



Biosorption of Cu(II) on extracellular polymers from *Bacillus* sp. F19

ZHENG Yan, FANG Xuliang, YE Zhilong, LI Yahong, CAI weimin*

School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, China. E-mail: zhengyan@sjtu.edu.cn

Received 3 December 2007; revised 15 January 2008; accepted 15 February 2008

Abstract

Biosorption can be an effective process for the removal of heavy metals from aqueous solutions. The adsorption of Cu(II) from aqueous solution on the extracellular polymers (EPS) from *Bacillus* sp. (named MBFF19) with respect to pH, incubation time, concentration of initial Cu(II), and biosorbent dose was studied. Biosorption of Cu(II) is highly pH dependent. The maximum uptake of Cu(II) (89.62 mg/g) was obtained at pH 4.8. Biosorption equilibrium was established in approximately 10 min. The correlation coefficient of more than 0.90 turned out that the adsorption process of Cu(II) on MBFF19 was in accordance with both Langmuir and Freundlich isotherms. The pseudo- first and second order models were applied to examine the kinetics of the adsorption, whereas the latter was found to be in harmony with the kinetic data better. Because of the outstanding uptake capacity of Cu(II), MBFF19 produced by *Bacillus* sp. was proved to be an excellent biosorbent for removing Cu(II) from aqueous solutions.

Key words: *Bacillus* sp.; biosorption; extracellular polymers; isotherms

Introduction

The presence of increasing concentrations of heavy metals in aqueous ecosystem has posed a major threat to aquatic lives and also is injuring people's well-being, arousing the importance of removing heavy metals. One metal that is targeted for the development of new removal technique is copper. With discharges from various industries such as mining, metal plating, dyes, and smelting, copper can be released into the natural ecosystem. Copper of low concentration was reported to be able to improve the performance of biological treatment. However, ecological impacts might be observed when copper concentrations exceed 0.2 mg/L (Lu and Gibb, 2007). Compared with the conventional methods for eliminating dissolved heavy metals including chemical precipitation, ion exchanges, adsorption, membrane processes, and evaporation (Naddafizi *et al.*, 2007), biosorption has the superiority on costs and effectiveness. Hence, biosorption has attracted many attentions as an alternative process for the removal of heavy metals from wastewaters in recent years (Bhatti *et al.*, 2007). A wide range of sorbents such as waste sludge (Yunus and Kargi, 2007), digested sludge (Tokcaer and Yetis, 2006), waste biomass (Chen *et al.*, 2005), and extracellular polymers (EPS) (Loaec *et al.*, 1997; Salehizadeh and Shojaosadati, 2003; Guibaud *et al.*, 2005) of microorganisms were used to abate heavy metal from aqueous solutions.

EPS is secreted by microorganisms such as algae, bacteria, fungi, and yeast. Generally, EPS has high molecular

weight with abundance of negatively charged functional groups referred to as ligands, for instance, carboxyl, hydroxyl, and uronic acids (Sobeck and Higgins, 2002). These ligands make it possible for EPS to capture metal ions through electrostatic interactions, forming multiple complex (Pulsawat *et al.*, 2003). Hence, EPS have been recommended as a kind of good metal absorbent because of their extensive complexing capacity for heavy metals (Gutnick and Bach, 2000). Adversely, until most recent time, only a few EPS were performed on metal biosorption and seldom are there the published works about the Cu(II) uptake by EPS.

The main focus of the present work was to investigate the potential ability of Cu(II) biosorption by biopolymer from *Bacillus* sp. F19. Of various factors affecting the adsorption, initial pH of solution, contact time, initial Cu(II) concentration, and biosorbent dose were examined. Moreover, kinetic and equilibrium models were used to fit experimental data.

