 40 mg/L. Then an elevated 2,4-DCP concentration from 40 to 160 mg/L led to a decrease in Cd(II) removal rate from 72.7% to 44.0% over the same period. However, another rapid rise in Cd(II) removal rate was observed, which could be attributed to the production of some alkaline substance, resulting in the formation of cadmium hydroxide complex. Consequently, the Cd(II) concentration in the solution decreased rapidly. In addition, the curves depicting Cd(II) removal rates were only observed bioaccumulation when the initial 2,4-DCP concentrations were 0 and 40 mg/L.

These observations showed that an increase in initial 2,4-DCP concentration boosted Cd(II) removal up to an optimum concentration of 40 mg/L during the bioaccumulation process. This could be attributed to that a low 2,4-DCP concentration might be used as carbon and energy sources by *P. chrysosporium* to improve its biological activity and promote the secretion of extracellular proteins, and then Cd(II) removal was reinforced. Similar results were found in our previous studies [25,37].

As illustrated in Fig. 3b, no significant difference was observed between the maximum 2,4-DCP degradation rates, which were 91.6%, 92.4%, 93.8%, 95.4%, 94.2%, 92.9%, 92.1%, and 91.8% at initial 2,4-DCP concentrations of 5, 10, 20, 40, 80, 100, 120, and 160 mg/L, respectively. It was found that the effect of Cd(II) toxicity on biological activity was higher than that of 2,4-DCP. However, the biological activity of PPBs was only slightly influenced by the initial Cd(II) concentration of 20 mg/L. The results suggested that 40 mg/L 2,4-DCP concentration was conducive to both Cd(II) removal and 2,4-DCP degradation. In addition, the high efficacy of 2,4-DCP degradation (Fig. 3b), with no significant difference after 24 h, might be attributed to the stability and activity of enzymes [25,38,39]. It was conformed that PPBs could significantly enhanced the resistance of *P. chrysosporium* to 2,4-DCP.

3.4. Changes in pH during the reaction

Changes in pH of the extracellular solution were monitored throughout the reaction process. A control group, a flask containing PPBs without Cd(II) and 2,4-DCP, was also performed during the experiment. The pH values of some solutions decreased with an increase in reaction time (Table 2). This could be associated with the production of organic acids during the process of 2,4-DCP degradation and fungal metabolism. Some organic acids, such as oxalic acid excreted by wood-rotting fungi, could act as a buffer and provide H_2O_2 during the degradation process of lignin and cellulose [25].

The change in the pH value was indistinctive in the first 36 h (Table 3). This was probably because the organic acid was consumed by *P. chrysosporium* as carbon and energy sources and the consumption rate was equal to its production rate. A similar result in an earlier study was reported by Song et al. [37]. However, the pH level increased during the later stage of the reactions, which might account for some alkaline substances produced during the reaction process. It is well known that the solubility of metal ions was affected by the pH of the solution. When the pH crossed 8.0, $Cd(OH)_2$ would appear due to the precipitation of Cd(II) ions. As shown in Table 3, the increased pH values of the solution could lead to the augmentation of adsorption capacity due to Cd(II) precipitation, which corresponded to the results in Fig. 3a. This validated the assumption that some alkaline substances were produced during the reaction. Further studies on the pH of the solution must be conducted to determine the mechanism of the produced alkaline substances.

The pH also influenced the ionization state of the functional groups on the cell wall components, such as carboxylic and amino groups [40]. At high pH, negatively charged groups on

Table 3
Changes of pH value with different initial 2,4-DCP concentrations during the reaction process.

