Bioresource Technology 101 (2010) 1668–1674

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Biosorption of cadmium by endophytic fungus (EF) *Microsphaeropsis* sp. LSE10 isolated from cadmium hyperaccumulator *Solanum nigrum* L.

Xiao Xiao [a], Shenglian Luo [a,b,c,*], Guangming Zeng [a], Wanzhi Wei [b], Yong Wan [a], Liang Chen [a], Hanjun Guo [a], Zhe Cao [a], Lixia Yang [a], Jueliang Chen [a], Qiang Xi [b]

[a] College of Environmental Science and Engineering, Hunan University, Changsha 410082, People's Republic of China
[b] State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, People's Republic of China
[c] School of Environment and Chemical Engineering, Nanchang Hangkong University, Nanchang 330063, People's Republic of China

ARTICLE INFO

Article history:
Received 9 July 2009
Received in revised form 18 August 2009
Accepted 25 September 2009
Available online 24 October 2009

Keywords:
Endophytics fungus
Hyperaccumulator
Biosorbent selection
Biosorption
Cadmium

ABSTRACT

A novel technology to obtain highly efficient biosorbent from the endophytes of a hyperaccumulator is reported. This technology is more convenient than the traditional method of obtaining biosorbents by experimentally screening many types of biomass by trial and error. Using this technology, endophytic fungus (EF) LSE10 was isolated from the cadmium hyperaccumulator *Solanum nigrum* L. It was identified as *Microsphaeropsis* sp. When cultured in vitro, the biomass yield of this EF was more than twice that of none-endophytic fungus (NEF) *Rhizopus cohnii*. Subsequently, it was used as a biosorbent for biosorption of cadmium from the aqueous solution. The results showed that the maximum biosorption capacity was 247.5 mg/g (2.2 mmol/g) which was much higher than those of other adsorbents, including biosorbents and activated carbon. Carboxyl, amino, sulphonate and hydroxyl groups on EF LSE10 surface were responsible for the biosorption of cadmium.

1. Introduction

Cadmium is known to be a harmful heavy metal. It is non-biodegradable and tends to accumulate in living organisms, causing significant threats to both the environment and public health (Chen et al., 2008). Therefore, a number of physicochemical strategies, such as filtration, chemical precipitation, electrochemical treatment, oxidation/reduction, ion exchange, membrane technology, reverse osmosis, and evaporation recovery, have been developed to remove heavy metals, including cadmium, from polluted water. However, most strategies appear to be expensive, inefficient, and labor-intensive, or the treatment process lacks selectivity (Chen et al., 2008; Tang et al., 2008).

Recently, heavy metal phytoremediation employing living plants, especially hyperaccumulators, to degrade and detoxify contaminants has attracted attention due to the efficiency and low cost (Garbisu and Alkorta, 2001). Heavy metal hyperaccumulators are plant species that can accumulate exceptionally high quantities of heavy metals (Brooks, 1998), exhibiting higher heavy metal tolerance and accumulating abilities compared to other plants. Many hyperaccumulators such as *Thlaspi caerulescens* (Baker et al., 1994), *Arabidopsis halleri* (Dahmani-Muller et al., 2000), and *Solanum nigrum* L. (Wei et al., 2005) have been utilized for the phytoremediation of cadmium. However, there are several disadvantages that limit the use of phytoremediation. First, it is difficult to find heavy metal hyperaccumulators (only about 500 plant species have been identified). Moreover, the phytoremediation efficiency is not only dependent on the metal accumulation capacity of the plants, but also on its biomass yields. Hyperaccumulators usually grow slowly and have a limited biomass since they are affected by the accumulated heavy metal. This makes the detoxify process much more time consuming than other strategies. Therefore, phytoremediation is not feasible for rapid heavy metal sewage treatment.

On the other hand, biosorption is considered to be an economic, eco-friendly and efficient option to solve the environmental pollution of heavy metals. Many scientists devote to finding good biosorbents which possess both the high sorption ability and high biomass yield. Some literatures report that many kinds of microorganisms are capable of removing heavy metals during sewage treatment, such as the byproducts of brown-rot fungus *Lentinus edodes* (Chen et al., 2008), white-rot fungus (Arıca et al., 2001; Huang et al., 2008), *Pseudomonas veronii* 2E (Vullo et al., 2008) and protonated *Sargassum* biomass (Yang and Volesky, 1999). However, until present we have to rely on experimentally finding good biosorbents by trial and error (screening many types of biomass). Some microorganisms are obtained from industrial or agricultural wastes usually with low treatment efficiency or need

* Corresponding author. Address: College of Environmental Science and Engineering, Hunan University, Changsha 410082, People's Republic of China. Tel./fax: +86 731 88821967.
E-mail address: sllou@hnu.cn (S. Luo).

0960-8524/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2009.09.083

pretreatment before application (Matheickal et al., 1999; Yang and Volesky, 1999; Yin et al., 1999). While most microorganisms are directly isolated from the contaminated environments (Pérez Silva et al., 2009; Puranik and Paknikar, 1999; Vullo et al., 2008) by an extremely tedious and time consuming process. These are not convenient at all. Thus, a simple and highly effective method to obtain biosorbents with high treatment efficiency is needed for biosorption.

It is known that endophytes inhabit virtually every plant on earth. They colonize the internal tissues of the host plant without an external sign of infection or negative impact on their host (Schulz and Boyle, 2006). Endophytes facilitate plant growth and biomass yield, and could act as a biocontrol agent (Backman and Sikora, 2008; Kuklinsky-Sobral et al., 2004). Endophytes can also be beneficial to their host by producing a series of natural products that may be potentially used in medicine, agriculture or industry (Strobel et al., 2004). In addition, it has been shown that they have the potential to remove soil contaminants by enhancing phytoremediation (Barac et al., 2004; Van Aken et al., 2004).

