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Studies on biosorption equilibrium and kinetics of Cd^{2+} by *Streptomyces* sp. K33 and HL-12

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ABSTRACT

The sorption of Cd^{2+} by *Streptomyces* sp. K33 and HL-12 was investigated. The removal efficiency increased with pH, but no obvious differences with different temperatures. Fourier transform infrared (FT-IR) was used to characterize the interaction between Cd^{2+} and K33 and HL-12. Results revealed that the presence of amino, carboxyl, hydroxyl and carbonyl groups were responsible for the biosorption of Cd^{2+}. Strain HL-12 had more changes in the functional groups than K33. Biosorption equilibrium was established earlier by strain K33 than that by HL-12, and K33 had higher adsorption ratio. Langmuir, Freundlich and Dubinin–Radushkevich (D–R) isotherms were used to describe the adsorption experiment, Langmuir model fitted the experiment data best. Strain K33 showed greater sorption capacities with 38.49 mg Cd^{2+}/g dry cells. Pseudo-first-order and second-order kinetic models were used to describe the kinetic data, and second-order kinetic model fitted better. About 70% recovery of Cd^{2+} could be obtained at pH $\leq$ 3 from metal-loaded biomass of strains HL-12 and K33.

1. Introduction

Biosorption is an innovative technology that employs inactive and dead biomass for the recovery of heavy metals from aqueous solutions. As an alternative to traditional methods, its promising results are now being considered for application by the scientific community. So, alternative methods of metal removal and recovery based on biological materials have been considered increasingly day by day. Certain types of microbial biomass can retain relatively high quantities of metals by means of a passive process known as biosorption, which is dependent on the affinity between the metallic species or its ionic forms and the binding sites on the molecular structure of the cellular wall [1]. Binding sites are present in cell wall, composed of lipopolysaccharide, peptidoglycan and phospholipids, and also present in EPS (exopolymeric substances) composed of the neutral sugar compounds, such as galactose and glucose, with minor amounts of mannose, xylose, arabinose, rhamnose, fucose and two *O*-methyl sugars [2]. In contrast to mineral surfaces, the microbial surface contains multiple reactive layers, each with a distinct structure and chemical composition. The use of biological materials, including living and non-living microorganisms, to remove and possibly recover toxic or precious metals from industrial wastewaters, has gained important credibility during recent years, because of the perfect performance and lower cost of these sorbent materials. The sorption of heavy metals on to these biomaterials is attributed to their constituents which are mainly proteins, carbohydrates and phenolic compounds containing functional groups such as carboxyl, hydroxyl and amine that are responsible for the binding of metal ions [3,4]. Therefore, identification of functional groups, responsible for binding the metals, is important. Spectroscopic examination of the dried microbial cells has suggested the presence of reactive functional groups.

Large numbers of microorganisms have been used as sorbents for heavy metals [5,6]. Some of these alternative adsorbent materials are algae, almond husk, clays, yeast biomass, perlite, maple sawdust, seaweeds, pine bark, fly ash, etc. for the removal of heavy metal from wastewater [7]. Although a lot of studies using different types of biomass have proved that biosorption is a more effective method for heavy-metal removal than the conventional ones, further investigation is still needed to optimize the maximum efficiency of heavy-metal removal, which is expected to lead to its large-scale exploitation [8]. Studies are indispensable on the testing capacities of metal loading of various types of streptomycetes, and identifying functional groups responsible for the metal binding as the information on biosorption of heavy metal by streptomycetes is still little until now and streptomycetes have abounding biomass

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and rapid growth capability. The cell wall of streptomycetes generally contains three components: peptidoglycan, teichoic acid and surface protein. These compounds may contain several functional groups (amino, carboxyl, sulphate, hydroxyl, etc.) which could play an important role in the biosorption process.

In this study, *Streptomyces* sp. K33, isolated from industrial metal mine and exhibited high resistance to a variety of heavy metals, and *Streptomyces* sp. HL-12 sensitive to heavy metals, isolated from a farmland uncontaminated by heavy metal on Huajiachi campus of Zhejiang University were tested for their biosorption. Cadmium (Cd) was used as the target metal pollutant in this work, which is frequently found in the industrial effluents and wastes in China.

Fourier transform infrared (FT-IR) analysis, equilibrium of biosorption, kinetics and desorption efficiency of Cd^{2+} from loaded biosorbents were used to further evaluate the feasibility of applying the two strains in practical heavy-metal removal processes. These results would contribute to a better understanding of biosorption phenomena and be beneficial in the development of potential biosorbents that possess high capacities for heavy-metal uptake from aqueous environments.

2. Materials and methods

2.1. Culture conditions of Streptomyces strains tested and preparation of biosorbents

Strain K33 and HL-12 were used in this study, which were isolated from industrial metal mine and a farmland uncontaminated by heavy metals, respectively. Strain K33 showed much higher metal resistance than another isolate HL-12. The bacterial cultures were typically incubated in LB broth at 28 °C. 150 rpm agitation was employed for the shake-flask culturing. Then the cells were harvested by centrifugation (10,000 × *g*, 8 min) from early stationary cultures with a cell density of approximately 2.0–2.5 g l^{-1}, and resuspended in Cd^{2+} solution for the biosorption experiments after twice rinsed with deionized water.

2.2. Measurements of cadmium uptake

The heavy-metal adsorbate used in this study was Cd ($CdCl_2{\cdot}2.5H_2O$). Heavy metal in solutions was measured with Polarized Zeeman Atomic Absorption Spectrometer (AAS, Shimadzu Model-AAA-6650, Japan). Before measured by AAS, the heavy-metal solutions were appropriately diluted with deionized water to ensure that the heavy-metal concentration in the sample was linearly dependent on the absorbance detected.

2.3. Effects of pH and temperature on biosorption

Effects of pH and temperature on biosorption were studied at pH values of 3–7 and temperatures of 20 to 40 ± 1.5 °C at 50 mg l^{-1} initial metal concentration and 24 h of incubation.

2.4. Batch adsorption experiments

The biomass was suspended in solutions containing Cd^{2+} concentrations of 0.5–100 mg l^{-1}. The cell concentration in the solutions was ranged from 1.5 to 2.5 g l^{-1}. The cell/metal suspension was gently agitated (125 rpm) at 28 °C. The pH of the solution was initially adjusted to 6.0 for Cd^{2+}, for avoiding precipitation of it in the form of metal hydroxides. Samples were taken from the solutions after 24 h of incubation (28 °C, 125 rpm) and the metal concentration in the supernatants was measured with AAS.

2.5. Sorption dynamics experiments

To determine the contact time required for the sorption equilibrium, the biosorbents were suspended in 100 ml of heavy-metal solutions (1.0, 10.0 and 50.0 mg l^{-1}) in a glass container, making a cell concentration of 1.5–2.5 g l^{-1}. The adsorption conditions (temperature, pH and agitation rate) were the same as those used in batch adsorption experiments. Samples were intermittently taken from the vessels for analyzing the Cd^{2+} concentration.

