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Removal of cadmium and lead from heavy metals loaded PVA–SA immobilized *Lentinus edodes*

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ABSTRACT

This study, relates to results of Cd(II) and Pb(II) adsorption and removal efficiency from polyvinyl alcohol–Na-alginate (PVA–SA) immobilizing cells of Shi-take mushroom (*Lentinus edodes*) by H_2SO_4, HNO_3, HCl, Na_2CO_3, $NaHCO_3$, and NH_4Cl solutions. Of these desorbing agents, H_2SO_4, HNO_3, and HCl had strong desorbing potential, while HCl and HNO_3 did better. The bio-sorbent particles, after desorption by both H_2SO_4 and HNO_3 were found to be corroded. H_2SO_4 solution was most severe and it caused formation of amorphous matter at the surface that wrapped bio-sorbent particles and caused their round-shape coagulation. This membranes formed, further blocked desorption of Cd(II)/Pb(II). After desorption by HNO_3, the particles were broken down due to strong oxidative power of the acid itself; while no obvious surface corrosion happened after desorption of the bio-sorbent particles when HCl was used as desorption agent. Since HCl solution had a weak influence on the bio-sorbent's surface features, this acid was used as desorbing agent. An optimal time at equilibrium for Pb(II) desorption by 1 mol/L HCl was 90 min and for Cd(II) by 0.1 mol/L HCl was 60 min, with maximum efficiency of 90% and 81%, respectively. The pseudo-second-order model well-fitted to Cd(II)/Pb(II) desorption rates and the correlation coefficients (R^2) were 0.9989 and 0.9969, respectively. From trial to trial as regeneration of bio-sorbent continued, its absorptive potential gradually weakened. Nevertheless, after three regeneration trials, the absorptive potential of reused bio-sorbent still reached 69% for Cd(II) and 85% for Pb(II), and what was displayed is that the immobilizing technology with the abandoned stipe of Shi-take mushroom can be feasible in practical application. After Pb(II) bio-sorption, the surface of bio-sorbent became rich in Pb(II) and energy spectra confirmed that the crystals found in scanning electron microscope (SEM) analysis were Pb(II) salts. On the other side, the spectral peak from Cd(II) was not as clear as from Pb(II). After desorption phase of Pb(II) and Cd(II), there were no spectral peaks from these heavy metals at the surface of the regenerated bio-sorbent.

Keywords: Immobilized *Lentinus edodes*; Pb^{2+}; Cd^{2+}; Desorption; SEM; ES analysis

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1. Introduction

An accumulation of heavy metals including cadmium and lead in the environment still takes place [1]. Wastewater clean-up by application of bio-sorbents to control toxic compounds is among sustainable environmental wastewater treatment technology priorities [2–8]. A fixed-microbial technology, apart from high efficiency of wastewater clean-up from heavy metals also enables metal recovery and bio-sorbent recycling. An example of such approach can be the application of bio-beads made of *Lentinus edodes* mushroom's waste material in wastewater treatment.

The *L. edodes* mushroom is known from the time of antique China as an oldest mushroom species cultivated by man. Its Chinese name is *xiang gu* and that means "mushroom that smells well"—especially if fresh. Nowadays, *L. edodes* is known worldwide under the name of Shi-take mushroom. This widely cultivated species is anticipated, due to its nutritional, sensory, and medicinal properties. In nature, this mushroom grows on dead wood of broad-leaved *Cyclobalanopsis* trees of evergreen forests in Asia. The trees of genus *Cyclobalanopsis* are also considered as subgenus to *Quercus* genus trees of *Fagaceae* family [9].

Apart from a large biomass of Shi-take mushroom cultivated and harvested annually for human consumption in China, available also is a waste material consisting of part of stipe, which is cut-off. Use of Shi-take mushroom waste material as bio-sorbent for reclamation of contaminated water bodies can be an interesting perspective. Higher mushrooms (*Macromycetes*) are known due to their high ability to fix and accumulate in fruit bodies metallic elements [10,11]. Among the genus *Agaricus,* the mushroom such as *Agaricus macrosporus* is even suggested as a potential species for bio-remediation of substrates contaminated with heavy metals [12].

First attempts to use the fixed-microbial technology were related to fermentation process, while for water treatment it dates back to the late 1970s and peaked-up recently [13]. The fixed-microbial technology enables to overcome some weakness, if only biological cells are used, which are too small in size to be effectively separated from the water medium and that causes secondary pollution problems. Fixed bio-sorbents (bio-beads), when compared to biological cells technology, had an advantage to be more efficient in sorption properties and stable. Hence, they can be purified and reused. This gives the fixed bio-beads technology a board application potential in the field of wastewater treatment [14,15].

A possibility to reuse of fixed bio-beads can mean a lot if they could find industrial applications [16]. This is because improper bio-beads disposal or lack of treatment after use affects not only recovery of heavy metals, but can cause secondary pollution problems. Efficient metal desorption rates not only lead to avoidance of secondary pollution, but also enable metals and bio-sorbent to recycle [17]. Fixed bio-beads are ideally suited for the recovery of non-destructive metal ions.

Heavy metals when enriched on fixed cell bio-beads can be further removed using a proper desorbent. Many desorbents can be used to remove a particular metallic element bound to bio-bead and to achieve selective metal recovery. If the fixed cells are alive, influences of desorbing agent on the cells' biological activity have to be considered. Desorbents that are nutritious to the cell system, should be the best. If the fixed cells in bio-bead are inactive, some common and cheap reagents can be used [18–22]. There is a battery of chemical reagents that could be utilized as heavy metals desorbing agents, including $CaCl_2$, HNO_3, $MgCl_2$, H_2SO_4, and others.

