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BIOSORPTION BEHAVIOR OF IMMOBILIZED
Phanerochaete chrysosporium FOR HEAVY METALS REMOVAL

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Abstract

Heavy metals are inorganic persistent pollutants with adverse health and environmental effects. In our study, self-synthesized iron oxide magnetic nanoparticles were encapsulated in the *Phanerochaete chrysosporium* (*P. chrysosporium*) hyphae. The prepared biosorbents possessed high efficiency for Pb(II) biosorption from single and binary metal systems. The maximum biosorption capacity was found to be 50.05 mg g^{-1} at pH 5.0. Environmental scanning electron microscope accompanied with energy disperse spectroscopy (ESEM-EDS) characterization showed Pb(II) ions were partially enriched via extracellular complexation and surface biosorption. MR analysis, defined as the ratio of heavy metals removed to H$^+$ released, confirmed the ion-exchange, surface complexation and extracellular chelation behavior of the biosorbents. Moreover, distinct increase in the interior of Pb(II) contents in the immobilized *P. chrysosporium* suggested that iron oxide magnetic nanoparticles promoted biosorption process. The proposed immobilized biosorbents, showing high efficiency and strong feasibility, exhibited the potential application in Pb-containing industrial wastewater treatment.

Key words: heavy metal, wastewater treatment, biosorption, immobilized *Phanerochaete chrysosporium*

1. Introduction

The discharge of heavy metals into aquatic ecosystems has become a global matter of concern over the last few decades, significantly as a result of rapid industrialization. The need for an effective and affordable technological solution for heavy metal removal is more pronounced nowadays, because of the acute toxicity of heavy metals (Anjum et al., 2013; Chellvarajoo et al., 2017; Huang et al., 2008; Xu et al., 2012). For instance, lead (Pb), which has been reported to be the most representative toxin, frequently encounters other metals in fine-chemical process or battery industry wastewater (Huang et al.,

2010). The application of biosorbents has emerged in the last decade as one of the most promising alternatives to conventional methods, based on metal binding capacities of various biological materials via metal–biomass interactions. Biosorption is a process that utilizes of a biological matrix to sequester toxic heavy metals and is being demonstrated as useful environmental biotechnology alternative to conventional systems for the heavy metals management for drinking and industrial water (Fomina and Gadd, 2014; Ghorbani and Younesi, 2016). The major advantages of biosorption over conventional treatment methods include high removal efficiency with low cost, regeneration of biosorbent

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avoiding secondary pollution and minimization of the volume of sludge (Arifa and Humaira, 2013; Malik, 2004). These advantages serve as the primary incentives for developing full-scale biosorption processes for heavy metal containing wastewater.

Actually, large-scale utilization of native form biosorbents has been hindered by problems associated with the poor mechanical strength/rigidity, which may cause significant mass loss after regeneration. Appropriate immobilization of the biomass can offer ideal size, increased biosorption capacity, mechanical strength and rigidity, exhibiting greater potential in large-scale wastewater treatment (Sudha and Abraham, 2003). Regarding limitations of free cells of *P. chrysosporium* application in biosorption process, conducting a satisfactory immobilization matrix to enhance and accelerate biosorption process becomes more feasible.

However, biosorption behavior and mechanism of immobilized biosorbents was scarcely studied, especially in immobilized biosorbents combining of iron oxide magnetic nanoparticles (Fe_3O_4 MNPs) and fungi. Therefore, the main objective of this study is to systematically analyze the biosorption behavior and mechanism of a novel immobilized *P. chrysosporium* embedded with Fe_3O_4 MNPs. Optimal biosorption condition was determined as a function of pH and its time-dependent variation, since their important roles in controlling the biosorbent-metal interface. Most importantly, biosorption behavior and mechanism was further studied by environmental scanning electron microscope accompanied with energy disperse spectroscopy (ESEM-EDS) characterization, pH variation analysis and Pb(II) biosorption contents in the interior of the immobilized *P. chrysosporium*.

2. Material and methods

2.1. Materials

P. chrysosporium (BKMF−1767) was maintained by subculturing on potato dextrose agar (PDA) slants at 4 °C. Spore suspensions were prepared in the sterile distilled water at a concentration of 2.0×10^6 CFU mL^{-1}. Reagents were of analytical reagent grade and obtained from Shanghai chemical reagent company of China national pharmaceutical industry corporation.

