Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2010, 22(7) 1049–1056
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Isolation, identification, Pb(II) biosorption isotherms and kinetics of a lead adsorbing *Penicillium* sp. MRF-1 from South Korean mine soil

Natarajan Velmurugan[1], Grim Hwang[2], Muthuswamy Sathishkumar[3], Tae Kie Choi[1], Kui-Jae Lee[4], Byung-Taek Oh[4], Yang-Soo Lee[1,*]

1. Department of Forest Science and Technology, Institute of Agriculture and Life Sciences, Chonbuk National University, South Korea. E-mail: velmmk@gmail.com
2. College of Agriculture and Life Sciences, Seoul National University, South Korea
3. Singapore-Delft Water Alliance, National University of Singapore, 2 Engineering Drive 2, Singapore 117577, Singapore
4. Division of Biotechnology, College of Environment and Bioresources Sciences, Chonbuk National University, South Korea

Received 17 August 2009; revised 04 November 2009; accepted 24 December 2009

Abstract

A heavy metal contaminated soil sample collected from a mine in Chonnam Province of South Korea was found to be a source of heavy metal adsorbing biosorbents. Chemical analyses showed high contents of lead (Pb) at 357 mg/kg and cyanide (CN) at 14.6 mg/kg in the soil. The experimental results showed that *Penicillium* sp. MRF-1 was the best lead resistant fungus among the four individual metal tolerant fungal species isolated from the soil. Molecular characterization of *Penicillium* sp. MRF-1 was determined using ITS regions sequences. Effects of pH, temperature and contact time on adsorption of Pb(II) by *Penicillium* sp. MRF-1 were studied. Favorable conditions for maximum biosportion were found at pH 4 with 3 hr contact time. Biosorption of Pb(II) gradually increased with increasing temperature. Efficient performance of the biosorbent was described using Langmuir and Freundlich isotherms. Adsorption kinetics was studied using pseudo first-order and pseudo second-order models. Biosorbent *Penicillium* sp. MRF-1 showed the maximum desorption in alkali conditions. Consistent adsorption/desorption potential of the biosorbent in repetitive cycles validated the efficacy of it in large scale. SEM studies given notes on surface modification of fungal biomass under metal stress and FT-IR results showed the presence of amino groups in the surface structure of the biosorbent. In conclusion, the new biosorbent *Penicillium* sp. MRF-1 may potentially be used as an inexpensive, easily cultivatable material for the removal of lead from aqueous solution.

Key words: heavy metal resistant fungus; *Penicillium* sp.; biosorption; isotherms; kinetics

DOI: 10.1016/S1001-0742(09)60216-3

Introduction

Heavy metal pollution in soil is a serious threat to human health. Through flooding or base level lowering, heavy metals in soils and sediments may enter the food web (Nriagu, 1999; La Force et al., 1998). Heavy metal discharge into the environment has significant impacts on human health and the life cycles of other living organisms (Arnason and Fletcher, 2003). Among the group of heavy metals, lead, cadmium and mercury are considered the most harmful pollutants and dangerous materials in the environment. Lead accumulation in the human body can generate various kinds of biological effects dependent on accumulation levels and concentrations. Recent reports indicate that adults in the UK consume daily lead concentrations of 1.6, 20, 28 μg from air, water and food, respectively (Lenntech Water Treatment and Air Purification Holding, 2008).

Heavy metals can be successfully removed from contaminated materials using biosorption technology. A number of microorganisms are known for their high heavy metal adsorption capacities. Various microbial biomasses have been used for the removal of heavy metal ions from industrial effluents, such as, bacteria (e.g., *Arthrobacter* sp., *Azotobacter*, *Bacillus subtillis*, *Bacillus megaterium*, *Pseudomonas marginalis*, *Staphylococcus* sp.), fungi (e.g., *Aspergillus niger*, *Basidomycetes*, *Rhizopus arrhizus*, *Penicillium* sp., *Mucor*), algae (e.g., *Sargassum wightii*, *Sargassum natans*) and yeast (e.g., *Saccharomyces cerevisae*) (Congeevaram et al., 2007; Dursun et al., 2003; Fan et al., 2008; Mishra and Roy Chaudhury, 1996; Sintuprapa et al., 2000; Sun and Shao, 2007; Vijayaraghavan et al., 2006). Fewer heavy metal adsorption investigations have been conducted with heavy metal resistant fungi than resistant bacteria, and dead fungi biomasses are more often examined and reported than live fungal biomasses. A few research articles have been published regarding the possible usage of *Penicillium* sp., as potential biosorbents (Fan et al., 2008; Li et al., 2008; Mishra and Roy Chaudhury, 1996). *Penicillium* sp. showed a greater adsorption capacity because of the extraordinary extracellular adsorption

* Corresponding author. E-mail: ysoolee@chonbuk.ac.kr

property of heavy metals to functional groups of cell walls. Also, heavy metals can precipitate on the surfaces of growing hyphal tips (Sintuprapa et al., 2000).

Although *Penicillium* sp. biosorption has been studied, the development of *Penicillium* biosorption models with isotherm prediction and kinetic studies are essential for the evaluation of biosorption performance. In addition, studies of microbial populations in heavy metal polluted regions (especially in soil and water) are necessary since the microbes have adapted to survive in high heavy metal concentrations. The development of biosorbents isolated from polluted areas could be used to aid in the advancement of alternative and cost effective adsorbent models.

The present research work was carried out to isolate fungal biosorbents from heavy metal contaminated soil. Identification of the potential fungal biosorbent was ascertained at the molecular level. Effects of pH, temperature and contact time on lead removal by *Pencillium* sp. were determined. The biosorption equilibrium was evaluated using Langmuir and Freundlich isotherms. Pseudo first-order and second-order kinetic were studied. Desorption capacities of biosorbent *Pencillium* sp. at various pH were also determined.

