

Immobilization study of biosorption of heavy metal ions onto activated sludge

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Abstract: Activated sludge was immobilized into Ca-alginate beads via entrapment, and biosorption of three heavy metal ions, copper(II), zinc(II), and chromium(II), from aqueous solution in the concentration range of 10–100 mg/L was studied by using both entrapped activated sludge and inactivated free biomass at pH ≤ 5. A biphasic metal adsorption pattern was observed in all immobilized biomass experiments. The biosorption of metal ions by the biosorbents increased with the initial concentration increased in the medium. The adsorption rate of immobilized pre-treated activated sludge(PAS) was much lower than that of free PAS due to the increase in mass transfer resistance resulting from the polymeric matrix. Biosorption equilibrium of beads was established in about 20 h and the adsorbed heavy metal ions did not change further with time. No significant effect of temperature was observed in the test for free biomass while immobilized PAS appeared to be strong temperature dependent in the test range of 10 and 40 °C. Besides, the content of activated sludge in the calcium alginate bead has an influence on the uptake of heavy metals. The sorption equilibrium was well modeled by Langmuir isotherm, implying monomolecular adsorption mechanism. Carboxyl group in cell wall played an important role in surface adsorption of heavy metal ions on PAS.

Keywords: biosorption; alginate beads; immobilization; heavy metal; activated sludge; wastewater treatment

Introduction

The hazard of heavy metals discharged from various industries has already been recognized for a long period. Several physical and chemical wastewater treatment techniques were developed and were used successfully at the large scale, to reduce the concentration of hazardous metals in effluents from higher to lower levels such as chemical precipitation, electrolytic process, ionic flotation process, electrodialysis, ferrite precipitation method and reverse osmosis treatment (Verma, 1996; Gadd, 1993). However, application of such treatment techniques either needs enormous cost and continuous input of chemicals or presents an unsatisfactory removal capacity for the wastewater with metal content lower than 100 mol/L. Since biosorption has been proven to be an ideal candidate for the treatment of low concentration wastewaters for its potential in sequestering dissolved metals with high efficiency and selectivity, and quickly rapid intrinsic kinetics, increasing attention has been paid on the research of various bio-adsorbent over worldwide in recent years (Tobin, 1994; Guibal, 1992; Delgado, 1998; Aksu, 1999; Kapoor, 1999; Say, 2001).

Unfortunately, application of freely suspended biomass for the heavy metal removal is plagued with serious problems. For example, the suspended biomass need to be separated from the aqueous solution, and there is high possibility for the pipelines clogged up with biomass. Since immobilization of biomass can help overcome such problems, various kinds

of natural and artificial polymers have been employed for the study on biomass immobilization via entrapment techniques (Eikmeier, 1984; Groboillot, 1994). Alginic acid, a heteropolysaccharide of α -L-glucuramic acid and β -D-manuromic acids which is found in many algal species, is a carboxylic polyelectrolyte precipitating in the form of a coacervate in the presence of multivalent metal ions. The nontoxic and cost effective characteristics of alginic acid ensure its wide application in biomass immobilization (Crist, 1994; Mofidi, 2000; Jianlong, 2000).

In this study, waste activated sludge, one of the major kinds of solid wastes resulting from domestic wastewater treatment plants, was entrapped using Ca-alginate as a natural polymer matrix. Immobilized heat-inactivated sludge was used to investigate heavy metal ion removal from aqueous solutions in a batch system. Since the Ca-alginate gel is known to bind metal ions strongly (Crist, 1994; Mofidi, 2000), metal removal efficiency of Ca-alginate was also taken into account. Besides, technological parameters, such as pH, the content of biomass and temperature were studied.

1 Experimental

1.1 Pretreatment of activated sludge

The waste activated sludge of pH 6.70 collected from the aeration tank in Nanjing Municipal Wastewater Treatment Plant. The harvested cells were washed thoroughly with double volume physiological saline solution for three times, centrifuged at 3000 r/min for 3 min, sterilized in an

autoclave at 121 °C and 0.1 MPa to make heat inactivate biomass and then stored in refrigerator at 4 °C until use. The MLSS of treated sludge was 14.32 g/L.

