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Simultaneous removal of Pb(II) and chemical oxygen demand from aqueous solution using immobilized microorganisms on polyurethane foam carrier

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Abstract−We studied the simultaneous removal of Pb(II) and chemical oxygen demand (COD) from synthetic solutions using immobilized microorganism. The immobilized microorganisms on polyurethane foam (IPUF) were successfully prepared by cultivating the microbe B350 in a mixture of culture medium and polyurethane foam (PUF). The adsorption of Pb(II) ion from aqueous solutions onto PUF and IPUF was studied by batch adsorption. IPUF exhibited high Pb(II) removal efficiency. When 0.12 g of IPUF was used to treat 50 mL of 20 mg/L P(II) solution at pH 7.0 and 25 °C for 120 mins, the removal ratio was 80%. The biosorption kinetics could be described by the pseudo-second-order model, and the adsorption isotherms could be described by Langmuir and Freundlich equations. In addition, for synthetic wastewater containing Pb(II) and $C_6H_{12}O_6$, the removal ratios of Pb(II) and COD after being treated by IPUF for 8 hours were 92.0% and 84.2%, respectively. The removal ratio of COD clearly decreased with the increase of Pb(II) concentration, meaning that Pb(II) was toxic to the mobilized microorganisms and lower Pb(II) concentration was preferred.

Key words: Immobilization, Adsorption, Pb(II), Polyurethane Foam, Wastewater Treatment

INTRODUCTION

With the development of batteries, printing, pigments, fuels, photographic materials and explosive industries etc., increasing amounts of industrial wastewater containing lead are produced. Because lead cannot be biodegraded, the discharged lead will have a chronically negative influence on people. Long-term consumption of water containing 5 ng/mL of Pb(II) can cause anemia, encephalopathy, hepatitis and nephritic syndrome [1]. Therefore, removing Pb(II) from wastewater before it is discharged to the environment is of great significance to keep human beings away from the harm of lead.

Many methods, including chemical precipitation, ion exchange, filtration, electrochemical treatment and reverse osmosis, have been used in the removal of heavy metals [2]. In the last few years, adsorption has become an important method to remove dissolved heavy metal ions from wastewater. Great efforts have been exerted in developing and finding new adsorbents such as polyurethane foam [3], activated carbons [4], microbial biomass [5], silica gels [6], zeolites [7] and clays [8], among which the most widely investigated adsorbent is microbial biomass. There are many functional groups such as carboxyl, hydroxyl, phosphate and amino groups on its surfaces, which play important roles in adsorption. In addition, these materials are cheap and available in large quantities. However, the use of freely suspended microbes for removal of heavy metal ions has many disadvantages, such as low biomass loading, easy washout and poor tolerance of heavy metal ions [9].

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Adsorption using immobilized microorganisms is ideal in engineering applications due to its high biomass loading, easy operation in solids-liquid separation, high metabolic activity and operation stability [10,11]. Polymeric carriers such as alginate [12], polyacrylamide (PAM) [13], polyvinyl alcohol (PVA) [14], polyethylene glycol (PEG) [15], polysulfone [16] and polyurethane foam (PUF) [17] have been widely used to immobilize microorganisms to remove heavy metal ions. However, few of these reports related to the chemical oxygen demand (COD) and organic pollutants removal efficiency of this method. On one hand, to cultivate the immobilized microorganism, carbon and nitrogen sources are usually added, which should be entirely or nearly entirely consumed to reduce the potential possibility to deteriorate eutrophication of water body. On the other hand, sometimes waste water containing Pb(II) also contains organic pollutants. If the immobilized microorganism can simultaneously adsorb heavy metal ions and degrade organic pollutants, the treatment cost will be decreased. Therefore, it would be an interesting topic to simultaneously eliminate organic pollutions and metal ions from wastewater using polyurethane foam immobilized microorganisms.