Time (h)	Initial 2,4-DCP concentration (mg/L)								
	0	5	10	20	60	80	100	120	160
0	6.49 ± 0.01c	6.50 ± 0.01f	6.51 ± 0.01d	6.51 ± 0.01d	6.51 ± 0.01d	6.49 ± 0.01d	6.51 ± 0.01d	6.50 ± 0.01e	6.49 ± 0.01d
12	6.77 ± 0.06c	6.74 ± 0.02e	6.71 ± 0.04c	6.65 ± 0.04d	6.55 ± 0.01d	6.50 ± 0.02d	6.47 ± 0.02d	6.44 ± 0.03e	6.40 ± 0.03d
24	6.74 ± 0.08c	6.74 ± 0.01e	6.71 ± 0.07c	6.65 ± 0.08d	6.86 ± 0.08c	6.52 ± 0.08d	6.54 ± 0.01d	6.47 ± 0.04e	6.43 ± 0.03d
36	6.75 ± 0.10c	6.71 ± 0.02e	6.67 ± 0.12c	6.62 ± 0.11d	6.84 ± 0.12c	6.55 ± 0.05d	6.56 ± 0.02d	6.51 ± 0.02e	6.38 ± 0.07d
48	6.79 ± 0.08c	7.60 ± 0.05d	7.91 ± 0.03b	7.81 ± 0.08c	7.89 ± 0.06b	7.52 ± 0.11c	7.51 ± 0.12c	6.58 ± 0.02e	6.43 ± 0.04d
60	7.35 ± 0.09b	7.90 ± 0.07c	7.86 ± 0.04b	7.79 ± 0.05c	7.90 ± 0.01b	7.63 ± 0.04c	7.60 ± 0.06c	7.40 ± 0.08d	6.57 ± 0.11d
72	7.56 ± 0.17b	7.92 ± 0.02c	7.93 ± 0.03b	7.92 ± 0.02c	7.89 ± 0.09b	7.80 ± 0.08b	7.82 ± 0.07b	7.77 ± 0.02c	7.55 ± 0.05c
84	7.52 ± 0.17b	7.95 ± 0.06c	7.92 ± 0.06b	7.91 ± 0.08c	7.97 ± 0.05b	7.90 ± 0.01ab	7.91 ± 0.02b	7.90 ± 0.04b	7.68 ± 0.10c
96	7.54 ± 0.23b	8.12 ± 0.04b	8.10 ± 0.05a	8.16 ± 0.01b	8.17 ± 0.11ab	8.02 ± 0.05a	8.10 ± 0.04a	7.99 ± 0.10b	7.91 ± 0.03b
108	7.83 ± 0.17a	8.31 ± 0.01a	8.19 ± 0.07a	8.31 ± 0.06a	8.23 ± 0.08a	8.08 ± 0.10a	8.11 ± 0.09a	8.10 ± 0.09a	8.29 ± 0.01a

Different lowercase letters in the same column indicate significant differences ($P < 0.05$) among different contact time.

