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BIOSORPTION OF PB(II) AND ZN(II) BY EXTRACELLULAR POLYMERIC SUBSTANCE (EPS) OF *RHIZOBIUM RADIOBACTER*: EQUILIBRIUM, KINETICS AND REUSE STUDIES

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Abstract: The extracellular polymeric substance (EPS) produced from *Rhizobium radiobacter* F2, designated as EPS_{F2}, was investigated as a biosorbent for the removal of Pb(II) and Zn(II) from aqueous solution. The optimum biosorption pH values were 5.0 for Pb(II) and 6.0 for Zn(II). Kinetics study revealed that the biosorption followed pseudo-first-order model well, and the equilibrium data fit the Langmuir model better. The adsorbed metal ions could be effectively desorbed by HCl. Desrobed EPS_{F2} regained 80% of the initial biosorption capacity after five cycles of biosorption-desorption-elution. These results demonstrated that EPS_{F2} could be a promising alternative for Pb(II) and Zn(II) removal from aqueous solution.

INTRODUCTION

Many industries (e.g. electroplating, metallurgy, textile, mining, ceramic, etc.) discharge aqueous effluents containing heavy metals into aquatic ecosystem [1]. These heavy metals are persistent environmental contaminants since they could not be degraded or destroyed [2]. Due to the toxic effects on humans, animals and environmental balances, heavy metal pollution has raised a great concern [3].

Lead and zinc are among those metals widely used in industry and their accumulation in the living tissues may pose serious health problems. It has been reported that constant exposure to lead can cause damage to organs (including the liver, kidney and heart) and disturbances of the immune system [4]. Zinc is an essential element for living organisms because of its important role in forming red blood cells and biosynthesis of nucleus acids/polypeptides, but it may cause accumulative poisoning, including dehydration, electrolyte imbalance, stomachache, nausea, dizziness and loss of muscle coordination [5]. Thus removal of these metals from aqueous phase is of great importance with respect to environmental and economic considerations [3].

Conventional separation techniques, including chemical precipitation/neutralization, chemical oxidation or reduction, membrane separation, filtration, ion exchange, and electrochemical methods etc., have been developed to respond this challenge [6]. Unfortunately, most of these methods have disadvantages such as low efficiency, poor regeneration, and yielding large amounts of metallic sludge [7]. Biosorption technology, one of the emerging methods for metal removal, has been regarded as a cheaper and more effective alternative [8]. Biological materials used as biosorbents include bacteria, algae, fungi, yeast, and their derivatives [9]. Recently, extracellular polymeric substances (EPSs), produced by microorganisms during their growth, are found to be effective for the removal of heavy metals (e.g. lead, copper, cadmium, manganese, and zinc) from wastewaters [10, 12]. The chemical structures of EPSs, being rich in functional groups of carboxyl, hydroxyl, and amino groups, are considered as the main contributor for metal removal [13, 14]. Besides the ability of binding with heavy metals, EPSs also possess advantage of aggregation of pollutant particles, stabilization of the floc structure, formation of a protective barrier, and retention of water [13]. Moreover, the EPSs are usually composed of a variety of easily biodegradable organic substances, such as carbohydrates, proteins, minor uronic acids, and nucleic acids, etc. [15, 16], which wouldn't cause a second pollution to the water quality. EPSs are therefore recommended as promising biosorbents for removal of heavy metals due to their extensive capacities [10].

In our previous study, the extracellular polymeric substance (EPS) produced from *Rhizobium radiobacter* F2 (EPS_{F2}), characterized to be insoluble and of high-molecular--weight, was found to show an excellent flocculating activity against solid particles in wastewater [17]. In addition, abundant functional groups with complexing ability, including hydroxyl, amino and carboxyl groups, were identified on the EPS_{F2} surface (Wang W, unpublished). So it is reasonable to expect that EPS_{F2} may have a good adsorption capability for metal ions in aqueous solutions. This study was implemented to investigate the applicability of EPS_{F2} as a new biosorbent for the removal of target metal ions (Pb(II) and Zn(II)) from aqueous solutions. Biosorption kinetics, isotherms, and thermodynamics were studied towards a better understanding of the biosorption process. Finally, experiments on desorption and cyclic utilization of EPS_{F2} were carried out to examine the possibility of reuse.