1 Material and methods

1.1 Microorganism cultivation

Bacillus sp. F19 used in this study was previously isolated from soil samples excavated on the campus of Shanghai Jiao Tong University, China. The pure culture, afterwards, was transplanted into the 1,000 ml optimized medium. The composition of the media (g/L) was as follows: glucose, 20; yeast extract, 2; KH_2PO_4 , 2; K_2HPO_4 , 5, and NaCl, 0.1 with the initial pH 9–10. The optimized medium was kept in the shaking table for 72 h, where temperate and stirring

* Corresponding author. E-mail: caiweimin@sjtu.edu.cn.

speed were, respectively, set at 30°C and 200 r/min.

1.2 MBFF19 purification

To extract the EPS, the culture broth of *Bacillus* sp. F19 was centrifuged for 15 min at 10,000 r/min and 4°C to settle cells. Afterwards, two volumes of ethanol previously stabilized at 4°C was added to the supernatant and agitated. The resultant precipitate was collected by centrifugation at 10,000 r/min for 3 min and purified with distilled water and ethanol for three times. After that, the EPS was dialyzed against deionized water overnight, and then, lyophilized to obtain purified EPS.

1.3 Biosorption studies

Copper standard stock solution of 1,000 mg/L was prepared from a ready made standard (SIMT, Shanghai). Working copper solution was extemporarily prepared by means of diluting stock solution as an appropriate ratio every time right before the experiment. In terms of initial pH of solution impacts on biosorption, the Cu(II) concentration of working solution was selected at 100 mg/L, and 1 mol/L NaOH and 1mol/L HCl were used to regulate the initial pH to the investigating range, from 1.1 to 4.8 standard values. The determination of the equilibrium time of Cu(II) removal was carried out with three working solutions with the same pH at 3.9 and different Cu(II) concentration, which are 50, 200, and 400 mg/L. Samples via reaction for 1, 2, 3, 4, 10, 20, 30, 60, and 90 min would be analyzed to determine the equilibrium time. In addition, metal solutions with various concentrations ranging from 50 to 500 mg/L were used to assess the effect of initial metal ion concentration on biosorption. The assessment was conducted separately at three different pH values of 3.2, 4.1, and 4.7. The effect of MBFF19 dose was examined with initial metal concentration of 100 mg/L. The reagents were blended in 100 ml Erlenmeyer flasks, which were being vibrated on an orbital shaker at 200 r/min and 25°C. After the reaction reaches equilibrium, two volumes of ethanol previously stabilized at 4°C were added to flasks. Then, the amalgam in the flasks was filtered by syringe driven filter (water, Φ 13 mm, 0.22 μm) to remove metal-EPS complex. Eventually, the residual metal ion concentration in the filtrate was analyzed for the assessment of impacts of initial Cu(II) concentrations. Meantime, controlling experiments (without adsorbents) were conducted under the same condition as the corresponding experiments. All the experiments were conducted in triplicates and mean values were used in the analysis.

Metal analysis was carried out by an inductively coupled plasma atomic emission spectroscopy (IRIS ADVANTAGE/1000, Thermo Jarrel Ash Corp., USA).

The metal adsorption uptake (q , mg/g) was calculated with Eq.(1):

$$q = \frac{V(C_0 - C_e)}{W} \quad (1)$$

where, V (L) is the volume of solution in flask, C_0 (mg/L) is the initial metal concentration, C_e (mg/L) is the residual

metal concentration, and W (g) is the mass weight of adsorbent.

2 Results and discussion

2.1 Chemical composition of MBFF19

MBFF19 produced by *Bacillus* sp. F19 was a sugar-protein derivative and its chemical composition was listed in Table 1 (the analysis process was illuminated by Zheng *et al.* (2008).

