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ENVIRONMENTAL BIOTECHNOLOGY

Simultaneous cadmium removal and 2,4-dichlorophenol degradation from aqueous solutions by *Phanerochaete chrysosporium*

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Abstract *Phanerochaete chrysosporium* has been recognised as an effective bioremediation agent due to its unique degradation to xenobiotic and biosorption ability to heavy metals. However, few studies have focused on the simultaneous removal of heavy metals and organic pollutants. The aim of this work was to study the feasibility of simultaneous cadmium removal and 2,4-dichlorophenol (2,4-DCP) degradation in *P. chrysosporium* liquid cultures. The removal efficiencies were pH dependent and the maximum removal efficiencies were observed at pH 6.5 under an initial cadmium concentration of 5 mg/L and an initial 2,4-DCP concentration of 20 mg/L. The removal efficiencies for cadmium and 2,4-DCP reached 63.62% and 83.90%, respectively, under the optimum conditions. The high production levels of lignin peroxidase (7.35 U/mL) and manganese peroxidase (8.30 U/mL) resulted in an increase in 2,4-DCP degradation. The protein content decreased with increasing cadmium concentration. The surface characteristics and functional groups of the biomass were studied by scanning electron microscopy and a Fourier-transformed infrared spectrometer. The results showed that the use of *P. chrysosporium* is promising for the simultaneous removal of cadmium and 2,4-DCP from liquid media.

Keywords Biosorption · Cadmium · Degradation · 2,4-Dichlorophenol · *Phanerochaete chrysosporium*

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Introduction

The contamination of aquatic systems with toxic heavy metal ions and organic pollutants is a problem of global concern. It is well recognised that the presence of heavy metals and aromatic compounds in the environment can be detrimental to a variety of living species, including man (Aksu and Gönen 2006). Cadmium has attracted much attention from environmentalists as one of the most toxic heavy metals (Chen et al. 2008). Many industries such as those involving electroplating, paint pigments, fertilisers, alloy preparation, mineral processing and battery manufacturing generate large quantities of wastewater containing various concentrations of cadmium (Lodeiro et al. 2005; Chen et al. 2008; Mashitah et al. 2008). The conventional remediation methods for water contaminated with cadmium include chemical precipitation, ion exchange, membrane filtration and activated carbon adsorption (Molinari et al. 2004; Fan et al. 2008). However, these treatment methods become ineffective and more expensive when the volumes of wastewater are high and metal concentrations are low (Say et al. 2001; Congeevaram et al. 2007). Bioremediation using microorganisms offers an attractive treatment option because this technology is cost-effective and environmentally compatible and has high removal efficiency for low metal concentrations (Chen et al. 2006; Song et al. 2009).

In fact, a wide range of organic pollutants, including chlorophenol, PAHs, phenol and trichloroethylene, have been found in water contaminated by cadmium. Cadmium and its organic co-pollutants are often produced from industrial processes such as leather tanning, photographic film manufacturing, wood preservation, car manufacturing, petroleum refining and agricultural activities (Nkhalambayausi-Chirwa and Wang 2001; Antizar-Ladislao and Galil 2004). The contamination of groundwater aquifers, lakes, river sediments, and soils by cadmium and its organic co-pollutants, e.g. chlorophenol, is widespread (Lin et al. 2009). Moreover, the combined toxicity of the co-pollutants is often higher than that of a single pollutant. Therefore, the simultaneous removal of cadmium and its co-pollutants is very important in water and wastewater treatment processes.

It has been reported that aromatic compounds can be used as carbon and energy sources for the removal of heavy metals in cultures of some microorganisms. Meanwhile, these microorganisms show different behaviours in terms of their ability to utilise metabolites as a carbon source for the removal of heavy metals during aromatic compound degradation (Kumar et al. 2005). Organic co-pollutants in a water system may support the growth and activity of microorganisms (Nkhalambayausi-Chirwa and Wang 2001). However, few studies are available on simultaneous cadmium removal and aromatic compound degradation by white-rot fungi—*Phanerochaete chrysosporium*—that is well known for its unique xenobiotic degradation (Kaçar et al. 2002; Baldrian 2003; Tang et al. 2009) and cadmium biosorption ability (Say et al. 2001; Li et al. 2004).

In this study, 2,4-DCP was chosen as a model aromatic co-pollutant of cadmium because of its relatively strong presence in cadmium-contaminated sites. The aim of this work was to investigate the removal potential of cadmium and 2,4-DCP by *P. chrysosporium*. The effect of factors such as pH, initial concentration and the dosage of biomass were examined. SEM micrographs and FT-IR spectra were measured to understand the removal mechanism. In this study, the feasibility of simultaneous cadmium removal and 2,4-DCP degradation using *P. chrysosporium* biomass was demonstrated.

Materials and methods

Microorganism and growth conditions

The *P. chrysosporium* BKM-F1767 (ATCC 24725) used in this study was purchased from the China Center for Type Culture Collection (Wuhan). Stock cultures were maintained on malt extract agar slants at 4 °C. Mycelial suspensions were prepared in sterile distilled water. The fungal concentration was measured and adjusted to 2.0×10^6 CFU/mL using a turbidimeter (WGZ-200, Shanghai, China). Aqueous suspensions of fungal spores were prepared for the inoculation of the liquid growth medium. Kirk's liquid culture medium (Kirk et al. 1978) (200 mL) was used in a 500-mL Erlenmeyer flask. Flasks containing 6×10^6 spores (v/v=1.5%) were incubated at 37 °C in an incubator under 150 r/min for 3 days.

Preparation of solutions

A stock solution containing 1.0 g/L Cd(II) was prepared by dissolving $Cd(NO_3)_2{\cdot}4H_2O$ in ultra-pure water. The exact concentration of the stock solution was determined by flame atomic absorption spectrometry (PerkinElmer AA700, USA). 2,4-DCP (Analytical Pure) was purchased from Tianjin Guangfu Fine Chemical Research Institute, Tianjin, China, and was used without further purification. All of the other inorganic chemicals were of analytical grade and were purchased from Shanghai First Reagent Co., China. A 2,4-DCP stock solution was prepared by dissolving 0.5 g 2,4-DCP in 1.0 L ultra-pure water. The stock solution of 2,4-DCP was stored in a brown glass bottle to avoid photodegradation. Test solutions were prepared by diluting the stock solutions to the desired concentrations. The pH value of the solution in this study (3.5–8.5) was adjusted to the required value by 0.1 mol/L NaOH or HNO_3 solutions. All of the solutions were stored in the dark at 4 °C prior to use.