EXPERIMENTAL

Microorganism

Strain F2 was originally screened from soil by State Key Laboratory of Urban Water Resource and Environment (China) and identified as *Rhizobium radiobacter* [18]. The strain was maintained at 4°C on slant culture medium which consisted of 3 g beef extract, 10 g peptone, 5 g NaCl, and 20 g agar per liter.

Preparation of the EPS_{F2}

The strain F2 was inoculated from slant culture medium into a 250 ml flask containing 100 ml liquid culture medium and cultivated on a shaker (140 rpm) for 24 h at 30°C. The composition of liquid culture medium was as follows (per liter): 10 g glucose, 5 g K_2HPO_4, 2 g KH_2PO_4, 0.5 g peptone, 0.5 g urea, 0.2 g $MgSO_4 \cdot 7H_2O$, and 0.2 g NaCl. The initial pH was adjusted to 7.2 ~ 7.5.

The fermentation broth of F2 was centrifuged at 5, 000 rpm for 20 min to remove cells of strain F2. Two volumes of 4°C cold ethanol were added to the remainder and EPS_{F2} precipitate formed. The mixture was stored at 4°C for 24 h and then EPS_{F2} precipitate was separated by centrifugation at 5000 rpm for 5 min. The EPS_{F2} was washed with deionized water several times and dried at 60°C for 24 h followed by storing in desiccator for further use.

Batch biosorption procedure

Stock solutions of Pb(II) and Zn(II) used in biosorption experiments were prepared by dissolving their nitric salts (Tianjin Chem. Ltd, China) in deionized water, respectively, with a concentration of 1000 mg·l⁻¹. The metal solutions with different concentrations in the biosorption experiments were prepared by proper dilution from the stock solutions.

Batch biosorption experiments were carried out in 50 ml Erlenmeyer flasks on an orbital shaker at 100 rpm. The EPS_{F2} suspension with a concentration of 5 g·l⁻¹ was prepared by magnetic stirrer in deionized water. Designed volume of metal stock solution and dispersed EPS_{F2} suspension were transferred into the flasks, and the solution was diluted with deionized water to 20 ml.

The effect of pH on biosorption was investigated in a pH range of 2.0 ~ 7.0 with initial metal ion concentration of 100 mg·l⁻¹. The pH of solution was adjusted using 1 M HCl and 1 M NaOH at the beginning of experiments and then not further controlled. The reaction mixture was shaken for 300 min which was sufficient for biosorption equilibrium. The effect of contact time was studied in the range of 10 ~ 300 min with initial metal concentration ranging from 25 to 400 mg·l⁻¹, and 5 ml of samples were taken from the biosorption media for analysis at definite time intervals (10, 20, 30, 40, 50, 60, 90, 120, 180, 240, and 300 min). Similarly, the effect of temperature was examined in the range of 5 ~ 55°C under an initial metal concentration of 100 mg·l⁻¹ and a proper contact time.

For each experiment, a blank, containing only the metal ions at proper concentration without EPS_{F2} added, was conducted to determine the adsorption of the metals onto the walls of Erlenmeyer flasks and the loss of metals in the filtration process. Leaching of metals from EPS_{F2} was also measured. Results indicated that EPS_{F2} did not release any metal ion into the aqueous solutions.

Desorption and reuse studies

Different chemicals, including deionized water, 0.05 M HCl, 0.1 M EDTA, 0.1 M $CaCl_2$, and 0.1 M NaCl solutions, were used to examine their desorption efficiency for the metals loaded onto EPS_{F2}. The desorption procedure was as follows: metal loaded EPS_{F2} after the biosorption experiment (under the conditions of initial metal concentration: 100 mg·l⁻¹, contact temperature: 25°C, contact time: 60 min, and initial pH: 5.0 for Pb(II) and 6.0 for Zn(II)) was first transferred into a 50 ml Erlenmeyer flasks containing 20 ml desorption solutions, and then the mixture was agitated on an orbital shaker at 100 rpm under 25°C for 2 h. Thereafter, the mixture was filtered for collection of the desorbed EPS_{F2} and the filtrate was measured for residual metal ion concentration.