For example, Wang et al. found that desorption efficiencies of Cu(II) from the bio-bead made of *Micrococcus luteus GC subgroup B.*, when being desorbed by use of $CaCl_2$, HNO_3, and H_2SO_4 were 62.1, 94.7, and 99.9%, respectively [23]. In a study by Xu et al. desorption of Cd(II) from a bio-bead that consisted of the yeast cells was far more efficient by use of 1 mol HCl solution than deionized water or NaOH [24]. Zyskova et al. found that microsoluble $CaSO_4$ and $CaCO_3$ not only exchanged heavy metals from the activated sludge, but also left a proper amount of the metals thus making possible, the use of sludge as crop fertilizer [25]. In another study, Xie et al. desorbed Pd(II) from the beer yeasts with the highest efficiency (98.7%) by 0.5 mol HCl solution, when compared to some other agents [26].

This study, relates to efficiency of Cd(II) and Pb(II) desorption and removal from polyvinyl alcohol–Na-alginate (PVA–SA) immobilizing cells of Shi-take mushroom (*L. edodes*) by H_2SO_4, HNO_3, HCl, Na_2CO_3, $NaHCO_3$, and NH_4Cl solutions. According to the study of Ma et al. the optimal immobilization condition for absorbing cadmium was 5%PVA + 1%SA + 3% *L. edodes* + 2% $CaCl_2$, and the optimal immobilization condition for absorbing was 8%PVA + 1%SA + 3% *L. edodes* + 2% $CaCl_2$. The balling property and mechanical strength are better, so we adopted their optimal formula to ensure the accuracy of this experiment [27]. Our practical goal was to find the best materials and parameters for removal of Cd and Pb from waste waters.

2. Materials and methods

2.1. Materials

2.1.1. Biotic component

An inedible part of stipe of the mature Shi-take mushroom (*L. edodes*), adhered to culture substrate were cut off and cleaned with distilled water, and further dried at 50 ± 2˚C in an electrically heated dryer to constant weight. A dried biomass was taken out of the dryer and cooled down to the ambient temperature. Next, to form a biotic component of sorbent, the biomass was crushed into powder using a grinder (ST-04A, 1,200 W, 2,800 rpm; Shuaito Tool Limited Company, Yong Koro city, China), crossed 120-mesh sieve, and kept in a glass jar.

2.1.2. Bio-sorbent for Cd(II)

Polyvinyl alcohol (PVA; 5 g; analytically pure) and sodium-alginate (SA; 1 g; analytically pure) were poured into distilled water (100 mL) in a beaker and the mixture was heated and stirred until dissolved. Next, the biotic component made of Shi-take mushroom stipes (3 g) was added to PVA–SA solution that was earlier cooled down to 45–50˚C. The biotic component was well stirred in the PVA–SA solution to make a mixture of epiphyte, alginate, and polyvinyl alcohol. Next, using a syringe (needle no. 7–9), the mixture was squeezed into saturated solution of H_3BO_3 (analytically pure; Merck), added $CaCl_2$ (2%, analytically pure; Merck), and stirred constantly to form the epiphyte, alginate, and polyvinyl alcohol bio-sorbent beads. Fresh bio-sorbent beads after standing for 24 h were washed-up using distilled water and next dried at 50 ± 2˚C in an electrically heated dryer to constant weight. In this way, the polyvinyl alcohol–Na-alginate (PVA–SA) immobilizing cells of Shi-take mushroom bio-sorbent for Cd was prepared and kept in a glass jar for further use.

2.1.3. Bio-sorbent for Pb(II)

The method of bio-sorbent preparation for Pb(II) was the same, as for Cd(II). The only exception was amount of PVA taken, which was 8 g.

2.1.4. Cd(II) solution

A stock solution containing 1,000 mg Cd/L was made by cadmium (100 mg, analytically pure; Merck) dissolving in a small volume (1:1) of concentrated HCl solution (38%, analytically pure; Merck). Next, 1 mL of HNO_3 solution (GB/T622-1989, specific gravity 1.42 g/cm^3, analytically pure; Merck) was added and solution was made up with distilled water to 100 mL and further shaken gently.

2.1.5. Pb(II) solution

A stock solution containing 1,000 mg Pb/L was made by dissolving of lead nitrate (1.5990 g, $Pb(NO_3)_2$, analytically pure; Merck) with an appropriate volume of distilled water, mixed with HNO_3 solution (specific gravity 1.42 g/cm^3; 100 mL). Next, the solution was transferred and made up with distilled water to 1,000 mL and further shaken gently.

2.1.6. Desorbing agents

1 mol/L solutions of HCl, HNO_3, and H_2SO_4 were prepared, respectively, by subsequent dissolving of concentrated solutions of HCl (38%; 85.9 mL), HNO_3 (68%; GB/T626-2006; 69.7 mL) and H_2SO_4 (95%; GB/T625-1989; 55.7 mL; analytically pure, Merck) in distilled water and made up to 1,000 mL.

1 mol/L solutions of sodium carbonate (Na_2CO_3; 105.99 g; analytically pure, Merck), sodium hydrocarbonate ($NaHCO_3$; 84.01 g; analytically pure, Merck), and ammonium chloride (NH_4Cl; 53.50 g; analytically pure, Merck) were prepared by dissolving reagents in distilled water and made up to 1,000 mL. 1 mol/L NaOH or HCl adjusts the pH value of desorbing agents to pH 6.

2.2. Methods

A 0.5 g portion of bio-sorbent were poured into water solution (25 mL) containing 10 mg/L of Cd(II)/Pb(II) and well shaken for the next 240 min. After the metal ions are absorbed, measure the concentration of metal ions by atomic absorption spectrometer (AAS, made in Australia, GBC 9322 AA). The measurement wavelength of Cd was 228.8 nm, the lamp current was 3.0 mA, and the spectral slit width was 0.5 nm; the measurement wavelength of Pb was 217.0 nm, the lamp current was 5.0 mA, and the spectral slit width was 1.0 nm. The bio-sorbent with bound Cd and Pb was repeatedly washed out with distilled water and kept wet, for the performance of the following sorbing-desorbing experiments.