Cadmium removal and 2,4-DCP degradation experiments

After 3 days of incubation, several millilitres of the cadmium and 2,4-DCP stock solutions were added to the flasks to ensure that the final concentrations were as desired. The total volume of mixture in each flask was adjusted to 200 mL. All of the cadmium removal and 2,4-DCP degradation experiments were conducted at 37±0.1 °C (a temperature which is beneficial to the growth and metabolism of *P. chrysosporium* as identified by many researchers (Kenealy and Dietrich 2004; Liu et al. 2008; Sedighi et al. 2009)) under 150 r/min. All of the experiments were performed in triplicate.

The effect of pH on the removal rate was investigated in the pH range of 3.5–8.5, which was adjusted with 0.1 mol/L HNO_3 or NaOH at the beginning of the experiment. Solutions containing 5 mg/L of cadmium and 20 mg/L of 2,4-DCP were shaken at 150 r/min. Aliquot samples were taken at specific time intervals (3, 6, 9, 12, 24, 36, 48, 60, 72, 84, 96 and 108 h) and then centrifuged at 10,000 r/min for 10 min. The supernatant was used for analysing the residual cadmium and 2,4-DCP. Two control treatments, i.e. a flask containing cadmium and 2,4-DCP without biomass and another containing biomass without cadmium or 2,4-DCP, were also used to detect the photodegradation of 2,4-DCP during the experiment and its release from the biomass.

It was observed that both photodegradation and release of 2,4-DCP could be negligible.

A series of solutions containing cadmium at 2, 5, 10, 15, 20 and 30 mg/L were prepared to determine the effect of the initial cadmium concentration on cadmium removal and 2,4-DCP degradation. The 2,4-DCP concentration in each of the flasks was adjusted to 20 mg/L. The mixtures were adjusted to pH 6.5 with 0.1 mol/L HNO_3 or NaOH and then shaken in the same incubator. The samples were taken from the flasks at different time intervals, and the residual Cd(II) and 2,4-DCP concentrations were analysed.

To determine the optimal initial 2,4-DCP concentration for the removal process, the initial 2,4-DCP concentration was adjusted to various values, including 5, 10, 20, 40, 60 and 100 mg/L, to evaluate the effect of the initial phenol concentration on cadmium removal and 2,4-DCP degradation. The initial cadmium concentration in each of the flasks was 2 mg/L. Flasks spiked with cadmium and 2,4-DCP solutions were also kept at pH 6.5 in an incubator using the method mentioned above.

The various amounts of inoculation in the experiments were of desired values, including 0.5%, 1.0%, 1.5%, 2.5% and 4.0% (*v*/*v*), to observe its influence on cadmium removal and 2,4-DCP degradation. The initial cadmium and 2,4-DCP concentrations were adjusted to 5 and 20 mg/L, respectively. *P. chrysosporium* pellets were contacted with cadmium and 2,4-DCP at 37 °C in a shaker. The pH in the flasks were adjusted to 6.5 as reported above. Samples were taken at intervals and analysed for residual cadmium and 2,4-DCP concentration.

Enzyme assay

The lignin peroxidase (LiP) activity in the extracellular medium was assayed by monitoring the oxidation of veratryl alcohol to veratraldehyde at 25 °C by hydrogen peroxide at $A_{310\ nm}$ (Liu et al. 2008). One unit of LiP activity was defined as the amount of enzymes that led to the production of 1 μmol veratryl aldehyde per minute under the assay conditions.

The manganese peroxidase (MnP) activity was measured by monitoring the change in the oxidation state of Mn^{2+} to Mn^{3+} at $A_{240\ nm}$ (Rogalski et al. 2006). The reaction was initiated at 25 °C. One unit of MnP activity was expressed as the amount of enzyme that led to the production of 1 μmol Mn^{3+} from the oxidation of Mn^{2+} per minute. Spectrophotometric measurements were performed with a UV–visible light spectrophotometer (Model UV-2550, Shimadzu Company, Tokyo, Japan).

Analytical procedure

The pH value during the experiment was measured with an FE20 laboratory pH meter (FE20 Mettler Toledo, Shanghai, China). The initial and residual concentrations of cadmium were determined using an atomic absorption spectrometer (Perkin-Elmer Analyst 700 AAS, USA). The 2,4-DCP concentration in the aqueous solution was determined using a UV–visible spectrophotometer (Model UV-2550, Shimadzu Company, Tokyo, Japan) at 306 nm. An aqueous solution of 2,4-DCP with a basic pH had a stronger absorbance at 306 nm than that with a natural pH, and the basification could eliminate the interference in the analysis (Wu and Yu 2007). Therefore, the sample was basified with a 2.0 mol/L NaOH solution, while the blank was the same sample but acidified using a 0.5 mol/L HCl solution. The concentration of protein in the extracellular medium was determined with a colorimetric method using a UV–visible spectrophotometer (UV754N, Shanghai, China) at 595 nm by reaction with Coomassie brilliant blue G250.

The amounts of cadmium and 2,4-DCP removed were calculated by the differences between the initial concentration of cadmium and 2,4-DCP added and the final concentrations of cadmium and 2,4-DCP in the filtrate. All of the data were obtained from triplicate assays and then analysed with Origin 8.0 software or mapped with Sigmaplot 10.0.

SEM and FT-IR studies

The pellets of *P. chrysosporium* were harvested through filtering after 3 days of incubation. The harvested pellets were then dried at −40 °C in a freezer dryer (FD-1, Boyikang, Beijing, China). The dry fungal pellets were used for SEM and FT-IR studies. Photomicrographs were taken before and after the experiment using a scanning electron microscope (SEM) (FEI QUANTA-200, Holland FEI Company, Holland) to determine the morphological changes in the mycelium. In order to determine which functional groups were responsible for cadmium adsorption, an FT-IR analysis of the biomass was carried out. The FT-IR spectra of the fungal pellets were obtained using a FT-IR spectrophotometer (WQF-410, Beijing, China). The dry fungal biomass (about 0.1 g) was mixed with KBr (0.1 g) and pressed into a tablet form. The FT-IR spectrum was then recorded.