1.2 Immobilization of activated sludge

The immobilization of pretreated activated sludge via entrapment was carried out as follows: Na-alginate solution of 3% was prepared by dissolving 0.4 g of Na-alginate in 20 ml of hot distilled water with constant stirring to avoid lump formation. The slurry was cooled to 40 °C and pretreated activated sludge was added in weight ratio of 1 : 2 under stirring condition to have a uniform mixture. The mixture was introduced into a 60 ml solution containing 4% CaCl₂ with a syringe coupled with a pinhead (1.5 mm, diameter) and stirred to prevent aggregation of the gel beads. The beads were allowed to cure for 24 h at 4 °C, washed twice with 200 ml distilled water and physiological saline solution, and then stored for following use.

1.3 Preparation of metal (II) solution

Stock metal (II) solution was prepared by dissolving 0.500 g of pure metal in 1 : 1 nitric acid and diluted with double distilled deionized water to 500 ml. The concentration of heavy metal (II) ions in the stock solution will be 1000 mg/L. Calculated quantities of this stock solution was measured and used for further experimental solution preparation. The range in concentration of metal (II) ion prepared from stock solution varied between 10 and 100 mg/L.

1.4 Biosorption studies

The biosorption of metal (II) ions on the both immobilized and free activated sludge was investigated in batch experiments. The effects of the medium pH, temperature and the initial concentrations of heavy metal ions

on the biosorption rate and capacity were studied. Samples of 1.5 ml were taken at given intervals from the mixed adsorption flasks, clarified by centrifugation and the supernatants were analyzed for metal ion content. The concentration of the metal ions in the aqueous phase were measured by a flame atomic absorption spectrophotometer (Angilent, Shanghai Analytic Inc.). The instrument response was periodically checked with known Cu(II), Zn(II) and Cr(II) ion solution standards. For each set of data present, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples in order to determine the margin of error.

The concentration of metal ions adsorbed per unit plain and alginate-immobilized activated sludge preparations (mg metal ions/g dry beads) was obtained using the following expression:

q = [C₀ - C]V/M, (1)

where, q is the amount of metal ions adsorbed onto the unit amount of the biosorbents (mg/g). C₀ and C are the concentration of the metal ions in the initial solution(mg/L) after biosorption, respectively. V is the volume of the aqueous phase(L) and M is the amount of the biosorbent (g).

A known quantity of wet free or immobilized biomass preparations was used in the adsorption test. After the adsorption process, the dry weight of the preparations was calculated by MLSS and used in the above equation. Each experiment was repeated three times and the results given are the average values. The specific experimental parameters of following tests are listed in Table 1.

Table 1 The specific experimental parameters of following tests

Parameter	Test name	Free PAS	Immobilized PAS
Initial heavy metal concentration	Isotherm	40 mg/L (Cu ²⁺ /Cd ²⁺ / Zn ²⁺)	20 mg/L (Cu ²⁺)
	pH effect	(WU, 2002)	20 mg/L (Cu ²⁺ /Cd ²⁺ / Zn ²⁺)
	T °C effect	(WU, 2002)	20 mg/L (Cu ²⁺)
	Contact time	(WU, 2002)	20 mg/L (Cu ²⁺)
	Biomass concn.	(WU, 2002)	20 mg/L (Cu ²⁺)
Biomass concentration	Isotherm	PAS/medium = 1:5 (v/v)	1 g/25 ml
	pH effect	(WU, 2002)	1 g/25 ml (PAS: CA = 1:2)
	T °C effect	(WU, 2002)	1 g/25 ml (PAS: CA = 1:2)
	Contact time	(WU, 2002)	1 g/25 ml (PAS: CA = 1:2)
	Biomass concn.	(WU, 2002)	-(PAS: CA = 1:2)
T, °C	Isotherm	20	20
	pH effect	(WU, 2002)	20
	T °C effect	(WU, 2002)	-
	Contact time	(WU, 2002)	20
	Biomass concn.	(WU, 2002)	20
pH	Isotherm	5	5
	pH effect	(WU, 2002)	-
	T °C effect	(WU, 2002)	5
	Contact time	(WU, 2002)	5
	Biomass concn.	(WU, 2002)	5
Contact time	Isotherm	1 h	20 h
	pH effect	(WU, 2002)	20 h
	T °C effect	(WU, 2002)	20 h
	Contact time	(WU, 2002)	-
	Biomass concn.	(WU, 2002)	20 h