2.2. Preparation of biosorbents

Iron oxide magnetic nanoparticles (Fe_3O_4 MNPs with size of 15-40 nm) and Ca−alginate were embedded in the *P. chrysosporium* hyphae pellets. First, mixed up Na−alginate (4%, 2 mL) and a series quality of MNPs (0.10 g), and then kept sterilization under 105 °C for 30 min. Next, 1.0 mL mycelium suspensions (2.0×10^6 CFU mL^{-1}) were inoculated into the mixture, and then drop wisely transferred to sterile CaCl$_2$ solution (0.2 M, 40 mL). After that, the microspheres containing *P. chrysosporium* formed

after 4 h stewing, and then rinsed twice with sterile distilled water. Finally, the mixtures were introduced into 100 mL growth medium and incubated at 30 °C at 120 rpm for 5 days, collected for biosorption study. The size of the biosorbents was about 4.5-5.0 mm and showed a larger size than free *P. chrysosporium* (about 3.0 mm).

2.3. Biosorption studies

Biosorption experiments were carried out at 35°C with shaking at 150 rpm in 250 mL conical flasks, which contained 50 mL of heavy metal containing solution at desirable concentrations. The batch biosorption experiments were performed by mixing 0.1 g (wet biomass) of immobilized P. chrysosporium in 50 mL of synthetic Pb(II)-containing solutions (100 mg L^{-1} Pb(II)) at pH 4.0, 5.0 and 6.0, respectively. The pH adjustment was done with the addition of either 0.1 M NaOH or 0.1 M HCl. Furthermore, biosorption of binary metals was conducted at the presence of Pb(II)/Cu(II), Pb(II)/Mn(II), Pb(II)/Zn(II), Pb(II)/Cd(II) and Pb(II)/Mg(II) binary systems for 24 h. Several 0.1 M of metal stock solutions were prepared by dissolving Pb(NO$_3$)$_2$, Cu(NO$_3$)$_2$·3H$_2$O, MnCl$_2$·4H$_2$O, Cd(NO$_3$)$_2$·4H$_2$O, MgCl$_2$·6H$_2$O, ZnSO$_4$·7H$_2$O, respectively. Each metal concentration was controlled at 100 mg L^{-1}. All batch experiments were conducted in triplicate and data presented were the mean values from these independent experiments.

2.4. Biosorption behavior analysis

*Environmental scanning electron microscope (*ESEM, FEI QUANTA 200) accompanied with energy disperse spectroscopy (EDS, EDAX genesis xm–2) were applied to analyze the component of the biosorbents before and after biosorption, after gold plating at an accelerating voltage of 20 kV.

A series of biosorption experiments were conducted at different initial pH (pH$_0$, 2.0–6.5) to study biosorption mechanisms. Define MR as the ratio of heavy metals removed to H$^+$ released (Zhan et al., 2001) (Eq. 1).

$$MR = (c_0 - c_e)/(10^{-pHe} - 10^{-pH0}) \qquad (1)$$

where: c_0 and c_e are initial and equilibrium Pb(II) concentrations (mmol L^{-1}), respectively, pH_0 and pH$_e$ is initial and equilibrium pH of solution, respectively.

2.5. Determination of metal concentrations

Pb(II), Cd(II), Zn(II), Cu(II), Mn(II) and Mg(II) concentrations were measured by an atomic absorption spectrometer (AAS, Agilent 3510, USA) at 283.3 nm, 228.8 nm, 213.9 nm, 324.7 nm, 279.5 nm and 285.2 nm, respectively. Meanwhile, the immobilized *P. chrysosporium* were collected after biosorption process and washed with phosphate solution (0.05 mM, pH7.0) for three times, and then

centrifuged at 5000 rpm for 10 min. Thereafter, the collected samples were homogenized in 5 mL of phosphate solution (0.05 M, pH 7.0). The homogenate was centrifuged at 10,000 rpm at 4 °C for 10 min, and the supernatant was filtered for the metal analysis to investigate the biosorption of Pb(II) in immobilized *P. chrysosporium.*