In our previous work, we successfully used immobilized microorganisms on PUF to remove COD and copper ions simultaneously [18]. In the present work, we studied the removal of Pb(II) and COD using immobilized microorganisms. Compared with Cu(II), which is beneficial to organisms in trace amount, Pb(II) is more harmful to the immobilized microorganisms, which might influence the degradation of organic compounds. Therefore, it is necessary to investigate the feasibility.

The immobilized microorganism on polyurethane foam (IPUF)

was obtained by immobilizing the microorganism on PUF. The effect of several operating parameters on adsorption of Pb(II), including pH, carrier amount, temperature and Pb(II) concentration, was investigated in batch mode. Biosorption data were analyzed using the Langmuir and Freundlich isotherm adsorption models. COD values before and after adsorption were also determined. The immobilized microorganisms were observed by using SEM.

MATERIALS AND METHODS

1. Microorganism and Immobilization

Macroporous PUF carrier was prepared according to the method described in our previous work [19]. The microorganism, B350, was purchased from Bio-Systems Corporation (USA). The composition of the medium used for microorganism immobilization was $C_6H_{12}O_6$ (500 mg/L), NH_4Cl (20 mg/L), $Pb(NO_3)_2$ (10 mg/L), KH_2PO_4 (10 mg/L) and $MgSO_4 \cdot 7H_2O$ (5 mg/L) [20].

According to the method described in reference [21], PUF was washed twice with distilled water and dried. One gram of B350 was added into a 2,000-mL bioreactor containing 10 g of PUF and 500 mL of culture medium at 30 °C, aerating at 1 vvm. The fresh medium was added once again after the PUF was consumed. After this operation was repeated six times, the biomass of immobilized microorganisms was up to 116 mg (dry wt.) per gram of carrier (dry wt.). The prepared IPUF was stored in a refrigerator at 4 °C for further researches.

2. SEM Observation

JSM-6380LV scanning electron microscope (Jeol, Akishima-Shi, Japan) was used to observe the morphologies of microorganisms. It was fixed in a 5.0% (w/v) glutaraldehyde solution for 2 h at 40 °C and dehydrated in an ascending (from 20 to 100%) water-ethanol series (the concentration being increased in 20% steps). The dried specimens were carefully mounted onto aluminum stubs by using conductive adhesive. The mounted specimens were sputter-coated with gold and examined by SEM.

3. Adsorption of Pb(II) ion by Immobilized Microorganisms

The batch biosorption experiments were performed using 250-mL beakers in a constant-temperature rotary shaker at 150 rpm. Effects of initial pH value, dosage of carrier, temperature, contact time, and initial Pb(II) concentration on the adsorption amount were investigated. After sorption, the immobilized microorganism carriers were filtrated off, and the Pb(II) concentrations in the filtrate were measured. The immobilization microorganism carriers were dried at 104 °C to obtain exact weight of carrier used. The removal ratio and adsorption amount of Pb(II) on the carrier were calculated as follows:

$$\text{Removal ratio (\%)} = 100 \times (C_0 - C)/C_0 \quad (1)$$

$$\text{Adsorption amount (mg/g): } Q = (C_0 - C)V/W \quad (2)$$

where C_0 is the Pb(II) concentration in solution before adsorption, C is the Pb(II) concentration in solution after adsorption, V is the volume of solution, and W is the net weight of the carrier. Negligible differences were found among the three Pb(II) concentration measurements under the same experimental conditions, resulting in less than 5% deviation from the average.

An experiment was also performed using blank carriers of an equal weight under the same conditions.

4. Determination of Pb(II) Concentration

The concentration of Pb(II) was determined colorimetrically according to the method based on rapid condensation with Phenanthraquinone monophenyl thiosemicarbazone at 520 nm in a Tween 80 micellar medium [22]. Before the determination of Pb(II), samples were filtered (0.22 μm) in a vacuum to ensure all biomass was removed.

5. COD Measurement

The COD was measured by the standard method based on potassium chromate ($K_2Cr_2O_7$) oxidization and sulfuric acid digestion.

6. Immobilized Biomass Concentration

Immobilized biomass concentrations were measured by weighing according to the method previously described [23].