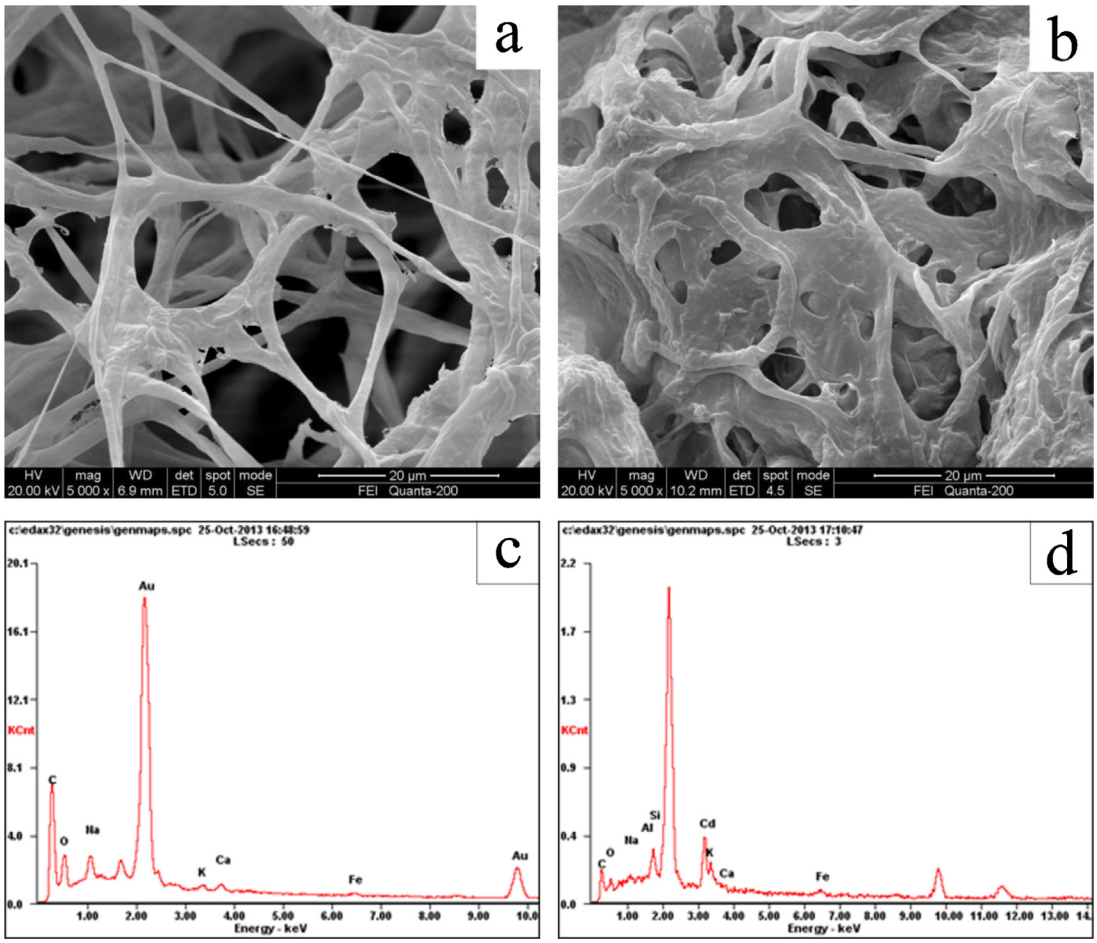

Fig. 5. SEM–EDAX micrographs of PPBs: (a) SEM image of PPBs in native form; (b) SEM image of PPBs after reaction; (c) EDAX of PPBs before reaction; and (d) EDAX of PPBs after reaction.

PPBs increased, leading to an increase in the uptake of positively charged Cd(II). It was found that Cd(II) adsorption was pH dependent.

3.5. Changes in protein concentration during the reaction

The influence of increasing concentrations of Cd(II) or 2,4-DCP in the medium on the secretion of extracellular proteins was investigated. The data indicated that Cd(II) and 2,4-DCP induced a general increase in the array of secreted proteins (Fig. 4). Extracellular protein concentrations related with different PPBs biomass dosages were shown in Fig. 4a, when the initial concentrations of Cd(II) and 2,4-DCP were 20 and 40 mg/L, respectively. The maximum concentration of extracellular proteins (86.17 μg/mL) was obtained at 4.1 g/L PPBs, demonstrating that the biomass dosage of 4.1 g/L was suited greatly for the removal of Cd(II) and 2,4-DCP among the other dosages evaluated, which was in agreement with the results in Fig. 1.

The concentrations of extracellular proteins secreted at high Cd(II) concentrations (120, 160, and 200 mg/L) were up to 79.63, 82.06, and 77.57 μg/mL, respectively, which were significantly higher than those at low Cd(II) concentrations (0, 5, 10, and 80 mg/L) with an initial 2,4-DCP concentration of 40 mg/L at 24 h (Fig. 4b). However, the maximum amount of extracellular proteins secreted per mass of Cd(II) decreased from 8.46 to 0.24 mg/mg when the initial Cd(II) concentration increased from 5 to 200 mg/L. After 24 h, no obvious variation in the concentration of extracellular proteins was noticed. It was assumed that larger quantities of proteins were secreted in response to the elevated Cd(II) concentration up to a certain limit. This protein could subsequently be utilized as a nitrogen source by PPBs, which assisted in Cd(II) removal. Additionally, protein was also well-known for its crucial role in the adsorption mechanisms of heavy metal ions, including extracellular complexation with metal-binding proteins, such as metallothioneins (MTs), phytochelatins (PCs), and glutathione (GSH) [40,41]. MTs have been confirmed to exhibit metal-binding property towards various metal ions, such as Zn, Cd, and Cu [42]. GSH was also reported to be the strong affinity to Cd(II) [7]. Changes in concentration of extracellular proteins presented the same trend with an increase in initial 2,4-DCP concentration (Fig. 4c).

3.6. Characterization of PPBs

3.6.1. Morphology analysis by SEM

To confirm the adsorption of Cd(II) ions onto PPBs, SEM technique was explored. The SEM images clearly showed that PPBs were surrounded by *P. chrysosporium* hyphae with a network surface structure, which was instrumental in reducing the diffusional resistance and facilitating mass transfer owing to their high internal surface area (Fig. 5a). After adsorption, the surface characteristics of PPBs underwent changes. The hyphae of PPBs became tight, rough, and were coated by a layer of Cd(II), resembling mud (Fig. 5b). A comparison between the EDAX spectra obtained before and after adsorption showed a distinct Cd peak in the after-adsorption spectrum, demonstrating that Cd(II) was adsorbed by PPBs (Fig. 5c and d).