2.6. Desorption experiments

Cd-loaded biosorbents were harvested from the cell/metal solutions with the Cd^{2+} concentration of 50.0 mg l^{-1} after biosorption experiments and then rinsed and resuspended with metal-free deionized water. Proper amounts of 0.1 mol l^{-1} HCl [9,10] were added into solutions containing metal-loaded biomass to adjust the pH value to 1, 2, 3, 4, 5, 6 and 7. Samples were taken from the suspensions after 24 h gentle agitation and centrifuged immediately and the metal concentration in the supernatant was detected. Thereafter, the desorbed cadmium was analyzed and the desorption efficiency was calculated as follows:

$$\text{The desorption efficiency}\,(\%) = \frac{\text{released Cd (mg)}}{\text{initially sorbed Cd(mg)}} \times 100 \quad (1)$$

2.7. FT-IR analysis

Infrared spectra of the Cd-loaded and Cd-non-loaded strains were obtained using a Fourier transform infrared spectrometer (FT/IR-300E, Jasco, Japan) in order to investigate the functional groups and the possible cadmium binding sites present in the strains.

3. Results and discussion

3.1. Effect of pH and temperature on the biosorption capacity

Experiment concerning the effect of pH on the sorption was carried out with the range of pH that was not influenced by the metal precipitation (as metal hydroxide). The calculation from the solubility product equilibrium constant (K_{sp}) demonstrated that the suitable pH range for Cd^{2+} is 1–8 [11]. Fig. 1 illustrated that in most cases, the removal efficiency increased steadily with pH. The sorption at the low pH range usually took place with low removal efficiency. This occurred because there was a high concentration of proton in the solution and this proton competed with metal ions in forming a bond with the active sites (the functional groups) on the surface of the strains. These bonded active sites

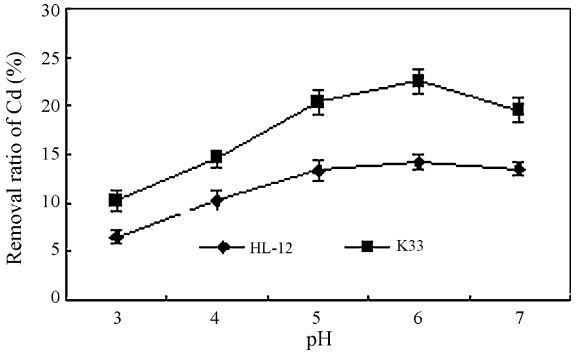


Fig. 1. Effect of pH on removal efficiency by *Streptomyces* sp. HL-12 and K33.

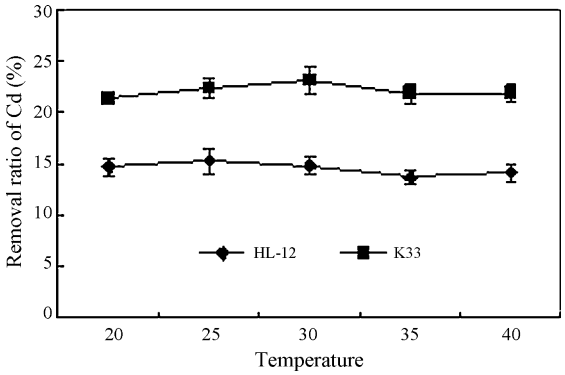


Fig. 2. Effect of temperature on removal efficiency by *Streptomyces* sp. HL-12 and K33.

thereafter became saturated and was inaccessible to other cations. These results were in good agreement with the findings of previous researchers [12–15]. On the other hand, an increase in pH meant a lower quantity of protons, which caused a decrease in the competition between proton and heavy-metal ions. Hence, an increase in the sorption capacity (or removal efficiency) could be observed. To ensure no interference from metal precipitation, subsequent experiments were carried out at pH $\leq$ 6.

The effect of temperature on the biosorption was studied at 20, 25, 30, 35 and 40 °C at 50 mg l^{-1} initial metal concentration, pH 6.0 and 24 h of incubation. The results were given in Fig. 2. Biosorption capacities had no obvious differences with increasing temperature from 20 to 40 °C. The following experimental temperature of biosorption was chosen to be 28 °C between the studied temperature ranges because the optimum growth temperature of the two strains tested was 28 °C (data not shown).

3.2. Biosorption isotherms

According to the adsorption, removal ratio (%) showed the contrary trend (Fig. 3). The removal ratio (%) fell with the increase of the experimental concentration of Cd^{2+}, but Antunes et al. [16] reported that when *Azolla filiculoides* was used to remove Au^{3+} in the solution, it also appeared that the removal ratio arose with the increase of the concentration when in low concentrations. The reason should be investigated farther. The removal ratio decreased faster when the test concentrations of cadmium exceeded 5.0 mg l^{-1}. By the way, the removal ratio of Cd^{2+} by strain K33 was higher than that of strain HL-12 in the tested concentrations range. It seems to suggest that the adsorption site of heavy metal by *Streptomyces* cell is finite, and biosorption is more availability and economical when treating low concentrations of heavy metal in wastewater.

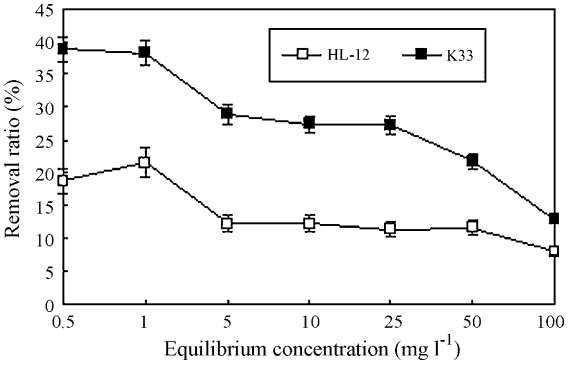


Fig. 3. Removal ratio of Cd^{2+} over initial concentration range of 0.5–100 mg l^{-1}.

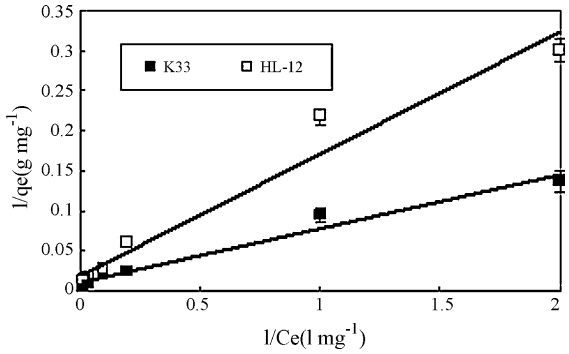


Fig. 4. Isotherms of Cd^{2+} biosorption by *Streptomyces* sp. HL-12 and K33: simulation with Langmuir isotherm model (symbols: experimental data; lines: model prediction).

