Asian Journal of Chemistry; Vol. 24, No. 11 (2012), 5313-5318

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Removal of Cadmium(II) by Mycelial Pellet of *Rhizopus oryzae* from Aqueous Solution

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(*Received*: 3 November 2011; *Accepted*: 16 June 2012) AJC-11625

Mycelial pellet of *Rhizopus oryzae* was applied as a potential biosorbent for the removal of cadmium(II) from aqueous solution. The effects of pellet diameter, pH, contact time, temperatures and initial metal concentration were studied. The optimum diameter and pH were 1.0-1.2 mm and 4.5. SEM study indicated that the structure of mycelial pellet of *Rhizopus oryzae* could enhance adsorption active sites and provided an advantageous condition for attracting more cadmium(II) around sites. Evaluation of the kinetics data showed that the adsorption followed the pseudo-second order dynamics well. The calculated thermodynamic parameters (ΔGº, ΔHº and ΔSº) showed that the adsorption was feasible, spontaneous and endothermic. The equilibrium data fitted well to the Langmuir adsorption model.

Key Words: Biosorption, Cadmium(II), Mycelial pellet, Kinetics, *Rhizopus oryzae*.

INTRODUCTION

Pollution by cadmium usually comes from several industrial processes such as electroplating, plastics manufacturing, nickel-cadmium batteries, fertilizers, pigments, mining and metallurgical processing[1,2]. The removal of cadmium from industrial wastewater is a problem of increasing concern that has been mostly solved by chemical and physical methods of treatment. However, the applications of such processes are often restricted because of technical and/or economic constraints[3]. Therefore, the search for clean and competitive technologies is strongly recommended.

Biological treatment is usually considered as an effective method and can significantly reduce the quantity of heavy metals in aqueous solutions. The use of microbial biomass as a potential metal adsorbent has caught great attention and much work has been devoted to the field for the past decades[4-7]. These works were mainly related to metal ion uptake either with the freely suspended/powdered microbial biomass or with immobilized microbial biomass[8,9]. However, they both have some major disadvantages, such as smaller particle size, poor mechanical strength and little rigidity for freely suspended/powdered microbial biomass and cost expensive, cell leakage strengths, instability at low pH, poor mechanical and rate-limitation in diffusion for immobilized microbial biomass[10,11], which made them impractical for application in industrial operation.

Pellets are often the preferable morphological forms of filamentous fungi in industrial fermentation processes and the pellets of *Rhizopus sp*. are applied more and more in organic acid industrial production[12]. As a potential adsorbent, it has lots of advantages such as facility to reuse and separation of solid biomass from the bulk liquid. There is no report on metal uptake by mycelial pellets at this time. In this work, the potential of the pellet biomass of *Rhizopus oryzae* TZ-F46, a fumaric acid producing strain, for cadmium(II) removal from the aqueous solution was investigated. The adsorption process was studied with regard to the effect of pellet diameter, pH level, contact time, temperature and initial cadmium(II) concentration. All the results obtained in this paper would provide a sound basis to the further exploration.

EXPERIMENTAL

Preparation of the metal ion stock: The stock solution was prepared by dissolving required amount of analytical grade $CdCl_2·2.5H_2O$ (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) in double distilled water to obtain a concentration of 1,000 mg L^{-1}. Further dilutions were made during the course of the experiment to obtain different concentrations as desired.

Preparation of biomass: *R. oryzae* TZ-F46 strain was grown aerobically in batch cultures at 35 ºC, as described previously[13]. The different diameters of mycelial pellets were harvested after the growth period. These collected pellets were boiled in 0.2 M NaOH solution (1:10 w/v) for 15 min and washed extensively with distilled water. The pellets were then pressed against filter paper to remove the absorbed water.