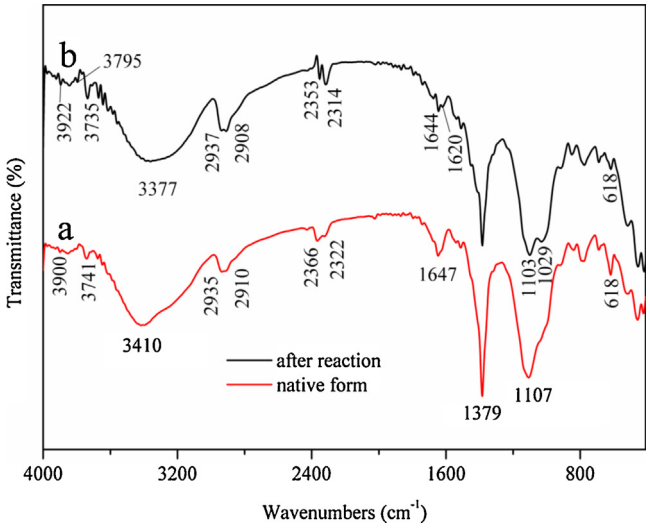

Fig. 6. FTIR spectra of PPBs (a) in native form and (b) after reaction.

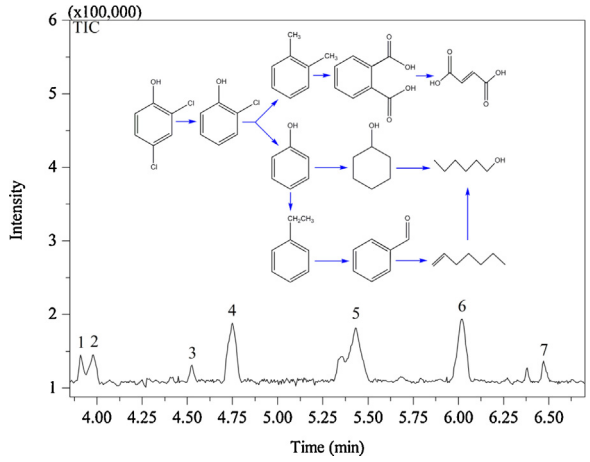

Fig. 7. Gas chromatograms of 2,4-DCP after the degradation by PPBs and the reaction scheme proposed based on its intermediates.

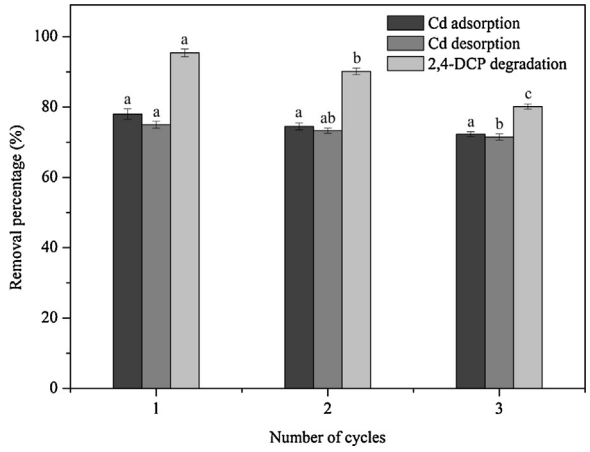

Fig. 8. Adsorption–desorption cycles of PPBs for the removal of Cd(II) and 2,4-DCP. Adsorption conditions: concentrations, 20 mg/L Cd(II) and 40 mg/L 2,4-DCP; pH 6.5; eluting agent: 10 mM HCl solution; and PPBs dosage: 4.1 g/L.

3.6.2. *Functional group analysis by FTIR*

FTIR was used to determine the functional groups responsible for Cd(II) binding. As observed in the spectra (Fig. 6), the broad peak appearing at around 3410 cm^{-1} could be characteristic of O—H stretching vibration bands arising from the hydroxyl group of PVA [14,43], while the band at 2935 and 2910 cm^{-1} could be ascribed to the asymmetric and symmetric methylene stretching vibrations of the hydrocarbons present in the fungal protein [44].