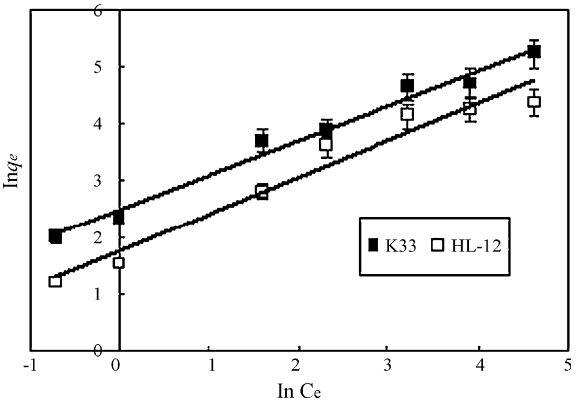


Fig. 5. Isotherms of Cd^{2+} biosorption by *Streptomyces* sp. HL-12 and K33: simulation with Freundlich isotherm model (symbols: experimental data; lines: model prediction).

The adsorption isotherm is the relationship between equilibrium concentration of solute in the solution and equilibrium concentration of solute in the sorbent at constant temperature. The data of sorption equilibrium in this work were tested with Langmuir, Freundlich and Dubinin–Radushkevich (D–R) isotherms. The adsorption isotherms for Cd^{2+} by biomass of *Streptomyces* sp. HL-12 and K33 were presented in Figs. 4–6. The equilibrium capacity of Cd^{2+} by strain K33 appeared to be significantly higher than that by strain HL-12 on the weight basis. Strain K33 was able to uptake Cd^{2+} up to nearly 38.49 mg (0.34 mmol) Cd/g dry cell, while the maximum biosorption of strain HL-12 was only around 24.24 mg (0.22 mmol) Cd/g dry cell.

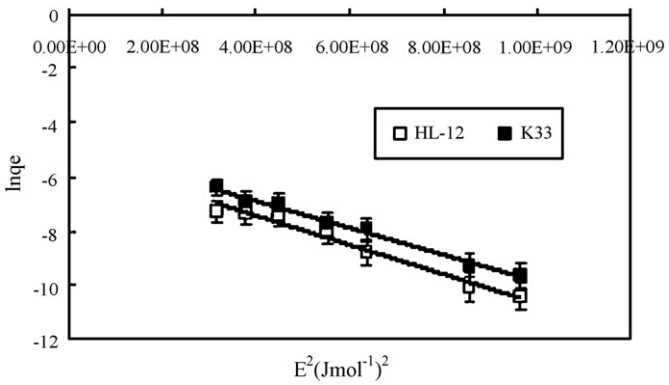


Fig. 6. Isotherms of Cd^{2+} biosorption by *Streptomyces* sp. HL-12 and K33 simulation with D–R isotherm model (symbols: experimental data; lines: model prediction).

Table 1
Adsorption constants estimated from simulations with Langmuir, Freundlich and D–R models for isotherms of Cd^{2+} using *Streptomyces* sp. HL-12 and K33 as biosorbents

Strains	Langmuir model				Freundlich model			D–R model			
	K_L (l g^{-1})	q_m (mmol g^{-1}/mg g^{-1})	R^2	R_L	n	K_F (l g^{-1})	R^2	q_m (mmol g^{-1}/mg g^{-1})	β (mol^{-2} kJ^{-2})	R^2	E (kJ mol^{-1})
	$q = q_m C_e/(K_L + C_e)$[a]				$q = K_F C_e \hat{1}/n$[a]			$\ln q_e = \ln q_m - \beta\varepsilon^2$ [a]			
HL-12	0.015	0.349/39.22	0.9922	0.402	1.203	0.603	0.9748	0.16/17.98	2.35×10^{-2}	0.9818	4.61
K33	0.024	0.436/49.02	0.9991	0.294	1.16	1.205	0.9786	0.29/32.6	2.29×10^{-2}	0.99	4.67

[a] Formula.

The Langmuir isotherm model assumes a monolayer sorption, which takes place at specific homogeneous sites within the biosorbent [17]. Studies on the biosorption of Pb^{2+} did by Çabuk et al. [17] using *Saccharomyces cerevisiae* as biosorbent showed that Langmuir isotherm model fitted the experiment data very well ($R^2 = 0.995$). The linear Langmuir isotherm is represented by the following equation [18]:

$$\frac{1}{q_e} = \frac{1}{q_{max}} + \left(\frac{1}{q_{max} K_L}\right) \frac{1}{C_e} \quad (2)$$

where q_e is the equilibrium Cd^{2+} concentration on the biosorbent (mg g^{-1}), C_e is the equilibrium Cd^{2+} concentration in the solution (mg l^{-1}), q_{max} is the monolayer maximum biosorption capacity of the biosorbent (mg g^{-1}), and K_L is the Langmuir adsorption constant (l mg^{-1}) and is related to the free energy of adsorption [19]. The plot of $1/q_e$ versus $1/C_e$ for the adsorption gives a straight line of slope $1/q_{max}K_L$ and intercepts $1/q_{max}$.

The Freundlich isotherm is an empirical equation employed to describe heterogeneous systems. The linear form of Freundlich equation is [20]:

$$\ln\ q_e = \ln K_F + \frac{1}{n} \ln C_e \quad (3)$$

where K_F (l g^{-1}) and n are Freundlich isotherm constants, being indicative of the extent of the biosorption and the degree of non-linearity between solution concentration and adsorption, respectively. The plot of $\ln q_e$ versus $\ln C_e$ for the biosorption was employed to generate K_F and n from the intercept and the slope values. The D–R isotherm is more general than the Langmuir and Freundlich isotherm. It was applied to distinguish the nature of biosorption as physical or chemical [21]. The linear form of D–R isotherm equation is [22]:

$$\ln\ q_e = \ln q_m - \beta\varepsilon^2 \quad (4)$$

where β is a constant related to the mean free energy of biosorption (mol^2 J^{-2}), q_m is the theoretical saturation capacity, and ε is the Polanyi potential, which is equal to $RT\ln(1 + (1/C_e))$, where R (J mol^{-1} K^{-1}) is the gas constant and T (K) is the absolute temperature. Hence by plotting $\ln q_e$ against ε^2 it is possible to generate the values of q_m (mol g^{-1}) from the intercept, and β from the slope.

The constant β giving an idea about the mean free energy E (kJ mol^{-1}) of biosorption can be calculated using the following relationship [23–25]:

$$E = \frac{1}{(2\beta)^{1/2}} \quad (5)$$

where E values give the information about biosorption mechanism as chemical ion-exchange or physical adsorption [17]. The numerical values of the mean free energy of biosorption were 4.61 and 4.67 kJ mol^{-1} for HL-12 and K33, respectively (Table 1), may correspond to a chemical ion-exchange mechanism [17].