However, to the best of our knowledge, there're not any reports about the utilization of endophytes as biosorbents for heavy metal sewage treatment. Microorganisms which possess the metal and radionuclide bioremediation capacity are ubiquitous in the environment, and their frequency is often increased in contaminated sites (Barkay and Schaefer, 2001). As the endophytes of a heavy metal hyperaccumulators can endure high concentrations of a certain heavy metal due to the hyperaccumulation ability of their hosts. Therefore, the endophytes in a hyperaccumulator are expected to be novel and promising biosorbents for a certain heavy metal sewage treatment.

Herein, endophytic fungus (EF) *Microsphaeropsis* sp. LSE10 which was isolated from a reported cadmium hyperaccumulator *S. nigrum* L. (Wei et al., 2004) whose bioaccumulation capacity is above 100 mg/kg were first employed as a biosorbent to remove its host's hyperaccumulation of heavy metal cadmium. The effect of pH, contact time, dosage and initial concentrations were studied. Moreover, the reusability was also tested.

2. Methods

2.1. Preparation of reagents and medium

All reagents used were of analytical grade and purchased from Shanghai Pharmaceutical Co. Ltd., in China. The 1000 mg/L Cd(II) stock solution was prepared by dissolving the exact quantities of the $3CdSO_4{\cdot}8H_2O$ in deionized-distilled water. The working concentration of Cd(II) solution was prepared from suitable serial dilution of the stock solution. The deionized-distilled water used in this experiment was obtained from a Milli-Q system (Millipore, USA).

Czapek's culture medium containing 3 g/L $NaNO_3$, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.5 g/L KCl, 0.01 g/L $FeSO_4{\cdot}7H_2O$, 1 g/L K_2HPO_4, 30 g/L sucrose and tryptic soy broth (TSB) medium comprising of tryptone, 15 g/L, soya, 5 g/L, beef extract, 5 g/L, NaCl, 5 g/L. The pH of the medium was adjusted to 7.2–7.4.

2.2. Isolation and identification of EF LSE10

The cadmium hyperaccumulator *S. nigrum* L. were collected at the sewage discharge canal bank of Zhuzhou Smeltery (27°52′N, 113°05′E). The plants were washed in tap water to remove soil, and divided into leaf, petiole, rhizome, and root portions. Subsequently, these parts were surface sterilized by 70% ethanol and sodium hypochlorite (2% available Cl^-) according to Kuklinsky-Sobral's method (Kuklinsky-Sobral et al., 2004). Each sterilized plant part was cut into 1 cm long segments as appropriate. The prepared plant segments were plated onto TSB agar plates and incubated at 303 K for 7 days.

The genomic DNA of EF LSE10 was extracted and 26S rDNA was amplified in polymerase chain reaction (PCR) using the genomic DNA as template and fungus universal primers, NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′) (O'Donnell, 1993). Amplification was performed for 30 PCR cycles with annealing at 328 K for 0.5 min, extension at 345 K for 1 min, and denaturation at 367 K for 0.5 min. The amplified DNA was purified with TaKaRa Agarose Gel DNA Purification Kit (TaKaRa, China) and sequencing was performed at TaKaRa Biotechnology Company, Limited (Dalian, China). Primers using for sequencing was Seq Reverse Primer 5′-GAGCGGATAACAATTTCACACAGG-3′ and Seq Forward Primer 5′-CGCCAGGGTTTTCCCAGTCACGAC-3′. The 26S rDNA sequence was compared against the GenBank database using the NCBI Blast program.

2.3. Biomass yield

The fungus was cultured on Czapek's argar medium until the mycelial colonies were approximately 3 cm in diameter. Such a colony was comminuted in 10 mL sterile water then inoculated to 200 mL Czapek's medium. After incubation for 3 days, the mycelia were collected and dried overnight at 343 K. The biomass yield was determined by weighting the dried biomass. None-endophytic fungus (NEF) *Rhizopus cohnii* an industrial fungus presented by Hunan Light Industry Research Institute was used as control. The experiments were done triplicate, yielding an experimental error of less than 5%.

2.4. Preparation of the adsorbents

The EF *Microsphaeropsis* sp. LSE10 and NEF *R. cohnii* were cultivated in the Czapek's culture medium at 303 K. After incubation for 3 days, the mycelia were washed several times with deionized-distilled water. The biomass were killed by autoclaving (15 lb, 394 K) for 20 min, and then dried at 343 K until weight constantly. Subsequently, the dried mycelia were crushed into fractions. The powdered biomass residues obtained (particle size between 0.45 and 1.0 mm) will be, respectively, referred to as "biosorbent EF LSE10" and "biosorbent NEF *R. cohnii* " in this paper. The activated carbon with similar diameter was obtained from Shanghai XingChang Activated Carbon Co., Ltd. The adsorbents were all stored in desiccators for the following experiments.

2.5. Analytical technique

The concentrations of Cadmium ions were determined by the flame atomic absorption spectrometry (FAAS) using Z2000 polarized zeeman atomic absorption spectrophotometer (Hitachi, Japan), The hollow cathode lamp was operated at 5 mA and the analytical wavelength was set at 228.8 nm.

2.6. Batch biosorption and desorption experiments

Experiments with known concentrations of cadmium solution at 20, 50 and 100 mg/L were conducted to determine optimal pH, contact time and dosage (dry weight). Biosorbent EF LSE10 was mixed with Cd(II) solution and agitated in an incubator at 150 rpm, 303 K. The effect of pH was investigated in the range of 1.5–6.5 at the dosage of 1.0 g/L and contact time 12 h (ensure equilibrium was reached). The pH values in the solutions were monitored by a FE20 pH electrode (Mettler Toledo, Shanghai, China). The kinetics of Cd(II) sorption on biosorbent EF LSE10 was also studied. The dosage was 1.0 g/L and the pH was adjusted to 6.5.