After desorption, the desorbed EPS_{F2} was eluted with deionized water until the pH of wash solution reached the range of 6.0 ~ 7.0. The EPS_{F2} eluted was dried at 60°C for 24 h for reuse in the batch biosorption experiments. Five cycles of biosorption-desorption--elution experiments were conducted to examine the potentiality for reuse of EPS_{F2}.

Analysis of metal ions

After biosorption under different conditions, samples taken from the tested solutions were filtered immediately using 0.45 μm cellulose acetate membrane and analyzed for residual metal ion concentration by ICP-OES (Optima 5300 DV, PE, USA) with working wavelengths at 220.35 nm for Pb(II) and 206.20 nm for Zn(II), respectively. All chemicals used in this study were of analytical grade without further purification and all the experiments were carried out in triplicate and results were given as average values.

RESULTS AND DISCUSSION

Effect of pH

The amount of adsorbed metal on per gram of EPS_{F2} was obtained by the following equation:

$$q_e = [(C_i - C_e) \cdot V]/M$$

where q_e is the amount of metal adsorbed on EPS_{F2} (mg·g⁻¹), C_i and C_e are the metal ion concentrations (mg·l⁻¹) before and after biosorption, respectively, V is the volume of the solution (l), and M is the EPS_{F2} dosage used in the reaction (g).

The pH of solution was considered as one of the most important environmental factors in the biosorption of heavy metals [1]. The pH profile of EPS_{F2} was investigated in a range of 2.0 ~ 7.0 with an increment of 1.0 pH unit (Fig. 1). pH higher than 7.0 was not studied because alkaline condition would result in insoluble hydroxide precipitates, making metal biosorption studies complicated [19].
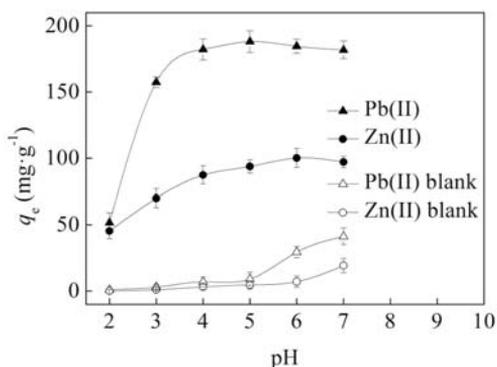

Fig. 1. Effect of pH on biosorption

Fig. 1 indicated that the biosorption of Pb(II) and Zn(II) on EPS_{F2} were strongly dependent on pH values. In general, the biosorption capacity of EPS_{F2} was low at pH 2.0 and a sharp increase was observed in the pH range of 3.0 ~ 5.0. At pH above 5.0, biosorption capacity for Pb(II) was found to decrease, while Zn(II) biosorption continued to increase till pH was 6.0. The maximum biosorption capacities were obtained at pH 5.0 for Pb(II) and 6.0 for Zn(II). These pH conditions were applied in the following biosorption experiments.

As can be seen in Fig. 1, loss of both metals due to adsorption onto the flask walls or/and filtration treatment of samples increased with pH. The adsorption of Pb(II) at pH above 5.0 and that of Zn(II) at pH 7.0 was significant, while the adsorption at pH 5.0 for Pb(II) and at pH 6.0 Zn(II) was negligible. If the precipitation contributed to the Pb(II) and Zn(II) removal, the biosorption capacity should increase and achieve higher values at higher pH conditions. These results indicated that the removal of metals is mainly biosorption.