2.2.1. Selection of desorbing agent

The desorbing agents tested valid to desorb Cd(II)/Pb(II) bound to bio-sorbent were 0.1 mol/L solutions of H_2SO_4, HNO_3, HCl, Na_2CO_3, $NaHCO_3$, and NH_4Cl.

These desorbing agents (50 mL) were added separately to the Cd(II)/Pb(II) enriched bio-sorbent portions (an original mass was 0.5 g dry weight) and the mixtures were shaken up evenly for 240 min. Cadmium and lead concentrations were tested by atomic absorption spectroscopy. A desorbing agent with the strongest desorbing potential was selected for further surveys. Each experiment was run in three replicates.

2.2.2. *Desorbing equilibrium time tests*

The 50 mL portions of 0.1 mol/L HCl as a final desorbing agent were added separately to the Cd(II)/Pb(II) enriched bio-sorbent portions (an original mass of each portion was 0.5 g dw). Next, the mixtures obtained were shaken up evenly for a period of 1, 5, 10, 15, 30, 60, 90, 120, 180, and 240 min. Each trial was run in three replicates.

2.2.3. *Selection of a final desorbing agent concentration*

The 50 mL portions of HCl solutions (0.01, 0.03, 0.05, 0.1, 0.5, and 1 mol/L) were added to the Cd(II)/Pb(II) enriched bio-sorbent portions (an original mass of each portion was 0.5 g dw). Next, the mixtures obtained were shaken up evenly for a period of 1, 5, 10, 15, 30, 60, 90, 120, 180, and 240 min. Each trial was run in three replicates.

2.2.4. *Desorbing agent efficiency assessment*

The Cd(II)/Pb(II) enriched bio-sorbent portions (an original mass of each portion was 0.5 g dw) were treated with 50 mL portions of 0.1 and 1.0 mol/L HCl solution, respectively. Each mixture obtained was shaken up evenly until equilibrium. Then the mixture was filtered through filter paper (type GB/T1914, manufactured by limited company of special paper production, Hangzhou, China) of medium filtering speed. These operations were repeated threefold and the metals concentrations were determined successively. The bio-sorbent material recovered was dried at 50 ± 2˚C in an electric oven until constant weight. This regenerated bio-sorbent can be further used to remove Cd(II)/Pb(II) from contaminated water media.

2.2.5. *Scanning electron microscope (SEM) and energy spectra (ES) analyses of the fixed bio-beads*

The SEM (type SEM JSM-5900LV, made in Japan) and energy spectra (ES) (type PV9100/65, made in Japan) analyses of bio-sorbent made of PVA–SA immobilizing cells of Shi-take mushroom were performed before and after sorption of Cd/Pb and after metals desorption. The bio-sorption material before the SEM and ES analyses was always dried and crushed. A scanning electron microscopy enabled examination of the microscopic structure of sample surface, while energy spectra enabled examination their elemental composition characteristics.

2.3. *Calculations*

Quantitative calculations and data processing followed the formula (a):

$$Q_e = (C_o - C_e)V/M \tag{1}$$

where q_e is adsorbed metal per unit mass (mg/g); C_o is an initial concentration of heavy metal ion (mg/L); C_e is concentration of heavy metal ion at adsorption equilibrium (mg/L); V is volume of the solution (L); M is a dry weight of bio-sorbent powder (g).

Calculations of adsorption ratios of metal ions followed the formula (b):

$$X = (C_o - C_e)/C_o \times 100 \tag{2}$$

where X is adsorption ratio of metal ions (%); C_o is an initial concentration of heavy metal ion (mg/L); C_e is concentration of heavy metal ion at absorption equilibrium (mg/L).

Calculations of desorption ratios of metal ions followed the formula (c):

$$Y = C_1/(C_o - C_e) \times 100\% \tag{3}$$

where Y is desorption ratio of metal ion (%); C_1 is metal ion concentration in solution after desorption (mg/L); C_o is an initial concentration of heavy metal

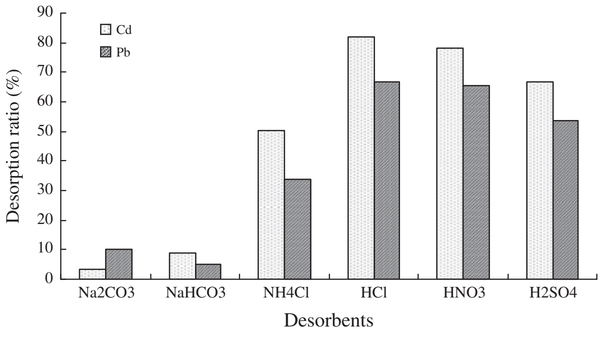

Fig. 1. Cd(II) and Pb(II) desorbing efficiency by various desorbing agents.

ion in solution before desorption (mg/L); C_e is concentration of heavy metal ion at desorption equilibrium (mg/L).

3. Results and discussion

3.1. Optimal desorbing agent selection

Among six desorbing agents tested (Fig. 1), H_2SO_4, HNO_3, and HCl had good desorbing potential, while HCl and HNO_3 did better. Their desorption efficiencies for Cd(II) and Pb(II) were 81.77% (HCl–Cd), 78.24% (HCl–Pb), 66.95% (HNO_3–Cd), and 65.91% (HNO_3–Pb). H_2SO_4, HNO_3, and HCl are strong acids and dissociate large amounts of H^+ ions, while an opposite situation is with weak acid salts such as Na_2CO_3, $NaHCO_3$, and NH_4Cl. Therefore, these experiments showed that H^+ at great concentration is able to exchange the metal ions bound to the bio-sorbent cells. The strong acid solutions contain large amounts of hydrogen ions (H^+) that compete with Cd(II)/Pb(II) ions for the active sites of bio-sorbent. In the cases where these active sites are occupied by hydrogen ions, the competing Cd(II)/Pb(II) lose their chance to be bound to bio-sorbent and return to the test solution.