The Langmuir, Freundlich and D–R parameters for the biosorption of Cd^{2+} are listed in Table 1. It is indicated that all of the isotherm models fit very well when the R^2-values are compared.

The essential feature of the Langmuir isotherm can be expressed by means of a separation factor or equilibrium parameter, R_L is calculated according to the following equation:

$$R_L = \frac{1}{1 + K_L C_0} \quad (6)$$

where C_0 is the highest Cd^{2+} concentration (mg l^{-1}). The parameter R_L indicates the shape of the isotherm and nature of the adsorption process ($R_L > 1$: unfavorable; $R_L = 1$: linear; $0 < R_L < 1$: favorable; $R_L = 0$: irreversible). As the R_L values lie between 0 and 1, the biosorption process is favorable [26,27]. The R_L values obtained from this study were 0.402 for strain HL-12 and 0.294 for strain K33 (Table 1), therefore, implying that biosorption of Cd^{2+} by strain K33 was more favorable.

For the cases of Freundlich isotherm, the adsorption feature is defined by both K_F and n values, where K_F value represents the adsorption coefficient and n value is related to the effect of concentration of metal ions. As shown in Table 1, the K_F value of K33 was 1.20, indicated a large biosorption capacity comparing to HL-12, where the K_F value is 0.603. Table 1 also showed that the n values of strains K33 and HL-12 were 1.16 and 1.203, respectively. It could be derived that there were no differences based effects from metal ions.

In general, all models had a good agreement with the data for Cd^{2+} biosorption, evidenced by the high R^2-values (all greater than 0.95) (Table 1). The prediction of Cd^{2+} adsorption by the two strains with Langmuir isotherm was better precisely with a higher R^2-value in contrast to the other two models. This seems to suggest that Cd^{2+} biosorption by *Streptomyces* sp. K33 was likely monolayer sorption, instead of heterogeneous surfaced adsorption and it was the same as strain HL-12. According to the two models, the prediction of Cd^{2+} adsorption by strain K33 is of higher R^2-value than that by strain HL-12. Moreover, there also exists a possibility that in addition to surface binding, other mechanisms may also contribute to the uptake of Cd^{2+} (e.g. intracellular uptake) [28]. From simulation with Langmuir isotherm, the predicted maximum adsorption capacity (q_{max}) was 39.22 mg g^{-1} (0.349 mmol g^{-1}) and 49.02 mg g^{-1} (0.436 mmol g^{-1}) dry cell of strain HL-12 and K33, respectively (Table 1). Strain K33 also had higher K_L value of 0.024 than that of HL-12 (0.015). Inspection of the two adsorption constants (q_{max} and K_L) points out that *Streptomyces* sp. K33 was higher efficient in adsorbing Cd^{2+} than the other strain HL-12.

3.3. Adsorption dynamics

The time-course adsorption data (Fig. 7) demonstrated that adsorption of Cd^{2+} occurred rapidly within the first 30 min. This is quite normal as biosorption is considered to be a spontaneous process and thereby often occurs very rapidly [29]. The ratio of adsorption of strain HL-12 was much lower than that of strain K33 when in low concentration of Cd^{2+}. Adsorption of Cd^{2+} sorption seemed to have a second adsorption phase, and it is suspected that the uptake of heavy metals by the *Streptomyces* strains were probably not only due to cell-surface binding, but also via intracellular accumulation.

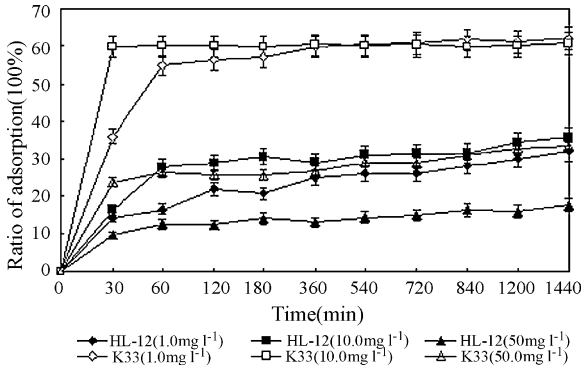


Fig. 7. The adsorption kinetic curves of Cd^{2+} by the biomass of *Streptomyces* sp. K33 and HL-12 at an initial metal concentration of 1.0, 10.0 and 50.0 mg l^{-1}.

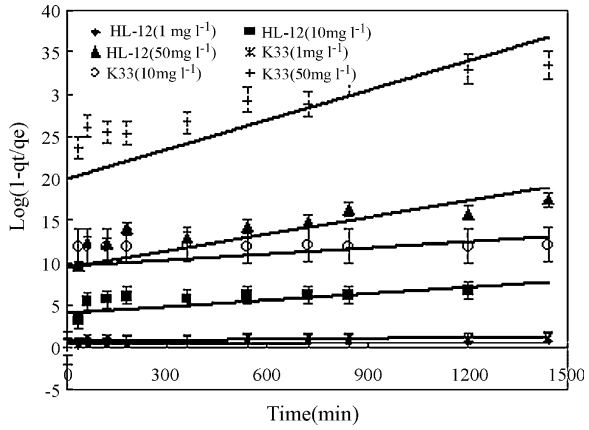


Fig. 8. The first-order plot for Cd^{2+} biosorption on the *Streptomyces* sp. K33 and HL-12 at an initial metal concentration of 1.0, 10.0 and 50.0 mg l^{-1} (symbols: experimental data; lines: model prediction).

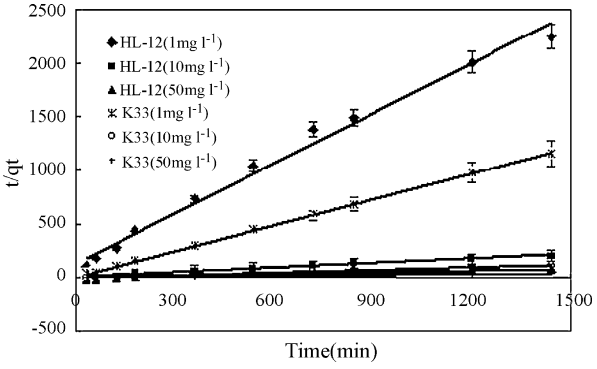


Fig. 9. The second-order plot for Cd^{2+} biosorption on the *Streptomyces* sp. K33 and HL-12 at an initial metal concentration of 1.0, 10.0 and 50.0 mg l^{-1} (symbols: experimental data; lines: model prediction).