Results

Effect of pH

The effects of the initial pH on cadmium removal and 2,4-DCP degradation by *P. chrysosporium* pellets at pH 3.5–8.5 are shown in Fig. 1a, b, respectively. It can be seen from Fig. 1 that the residual amounts of cadmium and 2,4-DCP decreased when the pH increased from 3.5 to 6.5 in the

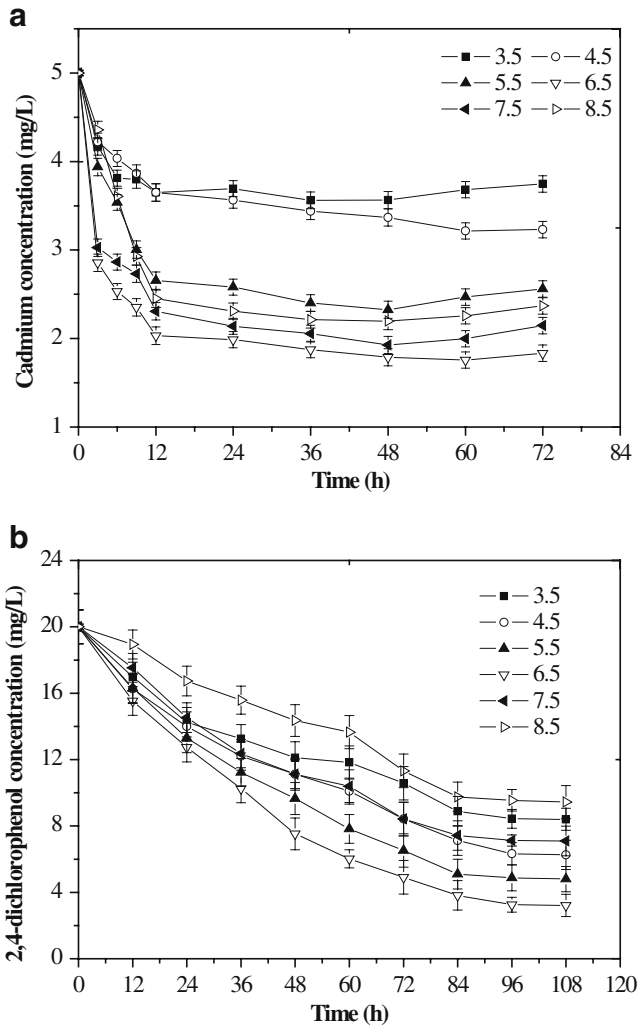

Fig. 1 Effect of initial pH on **a** cadmium removal and **b** 2,4-DCP degradation using *P. chrysosporium* at an initial cadmium concentration of 5 mg/L and an initial 2,4-DCP concentration of 20 mg/L; the initial pH in the medium was adjusted to 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5, respectively

solution. The data in Fig. 1a show that cadmium uptake was rapid in the first 12 h, accounting for about 91% of total cadmium sorption. There was a little more cadmium adsorbed with a further increase in contact time. The maximum metal removal rate was 62.41% at pH 6.5. The uptake of cadmium decreased when the pH rose above 6.5. The degradation of 2,4-DCP increased at pH values ranging from 3.5 to 6.5 and then declined sharply with a further increase in pH. The maximum degradation rate was 83.93% at pH 6.5 (Fig. 1b).

Effect of initial cadmium concentration

The effect of the initial cadmium concentration on the degradation of 2,4-DCP was examined using an initial 2,4-DCP concentration of 20 mg/L and a cadmium concentration range of 2–30 mg/L (Fig. 2a, b). The effect of the initial cadmium concentration on cadmium removal is shown in Fig. 2a. As can be seen in this figure, there was no complete cadmium removal by *P. chrysosporium* even under the lowest cadmium concentration. The percentages of cadmium removal were 32.29%, 63.62%, 59.88%, 58.60%, 56.40% and 55.89% at initial cadmium concentrations of 2, 5, 10, 15, 20 and 30 mg/L, respectively. The maximum value was found at an initial cadmium concentration of 5 mg/L. However, the maximum amounts of cadmium removal were 3.3, 17.8, 29.9, 42.3, 57.6 and 75.5 mg/g at initial cadmium concentrations of 2, 5, 10, 15, 20 and 30 mg/L, respectively. Thus, the adsorption capacity increased with an increase in the initial cadmium concentration from 2 to 30 mg/L.

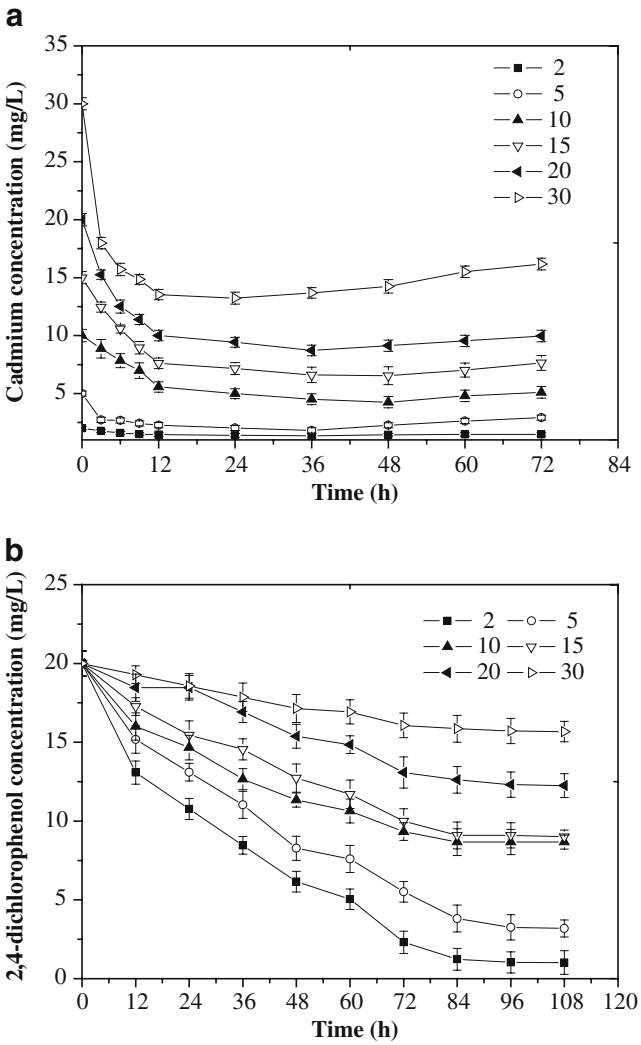

Fig. 2 Effect of initial cadmium concentration on **a** cadmium removal and **b** 2,4-DCP degradation using *P. chrysosporium* at an initial pH of 6.5 and an initial 2,4-DCP concentration of 20 mg/L; the initial cadmium concentration in the medium was adjusted to 2, 5, 10, 15, 20 and 30 mg/L, respectively