Table 1 Chemical composition of MBFF19

Chemical composition	Content
Total sugar (%)	66.4
Total protein (%)	16.4
Neutral sugar (%)	3.6
Uronic acid (%)	37.0
Amino sugars (%)	0.5
Mannose : glucose (molar ratio)	1.2:1
Elemental analysis (W/W) C : H : O : N : S	36.9 : 5.9 : 51.7 : 4.4 : 1.1

2.2 Adsorption equilibrium

Figure 1 indicates the relationship between contact time and Cu(II) uptake by the MBFF19 at different metal concentrations of 50, 200, and 400 mg/L. The results clearly showed that the uptake of Cu(II) by MBFF19 increased with the elapse of sorption time. No significant increase in the sorption was found after 10 min sorption, and the adsorption was rapid. Furthermore, the initial metal concentrations had no significant impact on equilibrium. This rapid initial biosorption required for biosorption equilibrium was consistent with the results of Pardo *et al.* (2003) and Dundar *et al.* (2007), in which the time required for equilibrium was 5 and 10 min, respectively. In this study, reaction time of 30 min was selected to ensure a complete metal uptake.

Because pH is one of the main controlling parameters affecting the adsorption process, the biosorption of Cu(II) on MBFF19 were measured at initial Cu(II) concentration of 100 mg/L, with pH ranging from 1.1 to 4.8 (Fig.2).

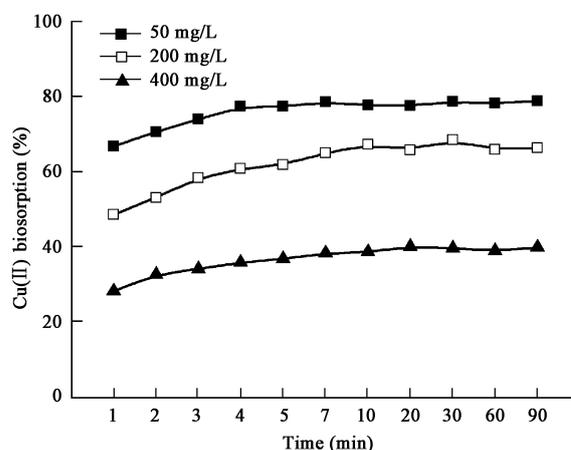


Fig. 1 Effect of contact time on Cu(II) biosorption by MBFF19 (pH: 3.9; biosorbent concentration: 1 g/L; 25°C; 200 r/min).

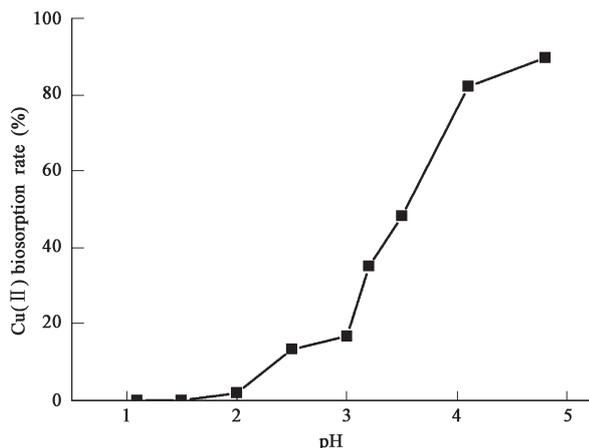
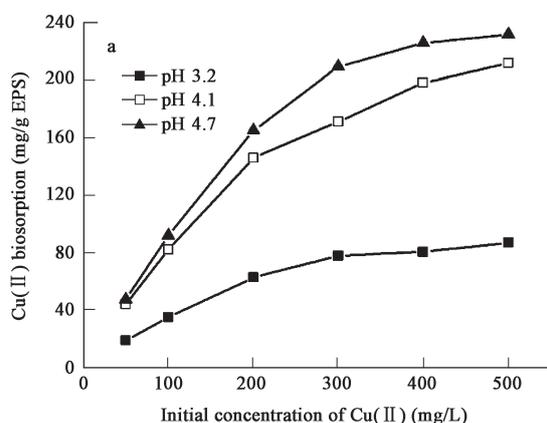


Fig. 2 Effect of pH on Cu(II) biosorption by MBFF19 (C_0 : 100 mg/L; biosorbent concentration: 1 g/L; 25°C; 200 r/min).

The increase of pH has a positive effect on metal uptake. The maximum adsorption of Cu(II) ions on MBFF19 was observed at pH 4.8.