To determine equilibrium time for adsorption, time intervals were assessed until no adsorption of strains HL-12 and K33 onto Cd^{2+} took place. Fig. 7 shows the extent of Cd^{2+} adsorption as a function of adsorption time. Results show that Cd^{2+} uptake increased with time and reached equilibrium value at about 30–60 min of the two strains and the concentrations tested, and became much less significant after that time.

The kinetic data were described using the pseudo-first-order and pseudo-second-order rate equations [30]. The pseudo-first-order model assumes that the rate of change of solute uptake with time is directly proportional to the difference in saturation concentration and the amount of solid uptake with time. This pseudo-first-order rate equation is

$$\log(q_e - q_t) = \log\ q_e - \frac{k_1 t}{2.303} \quad (7)$$

where q_t and q_e are the Cd^{2+} concentration on the biosorbent at any time and at equilibrium (mg g^{-1}), respectively, and k_1 is the rate constant of first-order adsorption (l min^{-1}). The pseudo-first-order equation has been extensively used to describe the adsorption kinetics.

The pseudo-second-order is another model for the analysis of adsorption kinetics and expressed as [31]:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (8)$$

where q_e and q_t are the amounts of heavy metal adsorbed on adsorbent (mg g^{-1}) at equilibrium and at time t, respectively, and k_2 (mg g^{-1} min^{-1}) is the rate constant of second-order adsorption [31].

The pseudo-first-order kinetic rate constant k_1 and q_e values were determined from the slope and intercept of Fig. 8. The correlation coefficients for the pseudo-first-order kinetic constants were much lower than the pseudo-second-order kinetic model (Table 2). As also can be seen from Table 2, the calculated q_e values of first-order did not give reasonable values, which were too low to compare with experimental q_e values in all experimental concentrations. It seemed that the biosorption of Cd^{2+} did not obey a pseudo-first-order.

If pseudo-second-order kinetic model is applicable, the plot of t/q_t versus t should show a linear relationship. The pseudo-second-order kinetic rate constant k_2 and q_e values were determined from the slope and intercept of Fig. 9. All of the correlation coefficients for the pseudo-second-order kinetic model were high ($R^2 > 0.99$) (Table 2). Also the q_e values fitted the experimental data better than the pseudo-first-order kinetic model. These suggested that the pseudo-second-order adsorption mechanism was predominant and that the overall rate of the Cd^{2+} adsorption process appeared to be controlled by chemical process [31].

Table 2
The first-order and second-order kinetics constants for the biosorption of Cd^{2+} on the strains

Strain	Concentration (mg l^{-1})	Experimental, q (mg g^{-1})	First-order kinetic			Second-order kinetic		
			q_e (mg g^{-1})	k_1 (min^{-1})	R^2	k_2 (mg g^{-1} min^{-1})	q_e (mg g^{-1})	R^2
K33	1	1.245	0.84	0.4×10^{-3}	0.2837	48.7×10^{-3}	1.25	0.9998
	10	12.15	9.69	2.29×10^{-3}	0.1157	46.9×10^{-3}	12.08	0.9999
	50	33.41	19.95	11.6×10^{-3}	0.3996	0.55×10^{-3}	33.78	0.9941
HL-12	1	0.64	0.294	0.29×10^{-3}	0.6172	17.5×10^{-3}	0.644	0.9904
	10	7.15	4.09	2.6×10^{-3}	0.3971	2.84×10^{-3}	7.097	0.9943
	50	17.45	9.5	6.5×10^{-3}	0.4625	0.96×10^{-3}	17.212	0.9911

3.4. Effect of pH on desorption efficiency

Regeneration of biosorbent for repeated uses is a critical issue in practical application of the biosorbent. The recovery of heavy metals from metal-laden biomass has been approached by utilizing various desorption agents, including HCl, H_2SO_4, Na_2CO_3, EDTA and mercaptoethanol [32–34]. The decrease of pH value by HCl was selected as desorption agent in this study as it appeared to have the best desorption efficiency among those approaches. The amount of metals released from the acid-treated biomass at different pH was monitored for determine the optimal pH for metal desorption. Basically, metal recovery was lower than 50% for pH > 4.0, below which the desorption efficiency increased rapidly as the pH kept decreasing, and desorption of Cd^{2+} was nearly complete at pH 3 for strain K33, while below 2.0 for strain HL-12 (Fig. 10). Therefore, it could be concluded that the optimal pH for strain desorption is 4.0 for K33 and 1.0 for HL-12 from the effect of pH on the desorption efficiency of Cd^{2+} from metal-loaded biomass. In addition, the results in Fig. 10 also elucidated that the binding strength of Cd^{2+} with strain K33 cell mass seems to be stronger than that with strain HL-12 cell mass. A possible mechanism of desorption of the experimental strain cells could be attributed to the adverse effect of HCl on the binding sites of the cell wall components and cell itself (i.e. hydrolysis of the cell macromolecules, cell disruption and solubilization) [38].

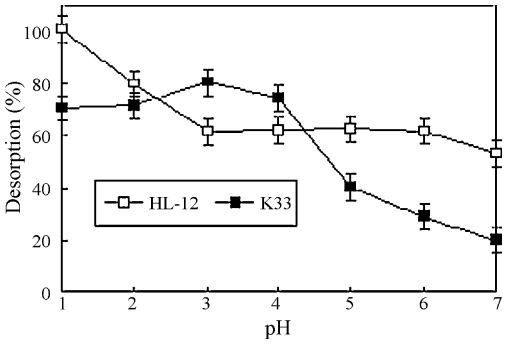


Fig. 10. The effect of pH on the desorption efficiency of Cd^{2+} from metal-loaded biomass of *Streptomyces* sp. K33 and HL-12.

3.5. IR spectrum analysis

The FT-IR analysis of dried strain cells was given in Figs. 11 and 12. The FT-IR spectra display a number of absorption peaks, indicating the complex nature of the biomass examined. The FT-IR spectra of metal unloaded and loaded forms of the two biosorbents in the range of 400–4000 cm^{-1} were taken and presented below. The FT-IR spectra of unloaded biomass showed several distinct and sharp absorptions at 3378 cm^{-1} (strain HL-12), 3400 cm^{-1}