The effect of cadmium concentration on 2,4-DCP degradation is shown in Fig. 2b. The 2,4-DCP degradation rate decreased with an increase in cadmium concentration (97.87%, 84.08%, 56.67%, 55.00%, 38.73% and 21.60% at initial cadmium concentrations of 2, 5, 10, 15, 20 and 30 mg/L, respectively). The average rate of 2,4-DCP degradation decreased slowly from 97.87% to 84.08% as the cadmium concentration was increased from 2 to 5 mg/L. However, a rapid decrease was observed when the initial cadmium concentration increased from 5 to 30 mg/L.

Effect of initial 2,4-DCP concentration

The effect of the initial 2,4-DCP concentration on cadmium removal and 2,4-DCP degradation was determined over a 2,4-DCP concentration range of about 5–100 mg/L, using a low cadmium concentration of 2 mg/L to reduce cadmium toxicity toward biological activity. As shown in Fig. 3, the initial 2,4-DCP concentration played an important role during cadmium removal and 2,4-DCP degradation. Cadmium removal occurred in all of the cell cultures. The cadmium removal rates were 21.59%, 23.05%, 32.88%, 27.17%, 19.72% and 12.80% at initial phenol concentrations of 5, 10, 20, 40, 60 and 100 mg/L, respectively. The maximum removal rate was found at an initial 2,4-DCP concentration of 20 mg/L (Fig. 3a). Meanwhile, an increase in the initial 2,4-DCP concentration enhanced cadmium removal until an optimum level of 20 mg/L was reached. As shown in Fig. 3b, the 2,4-DCP degradation rate increased from 65.31% to 98.15% when the 2,4-DCP concentration increased from 5 to 20 mg/L. Furthermore, an increase in the 2,4-DCP concentration from 20 to 100 mg/L led to a decrease in the 2,4-DCP degradation rate from 98.15% to 21.23%. These results suggest that an increase in the initial concentration of 2,4-DCP from 5 to 20 mg/L was helpful for both cadmium removal and 2,4-DCP degradation. The opposite result was obtained with a further increase in the initial 2,4-DCP concentration.

Effect of inoculum density

To understand the effect of inoculum density on cadmium removal and 2,4-DCP degradation, various inoculum densities (0.5%, 1.0%, 1.5%, 2.5% and 4.0% (*v*/*v*)) were studied with a level of cadmium concentration of 5 mg/L at an initial 2,4-DCP concentration of 20 mg/L. Based on Fig. 4a, the residual cadmium concentration decreased with an increased amount of inoculation of *P. chrysosporium*. The percentages of cadmium removal were 50.2%, 56.0%, 64.7%, 66.0% and 67.5% at various amounts of inoculation (0.5%, 1.0%, 1.5%, 2.5% and 4.0% (*v*/*v*), respectively), but if the uptake of cadmium was plotted against the weight of biomass (mg/g) it was quite different. Though the removal efficiency increased from 50.2% to 67.5%, when the inoculum density increased

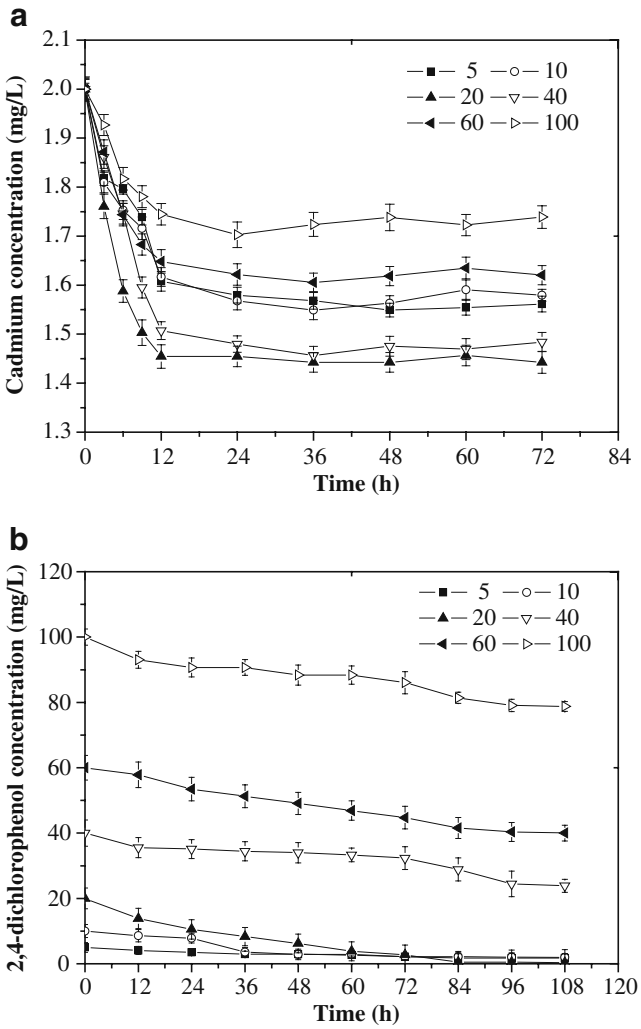

Fig. 3 Effect of initial 2,4-DCP concentration on **a** cadmium removal and **b** 2,4-DCP degradation using *P. chrysosporium* at an initial pH of 6.5 and an initial cadmium concentration of 2 mg/L; the initial 2,4-DCP concentration in the medium was adjusted to 5, 10, 20, 40, 60 and 100 mg/L, respectively

from 0.5% to 4.0%, the uptake of cadmium decreased from 33.5 to 5.63 mg/g. The removal efficiency increased with increasing inoculum density. However, the increased cadmium removal efficiency was not in proportion with the increase of inoculum density between 0.5% and 4.0%. The cadmium removed was not changed much with different inoculum densities ranging from 1.5% to 4.0%. Similar results were obtained for 2,4-DCP degradation (Fig. 4b). The 2,4-DCP concentration in the flasks decreased faster with increasing inoculum.