Fig. 2 has given a view of shape of PVA–SA *L. edodes* before and after Cd(II)/Pb(II) desorption by HCl (b), HNO_3 (c), and H_2SO_4 (d). It could be noted that particles of bio-sorbent after desorption of Cd/Pb ions with HCl were no obvious surface corrosion was present and the structure of particles was not broken. If HNO_3 acted as desorbing agent, the bio-sorbent particle structure was broken down by the strong oxidative power of acid itself. Hence, its action was largely destructive to bio-sorbent and it became unfavorable to reuse it. This observation on unsuitability of HNO_3 solutions as desorbing agent for biosorption, confirms the notes by Thezos and some other researchers [29].

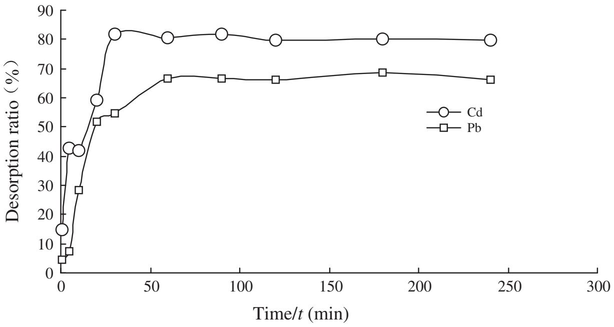

Fig. 3. Desorption equilibrium of Pb(II) and Cd(II) when using 0.1 molars HCl as desorption agent.

Corrosiveness of H_2SO_4 solution was most severe and it caused formation of amorphous matter at the surface that wrapped bio-sorbent particles and caused their round-shape coagulation. This membranes formed, further blocked desorption of Cd(II)/Pb(II). Because of these failures, H_2SO_4 was rejected. Since HCl solution had a feeble influence on the bio-sorbent's surface features, this acid was selected for further evaluations.

3.2. Equilibrium time determination and dynamic analysis

3.2.1. Determination of equilibrium time

A time-dependent data on desorption equilibrium of Pb(II)/Cd(II) with 0.1 mol/L HCl as desorbing agent are given in Fig. 3. As could be noted, desorption of Cd was rapid and 81.77% efficiency could be achieved within the first 30 min. After 30 min, efficiency was steady. Lead could be desorbed rapidly during the first 15 min. Within the next 45 min, efficiency slowed down and after further 15 min, steady state was reached but did not exceed 70% (Fig. 3).

Cadmium was desorbed with greater efficiency than lead and this difference was related to the different desorption mechanisms of these metals. As was

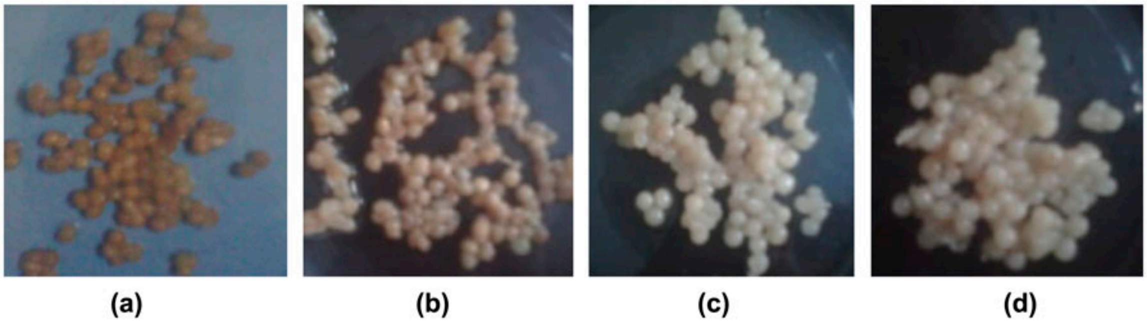

Fig. 2. The shapes of PVA–SA immobilized *L. edodes* bio-beads with bound Pb(II)/Cd(II) before desorption test (a), and after desorption by HCl (b), HNO_3 (c), and H_2SO_4 (d).

noted by Li et al. lead is bound to the functional groups of bio-sorbent and that causes its specific adsorption and resistance for desorption [28]. And opposite is with cadmium that is bound to bio-sorbent, due to electrostatic force, which is weaker, more active, and unsteady. It was suggested that if bio-sorbent is soaked in acidic solution for prolonged period of time, this will cause an impact on its functional groups. Hence, desorption time should not be too long [29]. Under the experiments in this study, an optimal desorption time for Cd(II) and Pb(II) was set as 60 and 90 min, respectively.

3.2.2. Analyses of desorption dynamic

To understand change in pattern of metals ion concentrations with time during the adsorption/desorption processes, the first and second order kinetic equations are anticipated to fit the best experimental data. A process of divalent heavy metal ions bio-sorption, could be described by the pseudo-second-order model (Eq. (d)) [30–33]:

$$t/q_t = 1/k_2 q_e 2 + t/q_e \tag{4}$$

where q_t and q_e are relative extent of adsorption at t time and equilibrium time (mg/g); k_2 is pseudo-second-order model adsorption rate coefficient.

As adsorption and desorption processes of metal ions are two sides of a reversible phenomenon and to fit changes in pattern of metals ion concentrations with time during desorption the pseudo-second-order model equation was used as well (*e*):

$$t/c_t = 1/k_{2d} c_e^2 + t/c_e \tag{5}$$

where c_t and c_e are contents of metal ions in the solutions at t time and equilibrium time (mg/L); k_{2d} is pseudo-second-order model desorption rate coefficient.

The pseudo-second-order model, well fitted to Cd(II)/Pb(II) desorption rates and determination coefficient R^2 were 0.9989 and 0.9969, respectively (Fig. 4). Taking into account a slope and intercept of the straight line, it may be inferred that theoretical content of Cd^{2+} at equilibrium time (c_e) was 2.91 mg/L and desorption rate constant (k_{2d}) was 0.0758. For Pb^{2+}, theoretical content at equilibrium time (c_e) was 3.39 mg/L and desorption rate constant (k_{2d}) 0.0335. As $k_{2d,\ \mathrm{Cd}} > k_{2d,\ \mathrm{Pb}}$, it could be concluded that desorption rate of Cd^{2+} was greater than of Pb^{2+}. For the same valence cation, the greater the ionic radius, the stronger the ionic sorption. The ionic radius of Pb^{2+} is greater than of Cd^{2+}, so the sorption of Pb^{2+} is stronger than of Cd^{2+}, while the desorption of Pb^{2+} is less than of Cd^{2+}.