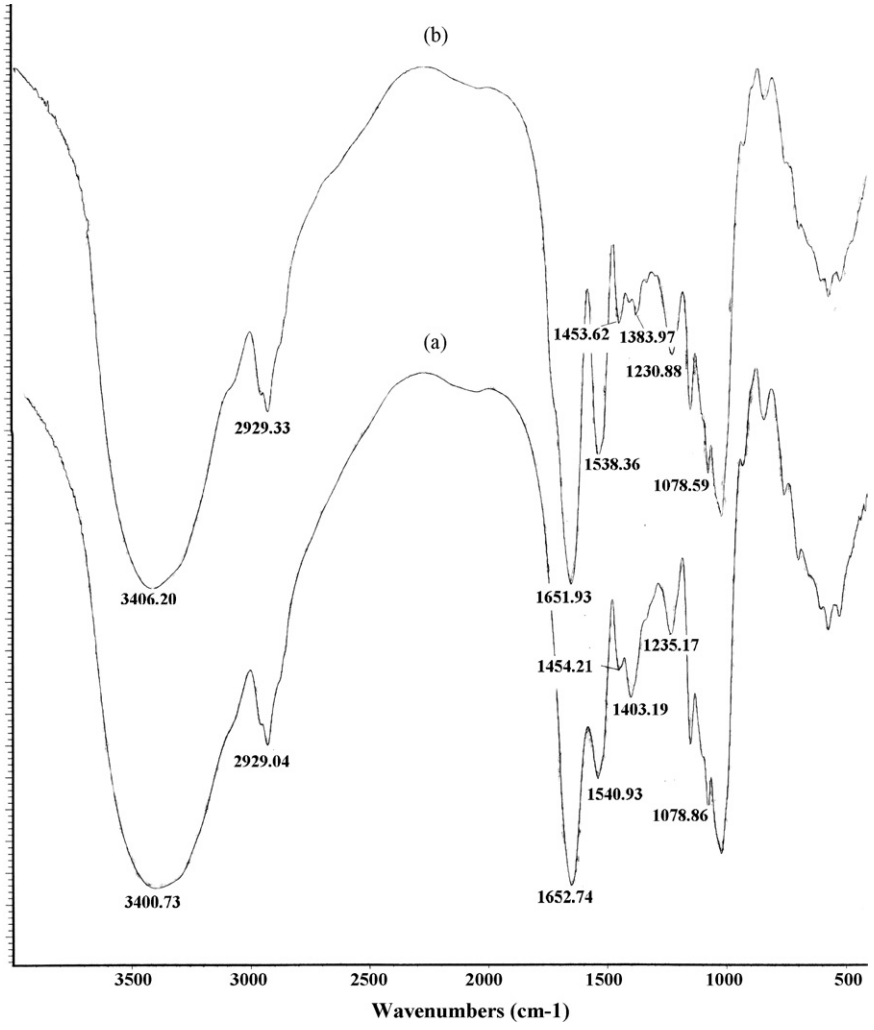


Fig. 11. Fourier transform infrared absorption spectrum of strain K33 and Cd-loaded strain K33 (a) native and (b) Cd^{2+} treated (Cd^{2+}: 50 mg l^{-1}).

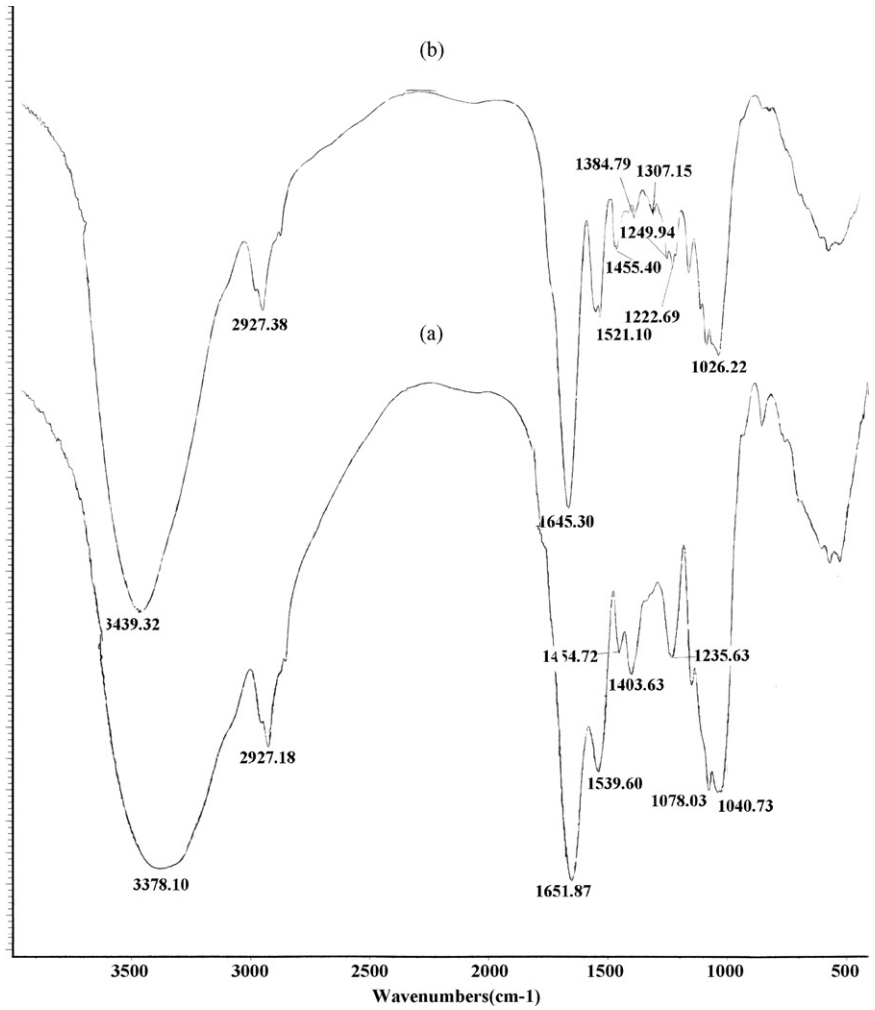


Fig. 12. Fourier transform infrared absorption spectrum of strain HL-12 and Cd-loaded strain HL-12 (a) native and (b) Cd^{2+} treated (Cd^{2+}: 50 mg l^{-1}).

(strain K33) (indicative of –OH and $–NH_2$ groups), 2927 cm^{-1} (HL-12), 2929 cm^{-1} (K33) (indicative of C–H group). The absorption bands at 1651 cm^{-1} (HL-12), 1652 cm^{-1} (K33) (mainly C=O stretch) and 1539 cm^{-1} (HL-12), 1540 cm^{-1} (K33) (mainly –NH, –CN stretch) can be attributed to the amide I and amide II bands of amide bond due to the protein–peptide bond. The moderately strong bands at 1078 cm^{-1} (HL-12) and 1078 cm^{-1} (K33) could be assigned to the –CN stretching vibration of the protein fractions. It was clear that the carboxylate ions gave a rise to two bands: C=O stretch at 1454 and 1403 cm^{-1} (HL-12), 1454 and 1403 cm^{-1} (K33). A band at about 1235 cm^{-1}, representing amide III stretching, was observed in both of the two FT-IR spectra. Bonds at about 1040 cm^{-1}, could be attributed to be the C–OH stretch of sugar. It seemed that the FT-IR spectra of the two strains are nearly the same with no obvious difference.