2 Results and discussion

2.1 Characterization and adsorption capacity of alginate based biosorbents

Four kinds of Ca-alginate (CA) beads ($\Phi 2-4$ mm) containing different amount of pretreated activated sludge (PAS) were prepared by the liquid curing method in the presence of Ca(II) ions. Compositions of these beads are listed in Table 2, while some physiochemical properties of prepared beads are illustrated in Table 3. The amount of entrapped activated sludge in the support was 15–45 mg dry weight per gram beads. Ca-alginate gel itself has a strong capacity of heavy metal removal. The adsorption contribution of PAS and CA was also investigated. The experimental condition is as follows: pH = 4, $T = 20^{\circ}\text{C}$, bead concentration = 1 g/25 ml, contact time = 24 h. Fig. 1 shows that the different PAS content in CA beads may exert a little influence on their biosorption capacity of Cu(II) ions. It seems that the more PAS CA beads contain, the higher equilibrium adsorption capacity they show. Obviously, high heavy metal removal efficiency may be obtained by using high PAS content CA beads. However, immobilization of PAS to Ca-alginate only marginally increased the biosorption yield since there is no significant difference(Aksu, 1998).

Table 2 Composition of CA-immobilized biomass beans

PAS:CA, w/w	Content of dry PAS, mg/g	CA content, mg/g	Water content, mg/g
1:1	42.85	15	942.15
1:2	28.57	20	951.43
1:3	21.43	22.5	956.08
1:4	17.14	24	958.86

