Insight into the influences of pH value on Pb(II) removal by the biopolymer extracted from activated sludge

Yun Zhou [a,b,c], Siqing Xia [a], Jiao Zhang [d], Binh T. Nguyen [c], Zhiqiang Zhang [a,b,*]

[a] State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[b] Key Laboratory of Yangtze River Water Environment, Ministry of Education, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[c] Swette Center for Environmental Biotechnology, Arizona State University, Tempe, AZ 85287, USA
[d] School of Civil Engineering and Transportation, Shanghai Technical College of Urban Management, Shanghai 200432, China

HIGHLIGHTS

- pH influences on Pb²⁺ removal by the biopolymer in activated sludge were disclosed.
- Total removal rate of Pb²⁺ increased with rising system pH value.
- Biosorption was predominant for Pb²⁺ removal under acid conditions.
- Biosorption and Pb(OH)₂ precipitation co-existed under alkaline conditions.
- Pb(OH)₂ excelled biosorption in capturing Pb²⁺ under alkaline conditions.

GRAPHICAL ABSTRACT
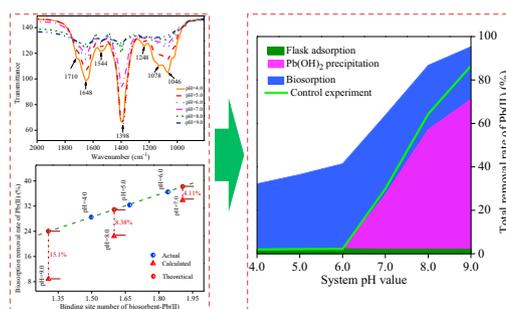
ABSTRACT

The influences of pH value on Pb(II) removal by the biopolymer extracted from activated sludge were investigated from various perspectives, including removal rate, functional groups, binding site number between the biopolymer and Pb(II), and removal distribution. With the system pH value rising from 4.0 to 9.0, the total removal rate of Pb(II) without and with adding the biopolymer increased from 1.94% to 86.5% and from 32.2% to 95.4%, respectively. From the analyses of Fourier transform infrared (FTIR) spectroscopy and two-dimensional correlation spectroscopy (2D-COS), the continuous dissociation and deprotonation of functional groups in the biopolymer with rising system pH value promoted the biosorption removal of Pb(II) via complexation and ion exchange. According to the three-dimensional excitation emission matrix (3D-EEM) fluorescence spectrum, two protein-like fluorescence peaks of A (Ex/Em = 280 nm/326–338 nm) and B (Ex/Em = 220–230 nm/324–338 nm) were identified in the biopolymer. The binding site numbers, which were obtained via fluorescence quenching titration experiments, increased first and then decreased with rising system pH value for both peaks A and B. Biosorption removal of Pb(II) was dominant under acid conditions, but biosorption and Pb(OH)₂ precipitation co-existed under neutral and alkaline conditions. The biosorption and the Pb(OH)₂ precipitation were interestingly found to interfere with each other under alkaline conditions. The decreases in both binding site number and biosorption removal rate indicated that Pb(OH)₂ precipitation excelled the biosorption in the competition of capturing Pb(II).

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* Corresponding author at: State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China.
   E-mail address: zhiqiang@tongji.edu.cn (Z. Zhang).

1. Introduction

Heavy metals are ubiquitous in the wastewater of China due to a large amount of environmental release, and it can bring high

hazard to both environment and human health [1]. Activated sludge system has been applied to treat wastewater over a hundred years, and shows good metal-sequestering property [2]. The biopolymer in activated sludge plays an important role in the removal of heavy metals, and it can also be extracted to serve as biosorbent for heavy metals [3]. The biopolymer in activated sludge is a complex of proteins, carbohydrates, nucleic acids, uronic acids, humic-like substances, lipids, and glycoproteins [4]. A number of factors can influence the removal of metal ions including biopolymer compositions [5], metal species and properties [6], as well as environmental conditions such as pH value, temperature, hydrodynamic status and solution ionic strength [7–10].