1 Materials and methods

1.1 Sampling site and characterization of soil

The soil samples were collected from Gongsanmyen Mine Sites in Naju City, Chonnam Province, South Korea during autumn 2008. The soil samples were collected 330 m from the entrance, placed in a sterile polythene bag and taken to the laboratory for analysis. Soil pH was determined and total soil metal concentration was analyzed using atomic absorption spectroscopy (AAS) (AA 6300, Shimadzu, Japan). The AAS parameters for determining Pb concentration were programmed as follows: wavelength of 283.3 nm; slit width of 0.7 nm; lamp mode BGR-SR; fuel gas flow rate of 2.0 L/min; support gas flow rate of 15.0 L/min; and flam type Air-C_2H_2. The CN concentration in the soil was determined at 620 nm using UV/Vis spectroscopy (2120UV Optizen, Mecasys, Korea).

1.2 Isolation identification and molecular characterization of metal resistant fungus *Penicillium* sp. MRF-1

To isolate metal resistant fungi from the contaminated soil sample, 1 g soil sample was dissolved in sterilized double distilled water. Two percent Czapek-Dox agar (CDA) plates (pH 5) were used with supplements of streptomycin sulphate (25 mg/100 mL) and cyclohexamide (6 mg/100 mL) for isolation of metal tolerant fungi. The diluted soil sample (0.1 mL) was inoculated onto CDA agar plates. Inoculated plates were incubated at 25°C for seven days. Control plates with inoculations of sterilized double distilled water were incubated under the same conditions. After the incubation period, fungal species grown on the sampling plates were transferred into fresh 2% CDA agar plates and incubated until growth was complete. Completely grown fungal samples were divided and considered pure fungal species. Division of fungal species was performed based on morphological appearances. Pure fungal cultures were maintained both on Czapek-Dox agar plates and in broths.

Heavy metal tolerances of isolated pure fungal cultures were determined. One milliliter spore suspensions of actively growing biomass of each pure fungal culture were obtained from Czapek-Dox Broths (CDB) and were inoculated into Czapek-Dox Broths (pH 5) containing different concentrations of lead (0.31, 0.62, 1.24, 3.1, 31 g/L). Four flasks were maintained for each lead concentration for each pure fungal culture. Controls were maintained without the addition of lead. All broths were incubated at 25°C for seven days at 200 r/min. Then actively growing fungal cultures were extracted by filtration using Whatman No. 1 filter papers and allowed to air dry in a laminar air flow chamber. Fungal biomasses obtained were weighted and results were registered. *Pencillium* sp. MRF-1 was the superior metal tolerant fungus based on these experiments.

Pencillium sp. MRF-1 was maintained in Czapek-Dox broth to isolate fungal genomic DNA. Genomic DNA was isolated from the actively growing fungal cultures after seven days. Total fungal genomic DNA extraction was carried out using a modified method of Graham et al. (1994). Isolated DNA fragment was observed on a 0.8% agarose gel with a 100-bp ladder (TaKaRa, Japan). Genomic DNA band was observed under UV light after EtBr staining. Isolated fungal genomic DNA was amplified using Primers ITS 1 and ITS 4. PCR reaction mixtures consisted of 10× PCR buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 8.3); 0.5 mmol/L dNTPs; ITS forward and ITS reverse primers, each 0.5 mmol/L; 2.0 mmol/L $MgCl_2$; 1 U AmpliTaq gold DNA polymerase (PerkinElmer); 2 μL DNA sample; each mixture was prepared up to a final volume of 30 μL using deionized sterile distilled water. PCR amplification was carried out in a thermocyler (ESCO, Swift Maxi Thermal Cycler, USA) with following reaction conditions of denaturation (95°C for 5 min), annealing (55°C for 1 min), extension (72°C for 1 min), these conditions were maintained for 40 cycles and followed by a final extension at 72°C for 7 min. After amplification, amplified fungal genomic DNA fragments were visualized on 1.5% agarose gel under UV light after EtBr staining.

PCR amplified fragment was cloned into a pGEM-T easy vector system (Promega, USA). Recombinant vector was transformed into JM109 high efficiency competent cells and transformants were selected based on the blue/white colony selection given in the manual (Promega, USA). Recombinant plasmid was isolated from the transformant cells using a Wizard plus plasmid DNA purification kit (Promega, USA). Recombinant plasmid was visualized on 1% agarose gel under UV light after EtBr staining.

The cloned fungal gene fragment was sequenced using a T7 promoter, with an automated DNA sequencer at MACROGEN Sequencing Company, South Korea. The obtained sequences of fungal genomic DNA fragment was analyzed using BLAST, DNASIS and GENETYX software, and the sequence arrangement and alignments

were carefully checked at individual base levels. GenBank BLASTn search was used to confirm the sequence identification. The fungal sequences obtained in this study were submitted to GenBank and accession numbers were obtained. Phylogenetic analysis was performed for *Pencillium* sp. MRF-1 using the neighbor-joining (NJ) method with ClustalW based on current experimental sequences and reference culture sequences obtained from GenBank.