These results revealed that the Cu(II) adsorption of MBFF19 was mainly attributed to the effort of ionic attraction. At low pH, a high concentration of protons resulted in the protonation of functional groups (such as carboxylate group), thus, the negative charged intensity on the binding was decreased (Yan and Viraraghavan, 2003). That was the reason why the attraction between Cu(II) and MBFF19 decreased. In contrast, at high pH, MBFF19 was more negatively charged because of the deprotonation of the metal binding sites, which was in favor of the Cu(II) biosorption. These results were similar to other adsorbent systems related to the biosorption of Cu(II) (Ekmekyapar *et al.*, 2006).

The effect of initial Cu(II) concentration on the biosorption capacity of MBFF19 was examined at pH of 3.2, 4.1, and 4.7. The Cu(II) concentrations varied from 50 to 500 mg/L. As shown in Fig.3a, the equilibrium sorption capacity on MBFF19 increased with increasing initial Cu(II) concentration. In the case of pH at 4.7, when initial Cu(II) concentration increased from 50 to 500 mg/L, the loading capacity increased from 46 to 232 mg/g EPS. The initial Cu(II) concentration also had significant influence on



removal efficiency (Fig.3b). The lowest efficiency turned up at the maximum Cu(II) concentration of 500 mg/L. That is because the number of metal ions increased to an extent that redundantly exceeded the available binding site in the MBFF19 for complexation. In other words, the metal ions competing for the available binding sites increased, which led to the lack of the binding sites for complexation of Cu(II) ions (Aksu and Balibek, 2007).

The Cu(II) biosorption on MBFF19 was studied at MBFF19 concentrations ranging from 500 to 1,500 mg/L. The results are graphically shown in Fig.4. The increase of MBFF19 concentration caused a decrease in the metal specific uptake and an increase in the biosorption removal efficiency. Indeed, the specific uptakes dropped from 142 to 61 mg/g EPS and the biosorption removal efficiency increased from 71.2% to 91.2%, respectively, as the biomass dose increased from 500 to 1,500 mg/L. Such a trend is mainly attributed to an increase in the sorptive surface area and the availability of more adsorption sites. Quite similar tendency was reported for Cu(II) adsorption onto *Bacillus subtilis* (Dogru *et al.*, 2007).

2.3 Adsorption isotherm

The experimental data are applied to the Langmuir and Freundlich isotherm models as they incorporate constants that estimate the quantity of the biosorption capacity

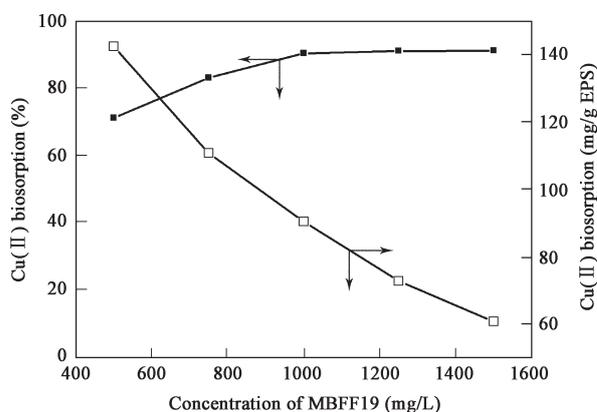


Fig. 4 Effect of adsorbent concentration on adsorption of Cu(II) by MBFF19 (pH: 4.7; initial Cu(II) concentration: 100 mg/L; 25°C; 200 r/min).