Batch biosorption studies: Cadmium(II) uptake was investigated in 250 mL Erlenmeyer flask. All experiments were conducted by mixing 100 mL of aqueous cadmium(II) solutions with 1 g (wet weight) of mycelial pellet of *Rhizopus oryzae*. The mixtures of mycelial pellet of *Rhizopus oryzae* and cadmium(II) solution were shaken in an environmental shaker at 150 rpm and desired temperature for an appropriate time. The pH values of solutions were adjusted with dilute NaOH or H_2SO_4 solution before introducing mycelial pellet of *Rhizopus oryzae* into the solution. At given time intervals, 1 mL of the suspensions were collected and centrifuged at 5,000 rpm for 5 min. The concentration of the residual cadmium(II) was analyzed by an atomic absorption spectrophotometer (PerkinElmer SIMAA 6000)[14]. In all the studies involving wet biomass, an equal amount of the same biomass (triplicate) was dried at 333 K over night to obtain the dry weight and the mean dry weight was used to calculate the cadmium(II) uptake (mg g^{-1} dry weight) as given below.

$$q = (C_i - C_q) * V / W \quad (1)$$

where, C_i and C_q refer to the initial and final cadmium(II) concentration (mg L^{-1}), respectively, V represents the volume (L) of the solution and W, the dry weight (g) of mycelial pellet of *Rhizopus oryzae* used in the study.

RESULTS AND DISCUSSION

Effect of pellet diameter and SEM analysis: The diameter of pellet determines the surface area of the biosorbent and therefore, is a key factor in all biosorption processes. It also determines the number of metal binding functional groups readily expose to the metal ions in solution. In this work, the effects of different diameter of pellet biomass (0.4-0.6 mm, 0.7-0.9 mm, 1.0-1.2 mm, 1.3-1.5 mm, 1.6-1.8 mm, > 2.0 mm) on cadmium(II) uptake were investigated in pH 4.5 at an initial cadmium(II) concentration of 100 mg L^{-1} (Fig. 1).

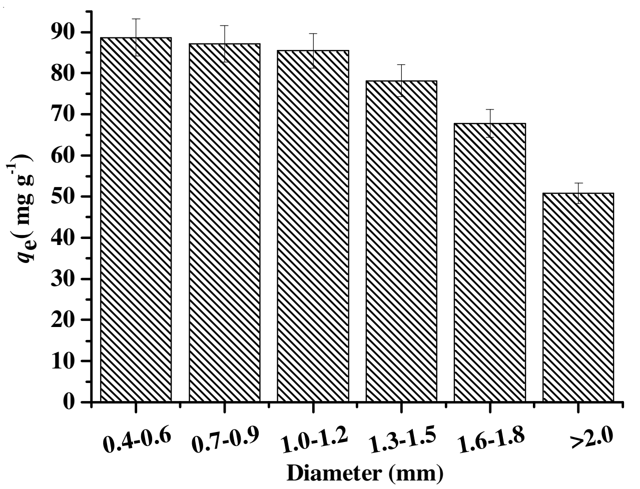

Fig. 1. Effect of pellet diameter on cadmium(II) uptake by mycelial pellet of *Rhizopus oryzae* (initial cadmium(II) concentration =100 mg L^{-1}, pH = 4.5, T = 308 K)

It could be clearly observed that the cadmium(II) adsorption capacity of mycelial pellet of *Rhizopus oryzae* decreased from 88.7 to 50.8 mg g^{-1} when the diameter of mycelial pellet of *Rhizopus oryzae* increased from 0.4 mm to above 2.0 mm, which corresponded to a 0.75-fold decrease in cadmium(II) uptake. This was possibly due to an increased surface area, which was responsible for a greater availability of exposed binding sites in the cell wall of smaller diameter[15]. However, the cadmium(II) uptake by mycelial pellet of *Rhizopus oryzae*, which diameter in the range of 1.0-1.2 mm was also reached 85.5 mg g^{-1}, which was only 3.6 % decrease compared to that in the range of 0.4-0.6 mm. It may be the smaller diameter of pellet (< 1.0 mm) was cultured in lower pH and it could inhibit the growth of cell. The immature pellet may not supply enough metal binding functional group in the cell wall. Meanwhile, the SEM image of mycelial pellet of *Rhizopus oryzae* (Fig. 2a) showed that mycelial pellet of *Rhizopus oryzae* had a compact central core with a fluffy (hairy), loosely packed filamentous, outer zone. There were many free mycelia flocculated at the outer zone and the diameter of the free mycelia was just 2-3 μm (Fig. 2b).