1.3 Biosorption experiments

To perform biosorption and desorption experiments, actively growing seven day old fungal mycelium was removed from Czapek-Dox Broth using Whatman No. 1 filter paper and washed using sterilized double distilled water to remove the media components from the mycelium. This type of mycelium was used for all biosorption and desorption experiments. Pb(II) stock solution 31 g/L was prepared by dissolving lead nitrate anhydrous into sterilized double distilled water. All biosorption and desorption experiments were carried out in the same kind of stock solution. The effect of pH on Pb(II) removal was determined using 0.31 g/L Pb(II) and 1 g biosorbent. Appropriate amounts of lead and biosorbent were added into a series of flasks containing 100 mL sterilized double distilled water. pH of water adjusted to 2, 3, 4, 5, 6, 7, 8, 9 and 10. All flasks were incubated in the shaker at 200 r/min for 24 hr. After the incubation period, supernatants were collected by centrifuge at 6000 r/min (4°C) for 30 min. The Pb(II) concentration in the supernatant was determined by using inductive coupled plasma (ICP, 7500 Agilent, USA). The same quantitative method was used in the subsequent experiments. The effect of contact time on the removal of Pb(II) was studied using 0.15, 0.31, 0.62 g/L Pb(II) and 1 g biosorbent at pH 4. Supernatants were collected from all active flasks to quantify the Pb(II) concentrations. Temperature effects on Pb(II) adsorption was determined using 0.31 g/L Pb(II) and 1 g biosorbent in 100 mL of sterilized double distilled water at pH 4. Flasks were incubated on shaker at 200 r/min for 24 hr at 20, 25, 30, 35, 40, 45, 50, 55 and 60°C, respectively. A separate control flask without an addition of Pb(II) was maintained for each experiment. The results of contact time test were used for the biosorption isotherm and kinetic studies. All experiments were carried out in triplicates, and average values were considered for data analyses. In addition, scanning electron microscopy (SEM) was used to understand the effect of Pb(II) on mycelial surface modification of biosorbents. One gram of active biosorbent was inoculated into the sterilized double distilled water containing 0.31 g/L Pb(II) at pH 4, and then incubated at 200 r/min (25°C) for 24 hr. A control without Pb(II) was also maintained. After 24 hr, the biosorbent was removed by centrifugation at 6000 r/min (4°C) for 30 min and the supernatant was discarded. The obtained pellet was dried at 60°C. The samples were coated with gold using ion sputtering (JFC-1200 fine coater, JEOL, Japan) and were used for scanning electron microscope analysis (JSM 5200, JEOL, Japan). FT-IR (Spectrum, GX, USA) was performed to investigate the surface structural groups of the biosorbent. FT-IR samples were prepared as per the SEM. Mycelia pellets were scanned from 4000 to 400 cm^{-1}.

1.4 Desorption studies

Fifteen grams biosorbents were added into pH 4 sterilized double distilled water containing 0.31 mg/L Pb(II). The working solution was incubated in the shaker at 200 r/min (25°C) for 24 hr. After the incubation period, the biosorbent was separated from the liquid by filtration and the filtrate was discarded. The biosorbent was gently washed with sterilized double distilled water to remove any unadsorbed Pb(II) on the surface of the biomass. A series of sterilized distilled water flasks adjusted to different pHs (2, 3, 4, 5, 6, 7, 8, 9 and 10) were used for the desorption experiment. One gram Pb(II) adsorbed biosorbent was inoculated separately into each flask and incubated at 200 r/min (25°C) for 24 hr. After incubation, the supernatants were collected by centrifugation at 6000 r/min (4°C) for 30 min, and Pb(II) concentration was determined using ICP. Five more cycles of desorption were performed at pH 10.

2 Results and discussion

2.1 Soil sample, isolation and identification of fungal strain

Several physicochemical properties of the mine soil sample were determined: the pH of the soil was 5.41, and the lead content was 357 mg/kg and CN content was 14.6 mg/kg. The experimental results showed that the soil was acidic and its salinity in low level; other characteristic parameters were also not similar to the average favorable conditions for the growth of microorganisms. It is known that the physical and chemical properties of soil directly influence the diversity and biological functioning of the microbial populations in soil (Azevedo et al., 2005). The presences of high concentrations of heavy metals are toxic to fungal communities in the soil. Few fungal strains have the potential to survive under non-favorable conditions that are extremely lethal for other microbial communities (Zapotoczny et al., 2007). Fungal microbes growing in acidic conditions (acidophilic) are more capable of bioremediation, especially heavy metal adsorption. The isolation, characterization and development of acidophilic microorganisms capable of specifically adsorbing heavy metals support the development of new technology for bioremediation.

Four morphologically different fungal species were isolated from the soil sample. Among the four fungal species, the MRF-1 strain was recognized as a potential metal resistant fungus and was identified as *Penicillium* sp. based on molecular data. Analyses of the ITS sequences of MRF-1 showed a 99.8% similarity with *Penicillium janthinellum* based on a GenBank BLASTn search. The ITS sequence length of *Penicillium* sp. MRF-1 was 482 bp. The ITS1-5.8S rDNA-ITS2 gene region sequence data of *Penicillium* sp. MRF-1 was deposited to GenBank and an accession number (AB490790) was obtained. GenBank reference sequence data were used for the phylogenetic analysis of

Penicillium sp. MRF-1. The current experimental data and heavy metal tolerant fungal reference species sequence data were used to construct a Neighbour-Joining (NJ) tree using ClustalW software. According to the results of the NJ tree, *Penicillium* sp. MRF-1 showed 99% similarity with the reported lead resistant fungus *Penicillium* sp. Psf1 isolated from deep sea sediments of the Pacific Ocean (Sun and Shao, 2007).