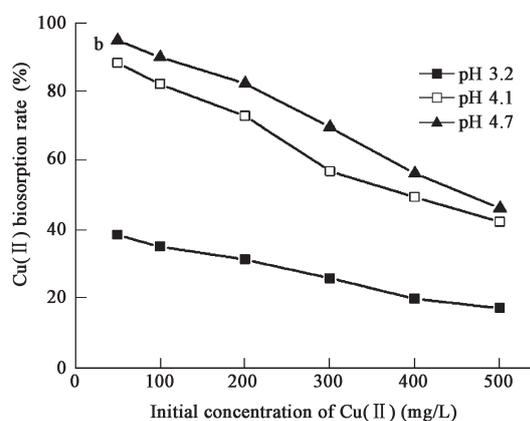


Fig. 3 Effect of initial Cu(II) concentration on biosorption capacity (a) and removal efficiency (b) of Cu(II) by MBFF19 (biosorbent concentration: 1 g/L; 25°C; 200 r/min).

of a biosorbent (Rmalli *et al.*, 2007). Traditionally, the Langmuir model is represented as

$$q_e = Q_{\max} b C_e / (1 + b C_e) \quad (2)$$

where, q_e (mg/g) is the amount of metal adsorbed per mass of adsorbent biomass. Q_{\max} (mg/g) is the adsorption capacity, defined as the maximum amount of metal ion forming a complete monolayer on the biomass surface, b (L/mg) is a constant related to the energy of adsorption, and C_e (mg/L) is the equilibrium concentration of the metal in the solution. After 30 min of adsorption reaction, the residual metal concentration that was considered as C_e was measured.

Equation (3) is the linearized form of the Langmuir model, and the plots of C_e/q_e versus C_e for the Cu(II) are shown in Fig.5a.

$$C_e/q_e = C_e/Q_{\max} + 1/bQ_{\max} \quad (3)$$

The Freundlich isotherm is expressed by Eq.(4), and this equation is linearized as shown in Eq.(5) as follows:

$$q_e = K_F C_e^{1/n} \quad (4)$$

$$\ln q_e = \ln K_F + 1/n \ln C_e \quad (5)$$

where, K_F and n are Freundlich biosorption isotherm constants, being indicative of the extent of the biosorption and the degree of nonlinearity between solution concentration and biosorption, respectively. K_F and $1/n$ are determined from the linear plot of $\ln q_e$ versus $\ln C_e$ (Fig.5b).

The relative parameters along with correlation coefficients resulting from the Langmuir and Freundlich plots are represented in Table 2. The Langmuir theory assumes homogeneous adsorption within the sorbent, meaning that once a Cu(II) ion occupies a site, nor can adsorption

take place at that site any further (Tunali *et al.*, 2006). The Langmuir constant Q_{\max} represents the monolayer saturation at equilibrium or the total capacity of MBFF19 for Cu(II) ions. From Table 2, the Langmuir constant Q_{\max} increased with increase of pH, indicating that high pH was of benefit to adsorption. This is in close agreement with earlier results. The values of the Q_{\max} about some biopolymer adsorbing Cu(II) from the Langmuir constant were listed in Table 3. The Cu(II) biosorption capacity observed in this study was superior to the other biopolymers which are shown in Table 3.

The Freundlich isotherm was hypothesized that a heterogeneous energetic distribution of the active binding sites on the biomass, and was considered that the molecules attached to surface site would have an effect on the next site (Sawalha *et al.*, 2006). From Table 2, the parameter K_F related to the sorption capacity increased with an increase in pH. The value of $1/n$ ($0.1 < 1/n < 1$) indicated that Cu(II) is favorably adsorbed by MBFF19 at the condition studied (Han *et al.* 2006).

As shown in Table 2, the correlation coefficient of the Langmuir isotherms was more than 0.91. With regard to the Freundlich, it was also over 0.90. This indicated that the data fits both Langmuir and Freundlich model well, although the former is better in the examined pH range. The fact that the biosorption data for Cu(II) fit with both isotherms implies that the binding of the Cu(II) takes place as a monolayer on the surface of the biomass.

2.4 Adsorption kinetics

Lagergren pseudo- first order and second order kinetic models were used to evaluate the kinetics of the Cu(II) biosorption on the MBFF19. The pseudo-first order model is expressed as Eq.(6) (King *et al.*, 2007).