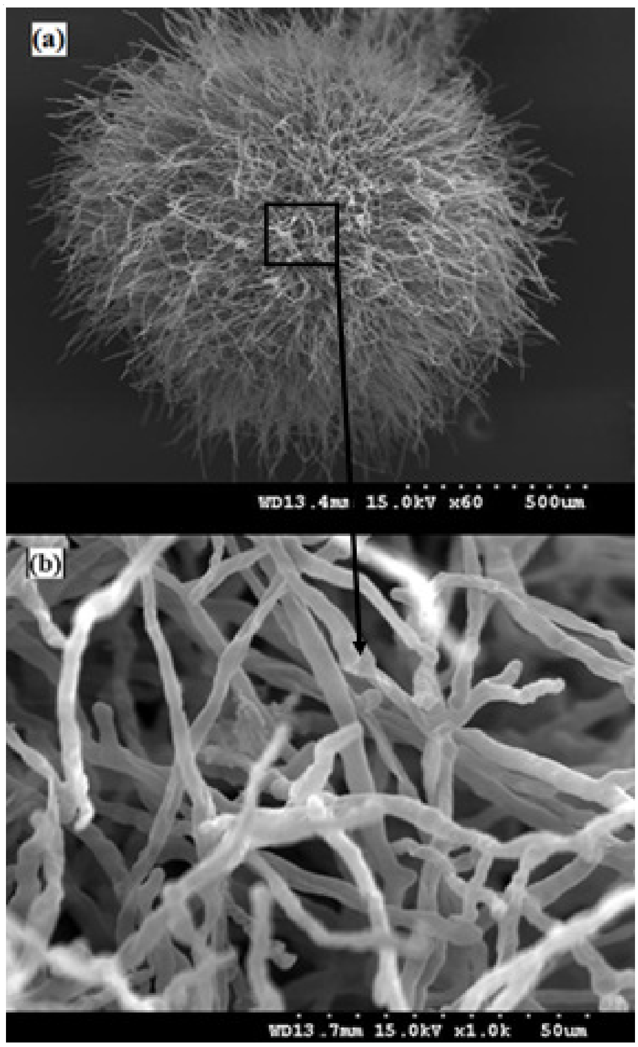

Fig. 2. SEM micrographs of mycelial pellet of *Rhizopus oryzae* (pellet diameter =1.0-1.2 mm) [(a) low magnification; (b) high magnification]

The structure of mycelial pellet of *Rhizopus oryzae* enhanced the number of metal binding functional groups readily exposed to the metal ions in solution when the diameter of mycelial pellet of *Rhizopus oryzae* increased. Although the smaller mycelial pellet had an increased surface area, it would not supply more metal binding functional group per unit volume of the cell wall than the bigger pellets. However, when the diameter continued to increase (> 1.2 mm), the pellets would be autolysis, which could decrease the metal binding functional groups for cadmium(II) uptake. The best pellet diameter for fumaric acid fermentation was in the range of 1.0-1.2 mm[13]. Thus, the diameter in the range of 1.0-1.2 mm was selected as the optimum diameter of mycelial pellet of *Rhizopus oryzae* for the following adsorption experiment.

Effects of pH: It has been consistently shown that pH is the dominant solution parameter controlling adsorption[3]. Fig. 3 shows the effect of pH on the adsorption of cadmium(II) by mycelial pellet of *Rhizopus oryzae*.

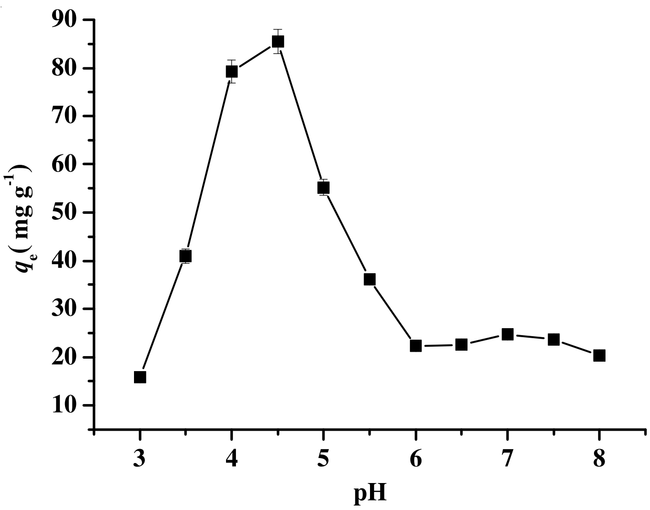

Fig. 3. Effect of pH on cadmium(II) uptake by mycelial pellet of *Rhizopus oryzae* (initial cadmium(II) concentration =100 mg L^{-1}, pellet diameter =1.0-1.2 mm, T = 308 K)