2.2 Heavy metal resistance assay

The metal resistant fungal strain MRF-1 was tested in CDB medium amended with 0.31 to 31 g/L $Pb(NO_3)_2$. The resistant strain *Penicillium* sp. MRF-1 showed a remarkable resistance to lead ions in the range from 0.31 to 1.24 g/L, compared with the control. A fungal biomass dry weight of 2900 mg (> 50%) was recovered from the 1.24 g/L lead solution (Fig. 1). To our knowledge, *P. lilacinus* is the best reported heavy metal tolerant fungus. *Paecilomyces lilacinus* has been shown to grow in medium containing 1434 mg/L Pb (about 7 mmol/L) (Zucconi et al., 2003). Sun and Shao (2007) also reported growth of *Pencillium* sp. Psf-2 strain in medium amended with 4 mmol/L lead ion. Other reports have also been published regarding the lead resistant capacity of fungi (Donmez and Aksu, 1999; Hasnain et al., 1993; Zanardini et al., 1997).

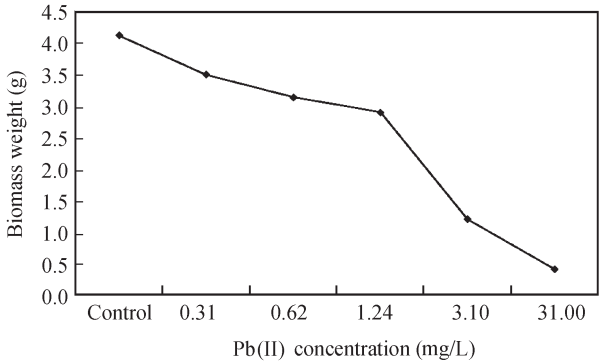

Fig. 1 Weight loss of *Penicillium* sp. MRF-1 at different Pb(II) concentrations.

Visible lead precipitation was observed at high level at 31 g/L and at lower level in 3.1 g/L of working solution. However, precipitates disappeared when fungus was growing in the solution. During fungal growth, heavy metal resistant fungus can produce organic chelators and acids. The solubilizing effects of these compounds could be a probable reason for the disappearance of lead precipitation in the working solutions (Sintuprapa et al., 2000).

2.3 SEM and FT-IR studies

Scanning electron microscope studies provided information on the changes in outer surface morphologies of fungal mycelia treated with lead ion (Fig. 2). The fungal mycelial morphologies of the outer surfaces were closely merged together (Fig. 2a), unlike those of lead treated fungal mycelium. The outer surface network of the treated fungal mycelium became more porous and flexible (Fig. 2b) than in the control. Thus, the physical strength of the treated mycelium was weaker under metal stress.

Figure 3 shows the surface structure and active groups of active compounds in both the control and the active mycelium of *Penicillium* sp. MRF-1. Surface groups of both mycelia were described. Groups at 3435, 2925, 1656, 1548 and 1149 cm^{-1} were observed in the control sample. Broad peaks observed between 3200 to 3600 cm^{-1} were attributed to OH and NH stretching modes. A strong peak appeared at 1149 cm^{-1} indicating C–N stretching vibration (Shringer et al., 1998). Bonds at 2925 and 1548 cm^{-1} were assigned to typical CH stretching vibrations and N–H absorption, respectively. The strong peak at 1656 indicated a C=O stretching in carboxyl or amide I and amide II groups (Deng and Ting, 2005). The presence of a common chemical structure for polysaccharides was found between 1200 to 800 cm^{-1} (Fischer et al., 2006), and the peak at 1149 cm^{-1} attributed to C–N stretching in the control sample shifted to 1152 cm^{-1} in the treated sample after lead uptake. Changes present in the region for N–H stretching between 1600 and 1500 cm^{-1} were also due to lead uptake (Deng and Ting, 2005).

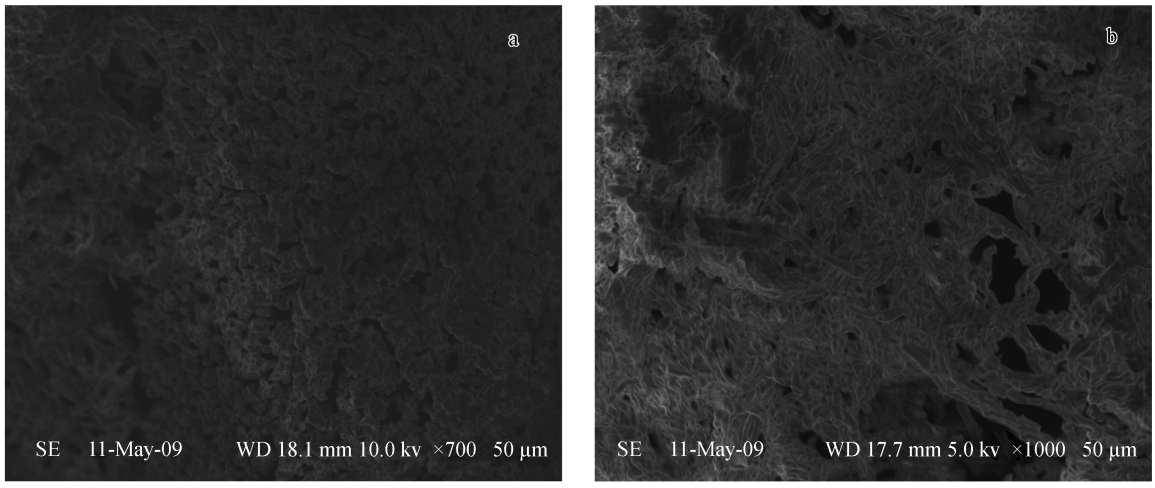

Fig. 2 SEM images of untreated (a) and treated (b) biomass of *Penicillium* sp. MRF-1.