2.2 Adsorption isotherms

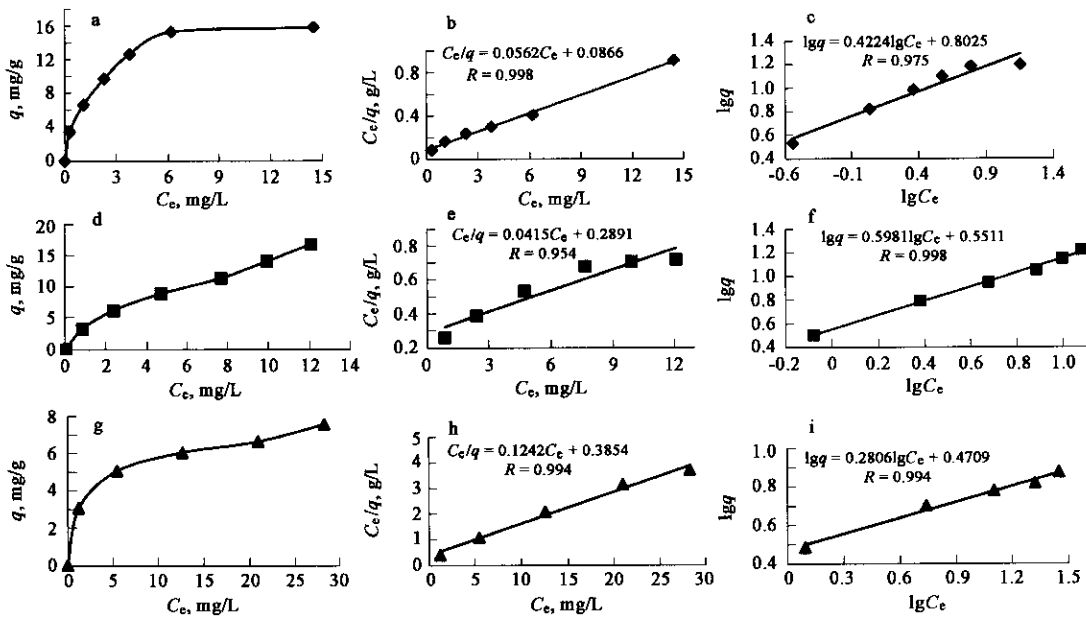


Fig. 2 Adsorption isotherms of free biomass

a. sorption isotherm of Cu^{2+} (of free sludge); b. relationship between C_e/q and C_e of Cu^{2+} ; c. relationship between $\lg q$ and $\lg C_e$ of Cu^{2+} ; d. sorption isotherm of Zn^{2+} ; e. relationship between C_e/q and C_e of Zn^{2+} ; f. relationship between $\lg q$ and $\lg C_e$ of Zn^{2+} ; g. sorption isotherm of Cd^{2+} (free sludge); h. relationship between C_e/q and C_e of Cd^{2+} ; i. relationship between $\lg q$ and $\lg C_e$ of Cd^{2+}

Generally, studies on adsorption can provide further understanding on mechanisms and adsorption capacity of sorbents at constant conditions, therefore, the two most commonly used adsorption isotherms for biosorption studies, the Langmuir and Freundlich adsorption isotherms, were investigated here. The experimental parameters are presented in Table 1, while Figs. 2 and 3 respectively show the adsorption isotherms for heavy metal ions by free PAS and immobilized PAS.

Table 3 Physiochemical properties of CA-immobilized biomass

Items of physiochemical property	Value
Water content, %	94–96
MLSS, %	1.6–4.5
CA content, %	1.5–2.4
Allowable temperature, $^{\circ}\text{C}$	0–40
Percent swell, %	400–500
Apparent gravity, g/ml	1.05–1.10
Absolute specific gravity, g/ml	1.35–1.40
Complete exchange capacity, mg/g	1.1–2.5
Operating exchange capacity, mg/g	0.2–1.0
Allowable pH	1–8

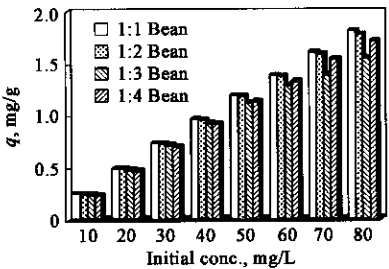


Fig. 1 Composition effect on biosorption

The Langmuir equation is valid for monolayer adsorption on to a surface containing finite number identical sites. The model is described by the following equation:

$$q_e = q_m \cdot C_e / (k_d + C_e), \tag{2}$$
where C_e is the residual metal concentration at equilibrium, q_e is the amount of metal adsorbed on the adsorbent at equilibrium, k_d is the apparent Langmuir constant of the system. The semi-reciprocal plot of C_e/q_e versus C_e was employed to generate the intercept of K_d/q_m and the slope of $1/q_m$, and the Langmuir constants (q_m and k_d) along with correlation coefficients (R^2) have been calculated from the plots (Figs. 2b, 2e, 2h, 3e—3h) for biosorption of Cu^{2+} , Cd^{2+} and Zn^{2+} on the biosorbents. The order of maximum capacity (q_m) of free PAS for heavy metal ion removal was found as: $\text{Zn}^{2+} > \text{Cu}^{2+} > \text{Cd}^{2+}$, while that of immobilized PAS was found as: 1:4 bead > 1:1 bead > 1:2 bead > 1:3 bead. It is clear that the increasing content of immobilized PAS in 1:1 alginate beads lead to a significant increase in adsorption active sites of about 54% for Cu^{2+} in the biosorbent with respect to 1:3 beads. Since k_d may acts as a measure of the stability of the complex formed between metal ions and adsorptive surface layer of the biosorbents under specified experimental conditions, small k_d values found in the study indicated that the metal ion has a high binding affinity for the biosorbents. Although maximum capacity (q_m)

for Zn^{2+} was the highest one in three metal ions, the binding affinity of Cu^{2+} and Cd^{2+} for PAS might lead to a higher affinity than that of the PAS with Zn^{2+} .

The empirical Freundlich equation is listed below:
$$q_e = K_F \cdot C_e^{1/n}, \tag{3}$$

where K_F and n are the Freundlich constants characteristic of the system. K_F and n are indicators of adsorption capacity and adsorption intensity, respectively. The slope and intercept of the linear Freundlich equation are equal to $1/n$ and $\ln K_F$, separately (Figs. 2c, 2f, 2i, 3i—3l). The n values were found high enough for separation, and the magnitude of K_F showed easy uptake of studied metal ions from aqueous medium with high adsorption capacity of biosorbents. Besides, the sorption equilibria of heavy metal ions were well modeled by Langmuir isotherms except that of Zn^{2+} on free PAS, which indicating a monolayer adsorption mechanism.