3.3. Determination of desorbing agent concentration

Concentration of desorbing agent could impact on bio-sorbent desorption rates. Some studies have shown that the rates of desorption increase in parallel with an increase of desorbing agent concentration, until it reaches a plateau and desorption is almost complete. Hence, in spite of further increase of agent concentration, desorption rates remain unchanged. For example, Qian et al. found that 0.1 mol/L HCl solution had greater desorption efficiency, compared to 0.01 mol/L or 1.0 mol/L HCl used to leach Cd(II) and Cu(II) from the fixed seaweed bio-sorbent [34].

As given in Fig. 5, initially the rates of Cd(II) and Pb(II) desorption increased with an increase in concentration of desorbing agent and for 0.01 mol/L HCl these rates were 22.2 and 35.0%, respectively. For Cd(II) and HCl, concentrations between 0.01 and

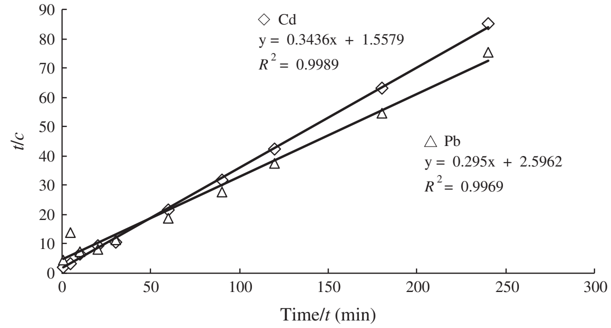

Fig. 4. The pseudo-second-order curve of Pb(II) and Cd(II) desorption.

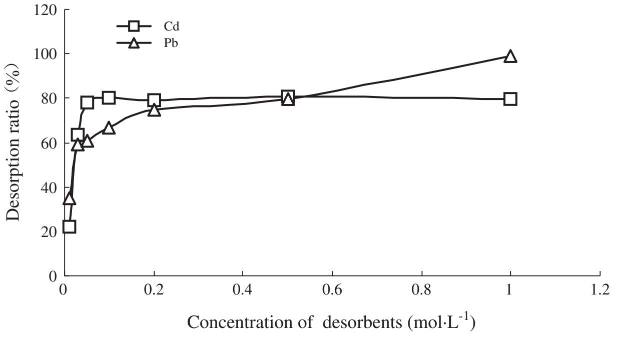

Fig. 5. The rates of Pb(II) and Cd(II) desorption for HCl as desorbing agent at its various molar concentration values.

0.05 mol/L the desorbing efficiency varied remarkably (between 22.2 and 77.9%) and afterwards at equilibrium reached 80%. Desorbing efficiency for Pb(II) increased rapidly when HCl concentration was between 0.01 and 0.03 mol/L and further increased gradually till 99% for 1.0 mol/L HCl (Fig. 5). Definitely, a subsequent increase of H^+ concentration in solution, enables it to be more competitive for active sites at the bio-sorbent's functional groups than of Cd (II)/Pb(II) ions and return metals into solution. The study of Tsezos under scanning electronic microscopy, found that high acid concentration destroyed the bio-sorbent ability of cell walls and was bad to reuse it [35]. An optimal concentration of HCl selected as best desorbing agent in this study, was 0.1 mol/L for Cd (II) and 1 mol/L for Pb(II) (Fig. 5).

3.4. *An influence of desorption process on absorptive potential of reused bio-sorbent*

A study aiming to assess an absorptive potential of reused bio-sorbent consisted of three regeneration trials. They included all procedural steps starting from the Cd(II)/Pb(II) adsorption and desorption processes using HCl, and followed bio-sorbent washing from acid with distilled water, drying, crushing, and re-absorbing phases, respectively. The results of these trials are summarized in Fig. 6.

From trial to trial as regeneration continued, an absorptive potential of regenerated bio-sorbent gradually weakened, but not dramatically. In detail, after first regeneration trial, absorption rates of Cd^{2+} and Pb^{2+} were 80.27 and 98.51%, respectively; and absorption rates of Cd^{2+} and Pb^{2+} in second trial were 73.37 and 89.11% and decreased by 6.9 and 9.4%, respectively; and in third trial decreased by 4.3 and 3.87% compared with the second trial, respectively and compared with the first trial decreased by 11.2 and 13.27%, respectively. Nevertheless, after these three regeneration trials, an absorptive potential of reused bio-sorbent still reached 69.07% for Cd(II) and 85.24% for Pb(II), and what displayed the Shi-take mushroom's abandoned stipe immobilizing technology was feasible in practical application.

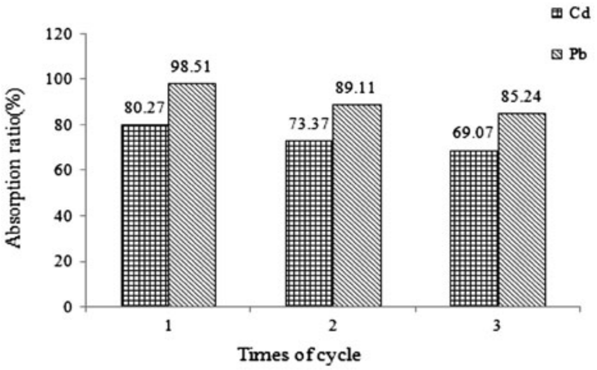

Fig. 6. Effect of bio-sorbent regeneration and reuse on its sorption efficiency for Cd(II)/Pb(II).