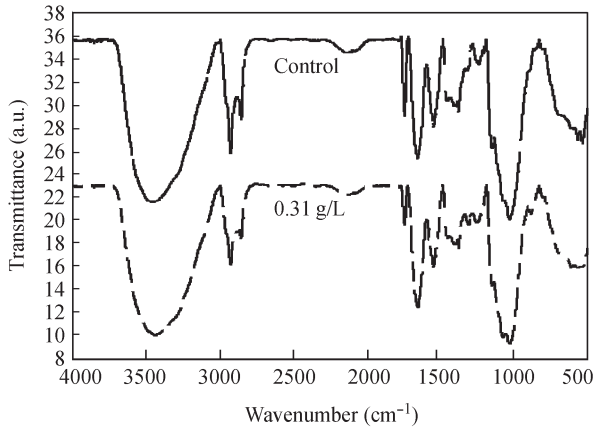

Fig. 3 FT-IR spectrum of Pb(II) treated (0.31 g/L) and untreated (control) mycelium of *Penicillium* sp. MRF-1.

2.4 Effect of pH, temperature and contact time on heavy metal removal by *Penicillium* sp. MRF-1

The study was performed over a pH range from 1 to 10 to optimize the favorable pH conditions for the removal of lead ions by *Penicillium* sp. MRF-1. Maximum removal of Pb(II) was observed between pH 6 and pH 10 according to Fig. 4. Biosorption of Pb(II) by *Penicillium* sp. MRF-1 gradually increased from pH 2 to pH 5 and then stabilized at pH 6. There was no difference in lead removal between pH 6 to pH 10 (Fig. 4). Thus, lead adsorption was highest at acidic pHs and was reduced in alkaline pHs. At low pH, fungal surfaces have a positive charge and prevented contact with metal ions. However, the increase in pH from 4 to 6 increased the removal of metal ions available in solution. Above pH 7 the metal ions precipitated out of solution and inhibited contact between the negatively charged fungal surfaces and the metal ions. Thus, metal ion removal stabilized above pH 7. Similar results were published (Fan et al., 2008) for Pb(II) removal by using *Penicillium simplicissimum*. Yan and Viraragavan (2003) also reported that the removal of Pb(II) by *Penicillium digitatum* and *Rhizopus nigricans* decreased with decreasing pH and increased with increasing pH. Other researchers have reported similar results regarding Pb(II) removal using fungus *Trametes versicolor* (Bayrmoglu et al., 2003) and the bacteria *Micrococcus luteus* (Leung et al., 200).

The effect of temperature on adsorption of Pb(II) by the fungal biomass was studied at ten different temperatures from 20 to 60°C. The removal percentage increased with increasing temperature (Fig. 5). Similar results reported by Fan et al. (2008) indicate that the biosorption of lead, cadmium and zinc by *P. simplicissimum* increase with increasing temperature from 20 to 40°C. They concluded that Pb(II) adsorption was endothermic. Energy dependent metal removal by microbes is greatly influenced by temperature since the adsorption process is physicochemical. Meanwhile, Mishra and Roy Chaudhury (1996) reported that the adsorption of Zn^{2+} by *Penicillium* sp. decreases with increasing temperature because temperature can affect the cell wall stability of fungus, leading to hydrolysis of the cell wall. In accordance with the report of Fan et al., (2008), the adsorption of Pb(II) by *Penicillium* sp. MRF-1 was endothermic.

The effect of contact time on Pb(II) removal by *Penicillium* sp. MRF-1 was studied using different concentrations of Pb(II) 0.15, 0.31 and 0.62 g/L. The uptake percentage of Pb(II) by *Penicillium* sp. MRF-1 at different contact times in different concentrations is given in Fig. 6. The maximum removal of Pb(II) by biosorbent was observed after two hours of exposure between the metal ion and the fungal biomass, and the equilibrium of metal removal was reached after three hours in all concentrations. Results indicated that metal ion removal increased with an increase in contact time. Similar results were also reported by Fan et al. (2008) on the removal of Pb(II) by *Penicillium* sp. Lead(II) removal began after 40 min of exposure to the biosorbent and the metal ions at 0.15 and 0.31 mg/L and

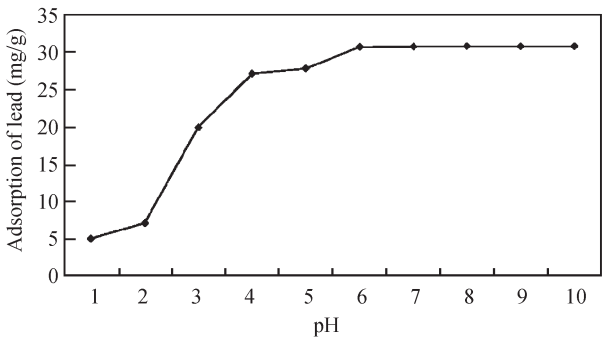

Fig. 4 Effect of pH on Pb(II) removal by *Penicillium* sp. MRF-1.

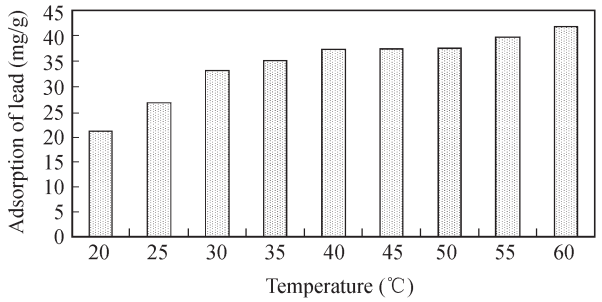

Fig. 5 Effect of temperature on Pb(II) removal by *Penicillium* sp. MRF-1.