Samples were taken and analyzed at the following time intervals: 0.25, 0.5, 1.0, 2.0, 4.0 and 6.0 h. The optimum dosage was examined in the range of 1.0–15.0 g/L.

The desorption study was conducted at the initial Cd(II) concentration of 20, 50 and 100 mg/L under previously determined optimal adsorption conditions. After the biosorption of Cd(II), the biosorbent was eluted with 0.1 M HNO_3 for 1 h at 150 rpm. Then, the biosorbent was washed with deionized water till the pH of the eluate was in the range of 5.0–5.5. This cycle was repeated five times. Samples were taken after every adsorption and desorption process by filtering through 0.45 μm filter units (Millipore, Ireland). The cadmium concentrations in the filtrate were analyzed with the methods mentioned above.

All experiments were done triplicate, yielding an experimental error of less than 5%. It is pertinent to mention that some error bars for the figures were smaller than the symbols used to plot graphs.

2.7. Effect of initial concentration and sorption capacities comparison

To estimate and compare the sorption capacities among activated carbon, biosorbent NEF *R. cohnii* and EF LSE10, the experiments were conducted at initial Cd(II) concentrations from 1 to 1000 mg/L, at previously determined optimum conditions (1 g/L dosage, pH 6.5 for biosorbent EF LSE10 and activated carbon, pH 4.0 for biosorbent NEF *R. cohnii*, at 150 rpm, 303 K) and contact for 12 h (ensure the equilibriums were reached), respectively. At the end of each experiment the mixtures were filtered through 0.45 μm filter units (Millipore, Ireland). The cadmium concentrations in the filtrate were analyzed with the methods mentioned above. The experimental data were processed via Langmuir and Freundlich isotherms.

All experiments were done triplicate, yielding an experimental error of less than 5%. It is pertinent to mention that some error bars for the figures were smaller than the symbols used to plot graphs.

2.8. Fourier transforms infrared analysis (FTIR)

To investigate the changes of functional groups during biosorption of cadmium by biosorbent EF LSE10, Fourier transform infrared analysis was employed to obtain the information associated with the biosorption mechanisms during the process.

Infrared spectra of the biosorbent before and after adsorption were acquired by a FTIR (Nicolet 5700 Thermo, USA). The sample/KBr mass ratio used for the preparation of the disks was 1:100.

3. Results and discussion

3.1. EF LSE10

By using a simple procedure, LSE10 was the only EF that isolated from the stem of cadmium hyperaccumulator *S. nigrum* L. It was identified as *Microsphaeropsis* sp. based on the 26S rDNA gene sequence analysis. The endophyte grew very rapidly when cultured in vitro. After being cultured in 200 mL of Czapek's medium for 3 days, the EF *Microsphaeropsis* sp. LSE10 yielded 0.89 g biomass. It was more than two times that of control NEF *R. cohnii* which has been used as a biosorbent (Li et al., 2008) at 5% (v/v) inoculation amount (data not shown).

Many kinds of biomass such as algae, fungi and microorganisms have been used to remove heavy metals (Arıca et al., 2001; Huang et al., 2008; Vullo et al., 2008; Yang and Volesky, 1999). However, the heavy metal biosorption assessment of an endophyte has never been reported. In this study, the *Microsphaeropsis* sp. LSE10 was the only EF obtained from cadmium hyperaccumulator *S. nigrum* L. It was not surprising that the EF *Microsphaeropsis* sp. LSE10 had such a high biomass yield when cultured in vitro. Endophytes inhabiting plants, especially heavy metal hyperaccumulators, always live under various pressures (such as competitive pressures from the host and other endophytes, as well as pressures caused by the heavy metals) with a limited nutrition supply. Thus, if the endophytes are released from such pressures and cultured with an abundant supply of nutrients in vitro, they grow rapidly and have high biomass yields. For practical and eventual biosorption process scale-up reasons, the ideal biomass type should not only possess high sorption ability but should also be available in large quantities (Volesky, 2007). When endophytes possess the preferable biosorption ability, their high biomass yield characteristics would make them advantageous in practical heavy metal treatments. Therefore, EF *Microsphaeropsis* sp. LSE10 was utilized as a biosorbent for the detoxification of cadmium, and its biosorption capacity was subsequently evaluated.

The distribution and diversity of microbes inhabiting contaminated sites and of the genes that code for phenotypes facilitating metal–microbe interactions are critical elements in metal and radionuclide bioremediation. Such microorganisms are ubiquitous in the environment, and their frequency is often increased in contaminated soils and water (Barkay and Schaefer, 2001). As a result, the biosorbents used in heavy metal biosorption are usually obtained after screening the heavy metal resistant/tolerant microorganisms from polluted environments. However, to obtain biosorbents with the potential of biosorption for specific kind of heavy metals the screening results always require further tests. Due to the complexity of polluted environments and the random screening methods, these tests determine the minimal inhibitory concentration (MIC) of each metal separately, making the process extremely tedious and time consuming.

On the contrary, obtaining potential biosorbents, endophytes from a hyperaccumulator, reported in this study was rapid, easy to manipulate and more efficient. Plants could recruit microorganisms that contain genotypes specific for toxicant degradation into the rhizosphere and root interior, and this selection should be contaminant specific (Siciliano et al., 2001). Hyperaccumulators accumulate huge amounts of heavy metals and can therefore provide a specific environment for screening endophytes that could be adapted to survive in high metal concentrations. In addition, endophytes which lived in hyperaccumulators may transport, transform or deposit heavy metal ions during the process of accumulating metals in inner tissues of plants. Consequently, the MIC test was not necessary to obtain potential biosorbents from the endophytes of a hyperaccumulator.

Moreover, EF was simply obtained by incubating the plate on which pieces of surface disinfected stem tissues had been previously plated. This suggests that this method of obtaining endophytes from hyperaccumulator is even more simple and convenient than other endophyte isolation methods which require more procedure steps (Kuklinsky-Sobral et al., 2004).