3. Results and discussion

3.1. Batch biosorption experiment

3.1.1 Biosorption from single metal system

Commonly, as proved by lots of studies, low initial pH resulting in low biosorption capacity, large quantities of protons competing with heavy metals for functional groups of cells and the approach of heavy metals were restricted as a result of the repulsive force (Kieu et al., 2011). While high pH leads to hydrolyzation of heavy metals, also restrains the biosorption efficiency. Therefore, the experiments were carried out with range of initial pH 4.0–6.0 at 35 °C in our study, to optimize pH and contact time on the biosorption of Pb(II).
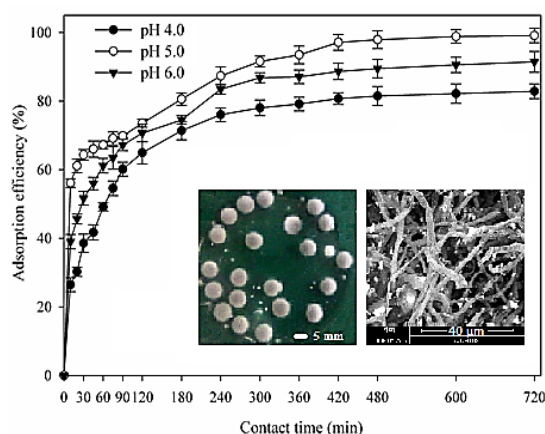
The effect of pH and contact time on biosorption efficiency is shown in Fig. 1. As depicted in Fig. 1, the biosorption rate was very fast at the beginning 1 h. The rapid uptake revealed a high affinity between the Pb(II) and biosorbent, which was mainly directed to the rapid biosorption via extracellular secreta or surface biosorption. Afterwards, a slower rates of uptake occurred from 90 min to 480 min, and no further significant biosorption were observed beyond this period, which might be attributed to the slower occurrence of biosorption intracellularly. It could be speculated that biosorption intracellularly might be enhanced with the embedding of MNPs, well-distributed in the interior of *P. chrysosporium* (Fig. 1). The equilibrium biosorption capacity was found to be 41.42, 50.05 and 45.70 mg g^{-1} at pH 4.0, 5.0 and 6.0, respectively. Maximum biosorption capacity was therefore found at pH 5.0. As widely reported, pH impacts on biosorption process might be associated with surface charges and functional groups of the biosorbents and also the formation of ion species. At low pH values, the *P. chrysosporium* cell surface tends to be positively charged, resulting the favorable attraction between Pb(II) and functional groups at the cell wall. However, the cell surface might tend to be negatively charged and inhibited the biosorption process when the pH increases (Gabr et al., 2008; Rivera-Utrilla et al., 2001). As a result, biosorption of heavy metals was partially controlled by the surface charge and biosorbent-metal interface, which might be correlated to solution pH controlling the dissociation of functional groups at the surface of biosorbents.

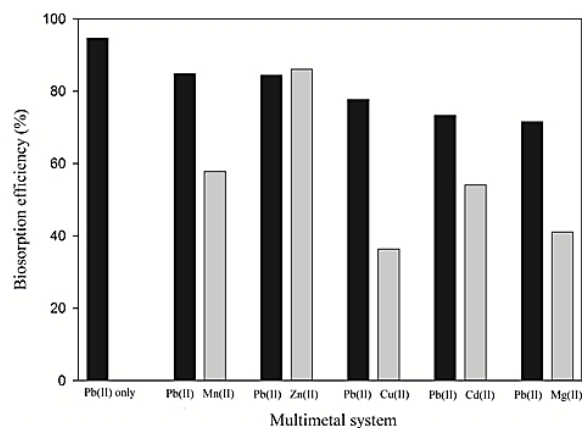### 3.1.2. Biosorption from binary metal solution

Commonly, there is more than one metal in the solution and, in this case, the assessment of the biosorption performance in binary metal systems

becomes necessary (Srivastava et al., 2008). The biosorption of Pb(II) from binary metal systems was therefore investigated. As seen in Fig. 2, it was evident that at the presence of co-existing ions, the biosorption efficiency for Pb(II) was lower than non-competitive condition, mainly ascribed to the competitive biosorption to Pb(II). Immobilized *P. chrysosporium* showed higher potential in Zn(II) and Mn(II) biosorption, while with the lowest affinity to Cu(II) in Pb(II)/Cu(II) system. The results might be related to the affinity variation to each heavy metal affecting by various factors, such as ion charges, molecular mass, hydrated ionic radius and hydration energy of the metals (Lv et al., 2005).