The reason why the bio-sorbent material weakened in its potential to adsorb heavy metals as regeneration continued was that, Cd(II) and Pb(II) might be not completely desorbed-out during desorption phases—thereby, followed a decrease in number of sites by which the bio-sorbent recombine with both metals. As the bio-sorbent regeneration trials continued in a parallel, the structure of the bio-sorbent could gradually change (degenerate) causing the number of active binding sites decreased.

3.5. *Scanning electron microscope observations and energy spectra analysis*

3.5.1. *Scanning electron microscope observations of bio-sorbent*

The microscopic features of bio-sorbent observed at various phases of its use are given in Fig. 7. A brand new bio-sorbent surface structure before absorption was rough and highly porous (Fig. 7(a)). After absorbing Pb(II) (Fig. 7(b)) and Cd(II) (Fig. 7(c)), the surface of bio-sorbent changed as the voids visible earlier disappeared and the crystals or precipitations emerged. These crystals could emerge because of precipitation of the inorganic salts such as $Pb(OH)_2$, $PbCO_3$ etc; in addition, when the cell or cell wall adsorbed Cd(II)/Pb(II), some cells macromolecules could rearrange and transform from the amorphous or incomplete crystalline forms to the crystalline state; other processes possibly can be due to deposition at the bio-sorbent surface of Cd(II)/Pb(II) combined with bio-molecules or small particles of bio-sorbent material.

In other words, the bio-sorbent material made of PVA–SA immobilizing cells of Shi-take mushroom could allow to heavy metal ions deposition at the surface of cell wall material by means of physical adsorption or by forming an inorganic precipitates, which agrees with some earlier reports [36]. After desorption phase, most of the heavy metal salts deposited in the voids were desorbed by HCl, the surface of bio-sorbent recovered were rough again and more porous (Fig. 7(d) and (e)). This observation further proved that 0.1 and 1.0 mol/L HCl solution desorbed heavy metals bound to bio-sorbent.

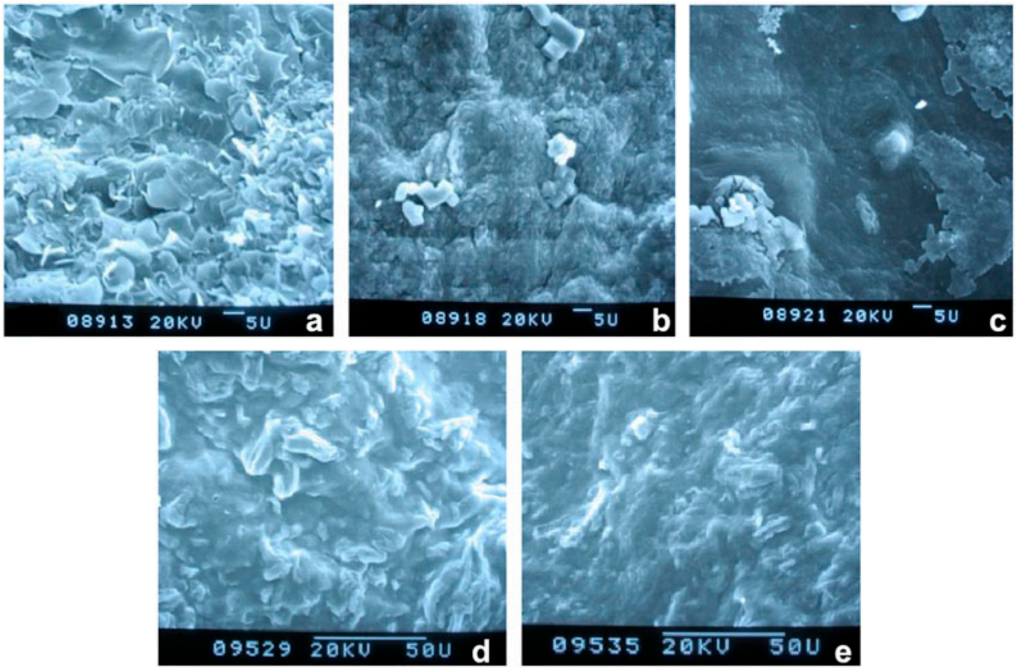

Fig. 7. The scanning electron microscope images of PVA–SA immobilized *L. edodes* mushroom bio-beads at various stages of use.

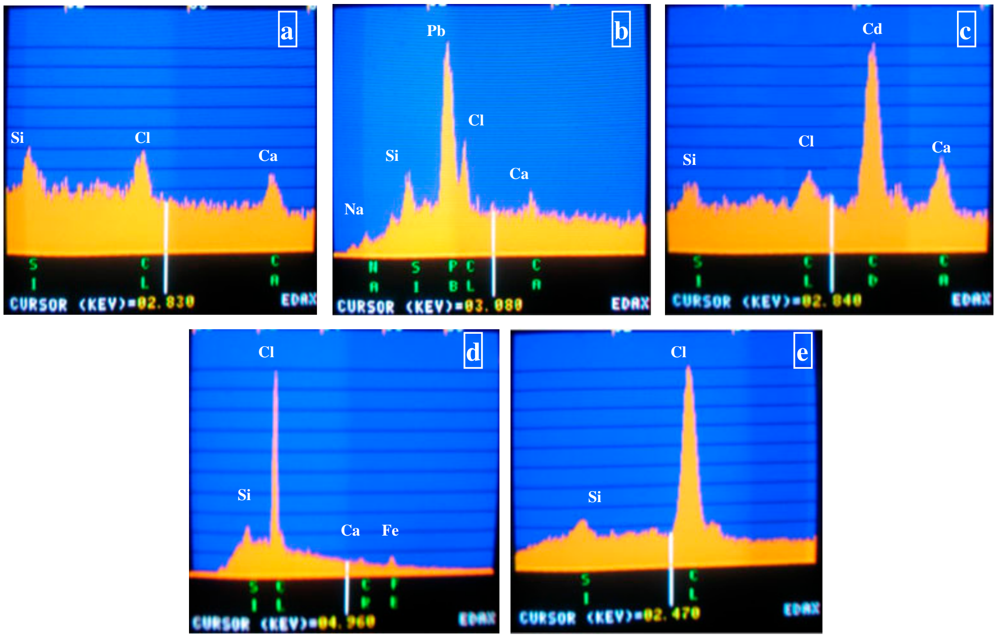

Fig. 8. The energy spectra analysis data of the PVA–SA immobilized *L. edodes* bio-beads at various stages of use.