3.2. Effect of pH

The pH values investigated were less than 7.0 since insoluble cadmium hydroxide starts precipitating from the solution at higher pH values, making true sorption studies impossible. In addition, there're no significant changes of pH values before and after sorption.

As shown in Fig. 1, the biosorption capacity strongly depends on the initial pH. Barely any biosorption was observed for a pH less than 2.0. As the initial pH of simulated cadmium wastewater increased from 3.5 to 5.5, the biosorption capacity increased sharply from 2.6, 4.2 and 8.6 mg/g to 10.5, 25.3 and 53.3 mg/g for initial cadmium concentrations of 20, 50 and 100 mg/L, respectively. After this the rate slowed down, and the highest biosorption capac-

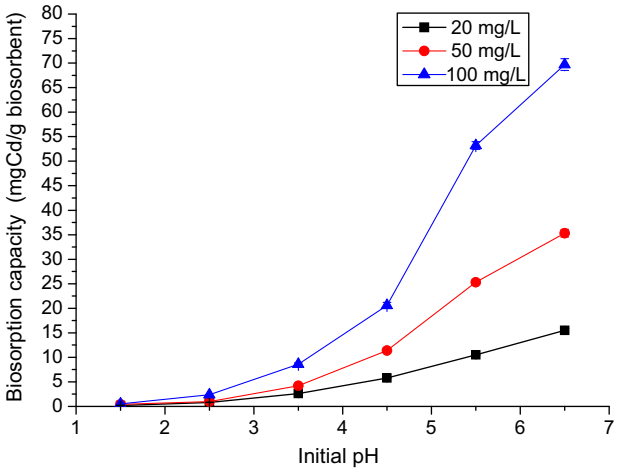

Fig. 1. Effect of pH on Cd(II) biosorption using biosorbent EF LSE10 at different cadmium concentrations, contact time 12 h, dosage of 1 g/L at 303 K (symbols style: square for 20 mg/L, circle for 50 mg/L and triangle for 100 mg/L).

ity occurred at a pH of 6.5. The biosorption capacity could reach 15.5 mg/g with an initial cadmium concentration of 20 mg/L, 35.3 mg/g for 50 mg/L and 69.7 mg/g for 100 mg/L, respectively.

A similar pH effect had also been observed when other kinds of biomass were employed as biosorbents, such as black gram husk (*Cicer arientinum*) (Saeed and Iqbal, 2003), fungus *Aspergillus niger* (Kapoor et al., 1999) and byproducts of *L. edodes* (Chen et al., 2008). The biosorption capacities of biosorbents depend on the available binding sites provided by the functional groups existing in the surface of the biosorbents. Negatively charged groups at the biosorbents' surface are necessary for the sorption process (Saeed and Iqbal, 2003). Such sites were not available due to competition between Cd^{2+} and H_3O^+ ions when pH < 2.5. With the acidity decreased in the solution, the deprotonation of acid functional groups, such as carboxyl, phosphonate and phosphodiester, were strengthen and the attraction increased between negative charge on biomass and positive metal cations (Chen et al., 2008).

3.3. Effect of kinetics

The changes over time in the residual metal concentrations at different initial cadmium concentrations and the optimum pH of 6.5 are shown in Fig. 2. The biosorption of cadmium reached equilibrium in 3 h. The residual cadmium concentrations were 5.4, 15.5 and 30.5 mg/L for the initial cadmium concentration of 20, 50 and 100 mg/L, respectively.

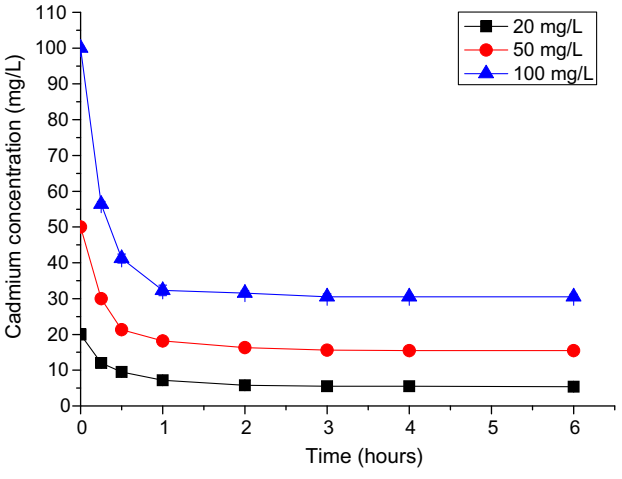

Fig. 2. Effect of sorption time on Cd(II) biosorption using biosorbent EF LSE10 at different initial concentrations at 303 K (symbols style: square for 20 mg/L, circle for 50 mg/L and triangle for 100 mg/L).

According to Fig. 2, the biosorption rate was independent of the initial cadmium concentration. The biosorption kinetics of heavy metal ions consisted of two phases; an initial rapid phase where the biosorption was rapid and contributed significantly to the equilibrium biosorption, and a slower second phase whose contribution to the total metal biosorption was relatively small. The first phase of biosorption kinetics lasted for almost an hour. The trend of Cd(II) biosorption was typical of metal binding to biomass by means of physicochemical interactions. Such particular behavior could have been due to the non-homogeneity of the biomass surface which possesses functional groups differing in dissociability and in cadmium adsorption rates (Matheickal et al., 1999).

The biosorption kinetics were investigated to better understand the adsorption dynamics of metal ions by the biosorbent EF LSE10, and to obtain predictive models that would allow an estimations of the amount of metal ions that would be adsorbed within a specific time. Thus, pseudo-first and second-order kinetics were applied to the experimental data obtained.