RESULTS AND DISCUSSION

1. SEM Observation

Samples of PUF and IPUF were observed by using SEM to identify the surface microstructures and determine sorption mechanism. SEM images of PUF and IPUF in Fig. 1(a) show that the carrier structure consists of some large pores with diameter of 0.5 mm. The large pore structure is helpful to increase the amount of immobilized microorganism and improve the transfer of heavy metal ions. Therefore, many micro-organisms were found clinging to PUF (Fig. 1(c), 1(d)).

2. Effect of pH Value on the Removal Ratio of Pb(II)

Fig. 2 shows the effect of pH value on Pb(II) removal using PUF and IPUF as adsorbents. The removal ratio of Pb(II) using IPUF was much higher than those using PUF, which should be attributed to the biosorption of immobilized microorganisms. The optimum pH for Pb(II) uptake was 7.0 for both PUF and IPUF. When the pH value decreased, the complexation between Pb(II) and adsorbents was weakened, which lowered the adsorption amount. For IPUF, an additional reason was that the degree of amino protonation in the protein of immobilized microorganisms increased with the decrease of pH value, which would reduce the adsorption sites

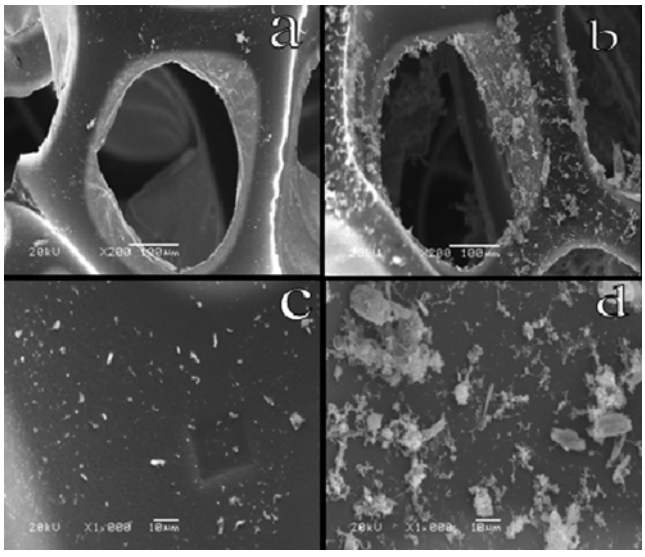

Fig. 1. SEM photos of PUF and IPUF ((a) PUF×200; (b) IPUF×200; (c) PUF×1,000; (d) IPUF×1,000).

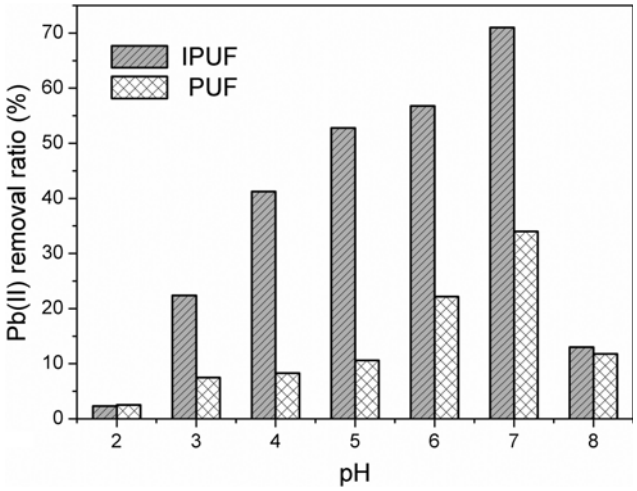

Fig. 2. Effect of pH value on the removal ratio of Pb(II) (initial Pb (II) concentration: 10 mg/L; solution volume: 50 mL; weight of carriers: 0.12 g; temperature: 25 °C; time: 8 h).