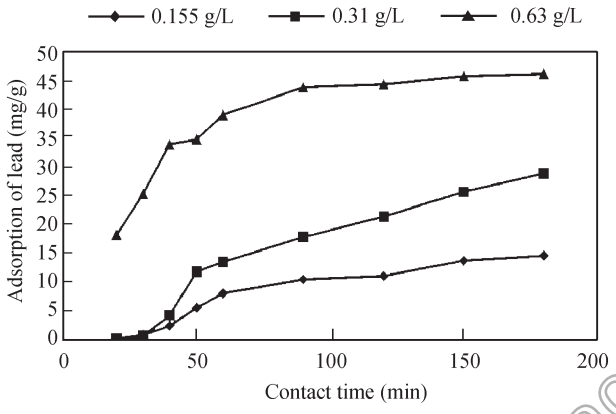

Fig. 6 Effect of contact time on Pb(II) removal by *Penicillium* sp. MRF-1.

simultaneously increased at 50, 60, 90, 120, 150 and 160 min. At 0.62 g/L, the removal of Pb(II) started during the first 20 min of exposure and reached higher concentrations more quickly than did the other concentrations (Fig. 6). This effect may be due to the greater availability of metal ions and free space on the biosorbent surfaces. The attraction between the metal ions and the absorbent might also be higher in the initial stages of exposure (Sathishkumar et al., 2007).

2.5 Biosorption isotherm studies

Two isotherm models were tested to evaluate the efficiency of *Penicillium* sp. MRF-1 as a biosorbent. Two-parameter models of Langmuir and Freundlich isotherms were used for modeling the adsorption data.

The performances of different biosorbents have traditionally been studied using the Langmuir isotherm. The Langmuir isotherm provides a clear explanation for the equilibrium relationships between a bulk liquid phase and a solid phase (Davis et al., 2003). The Langmuir isotherm describes strong monolayer sorption onto specific surface binding sites in the biomass. This can be described by Eq. (1):

$$Q_{eq} = \frac{Q_{max} b C_{eq}}{1 + bC_{eq}} \quad (1)$$

where, Q_{max} (mg/g) is the maximum metal adsorption capacity, Q_{eq} (mmol/g) is the amount of adsorption, b (L/mg) is the energy adsorption related constant and C_{eq} (mmol/L) is the residual concentration in the solution at equilibrium.

The Freundlich isotherm is based purely on natural empirical data on sorption, and is interpreted as sorption on heterogeneous surfaces. The Freundlich equation is usually represented as follows:

$$Q_{eq} = K_F C_{eq}^{1/n} \quad (2)$$

where, K_F (L/g) and $1/n$ are the Freundlich constants. Q_{eq} (mmol/g) is the amount adsorbed at equilibrium and C_{eq} (mmol/L) is the equilibrium concentration. Figure 7 shows the Langmuir and Freundlich plots with the experimental data of Pb(II) removal by *Pencillium* sp. MRF-1. The adsorption constants of both the Langmuir and Freundlich isotherms are shown in Table 1. Based on the modeling, the Langmuir isotherm better describes the adsorption of Pb(II) than does the Freundlich isotherm. The values of the constants related (b-values) to adsorption energy of adsorption much close to zero, indicating that the isotherm model for this study more strongly supported by Langmuir isotherm than by Freundlich isotherm. The

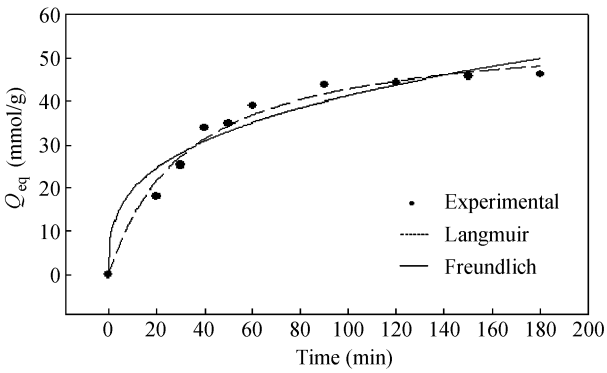

Fig. 7 Two-parameter isotherm models for adsorption of Pb(II) by *Penicillium* sp. MRF-1 (metal concentration 0.62 mg/L).

values of Q_{max} decreased with increasing concentrations of metal ions. According to the Freundlich isotherm, ranges of n values from 1 to 10 designate favorable adsorption. Our experimental results showed n value of 1.092, 1.0178, 3.101 for 0.15, 0.31, 0.62 mg/L, respectively (Table 1). Similar results were reported by Fan et al. (2008) on the removal of Pb(II) by *P. simplicissium*.

2.6 Biosorption kinetics

Kinetic modeling of Pb(II) adsorption by *Penicillium* sp. MRF-1 was described using Pseudo first-order and Pseudo second-order rate equations. The Pseudo first-order model assumes that the change of solute uptake rate with time is directly proportional to the difference in saturation concentration and the amount of solid uptake with time. Lagergren (1898) first proposed the Pseudo first-order rate equation based on solid capacity Eq. (3).

$$\log(q_e - q) = \log q_e - \frac{k_1}{2.303} t \quad (3)$$

where, the values of q_e (mg/g) and q (mg/g) are the quantity of metal ion adsorbed per unit mass of the adsorbent at equilibrium and at time t (min), respectively, and k_1 (min^{-1}) is the first-order kinetic constant of adsorption. The kinetic parameters of the Pseudo first-order model are given in Table 2. The Pseudo first-order kinetic equation is usually suitable for the initial 20–50 min of adsorption. Thus, Lagergren's first-order kinetic equation does not fit well over the entire adsorption period (Fan et al., 2008). The Pseudo second-order rate equation can be used to predict the behavior of adsorption for the entire adsorption processing period and is expressed as follows:

$$\frac{t}{q} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (4)$$

In the Pseudo second-order equation, the second-order constant k^2 ($g \cdot mg^{-1} \cdot min$) can be determined experimentally from the slope and intercept of a plot of t/q versus

Table 1 Adsorption isotherm constants of two-parameter models for the adsorption of Pb(II) by *Penicillium* sp. MRF-1