The pseudo-first-order kinetics considers that the occupation rate of adsorption sites is proportional to the number of unoccupied sites. Its equation (Cruz et al., 2004) can be expressed as

$$\ln(Q_e - Q_t) = -K_1 t + \ln Q_e$$

where Q_t and Q_e are the amount of metal ions adsorbed by the biosorbent at a given time of t and at equilibrium, respectively. K_1 is the biosorption rate constant. Linear plots of ln $(Q_e - Q_t)$ versus t indicate the applicability of this kinetic model.

The second model is based on the fact that cadmium ions displace alkaline-earth ions (Ca^{2+} or Mg^{2+}) from the biosorption sites of biomass and, therefore, with respect to the biosorption sites the metal ions sorption can be considered to be a pseudo-second-order reaction. The kinetics can be modeled assuming that the occupation rate of adsorption sites is proportional to the square of the number of unoccupied sites. Its equation (Cruz et al., 2004) can be expressed as

$$\frac{t}{Q_t} = \frac{t}{Q_e} + \frac{1}{K_2 Q_e^2}$$

where K_2 is the constant rate of second-order biosorption. The plot t/Q_t versus t should give a straight line if second-order kinetics are applicable.

As shown in Table 1, the relative coefficient (R^2) values of pseudo-second-order kinetics were all 0.99 which were better than that of pseudo-first-order kinetics (0.94 for 20 mg/L, 0.97 for 50 mg/L and 0.94 for 100 mg/L). Moreover, the Q_e values predicted from pseudo-second-order kinetics were 15.2, 35.5 and 71.1 mg/g with initial concentrations of 20, 50 and 100 mg/L, respectively. These were closer to the experimental Q_e values (14.6 mg/g for 20 mg/L, 34.5 mg/g for 50 mg/L and 69.6 mg/g for 100 mg/L) than the results from the pseudo-first-order kinetics, suggesting the cadmium biosorption mechanism was ion exchange. The cadmium ions displace alkaline-earth ions (Ca^{2+} or Mg^{2+}) from the biosorption sites of biomass (Cruz et al., 2004).

3.4. Effect of dosage and reusability

The economic possibility of a biosorbent is one of the major concerns in its application. This could be estimated by the dosage and reusability of the biosorbent.

The dosage effect was investigated at initial cadmium concentration of 20, 50 and 100 mg/L. Fig. 3 shows the biosorption capac-

Table 1
The biosorption rate constants and the Q_e values from the pseudo-first-order and pseudo-second-order kinetics for the biosorption of cadmium on biosorbent EF LSE10.

Cadmium concentration (mg/L)	Expt.[a] Q_e (mg/g)	Pseudo-first-order kinetic			Pseudo-second-order kinetic		
		Cal.[b] Q_e (mg/g)	K_1 (g/mg/min)	R^2	Cal.[b] Q_e (mg/g)	K_2 (g/mg/min)	R^2
20	14.6	10.6	0.027	0.94	15.2	0.0059	0.99
50	34.5	22.0	0.030	0.97	35.5	0.0036	0.99
100	69.6	37.8	0.033	0.94	71.1	0.0024	0.99

[a] Experimental data.
[b] Calculated or estimated from the model.

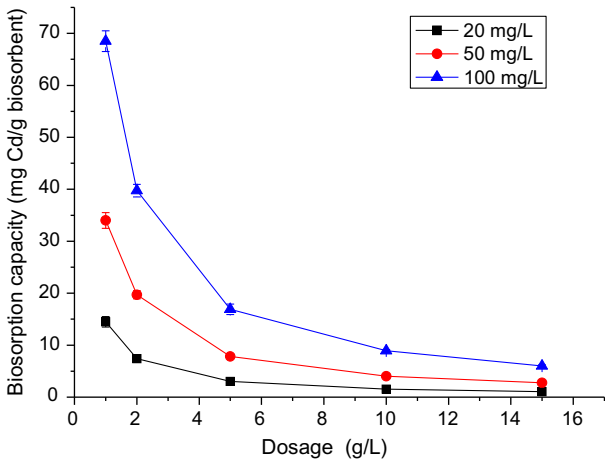

Fig. 3. Effect of dosage on Cd(II) biosorption capacity using biosorbent EF LSE10 at different initial concentrations at 303 K, pH 6.5 (symbols style: square for 20 mg/L, circle for 50 mg/L and triangle for 100 mg/L).

ities obtained at various dosages. The biosorbent EF LSE10 exhibited the maximum biosorption of cadmium to be 14.5, 34 and 67.5 mg/g at the dosage of 1 g/L.

The lower the dosage the more feasible it would be to use. The biosorption capacity decreased with the increasing dosage (Fig. 3). Similar results were observed when other biomasses were employed as biosorbents to remove heavy metals, such as lead, cadmium and zinc biosorption by *Citrobacter* strain MCMB-181 (Puranik and Paknikar, 1999), biosorption of cadmium by black gram husk (*C. arientinum*) (Saeed and Iqbal, 2003) and cadmium removal by a byproduct of *L. edodes* (Chen et al., 2008). The number of binding sites available for adsorption was determined by the dose of biomass added to the solution. A higher biosorption capacity at a lower dosage could be attributed to an increased ratio of metal to biosorbent, which decreases upon an increase in dosage (Puranik and Paknikar, 1999).

The desorption experiment was performed by 0.1 M HNO_3 after adsorption. The results showed that the sorption capacity of biosorbent EF LSE10 did not significantly decrease after five cycles. As shown in Table 2, nearly 90% of the sorption capacities remained after five adsorption–desorption cycles. The sorption capacity only decreased by 1.3, 4.1 and 8.6 mg/g at the initial Cd(II) concentrations of 20, 50 and 100 mg/L, respectively. Moreover, the desorption efficiencies were all above 95% during five cycles at different initial Cd(II) concentrations.