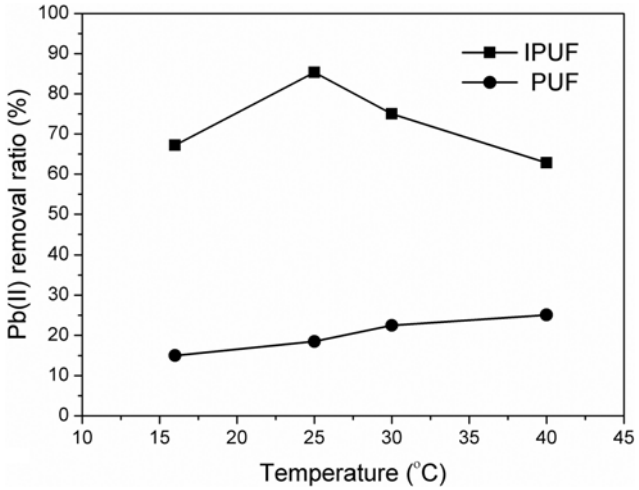

Fig. 4. Effect of temperature on the removal ratio of Pb(II) (initial Pb(II) concentration: 10 mg/L; solution volume: 50 mL; weight of carriers: 0.12 g; pH: 7.0; time: 8 h).

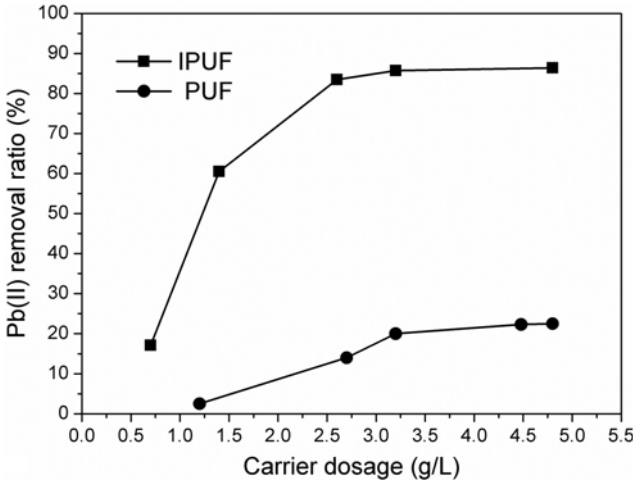

Fig. 3. Effect of carrier dosage on the removal ratio of Pb(II) (initial Pb(II) concentration: 10 mg/L; solution volume: 50 mL; temperature: 25 °C; pH: 7.0; time: 8 h).

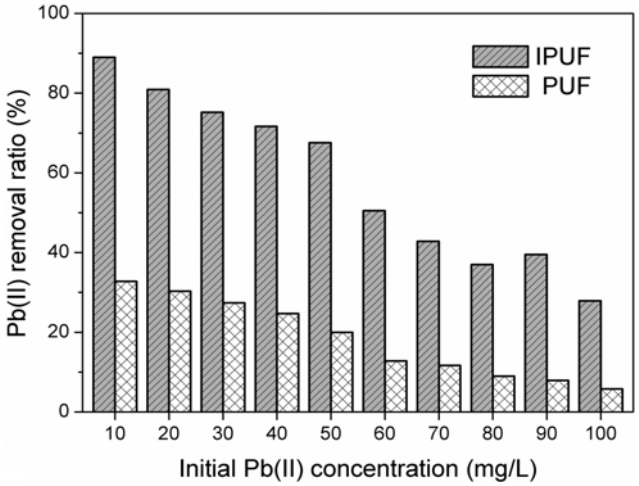

Fig. 5. Effect of initial Pb(II) concentration on the removal ratio of Pb(II) (Solution volume: 50 mL; weight of carriers: 0.12 g; pH: 7.0; temperature: 25 °C; time: 8 h).

for Pb(II). When the pH value was too high, the hydroxide generated from hydrolysis of lead ion would deposit on the surface of the microorganisms and influence the adsorption capacity of the immobilized microorganisms [24].