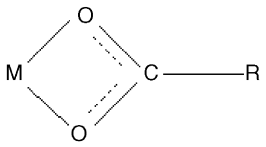
To confirm the differences between functional groups in relation to biosorption of Cd^{2+} in two strains, the FT-IR study was carried out. The absorption spectrum of Cd-loaded biomass was compared with that of pristine biomass. The Cd-loaded biomass was washed, dried and powdered after biosorption of Cd^{2+} under the same conditions used in the preparation of pristine biomass. Changes of absorption bands could be seen in the FT-IR spectra of Cd-loaded biomass comparing with that of pristine biomass (Figs. 11 and 12).

Fig. 11b shows the changes in the spectrum of strain K33 biomass after sorption of Cd^{2+}. There was a substantial increase in the absorption intensity of –NH bands at 1652 and 1540 cm^{-1}. Moreover, an interesting phenomenon was the sharp decrease and showed a bidentate in the band intensity at 1403 cm^{-1} corresponding to C=O stretching after metal binding.

In the case of strain HL-12, the spectral analysis before and after metal binding indicated that –NH, –OH, C=O was involved in Cd^{2+} biosorption (Fig. 12). There was also a substantial increase in the absorption intensity of –NH bands at 1651 and 1539 cm^{-1}, and it could be seen that a bidentate appeared at the 1539 cm^{-1}. C=O bands at 1454 cm^{-1} also showed the same case. The broad overlapping range for N–H and O–H stretching in the range 3200–3600 cm^{-1} also presents some changes, but it is difficult to determine the group that causes the shift. Differently from K33, the C=O stretching bands at 1403 cm^{-1} decreased a lot, almost disappeared. The bond intensity at 1035 cm^{-1} was decreased and showed some bidentates. Especially, the intensity of the band at 1040 cm^{-1} decreased and appeared some bidentates which was different from strain K33.

Comparing with the FT-IR spectra of two strains, it could be found that the FT-IR spectra of strain HL-12 performed more changes than strain K33. The C=O stretching band and amide I and amide II bands of amide bonds were involved in metal binding by both two strains. It seemed that protein was involved in the adsorption of Cd^{2+}. On the basis of variations of the bands, it was reasonable to assume that the peak value suggested the chelating

(bidentate) character of the Cd^{2+} bio-adsorption onto carboxyl groups [35]. The structure of the metal binding to carboxyl ligands on the bacterial cell is likely to take the form as mentioned by Figueira et al. [36]:



4. Conclusions

Two isolated *Streptomyces* strains had been successfully tested for the sorption of heavy-metal Cd^{2+}. The present investigation suggested that strain K33 appears as a low cost possible biosorbent and to be used for treating of Cd^{2+} bearing waste better than strain HL-12. The biosorption characteristic of Cd^{2+} had been examined with the variations in the parameters of Cd^{2+} concentrations and contact time. The maximum biosorption capacity was determined to be 38.49 mg l^{-1} (0.34 mmol g^{-1}) and 24.24 mg l^{-1} (0.22 mmol g^{-1}) at pH 6.0, 2.0–2.5 g l^{-1} biosorbent dosage, 28 °C and 24 h of incubation for strain K33 and HL-12, respectively. Biosorption equilibrium was established earlier by strain K33 than that by strain HL-12, and strain K33 has higher adsorption ratio. The equilibrium experimental data were evaluated by Langmuir, Freundlich and D–R isotherms and fitted well to all of the isotherm models with good regression coefficients with studied temperature and concentration ranges, and Langmuir isotherm was fitted best. Lu et al. [37] used *Enterobacter* sp. J1 as biosorbent with the maximum biosorption of 46.2 mg g^{-1} dry cell for Cd^{2+}, more or less the same as that obtained in this study. The values of R_L obtained were in between 0 and 1 indicate the favorable absorption of Cd^{2+}. As the values of K33 and HL-12 of $1/n$ <1, it indicates favorable absorption of Cd^{2+} on both strains.

The suitable kinetic models for the biosorption of Cd^{2+} were also discussed. The pseudo-second-order kinetic model was applicable for describing of adsorption system better than pseudo-first-order kinetic model in all the experimental concentrations which was the same as the biosorption of Cd^{2+} ions on the microalgae *Chlamydomonas reinhardtii* following second-order biosorption kinetics [38]. By the way, in this study, we achieved a desorption efficiency of over 70%, which could provide theory support for recovering heavy metals.

The interactions between Cd^{2+} and the functional groups on the biosorbent surface were examined by FT-IR analysis. It described the chelating characteristics of cadmium ion coordination to the functional groups on cell surface of both strains, and the possible functional groups of two strains responsible for the metal binding were O–H, N–H, C=O, C–O and so on, indicating mainly the protein and sugar. Choi and Yun [39] detected that marked change observed at carboxyl group peak (1384 cm^{-1}) in cadmium-loaded sewage sludge, carboxyl groups were the dominant species in the cadmium biosorption mechanism by sewage sludge, while much more functional groups on the surface of streptomycete were found in this study.

This work illustrated an alternative solution for the management of the unwanted biological materials where *Streptomyces* sp. K33 and HL-12, fast-growing streptomycete compared with fungi and algae, could be, at some extent, utilized as biosorbent for the removal of heavy metals from the low-strength wastewater, but, strain K33 represented more cost-effective.

Conventional technologies to clean up heavy-metal ions from contaminated waters have been utilized, but they remain cost ineffective. From this study, the use of streptomycetes with resistance to heavy metals for the removal of heavy metals from contaminated waters may be a novel and cost-effective alternative.