The application possibility of biosorbent depends not only on the sorptive capacity, but also on how well it can be reused. The reusability of biosorbent EF LSE10 was assessed by an adsorption–desorption experiment. The high desorption efficiency indicated that nitric acid was the efficient desorbent agent for cadmium desorption. Its efficiency is based on the competition between the protons and the cadmium ions adsorbed by the biosorbent, which will be released if the eluant concentration is high enough and there is not a steric impediment (Herrero et al., 2008). However, excessive amounts of hydrogen ions could reduce the biosorption capacity of the biomass (Kapoor et al., 1999). Therefore, the reuse of the biosorbent in biosorption after elution of biosorbed cadmium ions will require hydrogen ions to be removed from the biosorbent. In this case, the biosorbent was regenerated by being washed with deionized water until the pH of the wash solution was in the range of 5.0–5.5. It was noticed that the first acid treatment was responsible for more than half of the biosorption decrease. However, the decreases of sorption capacity at different initial Cd(II) concentrations were not significant after five cycles. Thus, the deleterious effect of the first acid treatment on the biosorbent resulted in these decreases, while it had less of an effect in the next four cycles.

Based on the results of dosage and reusability, it is clear that biosorbent EF LSE10 exhibits great potential of Cd(II) treatment.

3.5. Effect of initial concentration and sorption capacities comparison

Several isotherm equations have been used for equilibrium models of biosorption systems. The two most commonly used isotherm equations, the Langmuir and Freundlich, have been applied for this study.

Linearized Langmuir model (Chen et al., 2008): $\frac{C_e}{q} = \frac{1}{b \cdot Q_{max}} + \frac{C_e}{Q_{max}}$ where Q_{max} (mg/g) is the maximum amount of metal ion per unit weight of adsorbent to form a complete monolayer on the surface,

Table 2
Desorption of Cd(II) from biosorbent EF LSE10 at different Cd(II) initial concentrations.

Cycles	Cadmium concentration (mg/L)					
	20 mg/L		50 mg/L		100 mg/L	
	Sorption[a] (mg/g)	Desorption[b] (%)	Sorption (mg/L)	Desorption (%)	Sorption (mg/L)	Desorption (%)
1	15.1	98.7	34.2	98.8	70.4	99.1
2	14.2	98.6	31.8	97.9	65.8	98.6
3	14.2	97.9	31.4	97.1	64.9	97.4
4	14.0	97.1	30.7	96.4	63.5	96.7
5	13.8	95.7	30.1	95.7	61.8	96.0

[a] Sorption capacity.
[b] Desorption efficiency.

and b is the equilibrium adsorption constant, and is related to the affinity of the binding sites. Q_{max} represents a practical limiting adsorption capacity when the surface is fully covered with metal ions. It allows the comparison of adsorption performance, particularly in cases where the adsorbent was not fully saturated.

Linearized Freundlich model (Chen et al., 2008): $\ln q = \ln K + \frac{1}{n} \ln C_e$

where K and n are the Freundlich constants' system characteristics, indicating the adsorption capacity and adsorption intensity, respectively.

The initial concentration of metal ions in the solution plays a key role as a driving force to overcome the mass transfer resistance between the aqueous and solid phases (Dang et al., 2009). Therefore, the sorption capacity was expected to be higher with a higher initial concentration. As shown in Fig. 4, the sorption capacity of all three adsorbents increased with the equilibrium cadmium concentration in the solution. The activated carbon performed better sorption capacity than biosorbent NEF *R. cohnii* when the equilibrium cadmium concentration was below 200 mg/L. However, when the equilibrium cadmium concentration was higher than 200 mg/L, the situation was converse. No matter at what equilibrium cadmium concentration, the Cd(II) sorption of biosorbent EF LSE10 was much more than the others. The fact that the equilibrium adsorbed amounts increased with the metal concentrations indicated the great potential application of biosorbent EF LSE10 as a biosorbent to detoxify cadmium wastewater at high concentrations.

The maximum biosorption capacities (Q_{max}) of these three adsorbents for Cd(II) could be calculated from the Langmuir model. As can be seen in Table 3, the relative coefficient (R^2) revealed that the sorption data of the three adsorbents fit both models, especially the Langmuir model ($R^2 > 0.99$). The Q_{max} of biosorbent EF LSE10 was 247.5 mg/g which was 10 times more than that of biosorbent NEF *R. cohnii* (23.7 mg/g), and almost 20 times that of activated carbon (12.4 mg/g). In addition, it was much higher than the Q_{max} values of other reported cadmium treatment biosorbents (Table 4).

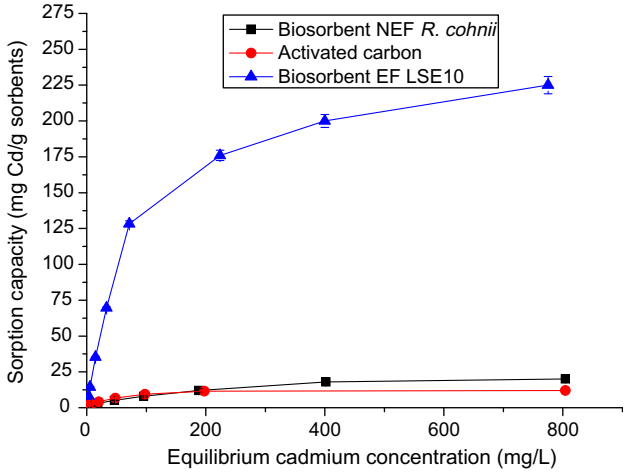

Fig. 4. Isotherm curves of Cd(II) sorption by three adsorbents at 303 K (symbols style: circle for activated carbon, square for biosorbent NEF *R. cohnii* and triangle for biosorbent EF LSE10).

Table 3
Langmuir and Freundlich isotherm parameters for Cd(II) biosorption on activated carbon, biosorbent NEF *R. cohnii* and EF LSE10 at 303 K.