SEM–EDAX and FT-IR analyses of *P. chrysosporium* pellets

SEM micrographs of the fungus are presented in Fig. 5a, b. Figure 5a clearly shows the hyphae on the cell surface.

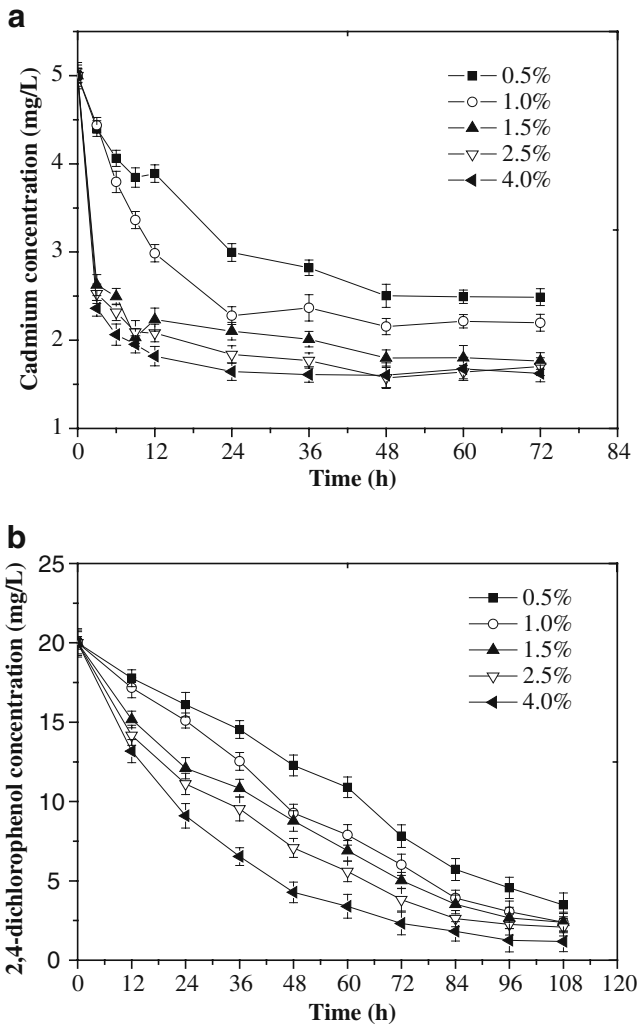

Fig. 4 Effect of inoculum density on **a** cadmium removal and **b** 2,4-DCP degradation using *P. chrysosporium* at an initial cadmium concentration of 5 mg/L and an initial 2,4-DCP concentration of 20 mg/L, with the amount of inoculation at 0.5%, 1.0%, 1.5%, 2.5% and 4.0% (*v*/*v*), respectively

Moreover, the hyphae on the surface of the cell are interwoven into the network structure, which was propitious to the binding of cadmium. The mycelium after incubation was very different from that before incubation. As clearly seen in Fig. 5b, the outline of the mycelium is no longer clear, and a large quantity of cadmium adhered to the surface of the mycelium. The energy-dispersive X-ray analysis (EDAX) spectrum of the *P. chrysosporium* pellets after incubation is shown in Fig. 5c. There is an obvious peak of cadmium in the EDAX spectrum, which also demonstrates that cadmium was adsorbed on the mycelium of *P. chrysosporium*.

The changes in the functional groups and the surface properties of the fungal pellets before and after the incubation were confirmed by the FT-IR spectra. The FT-IR spectra (Fig. 6) of the native and incubated fungus confirmed the presence of different characteristic peaks, in agreement with the possible presence of amino, carboxylic