Adsorption capacity was analyzed over a pH range 3-8. It could be seen that the adsorption capacity increased as the pH increased and reached the maximum at pH 4.5, then decreased as the pH continue increasing. It was observed that the adsorption capacity was relatively less at low pH value (pH 3). The less metal uptake at this pH condition may be explained based on binding sites being protonated, resulting in a competition between H^+ and cadmium(II) ions for occupancy of binding sites[16]. As the pH increased, the cell surface took more negative charges, thus attracting a greater number of metal ions[17]. With the pH further increased, OH^- itself had a tendency to combine with cadmium(II) and it competed with ligand on cytoderm for metal ions, leading to the decrease of adsorption capacity when the pH was higher than a certain value[18]. Thus, pH of 4.5 was selected as the optimum pH value of cadmium(II) solution for the following adsorption experiment.

Effect of contact time: Equilibrium time is another important parameter for heavy-metal wastewater treatment process. The cadmium(II) uptake by mycelial pellet of *Rhizopus oryzae* at different contact times has been shown in Fig. 4. This shows that the adsorption capacity increased with increasing contact time and almost 79.97 % of cadmium(II) was removed by mycelial pellet of *Rhizopus oryzae* in the first 60 min of contact time. Equilibrium was reached in a contact time of 360 min. This figure also verifies that the sorption took place in two stages: a very rapid surface adsorption and a slow intracellular diffusion[17].

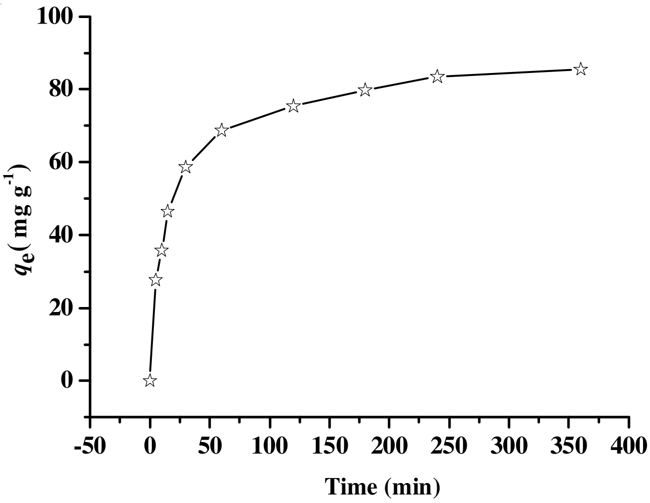

Fig. 4. Effect of contact time on cadmium(II) uptake by mycelial pellet of *Rhizopus oryzae* (initial cadmium(II) concentration =100 mg L^{-1}, pellet diameter =1.0-1.2 mm, pH = 4.5, T = 308 K)

To simulate the adsorption kinetics of cadmium(II) onto mycelial pellet of *Rhizopus oryzae*, Lagergren-first order eqn.(2) and pseudo-second order eqn.(3) models are mostly used[19].

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} \quad (2)$$

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

where, q_e and q_t are the amounts of cadmium(II) (mg g^{-1}) adsorbed on the adsorbent at equilibrium and at a given time t, respectively; k_1 is the rate constant (min^{-1}) of Lagergren-first order kinetic model, k_2 is the rate constant (g mg^{-1} min^{-1}) of pseudo-second order kinetic model.

For Lagergren-first-order plot[20], correlation coefficients were found to be 0.9794 (Fig. 5a), which suggested that the model was unsuitable to fit the kinetic data. Literature[4] also indicated that, in most cases, this model is not applicable to all experimental data throughout the whole biosorption process.