Adsorbents	Langmuir model			Freundlich model		
	Q_{max} (mg/g)	b (mM)	R^2	K	n	R^2
Activated carbon	12.4	0.23	0.99	1.35	2.67	0.95
Biosorbent NEF *R. cohnii*	23.7	1.33	0.99	0.3	1.46	0.96
Biosorbent EF LSE10	247.5	0.82	0.99	5.64	1.63	0.93

Table 4
Comparison of Cd(II) biosorption Langmuir estimated Q_{max} between different reported biosorbents.

Adsorbent	Q_{max}		References
	mg/g	mmol/g	
Wheat straw, *T. aestivum*	4.88	0.13	Dang et al. (2009)
P. veronii 2E	54	0.48	Vullo et al. (2008)
Rhizopus arrhizus	–	0.56	Yin et al. (1999)
Protonated *Sargassum*	–	0.994	Yang and Volesky (1999)
Dead *Sargassum* sp. biomass	—	1.07	Cruz et al. (2004)
White-rot fungus *T. versicolor*	—	1.47	Arıca et al. (2001)
Biosorbent EF LSE10	247.5	2.2	This study

Table 5
The association between the bands observed in FTIR spectra and the corresponding functional groups.

IR peaks	Wavenumber (cm^{-1})		Association
	Before Cd adsorption	After Cd adsorption	
1	3330	3400	Bonded–OH, –NH stretching
2	2920	2920	C–H stretching
3	2860	2860	H–C–H stretching
4	1740	Not observed	C=O stretch of COOH
5	1650	1630	Asymmetric C=O, amide bend
6	1450	Not observed	Symmetric C=O
7	Not observed	1370	Asymmetric–SO_3 stretching
8	1040	1080	C–O (alcohol)

3.6. Fourier transform infrared (FTIR) analysis

The early strong indications of ion exchange being at the root of biosorption metal uptake led us to examining the active chemical groups involved in the metal binding (Volesky, 2007).

Fourier transform infrared analysis (FTIR) is an important tool to identify the functional groups. The assignment of FTIR bands and detailed wavenumber shifts for the biosorbent EF LSE10 are summarized in Table 5. The shift of adsorption peak from 3330 to 3400 cm^{-1} indicated that the hydroxyl group had been changed from multimer to monopolymer or even dissociative state (Kellner et al., 1998), which meant that the degree of the hydroxyl polymerization in biosorbent surface was decreased by the addition of Cd(II). It offered more opportunity for Cd(II) to be bound to the hydroxyl groups. Another change in the spectrum was the carboxyl. The adsorption peak around 1740 and 1450 cm^{-1} were not observed after adsorption. In addition, the shift of adsorption peak from 1650 to 1630 cm^{-1}. These indicated that the carboxylic groups of cell wall biopolymers active in metal sequestering (Davis et al., 2003). Furthermore, the adsorption peak at 1370 cm^{-1} appeared after adsorption suggested that the sulphonate groups of biosorbent's polysaccharides were also responsible for the biosorption of cadmium.

4. Conclusions

EF *Microsphaeropsis* sp. LSE10 was isolated from cadmium hyperaccumulator *S. nigrum* L. It not only exhibits high biomass yield but also shows excellent biosorption efficiency. These confirm our hypothesis that the endophytes of a hyperaccumulator may be potential resources of highly efficient biosorbent for heavy

metal biosorption. Moreover, it is believed that this established technology of specifically obtaining potential biosorbents for a certain heavy metals from the original hyperaccumulator provide an alternative method to obtain the potential good biosorbents rather than the tedious traditional way (experimentally screening many types of biomass by trial and error).

Acknowledgements

This work was financially supported by a grant from National Science Fund for Distinguished Young Scholars (No. 50725825), the Key Program of National Natural Science Foundation of China (No. 50830301), Xiangjiang Water Environmental Pollution Control Project subjected to the National Key Science and Technology Project for Water Environmental Pollution Control (2008ZX07212-001) and the Key Special Science and Technology Project for Energy Saving and Emission Reduction of Hunan Province (No. 2008SK1002).

References

Arıca, M.Y., Kacar, Y., Genc, O., 2001. Entrapmen of white-rot fungus *Trametes versicolor* in Ca-alginate beads: preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution. Bioresour. Technol. 80, 121–129.

Backman, P.A., Sikora, R.A., 2008. Endophytes: an emerging tool for biological control. Biol. Control. 46, 1–3.

Baker, A.J.M., Reeves, R.D., Hajar, A.S.M., 1994. Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J.&C. Presl (Brassicaceae). New Phytol. 127, 61–68.

Barac, T., Taghavi, S., Borremans, B., Provoost, A., Oeyen, L., Colpaert, J.V., Vangronsveld, J., van der Lelie, D., 2004. Engineered endophytic bacteria improve phyto-remediation of water-soluble, volatile, organic pollutants. Nat. Biotechnol. 22, 583–588.

Barkay, T., Schaefer, J., 2001. Metal and radionuclide bioremediation: issues, considerations and potentials. Curr. Opin. Microbiol. 4, 318–323.

Brooks, R.R., 1998. Geobotany and hyperaccumulators. In: Brooks, R.R. (Ed.), Plants that Hyperaccumulate Heavy Metals. CAB International, Wallingford, pp. 55–94.

Chen, G.Q., Zeng, G.M., Tang, L., Du, C.Y., Jiang, X.Y, Huang, G.H., Liu, H.L., Shen, G.L., 2008. Cadmium removal from simulated wastewater to biomass byproduct of *Lentinus edodes*. Bioresour. Technol. 99, 7034–7040.

Cruz, C.C.V., da Costa, A.C.A., Henriques, C.A., Luna, A.S., 2004. Kinetic modeling and equilibrium studies during cadmium biosorption by dead *Sargassum* sp.. Biomass. Bioresour. Technol. 91, 249–257.

Dahmani-Muller, H., Van-Oort, F., Gélie, B., Balabane, M., 2000. Strategies of heavy metal uptake by three plant species growing near a metal smelter. Environ. Pollut. 109, 231–238.