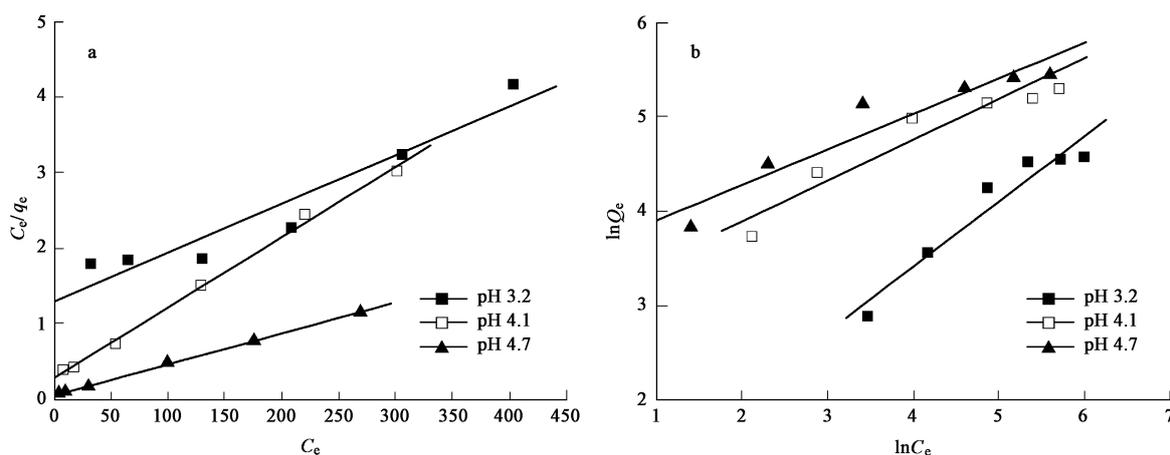


Fig. 5 C_e/q_e vs. C_e (a) and $\ln Q_e$ vs. $\ln C_e$ (b) for the adsorption of Cu(II) by MBFF19 from aqueous solution.

Table 2 Langmuir and Freundlich isotherm constants of Cu(II) adsorption on MBFF19 at different pH

pH	Langmuir constants			Freundlich constants		
	Q_{\max} (mg/g)	b (L/mg)	R^2	K_F ((mg/g)(mg/L) ⁿ)	$1/n$	R^2
4.7	244	0.0585	0.99	35.170	0.368	0.90
4.1	213	0.0322	0.99	22.369	0.404	0.91
3.3	154	0.0050	0.91	1.928	0.690	0.93

Table 3 Langmuir model parameters of Cu(II) sorption with biosorbent produced from different microorganisms

Microorganism	Q_{\max} (mg/g)	b (L/mg)	Operation condition		Reference
			pH	T ($^{\circ}\text{C}$)	
<i>Bacillus firmus</i>	860	NA	4	25	Salehizadeh and Shojaosadati, 2003
<i>Populus tremula</i> forest	29.761	0.186	4.5	45	Dundar <i>et al.</i> , 2007
	29.325	0.3574	4.5	60	Dundar <i>et al.</i> , 2007
	19.531	0.166	4.5	25	Dundar <i>et al.</i> , 2007
<i>Pseudomonas cepacia</i>	66	0.015	NA	NA	Savvaidis <i>et al.</i> , 2003
<i>Sargassum</i> sp.	77.4	0.288	6	30	Valdman and Leite, 2000
<i>Bacillus</i> sp. F19	244	0.0585	4.7	25	This work

NA: not available.

Table 4 Comparison of the first and second order kinetic model parameters obtained at different initial Cu(II) concentrations

Cu(II) concentration (mg/L)	First order kinetics			Experimental q_e (mg/g)	Second order kinetics		
	k_1 (min^{-1})	q_e (mg/g)	R^2		k_2 (g/(mg·min))	q_e (mg/g)	R^2
50	0.1073	2.2106	0.5488	39.3692	0.1556	39.355	1.0000
200	0.1126	25.174	0.5656	133.2200	0.0321	132.979	0.9995
400	0.0982	26.9029	0.8194	157.3870	0.0176	158.73	0.9998

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \quad (6)$$

where, q_t (mg/g) is the amount of adsorbed Cu(II) on the adsorbent at time t , and k_1 (min^{-1}) is the rate constant of first-order adsorption, respectively.