The value of the correlation coefficient (R^2) for the pseudo-second order model (Fig. 5b) was ≥ 0.999.

Moreover, the adsorption capacity calculated by the model (86.21 mg g^{-1}) was also close to that determined by experiment (85.5 mg g^{-1}). These results indicated that this adsorption model was suitable to describe the adsorption kinetics of cadmium(II) onto mycelial pellet of *Rhizopus oryzae*. The pseudo-second order model is based on the assumption that the rate-determining step may be a chemical sorption involving valence forces through sharing or exchange of electrons between adsorbent and sorbate[21]. In fact, the cell wall of mycelial pellet of *Rhizopus oryzae* contained 70-80 % carbohydrates, *i.e.* chitin, chitosan, β-1,3-D-glucans, β-1,6-D-glucans and small amounts of mannoproteins and lipids. These are abundant sources of different functional groups like carboxyl, amine, hydroxyl, phosphate and sulphonate for metal adsorption. Therefore, the cadmium(II) could be well adsorbed by interaction between the cadmium(II) ions and metal binding functional group in mycelial pellet of *Rhizopus oryzae*.

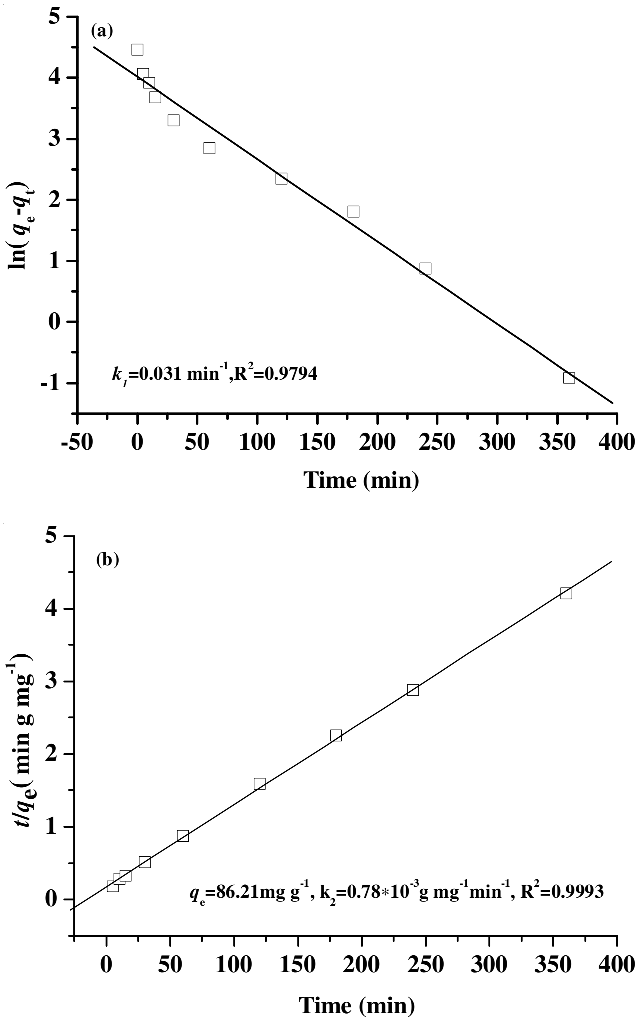

Fig. 5. Linear plot of the kinetic models for cadmium(II) biosorption by mycelial pellet of *Rhizopus oryzae*. (a) Lagergren-first order kinetic model; (b) pseudo-second order kinetic model

Effect of temperature and thermodynamics: Fig. 6 showed the effect of temperature on the equilibrium adsorption of cadmium(II) by mycelial pellet of *Rhizopus oryzae*.

As the temperature increased from 298 to 318 K, the adsorption capacity increased from 77.7 to 91.02 mg g^{-1}, indicating that the temperature might be an important factor for energy-dependent mechanisms in metal adsorption by mycelial pellet of *Rhizopus oryzae*. The increase in adsorption capacity at a higher temperature has been attributed to the endothermic nature of the adsorption process. Furthermore, the increase in kinetic energy of the cadmium(II) due to increased temperature might result in higher frequency of collision with the binding sites and improvement in the uptake[22].