3.5.2. *Energy spectra of bio-sorbent*

Fig. 8 displays the results of the energy spectra analysis of bio-sorbent at its various stages of its use. The surfaces of brand new bio-sorbent is free of Pb and Cd element, while weak spectral lines of Si, Cl, and Ca (Fig. 8(a)) are visible. They are due to utilization of immobilizing agents, for example preparations (PVA, $CaCl_2$, stipes of mushroom) with low content of these elements. After adsorption process of Pb(II), the spectral peak of Pb adsorbed by bio-sorbent is obvious (Fig. 8(b)). This finding confirms that the surface of bio-sorbent become rich in Pb(II) and that the crystals found in SEM analysis were Pb(II) salts. On the other hand, the spectral peak of Cd(II) was not as clear as of Pb(II) (Fig. 8(c)). The reason of this relatively low resolution for Cd(II) was its low absorbance. After desorption phase of Pb(II) (Fig. 8(d)) or Cd(II) (Fig. 8 (e)), there are no spectral peaks from these heavy metals at the surface of the bio-sorbent, but a peak of Cl from HCl is observed.

4. Conclusions

It has been shown in this study that HCl solution was used as desorbing agent. Desorbing Pb^{2+} and Cd^{2+} with HCl, the equilibrium time was 90 and 60 min, with maximum efficiency of 90 and 81%, respectively. The pseudo-second-order model well fitted to Cd(II)/Pb(II) desorption rates and the correlation coefficients (R^2) were 0.9989 and 0.9969, respectively. From trial to trial, as regeneration of bio-sorbent continued, its absorptive potential gradually weakened. Nevertheless, after three regeneration trials, the absorptive potential of reused bio-sorbent still reached 69% for Cd(II) and 85% for Pb (II) and what displayed that the immobilizing technology with the abandoned stipe of Shi-take mushroom can be feasible in practical application. After Pb(II) biosorption, the surface of bio-sorbent became rich in Pb (II) and energy spectra confirmed that the crystals found in SEM analysis were Pb(II) salts. On the other side, the spectral peak from Cd(II) was not as clear as Pb(II). After desorption phase of Pb(II) and Cd(II), there were no spectral peaks from these heavy metals at the surface of regenerated bio-sorbent. In conclusion, the bio-sorbent made of polyvinyl alcohol–Na-alginate (PVA–SA) immobilizing cells of Shi-take mushroom (*L. edodes*) abandoned stipe material can be effective medium to absorb and eliminate Cd(II) and Pb(II) from water contaminated with these metals. This created bio-sorbent, after use could be further regenerated and reused for Cd(II) and Pb(II) removal from contaminated water. In the future, we may find more kinds of fungus as biomaterals to remove heavy metals from wastewater. Otherwise, we can apply this mind to other environment pollutions, for example, soil pollution.

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References

[1] M. Rutgers, Field effects of contaminants: Experimental challenges, ecological significance of community shifts and risk assessment methodologies, in: The First Joint PSE-SETAC Conference on Ecotoxicology, 16–19 September 2009, Kraków, Poland.

[2] J. Greenman, A. Galvez, L. Giusti, I. Ieropoulos, Electricity from a landfill leachate using microbial fuel cells: Comparison with a biological aerated filter, Enzyme Microbial. Technol. 44 (2009) 112–119.

[3] H. Parab, S. Joshi, M. Sudersanan, N, Shenoy, A, Lali, U. Sarma, Removal and recovery of cobalt from aqueous solutions by adsorption using low cost lignocellulosic biomass-coir pith, J. Environ. Sci. Health Part A 45 (2010) 603–611.

[4] D. Inthorn, K. Tipprasertsin, P. Thiravetyan, E. Khan, Color removal from textile wastewater by using treated flute reed in a fixed bead column, J. Environ. Sci. Health Part A 45 (2010) 637–644.

[5] I. Tsushima, W. Yoochatchaval, H. Yoshida, N. Araki, K. Syutsubo, Microbial community structure and population dynamics of granules developed in expanded granular sludge bead (EGSB) reactors for the anaerobic treatment of low-strength wastewater at low temperature, J. Environ. Sci. Health Part A 45 (2010) 754–766.

[6] Y.J. Liu, M. Nikolausz, X.C. Wang, Biodegradation and detoxication of phenol by using free and immobilized cells of *Acinetobacter* sp. XA05 and *Sphingomonas* sp. FG03, J. Environ. Sci. Health Part A 44 (2009) 130–136.

[7] L. Bulgariu, M. Ratoi, D. Bulgariu, M. Macoveanu, Adsorption potential of mercury(II) from aqueous solutions onto Romanian peat moss, J. Environ. Sci. Health Part A 44 (2009) 700–706.

[8] K.K. Krishnani, X. Meng, L. Dupont, Metal ions binding onto lignocellulosic biosorbent, J. Environ. Sci. Health Part A 44 (2009) 688–699.

[9] M. Deng, Z.-K. Zhou, A. Coombes, Taxonomic notes on the genus Cyclobalanopsis (Fagaceae), Ann. Bot. Fennici. 43 (2006) 57–61.

[10] J. Falandysz, K. Lipka, M. Kawano, A. Brzostowski, M. Dadej, A. Jędrusiak, T. Puzyn, Mercury content and its bioconcentration factors in Wild mushrooms at Łukta and Morąg, J. Agric. Food Chem. 51 (2003) 2832–2836.