2.3 Effect of pH on the biosorption capacity of the immobilized biosorbents

Tests were undertaken with different initial pH values of unbuffered Cu^{2+} , Cd^{2+} and Zn^{2+} solutions in water. The adsorption medium was adjusted by HCl or NaOH at the

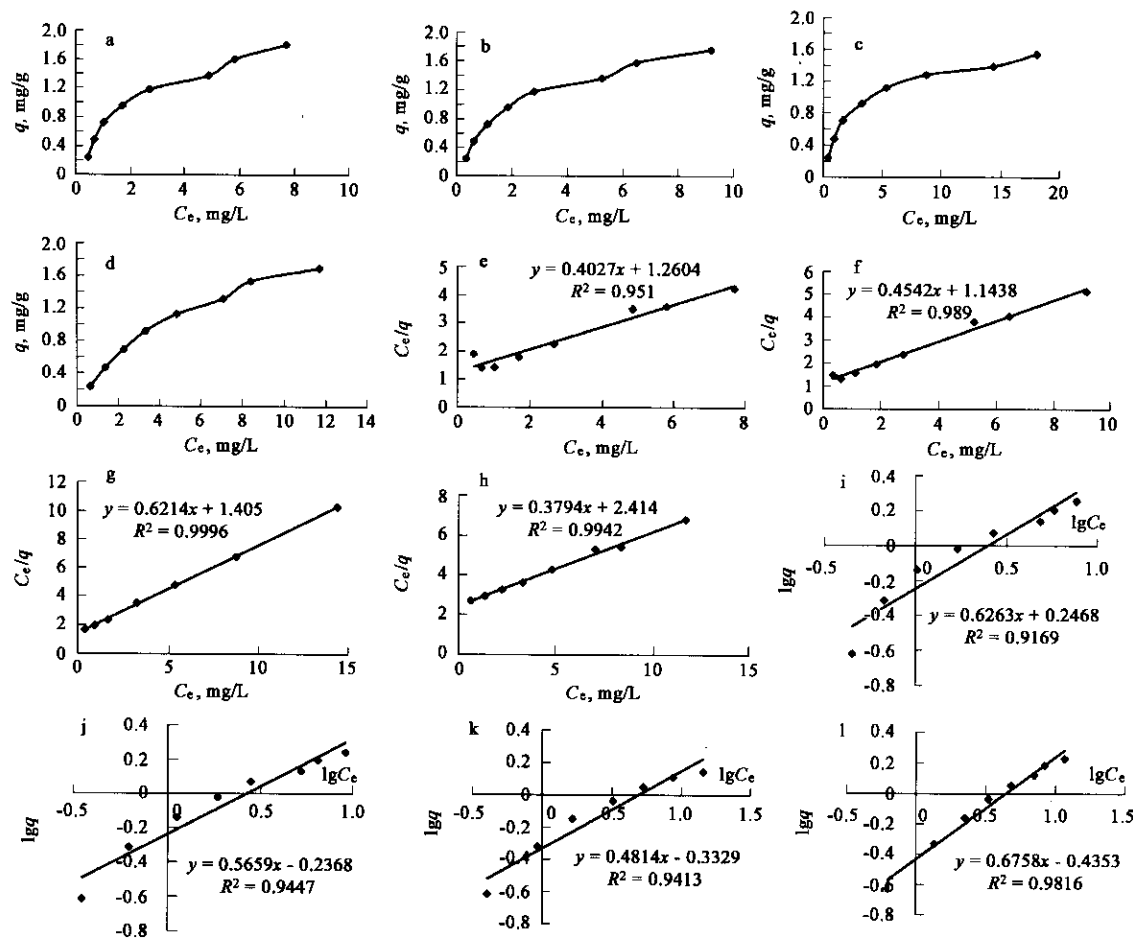


Fig. 3 Adsorption isotherms of immobilized biomass
a. sorption isotherm of 1:1 CA beans; b. sorption isotherm of 1:2 CA beans; c. sorption isotherm of 1:3 CA beans; d. sorption isotherm of 1:4 CA beans; e. Langmuir isotherm fitting of 1:1 CA beans; f. Langmuir isotherm fitting of 1:2 CA beans; g. Langmuir isotherm fitting of 1:3 CA beans; h. Langmuir isotherm fitting of 1:4 CA beans; i. Freundlich isotherm fitting of 1:1 CA beans; j. Freundlich isotherm fitting of 1:2 CA beans; k. Freundlich isotherm fitting of 1:3 CA beans; l. Freundlich isotherm fitting of 1:4 CA bean

beginning of the experiment and not controlled afterwards. During the biosorption-equilibrium experiments with PAS no significant changes in pH of the biosorption medium were observed.

The experimental results are presented in Fig. 4 for immobilized PAS. In all cases there was an increase in biosorption capacity with increasing pH from 1.0 to 3.0, which reached a plateau between pH 3.0 and 6.0. Precisely, the maximum Cu^{2+} , Cd^{2+} and Zn^{2+} biosorption occurred between pH 3.0 and 4.0. At acidic pH ($\text{pH} \approx 3$), protonation of the cell wall component adversely affected the biosorption capacity of the biomass, but its effect becomes minor with increasing pH in the medium. Since it has been proposed that the groups responsible for metal binding are carboxyl groups, which have pK_a 's between 3.0 and 4.0 (Shumate, 1985), it is reasonable to deduce that interaction of the ions with the alginate and immobilized fungal cell