The pH can affect the ionization state of the functional groups such as carboxylate, phosphate, imidazole, and amino groups on the surface of adsorbent, and thus influencing the absorption of metal ions [20]. At lower pH, the surface charge of the adsorbent became positive, which inhibited the approach of positively charged metal ions. In addition, protons in the solution competed with metal ions for binding sites, thereby decreasing the interaction of metal ions with the adsorbent [21]. When the pH was increased, protons began to be desorbed, and the metal ions hooked up the free binding sites. Hence, the metal adsorption increased on the surface of the adsorbent. Similar phenomenon was obtained by other researchers during their investigations. Rakhshaee et al. [22] observed that metals uptake on pre-treated *Lemna minor* biomass was negligible at pH 1.0 and increased continuously with pH from 2.0 to 7.0, 5.0 and 6.0 for Hg(II), Cr(II) and Cu(II), respectively. The study of Huang et al. [23] revealed that the percentage removal of Pb(II) by *Agaricus Bisporus* was enhanced with increasing solution pH and a maximum value was reached at around pH 4.0. Sarı and Tuzen [24] reported that the biosorption efficiency of macrofungus (*Amanita rubescens*) biomass was increased from 40% to 80% for Pb(II) and from 35% to 70% for Cd(II) with pH increasing from 2.0 to 4.0, and the maximum biosorption was found to be 98% and 97% for Pb(II) and Cd(II) at pH 5.

Biosorption kinetics

Fig. 2 showed biosorption of Pb(II) and Zn(II) on EPS_{F2} in the time range of $10 \sim 300$ min with different initial metal concentration. The biosorption process of each metal ion consisted of an initial rapid stage and a second slow stage. In the rapid stage, which lasted for approximately 60 min, q_e of both metal ions increased rapidly due to the abundance of active binding sites. The second stage accounted for a relatively small part of the total biosorption capacity. This could be explained by the occupancy of binding sites by metal ions absorbed in the initial rapid stage, which led to less efficient biosorption in the latter stage. The biosorption equilibrium was obtained in 60 min for both metal ions. The biosorption capacities at equilibrium were calculated as 188.25 ± 8.20 mg·g^{-1} for Pb(II) and 100.05 ± 6.88 mg·g^{-1} for Zn(II), which did not increase significantly up to 300 min.

The pseudo-first-order equation and the pseudo-second-order equation were employed for testing the experimental data [25]. The kinetic parameters of these two models are listed in Table 1, wherein k_1 is the pseudo-first-order reaction rate constant for adsorption (min^{-1}), and k_2 the pseudo-second-order reaction rate constant for adsorption (mg·g^{-1}·min^{-1}).

By applying the pseudo-first-order equation, the correlation coefficient (R^2) values for both metals were in the range of $0.94 \sim 0.99$, implying that the biosorption process followed the pseudo-first-order kinetic model well. The R^2 values of the pseudo-second-order kinetic model were in the range of $0.88 \sim 0.93$, which were lower than those of pseudo-first-order
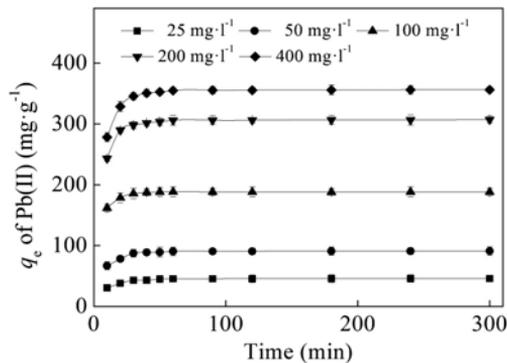
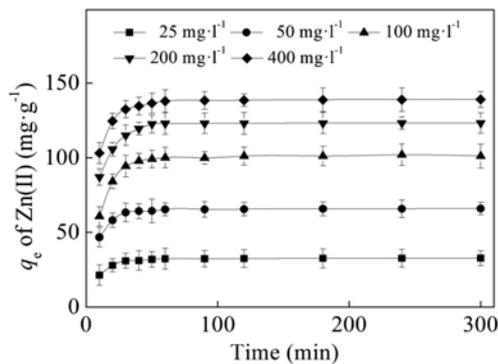Fig. 2(A). Effect of contact time and initial metal concentration of Pb(II)

Fig. 2(B). Effect of contact time and initial metal concentration of Zn(II)

equation. These results suggested that the biosorption of both metal ions on EPS_{F2} followed the pseudo-first-order model better than the pseudo-first-order mode.