Pb(II) concentration (g/L)	Langmuir			Freundlich		
	Q_{max}	b	R^2	K_F	n	R^2
0.15	72.5	0.0015	0.9664	0.133	1.092	0.9599
0.31	63.6	0.0280	0.9686	0.1864	0.0178	0.9673
0.62	56.8	0.0306	0.9909	9.39	3.101	0.9724

Table 2 Pseudo first-order and second-order kinetic parameters for the adsorption of Pb(II) by *Penicillium* sp. MRF-1

Pb concentration (g/L)	Pseudo first-order			Pseudo second-order		
	q_e	K_1	R^2	q_e	K_1	R^2
0.15	35.9	0.0039	0.9640	50.9	2.41	0.9637
0.31	37.9	0.019	0.9684	53.6	2.73	0.9685
0.62	43.5	0.0445	0.9740	67.8	4.67	0.9823

t. Values for the Pseudo second-order kinetics are given in Table 2. Significant q_e values variations were found between the Pseudo first-order and second-order kinetics (Table 2). The Pseudo second-order model correlation coefficient was higher (R^2 = 0.9823) than the first-order model. The Pseudo second-order model better fit the experimental data than the first-order model. Sathishkumar et al. (2007) reported similar results for the adsorption kinetics of Procion Blue H-B in an inactive mycelial biomass of *Panus fulvus*.

2.7 Desorption studies

The desorption capacity of any biosorbent validates its importance in commercial applications. Metal removal capacities of a biosorbent are determined using desorption studies that also help to elucidate the possible mechanisms of adsorption. To establish desorption capacity, the metal ion adsorbed biosorbent was treated at various pH values (from 2 to 10). The desorption results revealed that increases in pH increased the percentage release of metal ions into solution. The release of metal ion gradually increased from pH 2 to pH 10 (Fig. 8a). At pH 2, Pb(II) desorption above 94.4% was observed and desorption reached 96.53% at pH 7. Lead(II) desorption stabilized at pH 8, and there was no significant difference in desorption from pH 8 to pH 10 (Fig. 8a). Li et al. (2008) observed more than 98% Pb(II) desorption from *P. simplicissimum* immobilized within a loofa sponge after five cycles. Desorption at pH 10 was feasible, so more cycles of desorption were performed at pH 10. At the end of the fifth cycle, 51.45 mg/g desorption was found (Fig. 8b). Our results showed a higher than 96% metal release in the first cycle of desorption. Further cycles of desorption may increase metal ion release, thus strongly supporting the use of *Penicillium* sp. MRF-1 as a biosorbent.

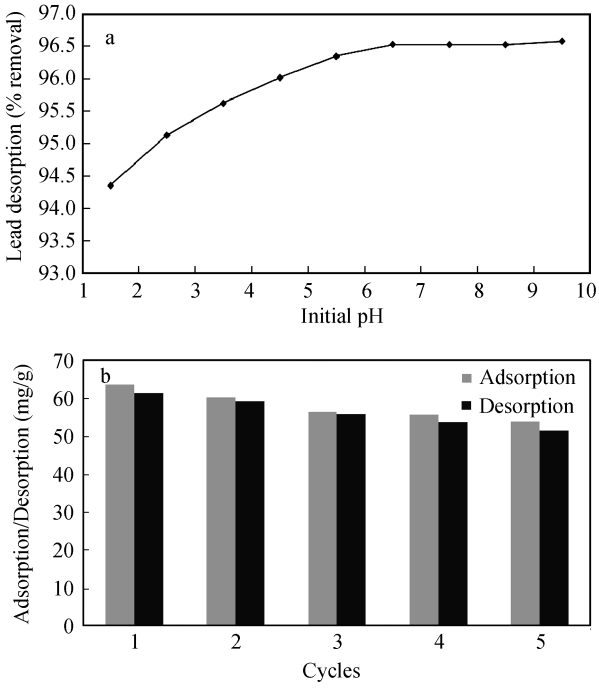

Fig. 8 Desorption of lead from *Penicillium* sp. MRF-1 (initial concentration 0.31 mg/g) (a) and multiple cycles of desorption at pH 10 (b).

3 Conclusions

A potential fungal biosorbent *Penicillium* sp. MRF-1 was isolated from heavy metal contaminated mine soil and was characterized at the molecular level. Experimental results showed the ability of *Penicillium* sp. MRF-1 remove Pb(II) from aqueous solution. The optimum pH and temperature conditions for Pb(II) removal by *Penicillium* sp. MRF-1 were determined in the study. The experimental data were well analyzed using isotherm models to evaluate the performance of the fungal biosorbent. Pseudo first-order model followed the Pseudo second-order model in the kinetic studies. The desorption experiment determined the desorption capacity of the isolated fungal biosorbent and showed that *Penicillium* sp. MRF-1 may be used as a more effective desorbent at alkaline pH 7. Finally, the results indicated that fungal microbes isolated from heavy metal contaminated mine soils could be used as cost-effective, easily cultivatable biosorbents for the removal of metal ions from heavy metal polluted environments.

Acknowledgments

Author N. Velmurugan thanks Mr. A. P. S. Suganth (India) for his valuable suggestions to improve this manuscript. This study was supported by Agricultural R&D Promotion Center, South Korea (No. 060101001).

References

Arnason J G, Fletcher B A, 2003. A 40+ year record of Cd, Hg, Pb and U deposition in sediments of Patroon Reservoir, Albany County, NY, USA. *Environmental Pollution*, 123: 383–391.

Azevedo M S, Teixeira K R S, Kirchof G, Hartman A, Baldani J I, 2005. Influence of soil and host plant crop on the genetic diversity of *Azospirillum amazonense* isolates. *Pedobiologia*, 49: 565–576.