3. Effect of Carrier Dosage on the Removal Ratio of Pb(II)

Fig. 3 shows the effect of carrier dosage on Pb(II) removal. The Pb(II) removal ratio of IPUF increased from 16.74% to 86.74% as the carrier dosage was raised from 0.07 to 0.5 g/L due to an increase in the number of binding sites and surface area of the carrier [25]. The minimum dosage of IPUF required for the removal of Pb(II) is 0.24 g/L, at which the removal ratio of IPUF was 85%, whereas that of PUF was less than 20%.

4. Effect of Temperature on the Removal Ratio of Pb(II)

Temperature affected the physiological metabolism and adsorption thermodynamics of microorganisms, and thus influenced the adsorption amount of heavy metal ions [26]. The results in Fig. 4 show that 25 °C was the optimal temperature for the adsorption of Pb(II) on IPUF, and the removal ratio was 85%. Therefore, the removal of Pb(II) from wastewater using IPUF could be carried out at room temperature (around 25 °C). For PUF, the adsorption amount increased with the increase of temperature in the range of 15-40 °C; however the influence was insignificant.

5. Effect of Initial Pb(II) Concentration on Pb(II) Removal Ratio

The removal ratios of Pb(II) under different initial Pb(II) concentrations are shown in Fig. 5. For IPUF, when the initial concentration of Pb(II) increased from 8.0 to 100.0 mg/L, the removal ratio of Pb(II) decreased from 90% to 30%.

6. Adsorption Kinetics

Fig. 6 shows the effect of contact time on Pb(II) adsorption amount. Equilibrium was achieved within 2 h. Obviously, the Pb(II) adsorption capacity on IPUF was higher than that on PUF. For IPUF, the Pb(II) adsorption amount increased rapidly in the first 20 mins and remained nearly constant after 120 mins' adsorption, which was

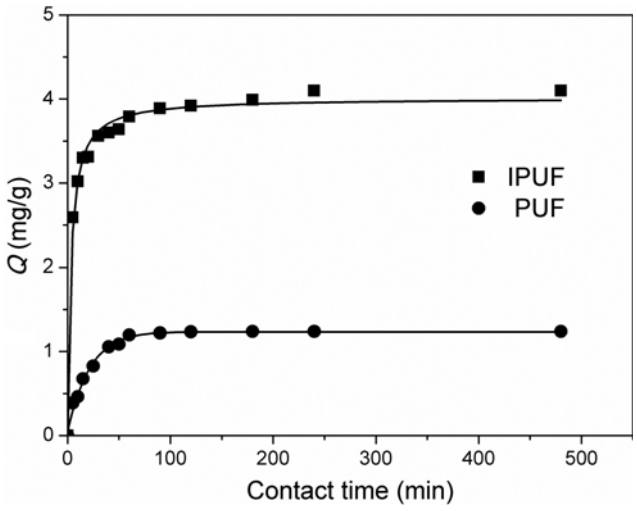

Fig. 6. Effect of contact time on the removal ratio of Pb(II) (initial Pb(II) concentration: 10 mg/L; solution volume: 50 mL; weight of carriers: 0.12 g; pH: 7.0; temperature: 25 °C).

attributed to biosorption, whereby metal ions were bound passively to the surface of the bacterial cell wall by physical/chemical processes. This process occurred rapidly, and subsequently the metal ions were slowly transferred into the interior of the cell by microbial energy systems normally associated with magnesium and potassium transport [27].

Furthermore, the Lagergren pseudo-first-order and the pseudo-second order kinetics models were used to elucidate the adsorption mechanism [28]. The linear forms of pseudo-first-order and pseudo-second-order equations are represented by Eqs. (3) and (4), respectively:

$$\log(q_{e_1} - q_t) = \log q_{e_1} - k_1/2.303 \quad (3)$$

$$t/q_t = 1/k_2 q_{e_2}^2 + t/q_{e_2} \quad (4)$$

where q_t (mg/g) is the adsorption amount at time t (min). In Eq. (3), q_{e_1} (mg/g) is the equilibrium adsorption capacity and k_1 (min^{-1}) is the rate constant of the pseudo-first-order adsorption, which can be obtained from the intercept and the slope of the linear plot of $\log(q_{e_1}-q_t)$ vs. t, respectively. In Eq. (4), q_{e_2} (mg/g) is the equilibrium adsorption capacity and k_2 (g/mg·min) is the rate constant, which can be determined experimentally by plotting t/q_t vs. t.