[11] J. Falandysz, K. Szymczyk, H. Ichihashi, L. Belawski, M. Gucia, A. Frankowska, S. Yamasaki, ICP/MS and ICP/AES elemental analysis (38 elements) of edible wild mushrooms growing in Poland, Food Addit. Contam. 18 (2001) 503–551.

[12] M.A. Garcia, J. Alonso, M.J. Melgar, Agaricus macrosporus as a potential bioremediation agent for substrates contaminated with heavy metals, J. Chem. Technol. Biotechnol. 80 (2005) 325–330.

[13] J.Z. Luo, X.X. Zhang, Q.X. Qiao, F.G. Xu, Application of immobilized cell technique to tertiary treatment of wastewater (in Chinese), Technol. Equip. Environ. Pollut. Control 3 (2006) 69–71.

[14] D. Zhang, H.J. He, W. Li, T.Y. Gao, P. Ma, Biosorption of cadmium(II) and lead(II) from aqueous solutions by fruiting body waste of fungus *Flammulina velutipe*, Desalin. Water Treat. 20 (2010) 160–167.

[15] D. Zhang, X.D. Zeng, P. Ma, H.J. He, J. Falandysz, The sorption of Cd(II) from aqueous solutions by fixed *Lentinus edodes* mushroom flesh particles, Desalin. Water Treat. 46 (2012) 21–31.

[16] Z.H. Li, H. Yin, J.S. Ye, H. Peng, H.M. Qin, B.Y. He, N. Zhang, Biosorption of heavy metals from nine mine wastewater by immobilized bacteria (in Chinese), Acta Sci. Circumst. 27 (2007) 1245–1250.

[17] X.X. Shen, X.M. Li, G.M. Zeng, Q. Yang, H.L. Guan, Development of research on adsorption of heavy metal ions by activated sludge (in Chinese), Ind. Water Wastewater 37 (2006) 7–11.

[18] W.R. Knocke, L.H. Hemphil, Mercurysorption by waste rubber, Water Res. 15 (1981) 275–282.

[19] Y. Sag, U. Ackel, Fully competitive biosorption of chromium (VI) and iron(II) ions from binary metal mixtures by *R. arrhizus* use of the competitive Langmuir model, Process Biochem. 31 (1996) 573–585.

[20] Z. Aksu, Equilibrium and kinetic modeling of cadmium(II) biosorption by *C. vulgaris* in a batch system: Effect of temperature, Sep. Purif. Technol. 21 (2001) 285–294.

[21] N. Chubar, J.R. Carvallo, M.N. Corriera, Cork biomass as biosorbent for Cu(II), Zn(II) and Ni(II), Colloid Surf. A 230 (2004) 57–65.

[22] C.M. Jansson, E. Guibal, J. Rousy, R. Surjous, P.L. Cloirec, Dynamic removal of uranium by chitosan influence operating parameters, Water Sci. Technol. 34 (1996) 169–170.

[23] Y.X. Wang, Y.L. Guo, R.X. Liu, Biosorption of heavy metals by bacteria isolated from activated sludge (in Chinese), Environ. Sci. 22 (2001) 72–75.

[24] H.J. Xu, S.Y. Liao, M.N. Long, J.B. Xu, Biosorption of cadmium(II) by *Saccharomyces cerevisiae* (in Chinese), Ind. Microbiol. 34 (2004) 10–14.

[25] J.V. Zykova, E.A. Alekseva, V.P. Panov, On the possibility of recovery of heavy metals from excess activated sludge at 6–37˚C, Russ. J. Appl. Chem. 74 (2001) 1965–1967.

[26] D.D. Xie, Y.Y. Liu, C.L. Wu, J.K. Fu, R. Xue, Studies on biosorption of Pd^{2+} by the immobilized *Saccharomyces cerevisiae* waste biomass (in Chinese), Microbiol. 30 (2003) 29–34.

[27] D. Zhang, X.D. Zeng, W. Li, H.J. He, P. Ma, Selection of optimal formulation for sorption of lead and cadmium from aqueous solution using PVA-SA immobilizing *Lentinus edodes* dry flesh particles, Desalin. Water Treat. 31 (2011) 107–114.

[28] Y. Li, G.Y. Zhang, J. Li, J. Wei, Adsorption and desorption characteristics of Cd and Pb in rhizosphere and non-rhizosphere (in Chinese), Ecol. Environ. Sci. 14 (2005) 208–210.

[29] W. Li, J. Wu. Q.B. Li, X. Deng, G. Liu, Desorption of Pb^{2+} from phanerochaete chrsosporium pellets (in Chinese), J. Xiamen Univ. (Nat. Sci.) 39 (2000) 49–53.

[30] M.Y. Arica, Y. Kacar, O. Genc, Entrapment of white-rot fungus trametes versicolorin Ca-alginate beads preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution, Biomass Technol. 80 (2001) 121–129.

[31] Z. Aksu, Equilibrium and kinetic modeling of cadmium(II) biosorption in batch system: Effect of temperature, Sep. Purif. Technol. 21 (2001) 285–294.

[32] F. Kargi, S. Cikla, Biosorption of zinc(II) ions onto powdered waste sludge (PWS): Kinetics and isotherms, Enzyme Microb. Technol. 38 (2006) 705–710.

[33] P. Vasudevan, V. Padmavathy, S.C. Dhingra, Kinetics of biosorption of cadmium on baker's yeast, Bioresour. Technol. 89 (2003) 281–287.

[34] A.H. Qian, H.Y. Qiu, S.H. Zheng, L.D. Chen, A study on desorption of metal ions by immobilized marine algae (in Chinese), J. Oceanogr. Taiwan Strait. 25 (2005) 54–58.

[35] M. Tsezos, Recovery of uranium from biological adsorption–desorption equilibrium, Biotechnol. Bioeng. 2 (1984) 973–981.

[36] D.B. Peng, Y.P. Tian, Isolation and composition analysis of a kind of extracts of antioxidant activity from *Lentinus edodes* (in Chinese), Food Res. Devel. 29 (2008) 89–93.