Among all the influencing factors, pH value can significantly affect the properties of both biopolymers and metal ions [11]. On the one hand, higher pH value can arouse dissociation of functional groups, deprotonation, and increasing negative charges of biopolymers, which can substantially enhance electrostatic attraction with metal ions [12]. Ozdemir et al. [13] mentioned that pH value affected the ionization state of the functional groups such as carboxylate, phosphate and amino groups of the cell wall and extracellular polymeric substances (EPS), all of which could become strong metal scavengers. Comte et al. [14] revealed that the number of EPS binding sites increased with rising pH value. On the other hand, pH value can also affect the solubility and speciation of metal ions. Pardo et al. [15] mentioned that alkaline conditions decreased the electronic charges and solubility of metal ions by forming hydroxylated complex. Comte et al. [14] also found that when the pH value was 4.0, the metallic species of Cu(II) and Pb(II) would exist as soluble divalent cations, but were transformed into insoluble hydroxylates at the pH value of 8.0. The hydroxylated complexes could not only enhance the removal of metal ions by surface precipitation, but also could compete with the active binding sites in biopolymer and decrease the adsorption capacity [3]. Gupta et al. [15] mentioned that positively charged Pb(II) species were dominant in case of low pH value (<6). In the case of higher pH values (pH 7–11), there were several Pb species with different charges, which included $Pb(OH)^+$ and $Pb(OH)_2$. To conclude, pH value significantly affects not only the dissociation and deprotonation of biopolymer, but also the speciation and solubility of metal ions. There is no doubt that these effects will be further transferred to influencing the removal of metal ions in activated sludge systems. However, the variations of adsorption mechanisms under acid and alkaline conditions were usually ignored in many researches about metal ions removed by adsorbents. Thus, it was difficult to theoretically explain the phenomenon that the removal performance markedly varied with pH value. To solve this problem, it is necessary to further investigate the different removal mechanisms of metal ions under acid and alkaline conditions.

In this work, the influences of system pH value on metal ions (Pb(II) as example) removal by the biopolymer extracted activated sludge were investigated via analyzing the changes of removal rate, functional groups, binding site number and removal distribution at different system pH values. The changes of Pb(II) removal rate at different system pH values were analyzed through the Pb(II) removal from the aqueous solution. The functional groups changes of the biopolymer at different system pH values were identified via Fourier transform infrared (FTIR) spectroscopy and two-dimensional correlation spectroscopy (2D-COS). The binding site number between the biopolymer and Pb(II) at different system pH values were analyzed according to the results of three-dimensional excitation emission matrix (3D-EEM) fluorescence spectroscopy of the fluorescence quenching titration process. The removal distribution of Pb(II) at different system pH values were analyzed via modelling linear relationships between the adsorption removal rate and binding site number between Pb(II) and the biopolymer.

2. Materials and methods

2.1. Biopolymer and reagents

Activated sludge samples were collected from the secondary settling tank back-flow sludge from a full-scale municipal WWTP in Shanghai, China. The main parameters of the sludge after gravity concentration were as following: pH 6.8–7.5, suspended solids (SS) 9.0 ± 1 g/L, and the ratio of volatile suspended solids to suspended solids (VSS/SS) $65 \pm 8\%$. The detailed extraction process of the biopolymer in activated sludge can be referred to our former studies [16,17]. The biopolymer consisted of protein (54.76%, w/w), polysaccharide (30.43%, w/w), and nucleic acid (14.81%, w/w).

All the used chemical agents, which were of analytical grades, were obtained from Runjie Chemistry Reagents Co. Ltd. (Shanghai, China). The stock solution of Pb(II) with an initial concentration of 1000 mg/L was prepared by dissolving AR grade of $Pb(NO_3)_2$ into distilled water.

2.2. Removing experiments of Pb(II) with and without biopolymer

Experiments were carried out by adding the biopolymer into 200-mL Erlenmeyer flasks containing 50 mL of $Pb(NO_3)_2$ solutions with 30 mg/L of metal ions. After adding the biopolymer with the biopolymer/Pb(II) weight ratio of 2.5/1, the pH values of the mixture system were adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 using 0.1 M NaOH and 0.1 M HNO_3, respectively. During the adjusting process, the solution pH values always varied. Adding acid or alkali was not stopped until the pH value basically kept stable. The flasks were sealed to prevent the change in volume of the solutions during the experiments, and shaken for 30 min at 35 °C in an isothermal shaker. Then the samples were taken out with syringes. After separating the removed Pb(II) from the samples via centrifugation at $10,800 \times g$ for 10 min, the residual Pb(II) concentrations in the supernatants were analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES, Agilent 720ES, USA). The control experiments without adding the biopolymer to $Pb(NO_3)_2$ solutions were simultaneously conducted under the same conditions. The removal rate of metal ions by the biopolymer was calculated using Eq. (1).

$$R_e = \left[\frac{(C_0 - C_e)}{C_0} \right] \times 100\% \tag{1}$$

where R_e is the equilibrium removal rate and adsorption quantity of metal ions, respectively; C_0 (mg/L) and C_e (mg/L) are the liquid phase concentrations of the metal ions at time 0 (min) and equilibrium status, respectively.