Comparing the FTIR spectra of the PPBs before and after Cd(II) adsorption, it was found that hydroxyl groups had undergone a change from polymer to monopolymer or even to a dissociative state. This could mean that the degree of hydroxyl polymerization in the PPBs had a decrease with the addition of Cd(II) and that Cd(II) now had a greater likelihood of being bound to the hydroxyl groups [26,28]. The absorption peak shifted from 1647 cm^{-1} to 1644 cm^{-1}, corresponding to the —C═O stretch of the sodium alginate polymeric chain in grafted PVA. The peak at 1107 cm^{-1}, corresponding to —C—O stretching of alcohols and carboxylic acids, underwent a shift to 1103 and 1029 cm^{-1} after Cd(II) adsorption. This indicated that carboxyl groups were converted to carboxylate, which occurred during the reaction between Cd(II) and carboxy1. Coupled with above results, it might be concluded that an important role was played by PVA as well as hydroxyl and carboxyl groups in the Cd(II) adsorption process.

3.6.3. *Analysis of metabolites by GC–MS*

To clarify the possible degradation pathways of 2,4-DCP by PPBs in the presence of Cd(II), the intermediates and end-products generated during the 2,4-DCP degradation process were identified with the aid of GC–MS. The mechanistic pathways (listed in Fig. 7) might account for the products of the 2,4-DCP degradation pathway encountered in this study. Mechanisms that resulted in metabolite formation were based on bond cleavage. The C—Cl and O—H bonds present in the 2,4-DCP ring were quite fragile, and could be further metabolized through the *ortho*-, or *para*-ring cleavage pathways. Chlorine atoms attached to the benzene rings were replaced, producing *o*-xylene, phthalic acid, and ethylbenzene, followed by cyclohexanol. Subsequently, linear chain organics with low toxicity, including 1-hexanol and fumaric acid, were generated due to the breakup of benzene rings (Fig. 7 and Table 4). In the end, all the carbon compounds were further decomposed and could be turned into CO_2 and H_2O by the biodegradation of *P. chrysosporium*. However, in the present study, the degradation of 2,4-DCP observed was incomplete, which could possibly be associated with the toxicity of intermediates involved in the degradation process.

Table 4
GC–MS spectral data of degradation products of 2,4-DCP after reaction with PPBs.

Peak no.	Retention time (min)	Molecular weight (m/z)	Intermediates	Molecular formula	CAS No.
1	3.91	106.16	Ethylbenzene	C_8H_{10}	100-41-4
2	3.98	106.16	*O*-xylene	C_8H_{10}	95-47-6
3	4.52	100.16	Cyclohexanol	$C_6H_{12}O$	108-93-0
4	4.75	166.13	Phthalic acid	$C_8H_6O_4$	88-99-3
5	5.43	102.18	1-Hexanol	$C_6H_{14}O$	111-27-3
6	6.02	116.07	Fumaric acid	$C_4H_4O_4$	110-17-8
7	6.47	163.00	2,4-Dichlorophenol	$C_6H_4Cl_2O$	120-83-2

3.7. Desorption and reusability

In order to demonstrate the reusability of PPBs, three consecutive adsorption–desorption experiments were carried out by using the same PPBs (4.1 g/L). Up to 98.9% of Cd(II) adsorbed onto PPBs was desorbed from the biosorbent. The Cd(II) adsorption capacity of PPBs remained fairly constant with the increased number of cycles (3% decline (maximum) was observed with the tested biosorbent; Fig. 8). The degradation rates of each cycle were 95.4%, 90.17%, and 80.14%, respectively. And the shape of PPBs had no visual deterioration, maintaining intact beads. This implied that PPBs could be repeatedly used for the removal of heavy metal, without detectable losses in their initial properties.

4. Conclusion

PVA-immobilized *P. chrysosporium* exhibited an excellent performance in the removal of Cd(II) and 2,4-DCP. 78% Cd(II) and 95.4% 2,4-DCP could be simultaneously removed under optimum conditions. 2,4-DCP and secreted proteins could be used as carbon and nitrogen sources by *P. chrysosporium* for Cd(II) removal. In the present study, Cd(II) removal was mainly associated with hydroxyl and carboxyl groups, and 2,4-DCP was broken up into 1-hexanol and fumaric acid by the degradation of PPBs. After three consecutive adsorption–desorption cycles, the desorption efficiency of Cd(II) adsorbed on PPBs was up to 98.9%, without detectable losses in Cd(II) uptake capacity. The results obtained suggested that PPBs, with good reusability, were effective in remediating composite-polluted wastewater containing heavy metals and organic compounds.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2015.02.043.

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