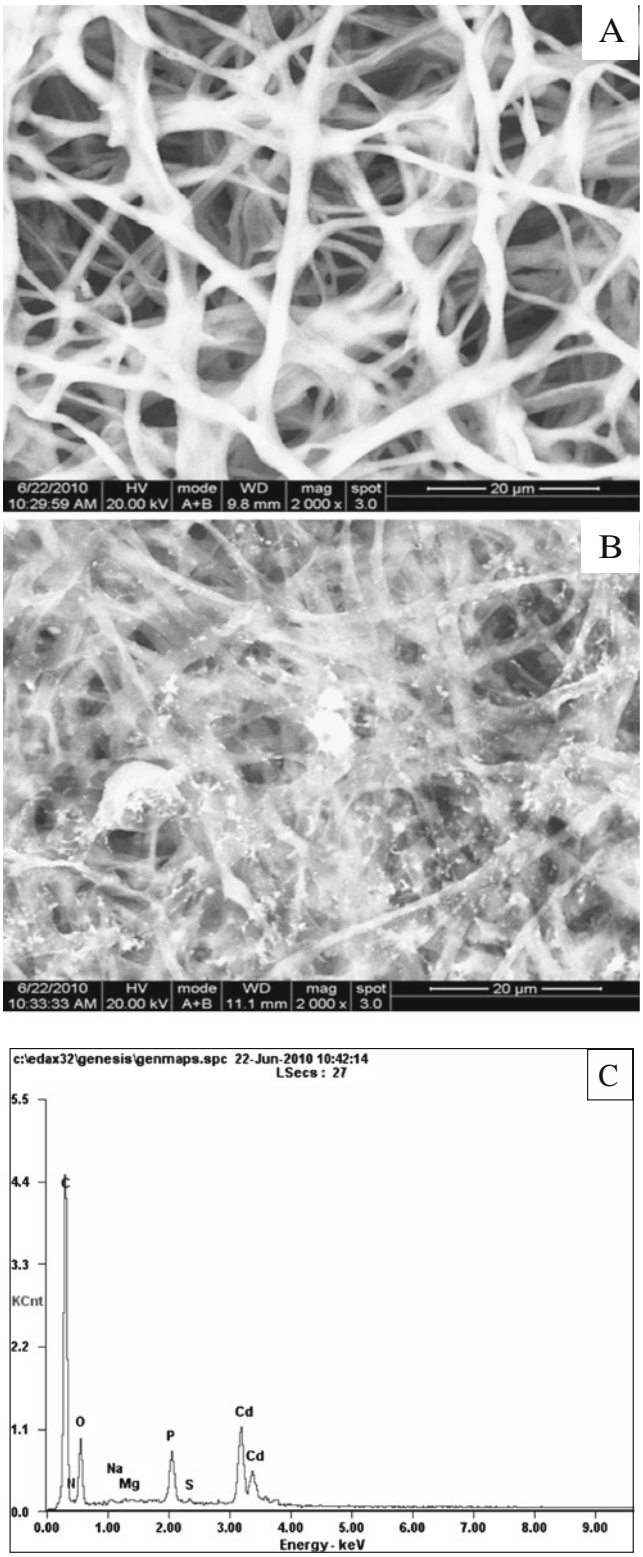

Fig. 5 SEM–EDAX micrographs of fungus: **a** native fungus, **b** fungus after incubation, **c** EDAX of fungus after incubation

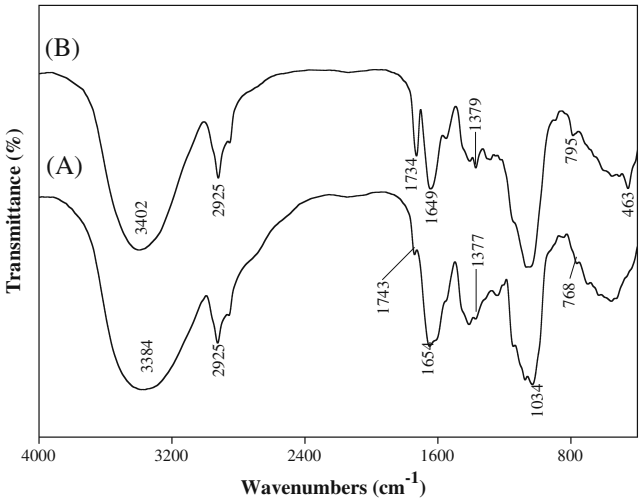

Fig. 6 FT-IR spectra of native (*a*) and incubated (*b*) fungal pellets

and hydroxyl groups. The peaks at around 3,400 cm^{-1} represent the –OH stretching of carboxylic groups and the stretching of –NH groups. The peak observed at 2,925 cm^{-1} could be assigned to the $–CH_2$ group bound by the stretching of the –OH groups. The peaks at 1,743 and 1,654 cm^{-1} were the characteristics of C = O group stretching from aldehydes and ketones. The peaks at 1,556, 1,414, 1,379 and 1,292 cm^{-1} represent N = O vibrations, C–H bending, $–CH_3$ wagging (deformation) and C–O stretching vibrations in the carboxylic acid dimer, respectively. The C–N stretching of aliphatic amines is evident from the peak at 1,034 cm^{-1} (1,090–1,020 cm^{-1}). The peak at 1,078 cm^{-1} representing = C–O–C is found in aromatic and vinyl structures. The peaks at 559 and 463 cm^{-1} representing P–S and P–S–P stretching are found in cell wall structures.

Analysis of metabolites

The changes in the pH values, amount of protein and LiP and MnP activities in the extracellular solution were monitored throughout the incubation process. The data in Table 1 shows that the pH values of the medium decreased in all of the cultures with an increase in the incubation time. Moreover, the rate of decrease in pH values decreased with an increase in the cadmium concentration. No significant change in the pH value was observed beyond the incubation time of 48 h. The representative protein data in Table 1 indicate that the secretion of extracellular proteins can be stimulated by cadmium. However, the concentration of extracellular proteins decreased with an increase in cadmium concentration.

The activities of LiP and MnP at an initial 2,4-DCP concentration of 20 mg/L and initial cadmium concentrations of 5, 15 and 30 mg/L are shown in Fig. 7. As seen in this figure, both the LiP and MnP activities increased with the incubation time and reached the maximum values at 60 or 72 h. The maximum enzyme activities were 7.35, 5.51 and 4.73 U/mL for LiP and 8.30, 4.69 and 3.31 U/mL for MnP under cadmium concentrations of 5, 15 and 30 mg/L, respectively. The enzyme activity detected in blank samples was low, probably because there was no 2,4-DCP as a substrate. Increases in cadmium concentration resulted in a reduction in enzyme activity, which was in agreement with the protein concentration in the medium.

Discussion

It is well known that metal ion adsorption on both non-specific and specific adsorbents is pH dependent (Bayramŏglu and

Table 1 Changes of pH value and protein concentration (μg/mL) during the incubation process. The initial 2,4-DCP concentration was 20 mg/L; the initial cadmium concentration in the medium was adjusted to 5, 15 and 30 mg/L, respectively

Cadmium concentration	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h
5 mg/L										
pH	6.52	5.14	3.95	3.61	3.57	3.57	3.57	3.58	3.61	3.58
Protein	13.10	10.46	8.05	22.87	38.97	46.21	52.87	46.21	53.56	58.48
15 mg/L										
pH	6.49	5.71	4.80	4.72	4.66	4.44	4.45	4.51	4.59	4.57
Protein	14.48	11.03	13.68	10.09	14.47	18.62	27.24	34.94	43.79	50.09
30 mg/L										
pH	6.51	6.31	5.98	5.81	5.79	5.71	5.71	5.76	5.77	5.75
Protein	16.74	12.53	14.02	11.25	13.10	12.51	16.21	20.02	29.08	39.84
Control[a]										
pH	6.48	4.86	4.57	4.54	4.53	4.54	4.47	4.39	4.37	4.37
Protein	17.36	10.57	9.66	6.09	8.85	11.95	11.38	10.80	10.00	11.08