However, even the Pb(II) biosorption efficiency decreased, the presence of other metal ions increased the total biosorption capacity of the biosorbents under experimental conditions. For example, the biosorption efficiency for Pb(II) and Zn(II) was 83.76% and 86.65%, respectively. It could be calculated that the total sorption capacity was 85.11 mg g^{-1} (41.88 mg g^{-1} for Pb(II) and 43.33 mg g^{-1} for Zn(II)), much higher than Pb(II) only (47.34 mg g^{-1} dry biosorbent).

Fig. 1. Effect of pH and contact time on Pb(II) biosorption efficiency (Pb(II) concentration: 100 mg L^{-1}; Biosorbent dosage: 2.0 g L^{-1}; Temperature: 35 °C; Biosorbents with the size of 4.5-5.0 mm)

Fig. 2. Biosorption of binary metals by immobilized *P. chrysosporium* (each metal ion concentration: 100 mg L^{-1}; pH of the solution: 5.0; contact time: 24 h; biosorbent dosage: 2.0 g L^{-1}; temperature: 35 °C)

The results showed that the biosorption of Pb-containing binary metals was competitive as well as cooperative. The biosorption capacity of Pb(II) and Cd(II) in *P. chrysosporium* without immobilization was 23.81 mg g^{-1} and 15.17 mg g^{-1} in our previous study (Li et al., 2011), whereas those in immobilized *P. chrysosporium* in this study was 36.66 mg g^{-1} and 27.07 mg g^{-1}, respectively. Nassar (2010) reported that the equilibrium biosorption capacity of Pb(II) and Cd(II) was about 9.9 and 2.1 mg g^{-1} in Pb(II)/Cd(II) binary system. The experimental results indicated that MNPs-Ca-alginate immobilized *P. chrysosporium* could be considered as a potentially useful material for the treatment of Pb(II) polluted wastewaters.

3.2. Extracellular complexation behavior

We applied ESEM–EDS of *P. chrysosporium* to reveal the extracellular complexation behavior (Fig. 3). The hyphae of immobilized *P. chrysosporium* were packed loosely with numerous tiny interspaces, in favor of the transmission and biosorption of Pb(II) ions. Meanwhile, ESEM image of immobilized fungal mycelia shows the formation of granular particle after biosorption, both on the cell wall surface and at interspaces of fungal hyphae (Fig. 3 a and b). Fig. 3(a′ and b′) represents the EDS results of biosorbents after biosorption.

After 24 h of biosorption, the *P. chrysosporium* hyphae existed a Pb-rich composition. It was obvious that Pb(II) ions existing in aqueous solution were biosorbed to the biosorbent surface (mainly *P. chrysosporium* hyphae). Accordingly, the extracellular secretion of organic acids by white rot fungi at the presence of heavy metals has been proved in our previous study, suggesting a role of surface precipitation in metal biosorption (Li et al., 2011). According to Huang et al (2008), one of the typical metal chelators produced by white-rot fungi is oxalic acid, facilitating the biosorption of heavy metals by chelating as insoluble oxalates. The secretion of oxalic acids therefore intimately involved in Pb(II) biosorption via production of oxalate–metal complexes.