Biosorption isotherms

Adsorption isotherms can indicate the relationship between the mass of the adsorbate on per unit mass of adsorbent and the solute concentration at equilibrium [26]. The general adsorption isotherms that can be used for describing the aquatic adsorption behavior include Langmuir and Freundlich models. The adsorption data in this work were analyzed using these two models.

The Langmuir and Freundlich isotherms of each metal ion are shown in Fig. 3. It can be seen that the experimental data fit the Langmuir isotherm well, while a deviation from linearity appeared when the Freundlich isotherm model was applied. The values of Langmuir and Freundlich parameters are tabulated in Table 2, wherein Q_0 (mg·g^{-1}) is the monolayer adsorption saturation capacity, b is the Langmuir constant related to adsorption energy, and K_F and n are Freundlich parameters denoting adsorption capacity and intensity, respectively.

Table 1. Pseudo-first-order and pseudo-second-order kinetics parameters for biosorption of Pb(II) and Zn(II)

| Metal	Initial metal concentration (mg·l⁻¹)	Pseudo-first-order model			Pseudo-second-order model		
		k_1(min⁻¹)	$q_{e,1}$(mg·g⁻¹)	R^2	k_2(mg·g⁻¹·min⁻¹)	$q_{e,2}$(mg·g⁻¹)	R^2
Pb(II)	25	0.24	45.09	0.97	4.09×10⁻³	47.97	0.93
	50	0.27	90.06	0.94	2.94×10⁻³	93.92	0.92
	100	0.45	187.50	0.96	3.21×10⁻³	191.56	0.90
	200	0.36	304.31	0.99	1.25×10⁻³	315.88	0.90
	400	0.34	354.12	0.98	1.09×10⁻³	365.32	0.88
Zn(II)	25	0.24	32.37	0.99	6.64×10⁻³	33.95	0.91
	50	0.27	65.41	0.98	4.16×10⁻³	67.91	0.90
	100	0.21	100.61	0.98	1.61×10⁻³	107.26	0.91
	200	0.26	122.01	0.95	1.90×10⁻³	128.07	0.93
	400	0.30	137.53	0.96	2.20×10⁻³	142.93	0.93
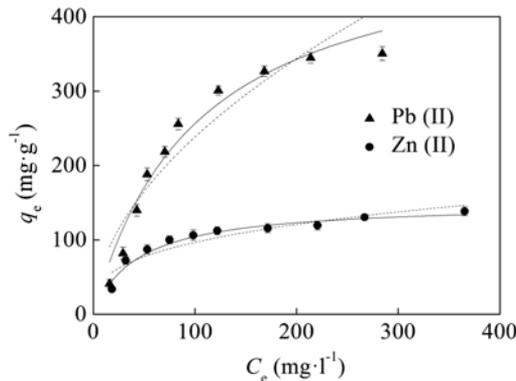
Fig. 3. Langmuir (solid line) and Freundlich (dash line) isotherms

Correlation coefficients (R^2) indicated that the biosorption data of both Pb(II) and Zn(II) were better described by the Langmuir isotherm model than the Freundlich model. A basic assumption of Langmuir theory is that the adsorption takes place at homogeneous surface with a finite number of identical sites [23] and the Langmuir equation is valid for monolayer adsorption [3]. The maximum monolayer biosorption capacities could be estimated as 461.56 mg·g⁻¹ for Pb(II) and 169.58 mg·g⁻¹ for Zn(II) from the Langmuir isotherm equation. Maximum adsorption capacities of different adsorbents for Pb(II) and Zn(II) collected from previous literatures along with the results of the present study