[a] A flask containing biomass without cadmium and 2,4-DCP

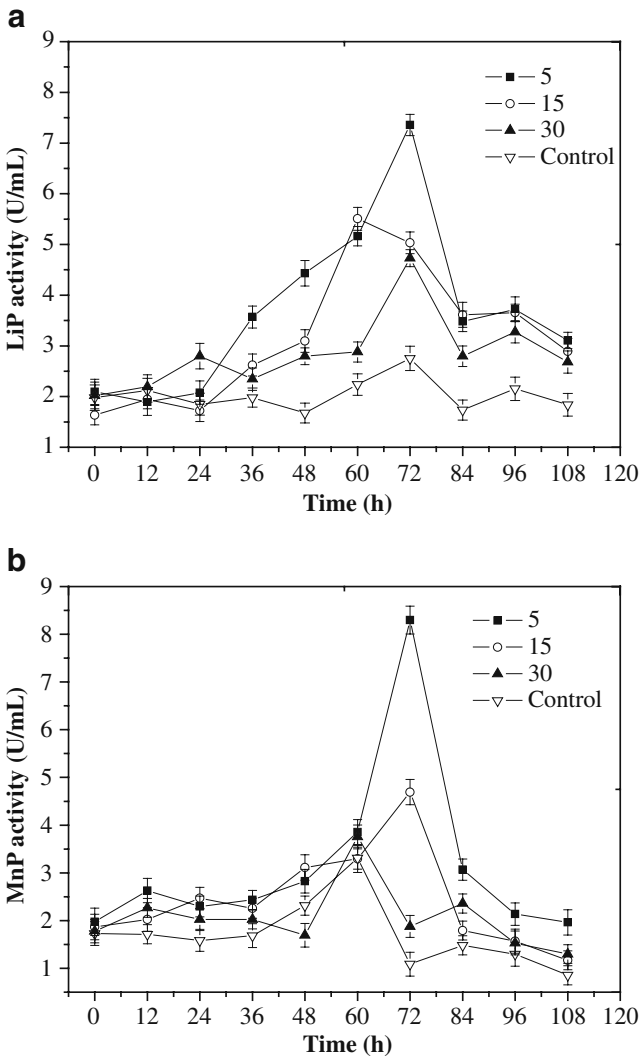

Fig. 7 Enzyme activity in the extracellular medium during the incubation process: **a** LiP, **b** MnP

Arıca 2008). The pH of the medium affects the solubility of metal ions and the ionisation state of the functional groups (i.e. carboxylate, phosphate and amino groups) on the fungal cell wall (Bayramŏglu and Arıca 2008; Fan et al. 2008). The effects of the initial pH on cadmium removal and 2,4-DCP degradation were evaluated within the pH range of 3.5–8.5. Studies beyond pH 8.5 were not attempted as the precipitation of the ions as hydroxides would have likely occurred (Vimala and Das 2009). In our study, the maximum metal removal rate was 62.41% at pH 6.5. This phenomenon was similar to that reported earlier by Say et al. (2001). The pH dependency of the removal efficiency could be explained by the functional groups involved in the metal uptake. At low pH values, protons occupy most of the biosorption sites on the sorbent surface, leading to the sorption of fewer cadmium ions because of the electric repulsion of the protons on the sorbent. When the pH values increased, the negative charge density on the cell surface increased because of the deprotonation of the metal binding sites. Therefore, the uptake of metal ions (positive charge) increased (Say et al. 2001). When the pH is higher than 6.5, the OH^- in the system and the functional groups on the surface of the cell wall compete for heavy metal ions which would decrease biosorption capacity (Arıca et al. 2001; Chen et al. 2011). The efficacy of the degradation of 2,4-DCP at pH 6.5 (Fig. 1b) might be attributed to the stability and activity of the enzymes. The enzymes secreted from *P. chrysosporium* are mainly stabilised by weak interactions such as hydrogen bonds, van der Waals forces, etc., which are largely influenced by the pH of the medium. An increase or decrease in the pH beyond a certain range adversely affected the stability and activity of the enzyme, thereby decreasing the rate of 2,4-DCP degradation (Zouari et al. 2002).

The adsorption capacity increased with an increase in the initial cadmium concentration. This could be attributed to the fact that the initial cadmium concentration was the driving force for overcoming the mass transfer limitation between the *P. chrysosporium* pellets and fluid phase. A high initial concentration of cadmium enhanced the mass transfer driving force and thus increased the adsorption capacity. In addition, an elevated initial cadmium concentration increased the number of collisions between the adsorbate and adsorbent, which also enhanced the adsorption process (Mashitah et al. 2008; Vimala and Das 2009; Rathinam et al. 2010). Results on the effect of initial cadmium concentration on 2,4-DCP degradation show that the degradation rate of 2,4-DCP was high only under a low initial cadmium concentration, indicating the inhibition of 2,4-DCP degradation by cadmium, which is in agreement with the results of an earlier study by Nkhalambayausi-Chirwa and Wang (2001). Toxic heavy metals can inhibit the growth, cause morphological and physiological changes and affect the reproduction of *P. chrysosporium*. Extracellular ligninolytic and cellulolytic enzymes are regulated by heavy metals on the level of transcription as well as during their action (Baldrian 2003). In this study, during the degradation of 2,4-DCP by *P. chrysosporium*, cadmium probably interfere with both the activity of extracellular enzymes involved in the process and fungal colonisation. Therefore, the 2,4-DCP degradation rate decreased with an increase in cadmium concentration.

An increase in the initial 2,4-DCP concentration enhanced cadmium removal and 2,4-DCP degradation until an optimum level of 20 mg/L was reached. This was probably because a low concentration of 2,4-DCP could be used as carbon and energy source during the cadmium removal process. Similarly, it has been shown that the removal of heavy metals and the degradation of organic pollutants are inhibited when the organic pollutant concentration is above an optimum value (Nkhalambayausi-Chirwa and Wang 2000). A high concentration of 2,4-DCP,

beyond the optimum level of 20 mg/L, resulted in decreased rates of cadmium removal and 2,4-DCP degradation, which could possibly be attributed to 2,4-DCP toxicity toward biological activity.

In the present study, the percentages of cadmium removal increased with an increase in the amount of inoculation of *P. chrysosporium*. However, if the uptake of cadmium against the weight of biomass (mg/g) is plotted, an opposite result was received. The higher cadmium uptake (mg/g) at a lower dosage of biomass was due to the higher ratio of metal to biosorbent. The number of binding sites available for adsorption was determined by the dose of biomass added into the solution. The low uptake capacity at a high dosage of biomass may be attributed to the reduction of the overall surface area of the biosorbent by the aggregation during sorption (Nuhoglu et al. 2002), while the increase of removal efficiency was due to the increase of the vacant adsorption sites with more biomass existing in solution (Kumar and Porkodi 2007; Chen et al. 2008).