3.3. Surface biosorption analysis

Accordingly, three possible surface biosorption mechanisms for heavy metals have been proposed in previous study, mainly ion-exchange, hydrolytic adsorption and surface precipitation (Zhan et al., 2001). In our study, a series of biosorption experiments were conducted at different initial pH to study surface biosorption mechanisms by detecting the pH variation (Fig. 4). Apparently, pH decreased during the whole biosorption process, at pH 4.0–6.0.
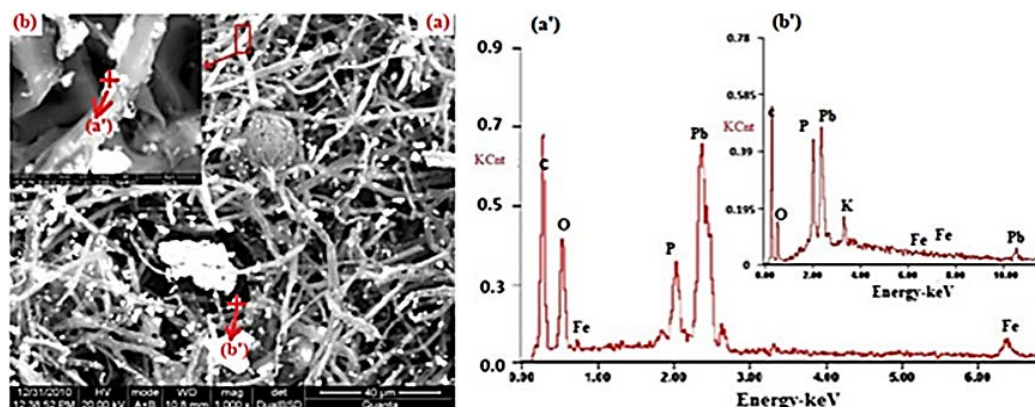

Fig. 3. ESEM images (a and b) and EDS spectra (a′ and b′) of the immobilized *P. Chrysosporium* hyphae after 24 h of biosorption (the cross area in (a and b))
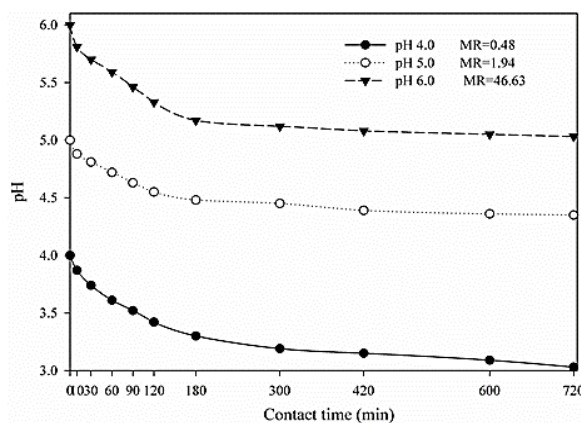

Fig. 4. Solution pH variation tendency during the whole biosorption process at pH 4.0, 5.0 and 6.0 (Pb(II) concentration: 100 mg L^{-1}; Biosorbent dosage: 2.0 g L^{-1}; Temperature: 35 °C

Accordingly, Pb(II) ions are positively charged, chelated on the free binding sites at the surface of cell walls. The decrease of pH might be conducted to the releasing of hydrogen ions at the surface of the biosorbents, depending on ion-exchange mechanism (Eqs. 2–3).

$$2RH + Pb^{2+} = R_2Pb + 2H^+ \qquad (2)$$

$$2R\text{-}OH + Pb^{2+} = 2(RO)Pb + 2H^+ \qquad (3)$$
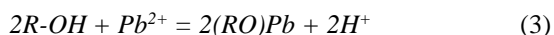
In order to further analyze surface biosorption mechanism, MR values calculated is shown in Table 1. At pH_0=2.0–3.0, the equilibrium pH_e values were slightly higher than initial pH_0. Accordingly, low pH of solutions increases H^+ concentration and intensifies the competition between H^+ and heavy metal ions for complexation sites, resulted in the slight increase of solution pH and negative MR value in the low pH range of 2.0–3.0 (Zhan et al., 2001). When pH was in the range of 4.0–5.0, MR was around 0.5, indicating the occurrence of ion exchange preferentially (Eq (3–4) (Xu et al., 2013). At pH 6.0–6.5, Pb^{2+} and $Pb(OH)^+$ were partly translated to $Pb(OH)_2$.