In general, the adsorption process depends on temperature and is associated with several thermodynamic parameters. The thermodynamic parameters such as Gibbs free energy change (ΔG°), enthalpy change (ΔH°) and entropy change (ΔS°) were calculated from the following equations:

$$\Delta G^0 = -RT \ln K_c \quad (6)$$

$$\ln K_c = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (7)$$

where, R is the universal gas constant (8.314 J mol^{-1} K^{-1}); T is the absolute temperature in Kelvin; and K_c(q_e/C_e) is the distribution coefficient. ΔG° is obtained from eqn.(6), ΔH° and ΔS° are estimated from the slope and intercept of the plot of $\ln K_c$ against 1/T. The values of these parameters are given in Table-1.

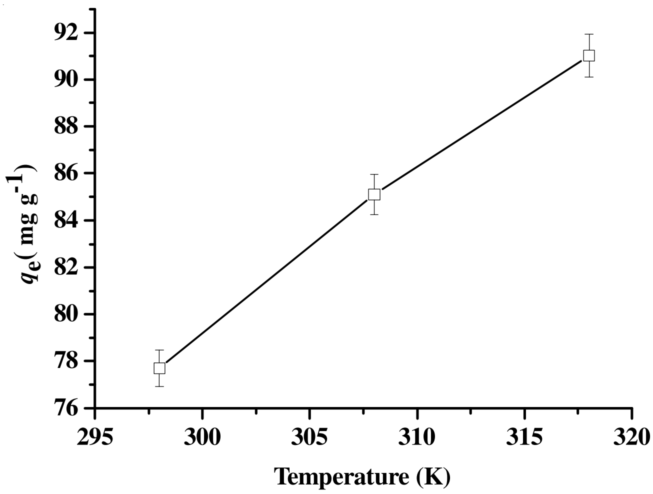

Fig. 6. Effect of temperature on cadmium(II) uptake by mycelial pellet of *Rhizopus oryzae* (initial cadmium(II) concentration =102.3 mg L^{-1}, pellet diameter = 1.0-1.2 mm)

TABLE-1
THERMODYNAMIC PARAMETERS FOR THE ADSORPTION OF CADMIUM(II) ON MYCELIAL PELLET OF *Rhizopus oryzae*

T(K)	Initial concentration (mg L^{-1})	Thermodynamic parameters		
		ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (kJ mol^{-1} K^{-1})
298	102.3	-17.011		
308	102.3	-17.879	8.033	0.084
318	102.3	-18.692		

The negative values of ΔG° implied that the biosorption of cadmium(II) onto mycelial pellet of *Rhizopus oryzae* was spontaneous. The ΔH° value was positive, suggesting that the biosorption process was endothermic. The positive value of ΔS° suggested the increased randomness at the solid/solution interface during the adsorption of cadmium(II) onto mycelial pellet of *Rhizopus oryzae*.

Effect of initial cadmium(II) concentration: The effects of initial cadmium(II) concentration on adsorption capacity and removal efficiency of cadmium(II) by mycelial pellet of *Rhizopus oryzae* were shown in Fig. 7.

The data showed that, with the increase of initial concentration from 11.8 to 403.6 mg L^{-1}, the adsorption capacity increased from 18.7 to 133.2 mg g^{-1}. However, the removal efficiency decreased from 99.4 to 31.8 %. The decrease in percentage biosorption with the increase of initial concentration may be attributed to a lack of sufficient available sites present on biomass surface to accommodate much more metal in the solution. In case of lower initial concentration, most of the cadmium(II) could interact with the active sites and thus the removal efficiency was higher. On the other hand, the biosorption capacity showed a decreasing trend due to the decrease in the diffusion coefficient and decreased mass transfer coefficient of cadmium(II) at lower concentration levels. With the increase of initial concentrations, the mass transfer driving force of the cadmium(II) between the aqueous solution and biosorbent phases increased, which led to an increase in cadmium(II) biosorption[23], while more unabsorbed cadmium(II) ions were left in solution due to the saturation of active sites, resulting in lower removal efficiency.