wall component could be primarily with the carboxylate groups of both alginate and cell wall components of the bacterium. In addition, it is possible for metal ions to hydrolyze in the case of $\text{pH} \geq 7.0$, and hydroxo species of the metals are not bound to the functional surface ligands. This could have caused a drop in the adsorption capacity of the biosorbent for biosorption of heavy metal ions. Therefore, $\text{pH} \leq 5.0$ was used for the remaining.

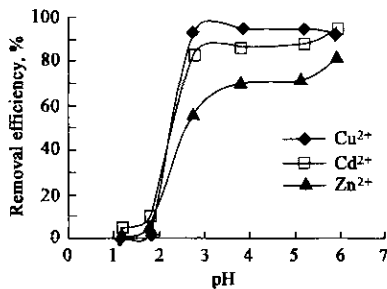


Fig. 4 Effect of pH on biosorption of sludge bean

2.4 Effect of temperature on the biosorption capacity of the immobilized PAS

The temperature could be important for energy dependent mechanisms in metal biosorption by microorganisms, while energy-independent mechanisms are less likely to be affected by temperature since the processes responsible for biosorption are largely physico-chemical in nature.

The biosorption of Cu^{2+} , Cd^{2+} and Zn^{2+} by free PAS presented to be temperature independent at 5–25°C (Wu, 2002) and indicated its physico-chemical nature. On the contrary, immobilized PAS was strong temperature dependent in the test range of 10 and 40°C. Moreover, the adsorption capacity had positive correlation with temperature (Fig. 5). The possible reason is the increase in mass transfer resistance due to the polymeric matrix caused by CA immobilization. High temperature may lead to a faster ion diffusion rate and lower viscosity of liquid film on bead surface, and thus

accelerate the adsorption process.

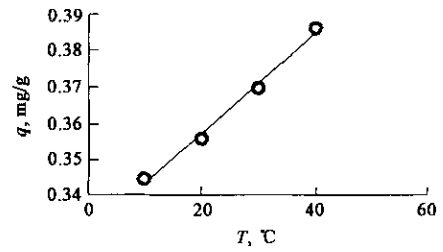


Fig. 5 Temperature effect on biosorption of sludge bead

2.5 Effect of contact time on metal removal

The role of contact time on biosorption of heavy metals by immobilized PAS was studied, and the observed results are shown in Fig. 6.