$Pb(OH)_2$ formed before biosorption thereafter can be hydrolyzed by the functional group of biosorbents as shown as Eq. (4) (Naiya et al., 2008).While pH_0 varied from 6.0–6.5, MR was much more than 0.5, and increased with the initial pH, indicating the dominant biosorption mode changed to hydrolysis adsorption (Zhou et al., 2013). Based on these findings, a hypothesis could be proposed that surface precipitation occurred predominantly at low pH range of 2.0–3.0, and ion-exchange preferentially takes place at pH 4.0–5.0, while at the relatively high pH range of 6.0–6.5, hydrolytic adsorption accounts for a large proportion.

$$RH + Pb(OH)_2 = R\text{-}Pb\text{-}OH + H^+ \qquad (4)$$
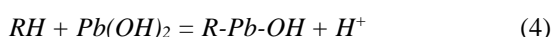
Table 1. Values of MR (being the ratio of heavy metals removed to H^+ released) at different initial pH

pH_0	2.0	3.0	4.0	5.0	6.0	6.5
c_e (mg/L)	36.8	26.74	17.16	4.97	8.56	20.08
pH_e	2.03	3.05	3.03	3.61	5.03	5.14
MR	-0.46	-3.25	0.48	1.94	46.63	64.28

3.4. Biosorption in the interior of biosorbents

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The content of Pb(II) in the interior of free *P. chrysosporium* was 1.33 mg g^{-1} (Fig. 5, pH=5.0), results were quite agreed well-known definition that biosorption is a physico-chemical process, including both passive interaction between biosorbents and biosorbates and metabolically-independent active transmission processes in case of living biomass (Fomina and

Gadd, 2014). As observed, Pb(II) content in the interior of immobilized *P. chrysosporium* reached at 6.08, 12.21 and 11.57 mg g^{-1} after 24 h of biosorption at pH 4.0, 5.0 and 6.0, respectively. Results indicated that the embedding of the MNPs and Ca-alginate yielded a distinct enhancement of biosorption, due to the large surface of iron oxide MNPs. Results demonstrated that metal ions biosorbed to the surface of the biosorbents were partly transmitted to the interior of biosorbents, which embedded in MNPs and Ca-alginate.

Therefore, a three-step process has been presented to describe the biosorption behavior of the immobilized *P. chrysosporium*. The first process involves the chelation of heavy metals by the excretive organic acid extracellularly, besides surface complexation onto cell walls and/or other outer layers occurred in a fast and reversible surface biosorption step, thereafter a much slower and complex overall accumulation mechanism occurred in the interior of biosorbents, confirmed by the promoted Pb(II) contents.
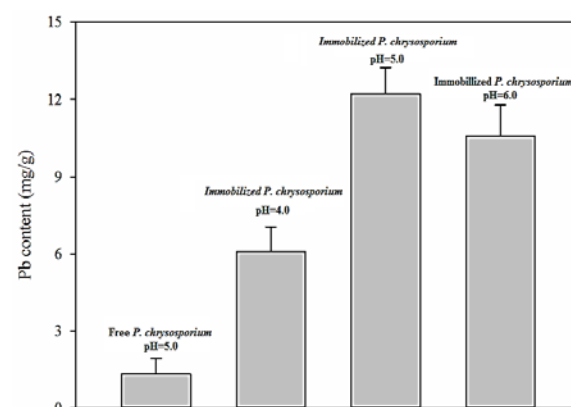
Fig. 5. Pb(II) contents in the interior of biosorbents at initial concentration of 100 mg L^{-1} Pb(II) (pH: 4.0−6.0; Contact time: 24 h; Biosorbent dosage: 2.0 g L^{-1}; Temperature: 35 °C)

4. Conclusions

The high potential of immobilized *P. chrysosporium* to remove Pb(II) and Pb(II)-containing binary metals was demonstrated in this study. ESEM-EDS characterization after biosorption indicated the extracellular complexation and surface biosorption behavior in the immobilized *P. chrysosporium*. Results based on MR analysis further suggested the occurrence of surface precipitation (at low pH range of 2.0–3.0), ion-exchange (pH 4.0–5.0) and hydrolytic adsorption (pH 6.0–6.5).

Moreover, Pb(II) contents in the interior of biosorbents was calculated at the value of 6.08, 12.21 and 11.57 mg g^{-1} at pH 4.0, 5.0 and 6.0, respectively. As a result, the development of the immobilized *P. chrysosporium* biosorbent is quite significant, which is expected to provide a potential application for Pb(II)-contaminated wastewater treatment.

Acknowledgements

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