The fitting results are shown in Table 1. The high correlation coefficients (R^2>0.99) of the pseudo-second-order for adsorption kinetics on the PUF and IPUF prove that the adsorption process is pseudo-second-order adsorption. Moreover, the rate constant k_2 (0.032 g/mg·min) of IPUF was relatively higher than that of PUF (0.023 g/mg·min), suggesting that IPUF adsorbed Pb(II) ions faster than PUF.

7. Sorption Isotherms

Freundlich and Langmuir isotherms were selected [29] to fit the experimental data. The Langmuir isotherms model assumes that the adsorption energy is equal in the whole surface. There is no transference in the surface plane and no interaction between the adsorbed species. Additionally, only chemical interactions are considered. Thus, only a monolayer of the adsorbates can form on the surface of the adsorbents. The Freundlich isotherm model does not assume equivalence of energy among the adsorption centers. The Langmuir equation can be written as:

$$Q_e = \frac{Q_{max} K_L C_e}{1 + K_L C_e} \quad (5)$$

The Freundlich equation can be written as:

$$Q_e = K_F C_e^{1/n} \quad (6)$$

where C_e (mg/L) is the concentration of the Pb(II) solution at equilibrium, Q_e (mg/g) is the adsorption amount at equilibrium. In the Langmuir equation, Q_{max} (mg/g) is the maximum sorption capacity and K_L (L/mg) is the Langmuir constant. In the Freundlich equation, K and 1/n are empirical constants which can be obtained from the intercept and the slope of the linear plot of $\ln Q_e$ versus $\ln C_e$, respectively. The Freundlich and Langmuir adsorption constants are listed in Table 2. The regression correlation coefficients for adsorption on PUF and IPUF were satisfactory, and the experimental equilibrium data fitted both models, which was consistent with previous results [30]. By comparison of the maximum adsorption capacity (7.63 mg/g) of IPUF with that of PUF (2.74 mg/g) using the Langmuir model, the adsorption capacity of IPUF was relatively higher. Moreover, the value of K_L (0.235) for IPUF appears to be significantly higher

Table 1. Kinetics fitting results of the adsorption data

Carrier	Model	Parameter	R^2	Equation
PUF	Pseudo-frist-order	q_{e_1}=1.78, k_1=0.027	0.9498	$\log(1.78-q_t)=0.2495-0.0116t$
	Pseudo-second-order	q_{e_2}=2.60, k_2=0.023	0.9942	$t/q_t=6.365+0.384t$
IPUF	Pseudo-frist-order	q_{e_1}=3.14, k_1=0.014	0.8728	$\log(3.14-q_t)=0.4952-0.0045t$
	Pseudo-second-order	q_{e_2}=4.95, k_2=0.032	0.9968	$t/q_t=1.293+0.202t$

Table 2. Isotherm model constants for Pb(II) adsorption

Carrier	Model	Parameter	R^2	Equation
PUF	Langmuir	Q_{max}=2.74 mg/g, K_L=0.076	0.9748	$1/Q_e=0.3655+4.8265/C_e$
	Freundlich	n=3.033, K_F=2.251	0.9848	$Q_e=1.731C_e^{0.330}$
IPUF	Langmuir	Q_{max}=7.63 mg/g, K_L=0.235	0.9893	$1/Q_e=0.131+0.5566/C_e$
	Freundlich	n=3.258, K_F=1.731	0.9259	$Q_e=2.251C_e^{0.307}$