The kinetic data were further analyzed through the pseudo-second order relation. The pseudo-second order kinetic model is expressed as (Malkoc, 2006):

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (7)$$

where, k_2 (g/(mg·min)) is the rate constant of second order adsorption. q_e and k_2 can be determined from the slope and intercept of the plot, respectively.

A comparison of the first and second order kinetic model parameters obtained at different ion concentrations is given in Table 4. The pseudo-first order model failed to estimate q_e because the correlation coefficient is always less than 0.8194 at different initial Cu(II) concentration. Furthermore, the experimental values of q_e are not in good agreement with the theoretical values of q_e , which can get through Eq.(6). With regard to second order kinetic model, nevertheless, the values of correlation coefficient were extremely high, even more than 0.999. Moreover, the experimental q_e was relatively close to the theoretical at different initial Cu(II) and adsorbent concentrations (Table 4). Therefore, it turned out that the pseudo-second order model was able to describe the kinetic behavior accurately. In accordance with the pseudo-second order reaction mechanism, the biosorption of Cu(II) onto MBFF19 may be controlled by the chemical processes (Dundar *et al.*, 2007).

3 Conclusions

In a conclusion, the studies illuminated that MBFF19 is an effective adsorbent for the removal of Cu(II) from aqueous solutions. The biosorption capacity of Cu(II)

was influenced by pH, ion concentration, contact time, and biosorbent dose. The experiment revealed that the adsorption data had an excellent comparability with both Langmuir and Freundlich isotherms. The pseudo-second order kinetic model, relative to the pseudo-first order, is suitable to depict the absorption process. Compared with other biosorbent produced from different microorganisms, the high affinity of MBFF19 for Cu(II) foresees the possibility of the MBFF19 application in removal of Cu(II) from aqueous solutions and thus, reducing aqueous environmental pollution.

References

- Aksu Z, Balibek E, 2007. Chromium(VI) biosorption by dried *Rhizopus arrhizus*: Effect of salt (NaCl) concentration on equilibrium and kinetic parameters. *J Hazard Mater*, 145(1-2): 210–220.
- Bhatti H N, Mumtaz B, Hanif M A, Nadeem R, 2007. Removal of Zn(II) ions from aqueous solution using *Moringa oleifera* Lam. (horseradish tree) biomass. *Process Biochem*, 42(4): 547–553.
- Chen G Q, Zeng G M, Tu X, Huang G H, Chen Y N, 2005. A novel biosorbent: characterization of the spent mushroom compost and its application for removal of heavy metals. *Journal of Environmental Sciences*, 17(5): 756–760.
- Dogru M, Gul-Guven R, Erdogan S, 2007. The use of *Bacillus subtilis* immobilized on Amberlite XAD-4 as a new biosorbent in trace metal determination. *J Hazard Mater*, 149(1): 166–173.
- Dundar M, Nuhoglu C, Nuhoglu Y, 2007. Biosorption of Cu(II) ions onto the litter of natural trembling poplar forest. *J Hazard Mater*, 151(1): 86–95.
- Ekmekyapar F, Aslan A, Kemal Bayhan Y, Cakici A, 2006. Biosorption of copper(II) by nonliving lichen biomass of *Cladonia rangiformis* hoffm. *J Hazard Mater*, 137(1): 293–298.
- Guibaud G, Comte S, Bordas F, Dupuy S, Baudu M, 2005. Comparison of the complexation potential of extracellular polymeric substances (EPS), extracted from activated sludges and produced by pure bacteria strains, for cadmium, lead and nickel. *Chemosphere*, 59(5): 629–638.