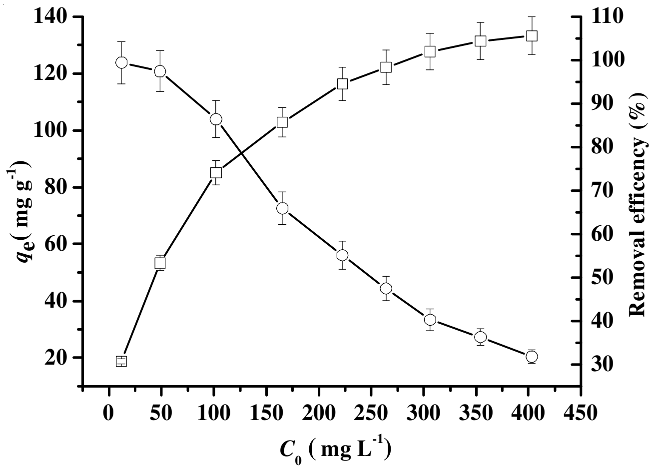

Fig. 7. Effect of initial cadmium(II) concentration on cadmium(II) uptake by mycelial pellet of *Rhizopus oryzae* (pellet diameter = 1.0-1.2 mm, pH = 4.5, temperature = 308 K)

Adsorption isotherms: In order to examine the biosorption mechanism and surface properties of mycelial pellet of *Rhizopus oryzae*, the linearized Langmuir eqn. (4) and Freundlich isotherm eqn. (5) models were employed to analyze the experimental data.

$$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e \quad (4)$$

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{K_L q_m} \quad (5)$$

where, q_e is the amount of cadmium(II) (mg g^{-1}) adsorbed on the adsorbent at equilibrium, C_e (mg L^{-1}) is the cadmium(II) concentration at equilibrium, K_F (mg$^{1-1/n}$L$^{1/n}$ g^{-1}) and n is the Freundlich constants. q_m and K_L are Langmuir constants related to theoretical maximum adsorption amount of cadmium(II) on per gram of biomass (mg g^{-1}) and bonding energy of adsorption (L mg^{-1}), respectively. The results are presented in Fig. 8a and 8b.

It was found that the adsorption of cadmium(II) onto mycelial pellet of *Rhizopus oryzae* fitted better with the Langmuir model (R^2 = 0.9959) as compared to the Freundlich model (R^2 = 0.9771) under the concentration range studied. The basic assumption of the Langmuir model was based on monolayer coverage of the adsorbate on the surface of adsorbent[24], which was an indication of the fact that the adsorption of cadmium(II) onto mycelial pellet of *Rhizopus oryzae* generates monolayer formation. The maximum adsorption capacity of mycelial pellet of *Rhizopus oryzae* for cadmium(II) (q_m) calculated from Langmuir isotherm was 135.14 mg g^{-1}.