Table 2. Isotherm parameters for biosorption of Pb(II) and Zn(II) on EPS_{F2}

Metal	Langmuir parameters			Freundlich parameters		
	Q_0(mg·g^{-1})	b(mg·g^{-1})	R^2	n	K_F	R^2
Pb(II)	461.56	0.01	0.97	2.48	36.73	0.84
Zn(II)	169.58	0.07	0.97	3.62	27.09	0.87

were summarized in Table 3. Despite direct comparison of EPS_{F2} with other adsorbents has a relative meaning due to the different operating conditions, this comparison was useful to evaluate the applicability of EPS_{F2} for heavy metal removal. According to Table 3, it could be found that EPS_{F2} presented higher biosorption capacity than most of the biosorbents, reflecting a promising future for EPS_{F2} utilization in Pb(II) and Zn(II) removal from aqueous solutions.

Biosorption thermodynamics

The effect of temperature on the biosorption of Pb(II) and Zn(II) was carried out at a temperature range of 5 ~ 55°C (data not shown). At 45°C, the biosorption capacity reached a maximum, 197.80 ± 8.73 mg·g^{-1} for Pb(II) and 119.25 ± 6.11 mg·g^{-1} for Zn(II). Temperature affected the biosorption usually by influencing the stability of the metal-biosorbent complex and the ionization of the functional groups [21]. Elevated biosorption capacity at higher temperature indicated that the biosorption of Pb(II) and Zn(II) on EPS_{F2} may be endothermic processes. However, a decrease in biosorption capacity occurred with increasing temperature up to 55°C, which may be resulted from

Table 3. Comparison between EPS_{F2} with biosorbent produced from other microorganisms

Metal	Microorganism	q_e (mg·g^{-1})	Operating conditions			Reference
			pH	T (°C)	C_i(mg·g^{-1})	
Pb(II)	*Bacillus. sp.*	92.27	3.0	25	250	[1]
	Mucor rouxii	35.69	5.0	-	10	[9]
	Gloeocapsa gelatinosa	82.22	4.0	25	10	[11]
	Acacia leucocephala	185.20	4.0	30	100	[25]
	Rhizobium radiobacter	461.56	7.0	25	100	present study
Zn(II)	*Geobacillus thermoleovorans*	20.30	4.0	70	292.9	[3]
	Mucor rouxii	47.75	5.0	-	10	[9]
	Egeria densa	41.86	5.0	30	270	[26]
	Bacillus firmus	23	6.9	25	50	[10]
	Rhizobium radiobacter	169.58	7.0	25	100	present study

either the damage of active binding sites of EPS_{F2} or the increasing tendency to desorb metal ions from the interface to the solution. These results indicated EPS_{F2} possessed excellent thermostability and could be used in a wide temperature range, which was advantageous for its application in industry.

In order to describe thermodynamic behavior of the biosorption of Pb(II) and Zn(II) on EPS_{F2}, thermodynamic parameters including the change in free energy ($\Delta G°$) and enthalpy ($\Delta H°$) were studied [27]. The $\Delta G°$ values were calculated as -0.15, -0.46, -0.76, -1.09, -1.40, and -1.72 kJ·mol^{-1} for Pb(II) and -0.08, -0.28, -0.49, -0.72, -0.95, and -1.19 kJ·mol^{-1} for Zn(II), respectively. The negative $\Delta G°$ value was an indicator of thermodynamically feasible and spontaneous nature of the biosorption [26]. Namely, the biosorption was largely driven towards the products, with the sequence of preference being Pb(II) > Zn(II). The decrease in $\Delta G°$ values with increasing temperature suggested an increase in feasibility of biosorption at higher temperatures. It could be speculated that the biosorption of Pb(II) on the EPS_{F2} was easier than that of Zn(II) because of the more negative $\Delta G°$ values at the same temperature.

The positive $\Delta H°$ values (28.41 kJ·mol^{-1} for Pb(II)and 25.54 kJ·mol^{-1} for Zn(II)) demonstrated that the biosorption processes were endothermic for both Pb(II) and Zn(II), which confirmed that higher temperatures were preferred for biosorption, with the same sequence of preference as given above.