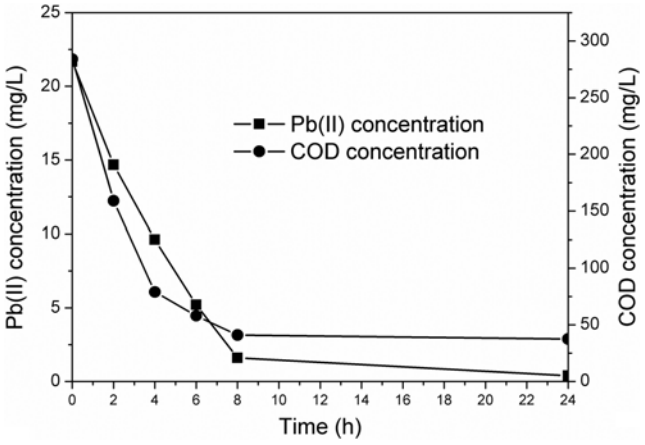

Fig. 7. Effect of contact time on the removal of Pb(II) and COD by IPUF (initial Pb(II) concentration: 20 mg/L; initial $C_6H_{12}O_6$ concentration: 280 mg/L; initial NH_4Cl concentration: 20 mg/L; weight of carriers: 3 g; pH: 6.0; temperature: 2 °C; dissolved oxygen=3.0-6.0 mg/L).

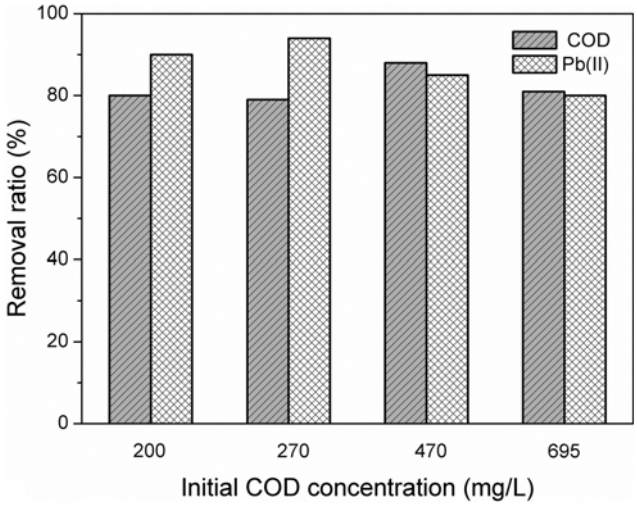

Fig. 8. Effect of initial COD on the removal of Pb(II) and COD by IPUF (initial Pb(II) concentration: 10 mg/L; initial NH_4Cl concentration: 20 mg/L; weight of carriers: 3 g; pH: 6.0; temperature: 2 °C; dissolved oxygen=3.0-6.0 mg/L; time: 8 h).

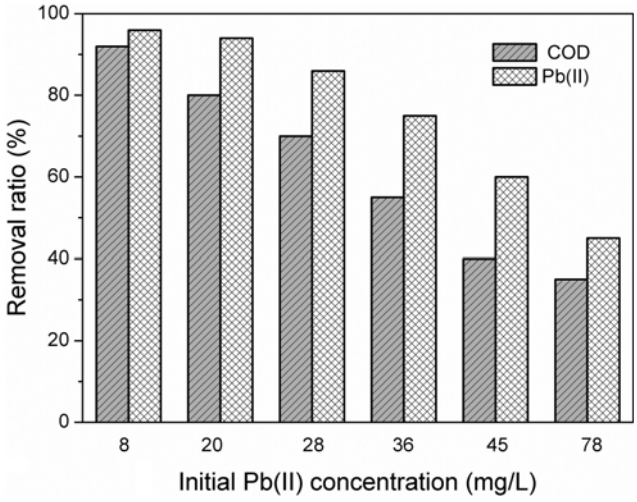

Fig. 9. Effect of initial Pb(II) concentration on removal of Pb(II) and COD by IPUF (Initial $C_6H_{12}O_6$ concentration: 280 mg/L; initial NH_4Cl concentration: 20 mg/L; weight of carriers: 3 g; pH: 6.0; temperature: 20 °C; dissolved oxygen=3.0-6.0 mg/L; time: 8 h).

than that for PUF (0.076), which also means IPUF adsorbed Pb(II) ions more rapidly than PUF [17].