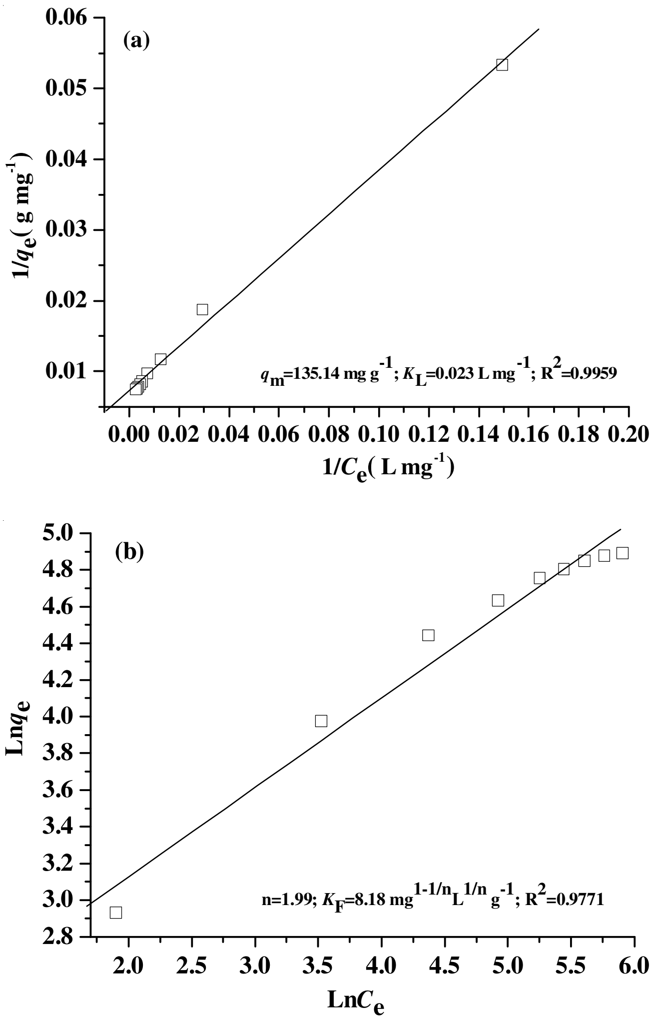

Fig. 8. Cadmium(II) biosorption isotherms by mycelial pellet of *Rhizopus oryzae*. (a) Langmuir model; (b) Freundlich model

Another dimensionless parameter (R_L) that referred to the constant separation factor was derived according to Hall *et al.*[25]. The parameter R_L could be defined by the relationship as given in eqn. (8).

$$R_L = \frac{1}{(1 + K_L C_i)} \quad (8)$$

where, K_L is the Langmuir constant (L mg^{-1}) and C_i is the initial concentration (mg L^{-1}). The parameter indicated the type of isotherm to be irreversible (R_L = 0), favourable (0 < R_L < 1), linear (R_L = 1) or unfavourable (R_L > 1). As seen from Table-2, the values of R_L at the different initial concentrations were between 0 and 1.0, indicating that the adsorption of cadmium(II) onto mycelial pellet of *Rhizopus oryzae* was all favourable.

The 'n' value of Freundlich equation could give an indication on the favourability of sorption. It was generally stated that value of 'n' in the range of 1-10 indicated favourable biosorption and less than 1 poor sorption characteristics[26]. The

TABLE-2
SEPARATION FACTOR (R_L) OF CADMIUM(II) UPTAKE BY MPRO BASED ON LANGMUIR ISOTHERM AT VARYING INITIAL CONCENTRATION OF CADMIUM(II)

C_i (mg L^{-1})	11.7	48.4	102.5	165.3	222.4
R_L	0.810 ± 0.0405	0.508 ± 0.0254	0.327 ± 0.0164	0.232 ± 0.0116	0.184 ± 0.0092

result showed that the value of 'n' was greater than 1, which indicated that the cadmium(II) was favourably adsorbed by mycelial pellet of *Rhizopus oryzae*.

Conclusion

The aim of this work was to find the possible application of mycelial pellet of *Rhizopus oryzae* as a biosorbent for the removal of cadmium(II) from aqueous solutions. It was seen that pellet diameter, pH, contact times, temperature and initial cadmium(II) concentration highly affected the adsorption capacity. The optimum diameter and pH for biosorption were found to be 1.0-1.2 mm and 4.5. The SEM images of mycelial pellet of *Rhizopus oryzae* showed that the surface structure of mycelial pellet of *Rhizopus oryzae* could enhance adsorption active sites and provided an advantageous condition for attracting more cadmium(II) ions around sites. The pseudo-second order dynamic model agreed very well for the adsorption of cadmium(II) onto mycelial pellet of *Rhizopus oryzae*. Biosorption capacity increased as temperature and initial cadmium(II) concentration increased. The calculated thermodynamic parameters (ΔGº, ΔHº and ΔSº) showed that the a dsorption of cadmium(II) onto mycelial pellet of *Rhizopus oryzae* were feasible, spontaneous and endothermic at the temperature ranges of 298-318 K. The equilibrium behaviour of adsorption of cadmium(II) was tested using Langmuir and Freundlich models. The Langmuir equation was found to be the better model that represented the equilibrium data. These results showed that mycelial pellet of *Rhizopus oryzae* could be effectively used as a low-cost and alternative biosorbent for the removal of cadmium(II) from aqueous solutions.

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 21007044, 21106091) and the National High Technology Research and Development Program of China (Grant No. 2011AA02A206).

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