Desorption and reuse studies

0.05 M HCl, 0.1 M EDTA, 0.1 M $CaCl_2$, and 0.1 M NaCl were used to desorb Pb(II) and Zn(II) loaded on the EPS_{F2}. The desorption efficiency was expressed as a percentage of metal ion desorbed from metal loaded EPS_{F2} out of that adsorbed onto the EPS_{F2}. The results of desorption studied of these chemicals are presented in Fig. 4.
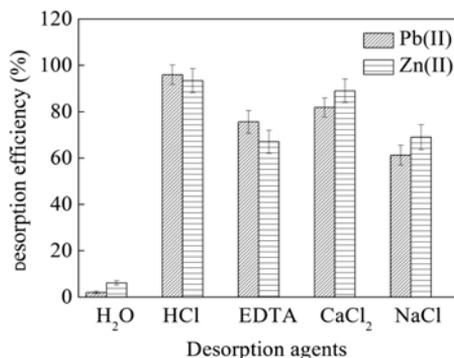

Fig. 4. Desorption efficiency of different agents

Compared to $CaCl_2$, NaCl and EDTA, HCl exhibited higher desorption efficiency for both metal ions with a desorption efficiency of above 90%, while the desorption capability of deionized water was negligible. Yan and Viraraghavan [9] reported that more than 90% of Pb(II), Cd(II), Ni(II), and Zn(II) could be desorbed from dead *Mucor rouxii* biomass. According to Zhang et al.[28], HCl and HNO_3 showed desorption efficiency higher than 80% for lead from dead *R. nigricans*.

Biosorption capacity EPS_{F2} and its reuse possibility could affect its potential as a biosorbent in application. After desorption by HCl, EPS_{F2} was eluted by deionized water followed by drying. The EPS_{F2} obtained was used for batch biosorption experiment. Fig. 5 shows the results of 5 cycles of biosorption-desorption-elution. It can be seen that over 80% of the Pb(II) biosorption capacity of EPS_{F2} was kept within 4 cycles, and EPS_{F2} lost some of its metal binding capability significantly in cycle 5. In the case of Zn (II), the biosorption capacity of EPS_{F2} was kept in a high level. This result suggests that the EPS_{F2} in this study could be used repeatedly.
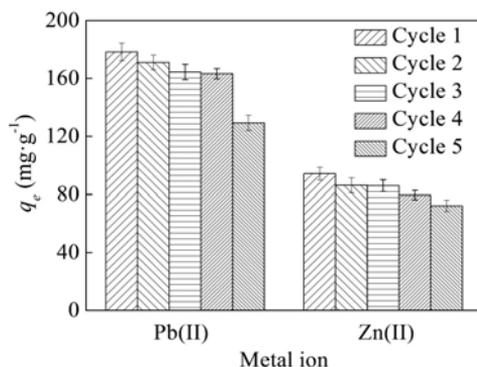

Fig. 5. Biosorption efficiency of EPS_{F2} for Pb(II) and Zn(II) in five cycles

CONCLUSIONS

The EPS_{F2} was used as a biosorbent for the removal of Pb(II) and Zn(II) from aqueous solution. The pH was found to be a critical parameter for the biosorption process, and the optimum pH was 5.0 for Pb(II) and 6.0 for Zn(II). Adsorption equilibrium was reached within 60 min for both metal ions, and the kinetics of both metal ions obeyed the pseudo-first-order equation. The biosorption data were in good agreement with the Langmuir isotherm model. The theoretic maximum biosorption capacities of EPS_{F2} for investigated metal ions calculated by Langmuir equation showed outstanding advantage over similar biosorbents in previous work. The thermodynamic analysis illustrated that the biosorption process was spontaneous and endothermic in nature. After desorption by HCl, EPS_{F2} retained 80% of the initial biosorption capability within four cycles. The present study proved that EPS_{F2} could be used as a potential alternative for the removal of Pb(II) and Zn(II). Furthermore, some investigations such as the stabilization of EPS_{F2} and the analysis of biosorption mechanism were also in progress.

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