8. Study of the Removal of Pb(II) and COD

It is expected that the immobilized microorganisms could metabolize by utilizing glucose as carbon source and the regenerated microorganisms could still adsorb Pb(II). Based on this hypothesis, the simultaneous removal of Pb(II) and COD was studied. The experiments were carried out using a small column (internal diameter=20 mm; length=60 mm) packed with 3 g of IPUF, containing approximately 348 mg (dry wt.) of biomass. Air was supplied at the bottom of the column.

Fig. 7 shows the change of Pb(II) and COD concentrations with time by batch-wise operation. Within the first 8 h, the concentrations of Pb(II) and COD decreased from 20.6 mg/L and 283.75 mg/L to 1.66 mg/L and 42.44 mg/L, respectively. The removal efficiencies of Pb(II) and COD were 92.0% and 85.0%, respectively. After 24 h, the concentration of Pb(II) further decreased to 0.33 mg/L, reaching the I-class criteria specified in Integrated Wastewater Discharge Standard (GB 8978-1996) of China. Meanwhile, the COD further decreased to 36.4 mg/L. Therefore, the immobilized microorganisms are able to endure Pb(II) ions and remove COD of wastewater simultaneously. The reasons are illustrated as follows [31,32]: (i) Pb(II) ions as a selective pressure on microbial growth can inhibit most random mutations of the microbial colony during immobilization of microorganisms and domestication process. Therefore, the profitable spontaneous mutation of the entire microorganism colony could be accumulated, and ultimately change the structure of a variety of cells so as to adapt to toxic heavy metal ions. (ii) The multiple porous structure of carrier provided the microbes with a tempered micro-environment and enhanced the anti-toxic ability of the microbes against Pb(II) ions. Moreover, the adsorption of the carrier's reactive group to Pb(II) ions alleviated the toxicity of Pb(II) ions to the microbes.

The effects of initial COD on the removal ratios of Pb(II) and COD are presented in Fig. 8. The initial COD concentration varied from 200 to 695 mg/L and the initial Pb(II) concentration was 10.0 mg/L, NH_4^+-N=20 mg/L. It can be seen that the proper COD concentration is 270 mg/L, at which the high removal ratios of COD and Pb(II) can be achieved. This would be attributed to the metabolism of the immobilized microorganisms. When COD concentration is 270 mg/L (COD/NH_4^+-N=13.5 : 1), it would be suitable for microorganisms to grow and reproduce and facilitate metal biosorption [33,34].

The effect of the initial Pb(II) concentration on the removal of Pb(II) and COD was investigated and the results shown in Fig. 9. The respective removal efficiencies of Pb(II) and COD were higher than 85% and 70% when the concentration of Pb(II) was lower than 30 mg/L. However, the removal ratio of COD obviously decreased

with the increase of the initial Pb(II) concentration, meaning that high Pb(II) concentration did influence the metabolism of the mobilized microorganisms and lower Pb(II) was preferred. For conventional activated sludge, Pb(II) concentration endured by microbe is 1.0 mg/L [35]. When being immobilized on PUF, the microbes could endure 30.0 mg/L of Pb(II). Therefore, having immobilized microorganisms on PUF provides a new way to remove COD and toxic heavy metal ions simultaneously.

CONCLUSIONS

IPUF can be used to remove Pb(II) ion from the aqueous solution effectively. More than 80% of Pb(II) in 20 mg/L Pb(II) solution could be removed when the dosage of IPUF was 2.4 g/L at pH 7.0 and 25 °C. IPUF has higher sorption capacity and speed than PUF. The adsorption kinetics was consistent with the pseudo-second-order adsorption model, and the adsorption isotherm could be described by Langmuir and Freundlich adsorption model. For synthetic wastewater containing Pb(II), the removal ratios of Pb(II) ion and COD were 92.0% and 84.2%, respectively, after 8 hours treatment by IPUF. The immobilized microorganisms could remove COD and Pb(II) simultaneously, and the method may be used for wastewater treatment in large volume with low concentration heavy metal ions.

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