Dang, V.B.H., Doan, H.D., Dang-Vu, T., Lohi, A., 2009. Equilibrium and kinetics of biosorption of cadmium(II) and copper(II) ions by wheat straw. Bioresour. Technol. 100, 211–219.

Davis, T.A., Llanes, F., Volesky, B., Mucci, A., 2003. Metal selectivity for *Sargassum* spp. and their alginates in relation to their a-L-guluronic acid content and conformation. Environ. Sci. Technol. 37 (2), 261–267.

Garbisu, C., Alkorta, I., 2001. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour. Technol. 77, 229–236.

Herrero, R., Lodeiro, P., Rojo, R., Ciorba, A., Rodríguez, P., Manuel, E., Sastre de Vicente, 2008. The efficiency of the red alga *Mastocarpus stellatus* for remediation of cadmium pollution. Bioresour. Technol. 99, 4138–4146.

Huang, D.L., Zeng, G.M., Feng, C.L., Hu, S., Jiang, X.Y., Tang, L., Su, F.F., Zhang, Y., Zeng, W., Liu, H.L., 2008. Degradation of lead-contaminated lignocellulosic waste by *Phanerochaete Chrysosporium* and the reduction of lead toxicity. Environ. Sci. Technol. 42, 4946–4951.

Kapoor, A., Viraraghavan, T., Roy Cullimore, D., 1999. Removal of heavy metals using the fungus *Aspergillus niger*. Bioresour. Technol. 70, 95–104.

Kellner, R., Mermet, J.M., Otto, M., 1998. Analytical Chemistry. Wiley-VCH Verlag GmbH Press, New York. pp. 824.

Kuklinsky-Sobral, J., Araújo, W.L., Mendes, R., Geraldi, I.O., Kleiner, A.A.P., Azevedo, J.L., 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ. Microbiol. 6, 1244–1251.

Li, H.D., Zhao, L., Liu, T., Xiao, X., Peng, Z.H., Deng, L., 2008. A novel technology for biosorption and recovery hexavalent chromium in wastewater by bio-functional magnetic beads. Bioresour. Technol. 99, 6271–6279.

Matheickal, J.T., Yu, Q., Woodburn, G.M., 1999. Biosorption of cadmium(II) from aqueous solution by pre-treated biomass of marine algae *Durvillaea potatorum*. Water. Res. 33, 335–343.

O'Donnell, K., 1993. *Fusarium* and its near relatives. In: Reynolds, D.R., Taylor, J.W. (Eds.), The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics. CAB International, Wallingford, pp. 225–233.

Pérez Silva, R.M., Rodríguez, A.Á., Montes De Oca, J.M.G., Moreno, D.C., 2009. Biosorption of chromium, copper, manganese and zinc by *Pseudomonas aeruginosa* AT18 isolated from a site contaminated with petroleum. Bioresour. Technol. 100, 1533–1538.

Puranik, P.R., Paknikar, K.M., 1999. Biosorption of lead, cadmium and zinc by Citrobacter strain MCMB-181: characterization studies. Biotechnol. Prog. 15, 228–237.

Saeed, A., Iqbal, M., 2003. Bioremoval of cadmium from aqueous solution by black gram husk (*Cicer arientinum*). Water. Res. 37, 3472–3480.

Schulz, B., Boyle, C., 2006. What are endophytes? In: Schulz, B.J.E., Boyle, C.J.C., Sieber, T.N. (Eds.), Microbial Root Endophytes. Springer-Verlag, Berlin, pp. 1–13.

Siciliano, S.D., Fortin, N., Mihoc, A., Wisse, G., Labelle, S., Beaumier, D., Ouellette, D., Roy, R., Whyte, L.G., Banks, M.K., Schwab, P., Lee, K., Greer, C.W., 2001. Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. Appl. Environ. Microbiol. 6, 2469–2475.

Strobel, G., Daisy, B., Castillo, U., Harper, J., 2004. Natural products from endophytic microorganisms. J. Nat. Prod. 67, 257–268.

Tang, L., Zeng, G.M., Shen, G.L., Li, Y.P., Zhang, Y., Huang, D.L., 2008. Rapid detection of picloram in agricultural field samples using a disposable immunomembrane-based electrochemical sensor. Environ. Sci. Technol. 42, 1207–1212.

Van Aken, B., Peres, C., Doty, S., Yoon, J., Schnoor, J., 2004. *Methylobacterium populi* sp. nov., a novel aerobic, pink-pigmented, facultatively methylotrophic, methane-ultilising bacterium isolated from poplar trees (*Populus deltoides* × nigra DN34). Evol. Microbiol 54, 1191–1196.

Volesky, B., 2007. Biosorption and me. Water. Res. 41, 4017–4029.

Vullo, D.L., Ceretti, H.M., Daniel, M.A., Ramírez, S.A.M., Zalts, A., 2008. Cadmium, zinc and copper biosorption mediated by *Pseudomonas veronii* 2E. Bioresour. Technol. 99, 5574–5581.

Wei, S.H., Zhou, Q.X., Wang, X., 2004. A newly found Cd-hyperaccumulator *Solanum nigrum* L.. Chin. Sci. Bull. 49, 2568–2573.

Wei, S.H., Zhou, Q.X., Wang, X., 2005. Cadmium-hyperaccumulator *Solanum nigrum* L. and its accumulating characteristics. Environ. Sci. 26, 167–171.

Yang, J.B., Volesky, B., 1999. Cadmium Biosorption Rate in Protonated *Sargassum* Biomass. Environ. Sci. Technol. 33, 751–757.

Yin, P., Yu, Q., Jin, B., Ling, Z., 1999. Biosorption removal of cadmium from aqueous solution by using pretreated fungal biomass cultured from starch wastewater. Water. Res. 33, 1960–1963.