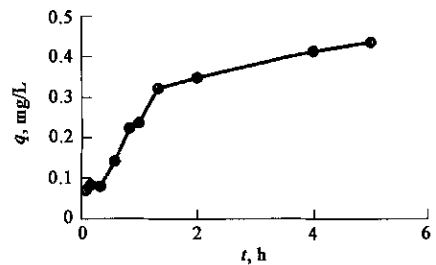


Fig. 6 Effect of contact time on biosorption of beans

One of the most important disadvantages of cell immobilization is the increase in mass transfer resistance due to the polymeric matrix. From this point of view, alginate beads have advantages when compared with support materials such as polyvinyl alcohol and 2-hydroxyethylmethacrylate, because the presence of carboxylic groups in the alginate structure enhanced heavy metal ions adsorption (Park, 1999; Khoo, 2001). From Fig. 6, it is evident that the adsorption rate of immobilized PAS is much lower than that of free PAS (Wu, 2002). For free biomass, saturation levels were obtained after 10 min. After this period, the concentration of adsorbed metal ions did not significantly change further. These are attributed to the surface adsorption resulting from the ion exchange action with the participation of the carboxyl groups of uronic acids presented in cell structure as metal sequestering sites. As for immobilized biomass, the results were different. The data presented in Fig. 6 depicted that the heavy metal adsorption on CA-immobilized PAS occurred in two phases: initial fast phase which lasted for about 2 h followed by slower second phase which continued till the end of test. The second phase represented the diffusion of metal ions into beads need more time because of high mass transfer resistance.

2.6 Effect of biomass concentration on the biosorption capacity

It can be seen that the metal removal efficiency increased with the addition of biosorbents, while the adsorption capacity per unit biosorbent decreased with

increasing sorbents. Considering both removal efficiency and economic factor, the biosorbent concentration around the intersection points in Fig. 7 were recommended for the following study.

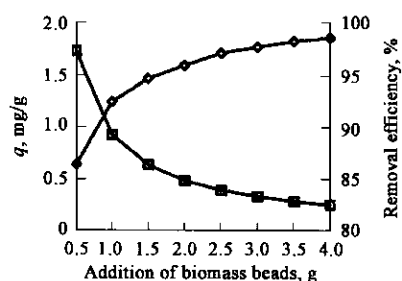


Fig. 7 Effect of biomass concentration on bead adsorption

2.7 Effect of initial metal concentration on the biosorption

The biosorption of copper ions was carried out at different copper ion concentration of 10–80 mg/L at pH 5 with 20 h of contact time using 1 g immobilized biomass [PAS:CA = 1:2 (w/w)] in 25 ml adsorption medium. The results are presented in Fig. 8. Both adsorption capacity of 1:2 beads and remained concentration of copper(II) ions in aqueous phase increased with initially concentration, while the latter plot showed two phases: initial slow phase from 10 to about 50 mg/L followed by fast second phase from 50 mg/L to 80 mg/L of initial experimental concentration. Thus, initial concentration under 40 mg/L was chosen as recommended concentration to ensure the outlet water quality of biosorption process.

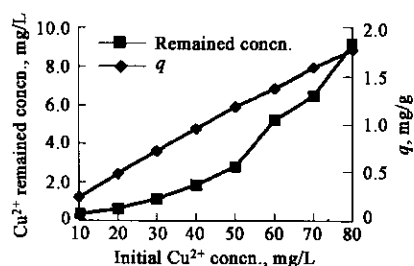


Fig. 8 Effect of initial metal concentration on biosorption

3 Conclusions

Immobilized pretreated activated sludge was successfully used as biosorbent for removal of heavy metal ions from aqueous medium, and the effects of performance parameters such as pH, temperature and contact time on it were analyzed. The biosorption of heavy metals depended on the experimental conditions, particularly acidic pH and the concentration of metals ion in the medium. No significant effect of temperature has been observed in the test for free biomass, while immobilized PAS was strong temperature dependent at 10–40 °C. The adsorption rate of immobilized PAS was much lower than that of free PAS due to the increase in mass transfer resistance resulting from the polymeric

matrix. Besides, the sorption process of most heavy metal ions was in accordance with Langmuir isotherm, which implying monomolecular adsorption mechanism. Carboxyl group in cell wall played an important role in surface adsorption of heavy metal ions on PAS. Compared with the free PAS, the entrapped sterilized PAS showed improved performance in the batch system and could be used as an efficient biosorbent system for the treatment of metal-containing wastewaters.