The functional groups for heavy metal ions binding on the fungal cell walls are carboxyl (−COOH), phosphate (PO_4^{3-}), amide ($-NH_2$), thiol (−SH) and hydroxide (−OH) (Bayramŏglu and Arıca 2008). In fungal cell walls, chitin and its associated proteins contain many carboxyl groups with pK_a values in the range of 4.0–5.0 (Rostami and Joodaki 2002; Akar and Tunali 2006). Phosphate groups are present mainly in glycoproteins and are believed to play an important role in biosorption because they can exhibit a negative charge above pH 3.0 (Zeng et al. 2007). The FT-IR spectra (Fig. 6) of the native and incubated fungus show that the significant change was the move of vibrancy frequency of –OH. The adsorption peak of 3,384–3,402 cm^{-1} indicated that the hydroxyl group had been changed from a multimer to a monopolymer or even a dissociative state, which showed that the degree of the hydroxyl polymerization in lignocellulose was decreased by the addition of cadmium cations (Chen et al. 2008). It offered more opportunity for cadmium to be bound to the hydroxyl groups. Another change in the spectrum was the –CO–NH–. The adsorption peak around 1,743 cm^{-1} shifted to 1,734 cm^{-1}, and the intensity was strengthened. This indicated the reaction of cadmium and peptide bond. After the cadmium ions were adsorbed onto the biomass, the carbonyl double-bond stretching band exhibits a clear shift to a lower frequency at 1,649 cm^{-1}, corresponding to the complexation of cadmium to C = O bonds. This indicates the typical carboxylic absorption. The intensity of the peak representing the $-COO^-$ groups after cadmium sorption becomes much lower than that for the $-COO^-$ groups before the sorption (comparing the peak at 1,654–1,649 cm^{-1}). The results indicate that the $-COO^-$ groups are involved in the metal binding.

The pH values in the solutions decreased during the incubation process. This could probably be attributed to the production of organic acid during the metabolic process of the fungus (Kenealy and Dietrich 2004) and 2,4-DCP degradation. Some kinds of organic acids such as the oxalic acid secreted by wood-rotting fungi have several roles in cellulose and lignin degradation, e.g. the chelation and stabilisation of Mn(III), providing H_2O_2 and buffering the environment, all of which are important factors in the performance of lignin-degrading peroxidases (Ogawa et al. 2004). Low concentrations of heavy metals can boost the fungal production of organic acids (Jarosz-Wilkolazka and Gadd 2003). However, when the heavy metal concentration exceeded a certain value, this trend would be inhibited. No significant change in the pH value was observed beyond the incubation time of 48 h. This is probably attributable to the consumption rate of the organic acid (the organisms consumed organic acid as a carbon and energy source) which was equivalent to its production rate. A similar phenomenon was observed in phenol degradation by *P. aeruginosa* (Song et al. 2009).

The concentration of extracellular proteins decreased with an increase in cadmium concentration, which could possibly be attributed to cadmium toxicity toward biological activity. Cadmium is in general the most toxic metal for all white-rot fungi (Baldrian 2003). After cadmium enters the cell during the uptake process by *P. chrysosporium*, it affects both individual reactions and complex metabolic processes. The growth and production of protein were inhibited. It is well known that protein plays a crucial role in the process of heavy metal intake by microbial cells. The transmembrane protein on the cell membrane carries the heavy metal ions into the cell through ion channels. Oxygenous or nitrogenous functional groups such as carboxyl, amino and peptide bonds in the proteins of microbial cell walls have been suggested as responsible for the binding of metal ions in microorganisms (Lin et al. 2005). The protein in the medium was beneficial to the removal of the cadmium.

Previous studies on the degradation of xenobiotics by *P. chrysosporium* showed that LiP and MnP are involved in the mineralization of most organic compounds (Sedighi et al. 2009) and that LiP plays an important role in the dechlorination of polychlorinated phenols (Tuomela et al. 2002; Zouari et al. 2002). The relationship between cadmium and enzyme activity was also studied. Heavy metals in general are potent inhibitors of enzymatic reactions. The inhibition of proteolytic enzymes by metals can change the turnover rate of extracellular enzymes (Palmieri et al. 2001). Cadmium can cause an oxidative damage of proteins by the induction of oxidative stress associated with the production of reactive oxygen species like hydroxyl or superoxide radicals (Stohs and Bagchi 1995). Moreover, cadmium has been shown to affect hydrogen peroxide concentration in vitro. Hydrogen peroxide is one of the substrates for several lignin-degrading enzymes but, when produced in higher concentrations, it can inactivate Mn-peroxidase (Cabaleiro et al. 2001). Therefore,

the enzyme activity decreased with increasing cadmium concentration. LiP and MnP are the secondary metabolites of *P. chrysosporium*. The secondary metabolism of *P. chrysosporium* can be only triggered by C or N limitation. After 3 days of incubation, C and N in the culture medium were depleted gradually. Thus, LiP and MnP activities increased with the incubation time and reached the maximum value at 60 or 72 h. With a further increase in the incubation time, the enzyme activity decreased. This was probably due to the fact that the biology activity decreased since the prolonged lack of nutrients. The maximum activity at 60 or 72 h was in agreement with the previous studies carried out by Zouari et al. (2002) and Radha et al. (2005).

In conclusion, *P. chrysosporium* was successfully used for the removal of cadmium and 2,4-DCP from artificial wastewaters. The removal rates were dependent on the experimental conditions, particularly the pH of the medium, initial concentrations of the two pollutants and the amount of inoculation. Optimum cadmium removal and 2,4-DCP degradation were observed at a cadmium concentration of 5 mg/L and a 2,4-DCP concentration of 20 mg/L under pH 6.5. The concentration of cadmium is a critical factor affecting the removal of cadmium and 2,4-DCP. The extracellular secretions including organic acid, protein and enzyme are regulated by cadmium. A low concentration of 2,4-DCP could be used as a carbon and energy source by *P. chrysosporium*, which is beneficial to cadmium removal. The FT-IR study shows that the hydroxyl, carboxyl and –CO–NH– functional groups play an important role in cadmium binding. This paper indicates the potential for using a single microorganism, *P. chrysosporium*, to remove both heavy metals and organic pollutants.

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