Bayramoglu G, Bektas S, Arica M Y, 2003. Biosorption of heavy metal ions on immobilized white-rot fungus *Trametes versicolor*. *Journal of Hazardous Materials*, 101B: 285–300.

Congeevaram S, Dhanarani S, Park J, Dexilin M, Thamaraiselvi

K, 2007. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *Journal of Hazardous Materials*, 146: 270–277.

Davis T A, Volesky B, Mucci A, 2003. A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research*, 37: 4331–4330.

Deng S, Ting Y P, 2005. Characterization of PEI-modified biomass and biosorption Cu(II), Pb(II) and Ni(II). *Water Research*, 39: 2167–2177.

Donmez G, Aksu Z, 1999. The effect of copper(II) ions on the growth and bioaccumulation properties of some yeasts. *Process Biochemistry*, 35: 135–142.

Dursun A Y, Uslu G, Cuci Y, Aksu Z, 2003. Bioaccumulation of copper(II), lead(II) and chromium(VI) by growing *Aspergillus niger*. *Process Biochemistry*, 38: 1647–1651.

Fan T, Liu Y G, Feng B Y, Zeng G M, Yang C P, Zhou M et al., 2008. Biosorption of cadmium(II), zinc(II) and lead(II) by *Penicillium simplicissimum*: Isotherms, kinetics and thermodynamics. *Journal of Hazardous Materials*, 160: 655–661.

Fischer G, Braun S, Thissen R, Dott W, 2006. FT-IR spectroscopy as a tool for rapid identification and intra-species characterization of airborne filamentous fungi. *Journal of Microbiological Methods*, 64: 63–77.

Graham G C, Mayers P, Henry R J, 1994. A simplified method for the preparation of fungal genomic DNA for PCR and RAPD analysis. *Biotechniques*, 16: 48–50.

Hasnain S, Yasmin S, Yasmin A, 1993. The effects of lead resistant *Pseudomonads* on the growth of *Triticum aestivum* seedlings under lead stress. *Environmental Pollution*, 81: 179–184.

La Force M J, Fendorf S E, Li G, Rosenzweig R F, 1998. A laboratory evaluation of trace element mobility from flooding and nutrient loading of Coeu d'Alene River Sediments. *Journal of Environmental Quality*, 27: 318–328.

Lagergren S, 1898. About the theory of so-called adsorption of soluble substances. *Kungliga Svenska Vetenskapsakademiens Handlinger*, 24: 451–465.

Lenntech Water Treatment and Air Purification Holding B V, 2008. Lenntech Heavy Metals. http://www.lenntech.com/heavy-metals.htm.

Leung W C, Wong M F, Chua H, Lo W, Yu P H F, Leung C K, 2000. Removal and recovery of heavy metals by bacterial isolated from activated sludge treating industrial effluents and municipal wastewater. *Water Science Technology*, 12: 233–240.

Li X M, Liao D X, Xu X Q, Yang Q, Zeng G M, Zheng W et al., 2008. Kinetic studies for the biosorption of lead and copper ions by *Penicillium simplicissimum* immobilized within loofa sponge. *Journal of Hazardous Materials*, 159: 610–615.

Mishra S P, Roy Chaudhury G, 1996. Kinetics of Zn^{2+} adsorption by *Pencillium* sp. *Hydrometallurgy*, 40: 11–23.

Nriagu J O, 1999. Global climate change and cycling of toxic metals. *Abstracts with Programs-Geological Society of America*, 31: 218–219.

Sathishkumar M, Binupriya A R, Vijayaraghavan K, Yun S I, 2007. Two and three parameter isothermal modeling for liquid-phase sorption of Procion Blue H-B by inactive mycelial biomass of *Panus fulvus*. *Journal of Chemical Technology Biotechnology*, 82: 389–398.

Shringer R L, Hermann, C K F, Morrill T C, Curtin D Y, Fuson R C, 1998. The Systematic Identification of Organic Compounds (7th ed.). Wiley, New York. 324.

Sintuprapa W, Thiravetyan P, Tanticharoen M, 2000. A possible mechanism of Zn^{2+} uptake by living cells of *Penicillium* sp. *Biotechnology Letters*, 22: 1709–1712.

Sun F, Shao Z, 2007. Biosorption and bioaccumulation of lead by *Penicillium* sp. Psf-2 isolated from the deep sea sediment of the Pacific Ocean. *Extremophilies*, 11: 853–858.

Vijayaraghavan K, Padmesh T N, Palanivelu K, Velan M, 2006. Biosorption of nickel(II) ions onto *Sargassum wightii*: Application of two-parameter and three-parameter isotherm models. *Journal of Hazardous Materials*, 133: 304–308.

Yan G, Viraraghavan T, 2003. Heavy metal removal from aqueous solution by fungus *Mucor rouxii*. *Water Research*, 37: 4486–4496.

Zanardini E, Andreoni V, Borin S, Cappitelli R, Daffonchio D, Talotta P et al., 1997. Lead resistant microorganisms from red stains of marble of the Certosa of Pavia, Italy and use of nucleic acid-based techniques for their detection. *International Biodeterioration Biodegradation*, 40: 71–82.

Zapotoczny S, Jurkiewicz A, Tyloko G, Anielska T, Turnaud K, 2007. Accumulation of copper by *Acremonium pinkertoniae*, a fungus isolated from industrial wastes. *Microbiological Research*, 162: 219–228.

Zucconi L, Ripa C, Alianiello F, Benedetti A, Onofri S, 2003. Lead resistance, sorption and accumulation in a *Paecilomyces lilacinus* strain. *Biology and Fertility of Soils*, 37: 17–22.