References

- Aksu Z C, Alik A, Dursun A Y *et al.*, 1999. Biosorption of iron(III)-cyanide complex anions to *Rhizopus arrhizus*: application and adsorption isotherms [J]. *Process Biochem*, 34: 483–491.
- Aksu Z, Egrelti G, Kutsal T, 1998. A comparative study of copper(II) biosorption on Ca-alginate, agarose and immobilized *C. vulgaris* in a packed-bed column[J]. *Process Biochem*, 33: 393–400.
- Delgado A, Anselmo A M, Novais J M, 1998. Heavy metal biosorption by dried powdered mycelium of *Fusarium flocciferum* [J]. *Wat Environ Res*, 70: 370–375.
- Eikmeier H, Rehm H J, 1984. Production of citric acid with immobilized *Aspergillus niger* [J]. *Appl Microbial Biotechnol*, 39: 257–264.
- Gadd G M, 1993. Interaction of fungi with toxic metals [J]. *New Phytol*, 124: 25–60.
- Crist R H, Martin J R, Carr D *et al.*, 1994. Interaction of metals and protons with algae. 4. Ion exchange vs adsorption models and a reassessment of Scatchard plots; ion-exchange rates and equilibrium compared with calcium alginate [J]. *Environ Sci Technol*, 28: 1859–1866.
- Grobillot A, Boadi D K, Poncelet D *et al.*, 1994. Immobilization of cells for application in industry [J]. *Crit Rev Biotechnol*, 14: 75–107.
- Guibal E, Rouilh C, Le Cloirec P, 1992. Uranium biosorption by a filamentous fungus *Mucor miehei* pH effect on mechanism and performances of uptake [J]. *Wat Res*, 26: 1139–1145.
- Jianlong W, Horan N, Stentiford E *et al.*, 2000. The radial distribution and bioactivity of *Pseudomonas* spp. immobilized in calcium alginate gel beads [J]. *Process Biochem*, 35: 465–469.
- Kapoor A, Viraraghavan T, Cullimore D R, 1999. Removal of heavy metals using the fungus *Aspergillus niger* [J]. *Biores Technol*, 70: 95–104.
- Khoo K M, Ting Y P, 2001. Biosorption of gold by immobilized fungal biomass [J]. *Biochem Engineer J*, 8: 51–59.
- Mofidi N, Aghai-Moghadam M, Sarbolouki M N, 2000. Mass preparation and characterization of alginate microspheres [J]. *Process Biochem*, 35: 885–888.
- Park J K, Jin Y B, Chang H N, 1999. Reuseable biosorbents in capsules from *Zoogloea ramigera* cells for cadmium removal [J]. *Biotechnol Bioeng*, 63: 116–121.
- Say R, Denizli A, Arica M Y, 2001. Biosorption of cadmium(II), lead(II) and Copper(II) with the filamentous fungus *P. Chrysosporium* [J]. *Biores Technol*, 76: 67–70.
- Shumate S E, Strandberg G M, 1985. Accumulation of metals by microbial cells [M]. *Comprehensive biotechnology* (Young M., Robinson C. N., Howell J. A. ed.). New York: Pergamon Press.
- Tobin J M, White C, Gadd G M, 1994. Metal accumulation by fungi: application in environmental biotechnology [J]. *J Ind Microbial*, 13: 126–130.
- Verma N, Rahal R, 1996. *J Industrial Poll Cont* [J]. 12: 55–63.
- Wu H S, Zhang H, Zhang A Q *et al.*, 2002. Biosorption of heavy metal mixture by activated sludge biosorption of heavy metal mixture by activated sludge [J]. *Environ Chem*, 21(6): 528–532.
- Zumriye Aksu, Ferda Gonen, Zafer Demircan, 2002. Biosorption of chromium (VI) ions by Mowital® B30H resin immobilized activated sludge in a packed bed: comparison with granular activated carbon [J]. *Process Biochem*, 38: 175–186.