Acknowledgement

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References

[1] R.A. Manriquez, P.I. Magana, V. Lopez, R. Guzman, Biosorption of Cu by *Thiobacillus ferrooxidans*, Bioprocess Eng. 18 (1997) 113–118.
[2] N. Yee, L.G. Benning, V.R. Phoenix, F.G. Ferris, Characterization of metal–cyanobacteria sorption reactions: a combined macroscopic and infrared spectroscopic investigation, Environ. Sci. Technol. 38 (2004) 775–782.
[3] J.M. Tobin, D.G. Cooper, R.J. Neufeld, Investigation of the mechanism of metal uptake by denatured *Rhizopus arrhizus* biomass, Enzyme Microb. Technol. 12 (1990) 591–595.
[4] S. Al-Asheh, Z. Duvnjak, Binary metal sorption by pine bark: study of equilibria and mechanisms, Sep. Sci. Technol. 33 (1998) 1303–1329.
[5] B. Volesky, Biosorption of Heavy Metals, CRC Press, Boca Raton, FL, 1990 (Chapter 1).
[6] B. Volesky, Removal and recovery of heavy metals by biosorption, in: B. Volesky (Ed.), Biosorption of Heavy Metals, CRC Press, Boca Raton, FL, 1990, pp. 7–43.
[7] O. Gulnaz, S. Saygideger, E. Kusvuran, Study of Cu(II) biosorption by dried activated sludge: effect of physico-chemical environment and kinetics study, J. Hazard. Mater. B 120 (2005) 193–200.
[8] B. Volesky, Z.R. Holan, Biosorption of heavy metal, Biotechnol. Prog. 11 (1995) 235–250.
[9] J.S. Chang, R. Law, C.C. Chang, Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21, Water Res. 31 (1997) 1651–1658.
[10] P.K. Wong, K.C. Lam, C.M. So, Removal and recovery of Cu(II) from industrial effluent by immobilized cells of *Pseudomonas putida* II-11, Appl. Microbiol. Biotechnol. 39 (1993) 127–131.
[11] C.N. Sawyer, P.L. McCarty, G.F. Parkin, Chemistry for Environmental Engineering, fifth ed., McGraw-Hill Professional, 2002.
[12] T.J. Matheickal, Q. Yu, Biosorption of lead(II) and copper(II) from aqueous solution by pre-treated biomass of Australian marine algae, Bioresour. Technol. 69 (1999) 223–229.
[13] P. Yin, Q. Yu, B. Jin, Z. Ling, Biosorption removal of cadmium from aqueous solution by using pretreated fungal biomass cultured from starch wastewater, Water Res. 33 (1999) 1960–1963.
[14] A. Kapoor, T. Viraraghavan, R.D. Cullimore, Removal of heavy metals using the fungus *Aspergillus niger*, Bioresour. Technol. 70 (1999) 95–104.
[15] P. Kaewsarn, Single and multi-component biosorption of heavy metal ions by biosorbents from marine alga *Durvillaea potatorum*, Ph.D. Dissertation, Environmental Engineering, Griffith University, Queensland, 2000.
[16] A.P.M. Antunes, G.M. Watkins, J.R. Duncan, Batch studies on the removal of gold(III) from aqueous solution by *Azolla filiculoides*, Biotechnol. Lett. 23 (2001) 249–251.
[17] A. Çabuk, T. Akar, S. Tunali, S. Gedikli, Biosorption of Pb(II) by industrial strain of *Saccharomyces cerevisiae* immobilized on the biomatrix of cone biomass of *Pinus nigra*: equilibrium and mechanism analysis, Chem. Eng. J. 131 (2007) 293–300.
[18] I. Langmuir, The adsorption of gases on plane surfaces of glass, mica and platinum, J. Am. Chem. Soc. 40 (1918) 1361–1403.
[19] S.W. Won, G.P. Wu, et al., Adsorption performance and mechanism in binding of Reactive Red 4 by coke waste, J. Hazard. Mater. B 138 (2006) 370–377.
[20] H.M.F. Freundlich, Über die adsorption lösungen, Z. Phys. Chem. 57 (1906) 385–470.
[21] A. Benhammou, A. Yaacoubi, L. Nibou, N. Tanouti, Adsorption of metal ions onto Moroccan stevensite: kinetic and isotherm studies, J. Colloid Interf. Sci. 282 (2005) 320–326.
[22] M.M. Dubinin, L.V. Radushkevich, Proc. Acad. Sci. U.S.S.R. Phys. Chem. Sect. 55 (1947) 331.
[23] J.P. Hobson, Physical adsorption isotherms extending from ultrahigh vacuum to vapor pressure, J. Phys. Chem. 73 (1969) 2720–2727.
[24] S.M. Hasany, M.H. Chaudhary, Sorption potential of Hare river sand for the removal of antimony from acidic aqueous solution, Appl. Radiat. Isotopes 47 (1996) 467–471.

[25] S.S. Dubey, R.K. Gupta, Removal behavior of Babool bark (*Acacia nilotica*) for submicro concentrations of Hg^{2+} from aqueous solutions: a radiotracer study, Sep. Purif. Technol. 41 (2005) 21–28.
[26] K.R. Hall, L.C. Eagleton, A. Acrivos, T. Vermeulen, Pore and solid-diffusion kinetics in fixed-bed adsorption under constant-pattern conditions, Ind. Eng. Chem. Fundam. 5 (1966) 212–223.
[27] T.W. Weber, R.K. Chakravorti, Pore and solid diffusion models for fixedbed adsorbers, J. Am. Inst. Chem. Eng. 20 (1974) 228–238.
[28] D. Purchase, R.J. Miles, T.W.K. Young, Cadmium uptake and nitrogen fixing ability in heavy metals resistant laboratory and field strains of *Rhizobium leguminosarum* biovar trifolii, FEMS Microbiol. Ecol. 22 (1997) 85–93.
[29] B. Volesky, Detoxification of metal-bearing effluents: biosorption for the next century, Hydrometallurgy 59 (2001) 203–216.
[30] Y.S. Ho, G. McKay, Comparative sorption kinetic studies of dyes and aromatic compounds onto fly ash, J. Environ. Sci. Health A 34 (1999) 1179–1204.
[31] Y.S. Ho, G. McKay, Pseudo-second-order model for sorption processes, Process Biochem. 34 (1999) 451–465.
[32] A. Nakajima, T. Sakaguchi, Accumulation of uranium by basidiomycetes, Appl. Microbiol. Biotechnol. 38 (1993) 574–578.
[33] M.P. Pons, M.C. Fuste, Uranium uptake by immobilized cells of *Pseudomonas* strain EPS 5028, Appl. Microbiol. Biotechnol. 39 (1993) 661–665.
[34] J.S. Chang, J. Hong, Biosorption of mercury by the inactivated cells of *Pseudomonas aeruginosa* PU21(Rip64), Biotechnol. Bioeng. 44 (1994) 999–1006.
[35] N. Yee, J.B. Fein, Cd adsorption onto bacterial surfaces: a universal adsorption edge, Geochim. Cosmochim. Acta 65 (2001) 2037–2042.
[36] M.M. Figueira, B. Volesky, H.J. Mathieu, Instrumental analysis study of iron species biosorption by *Sargassum* biomass, Environ. Sci. Technol. 33 (1999) 1840–1846.
[37] W.B. Lu, J.J. Shi, et al., Biosorption of lead, copper and cadmium by an indigenous isolate *Enterobacter* sp. J1 possessing high heavy-metal resistance, J. Hazard. Mater. B 134 (2006) 80–86.
[38] İlhami Tüzün, Gülay Bayramoğlu, et al., Equilibrium and kinetic studies on biosorption of Hg(II), Cd(II) and Pb(II) ions onto microalgae *Chlamydomonas reinhardtii*, J. Environ. Manage. 77 (2005) 85–92.
[39] S.B. Choi, Y.S. Yun, Biosorption of cadmium by various types of dried sludge: an equilibrium study and investigation of mechanisms, J. Hazard. Mater. B 138 (2006) 378–383.