- Gutnick D L, Bach H, 2000. Engineering bacterial biopolymers for the biosorption of heavy metals; new products and novel formulations. *Appl Microbiol Biotechnol*, 54: 451–461.
- Han R P, Li H K, Li Y H, Zhang J H, Xiao H J, Shi J, 2006. Biosorption of copper and lead ions by waste beer yeast. *J Hazard Mater*, 137(3): 1569–1576.
- King P, Rakesh N, Beenalahari S, Kumar Y P, Prasad V S R K, 2007. Removal of lead from aqueous solution using *Syzygium cumini* L.: equilibrium and kinetic studies. *J Hazard Mater*, 142(1-2): 340–347.
- Loač M, Olier R, Guezennec J, 1997. Uptake of lead, cadmium and zinc by a novel bacterial exopolysaccharide. *Water Res*, 31(5): 1171–1179.
- Lu S G, Gibb S W, 2007. Copper removal from wastewater using spent-grain as biosorbent. *Bioresource Technol*, 99(6): 1509–1517.
- Malkoc E, 2006. Ni(II) removal from aqueous solutions using cone biomass of *Thuja orientalis*. *J Hazard Mater*, 137(2): 899–908.
- Naddafi K, Nabizadeh R, Saeedi R, Mahvi A H, Vaezi F, Yaghmaeian K, Ghasri A, Nazmara S, 2007. Biosorption of lead(II) and cadmium(II) by protonated *Sargassum glaucescens* biomass in a continuous packed bed column. *J Hazard Mater*, 147(3): 785–791.
- Pardo R, Herguedas M, Barrado E, Vega M, 2003. Biosorption of cadmium, copper, lead and zinc by inactive biomass of *Pseudomonas putida*. *Anal Bioanal Chem*, 376(1): 26–32.
- Pulsawat W, Leksawasdi N, Rogers P L, Foster L J R, 2003. Anions effects on biosorption of Mn(II) by extracellular polymeric substance (EPS) from *Rhizobium etli*. *Biotech Lett*, 25(15): 1267–1270.
- Rmalli S W, Dahmani A A, Abuein M M, Gleza A A, 2007. Biosorption of mercury from aqueous solutions by powdered leaves of castor tree (*Ricinus communis* L.). *J Hazard Mater*, 152(3): 955–959.
- Salehizadeh H, Shojaosadati S A, 2003. Removal of metal ions from aqueous solution by polysaccharide produced from *Bacillus firmus*. *Water Res*, 37(17): 4231–4235.
- Savvaidis I, Hughes M N, Poole R K, 2003. Copper biosorption by *Pseudomonas cepacia* and other strains. *World J Microbiol Biot*, 19(2): 117–121.
- Sawalha M F, Jose R P, Jaime R, Gardea-Torresdey J L, 2006. Biosorption of Cd(II), Cr(III), and Cr(VI) by salt-bush (*Atriplex canescens*) biomass: Thermodynamic and isotherm studies. *J Colloid Interf Sci*, 300(1): 100–104.
- Sobeck D C, Higgins M J, 2002. Examination of three theories for mechanisms of cation-induced bioflocculation. *Water Res*, 36(3): 527–538.
- Tokcaer E, Yetis U, 2006. Pb(II) biosorption using anaerobically digested sludge. *J Hazard Mater*, 137(3): 1674–1680.
- Tunali S, Akar T, Ozcan A S, Kiran I, Ozcan A, 2006. Equilibrium and kinetics of biosorption of lead(II) from aqueous solutions by *Cephalosporium aphidicola*. *Sep Purif Technol*, 47(3): 105–112.
- Valdman E, Leite S G F, 2000. Biosorption of Cd, Zn, and Cu by *Sargassum* sp. waste biomass. *Bioprocess Eng*, 22(2): 171–173.
- Yan G Y, Viraraghavan T, 2003. Heavy-metal removal from aqueous solution by fungus *Mucor rouxii*. *Water Res*, 37(18): 4486–4496.
- Yunus P M, Kargi F, 2007. Effects of operating parameters on kinetics of copper(II) ion biosorption onto pre-treated powdered waste sludge (PWS). *Enzyme Microbiol Technol*, 42(1): 76–82.