2.3. FTIR spectroscopy and 2D-COS analyses

The FTIR spectroscopy results of the biopolymer before and after adsorbing Pb(II) (Fig. S1) showed that the functional groups responsible for binding Pb(II) contained hydroxyl, amino, carboxyl and amide groups. To determine complexation and proton dissociation of the functional groups, the FTIR spectra of the biopolymer at different system pH values were analyzed with a Nicolet 5700 FTIR (Thermo Electron Co., USA). The pH values of the biopolymer were adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 using 0.1 M HCl or NaOH solution, and we stop added the acid or alkali when the solution pH value basically keep stable as the deviation of pH value is less than 0.1 within 5 min. Each sample was lyophilized and then mixed with dried KBr (FTIR grade, Aldrich) in the ratio of 1:100. The mixtures were then dried by heating and they were kept under vacuum in a desiccator prior to use. The pellets of 12 mm diameter were prepared by using 200 mg of mixture at 7×10^3 kg/cm² pres-

sure for 90 s. The control experiment was conducted using a clean KBr pellet. The samples were scanned between the region of 4000 and 400 cm^{-1} at a resolution of 5 cm^{-1} with 16 scans, and the FTIR spectra between the region of 2000 and 800 cm^{-1} were extracted to reflect the changes of the major functional groups in the biopolymer [18].

In order to enhance the FTIR spectrum resolution and elucidate the changes in chemical structures at different system pH values, the 2D-COS was applied in this study and the detailed steps were previously described by Noda et al. [19]. The obtained FTIR spectra were converted into absorbance for the 2D-COS analysis. The reconstructed data matrix was then progressed using the "2D shige" software released by Kwansei-Gakuin University, Japan.

2.4. Binding site number analysis of biopolymer-Pb(II) complex

3D-EEM fluorescence spectroscopy can be applied as a reliable technique to understand the biopolymer features [20–23]. According to the 3D-EEM fluorescence spectrum (Fig. S2), peak A (Ex/Em = 280 nm/326–338 nm) and peak B (Ex/Em = 220–230 nm/324–338 nm) were identified in the biopolymer. Both peaks A and B could be assigned to protein-like fluorescence [24,25].

In order to obtain the binding site number between the biopolymer and Pb(II) at different system pH values, the fluorescence quenching titration experiments of the biopolymer by Pb(II) were carried out. The fluorescence intensities of peak A and peak B at different values (4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) were shown in Fig. S3. Based on the data obtained from the fluorescence quenching titration experiments, the binding site number between biopolymer and Pb(II) could be calculated from the following modified Stern-Volmer Eq. (2) [26]:

$$\lg\left[\frac{F_0 - F}{F}\right] = \lg K_b + n\lg[\text{Pb(II)}] \qquad (2)$$

where F_0 and F are the fluorescence intensities of fluorophore in the absence and presence of Pb(II), respectively; K_b is the binding constant, and n is the binding site number. The values of n and K_b were obtained from the slope and intercept of the modified Stern-Volmer plot, respectively.

3. Results and discussion

3.1. Influence of system pH value on the removal rate of Pb(II) by the biopolymer

Fig. 1 shows the total removal rate of Pb(II) without and with adding the biopolymer at the noted system pH values. Without adding the biopolymer, the total removal rate of Pb(II) was very low as the initial system pH value lower than 6.0. It should be refer to the Erlenmeyer flask adsorption resulted from its silicon-oxygen group which has good adsorption ability for heavy metals [27]. It was reasonable that we could consider the stable removal rate of Pb(II) by the Erlenmeyer flask at different system pH values. Then, the total removal rate dramatically increased from 2.08% to 86.5% with the pH value rising from 6.0 to 9.0, and Pb(OH)$_2$ precipitates were observed at the pH values higher than 6.0. After adding the biopolymer, the total removal rate of Pb(II) rapidly increased to 32.2% at pH 4.0, and then further increased to 41.5% at pH 6.0. As the pH value was higher than 6.0, the total removal rate dramatically increased and reached to 95.36% at pH 9.0. The results confirmed that the biopolymer should be responsible for the removal of heavy metals in activated sludge system [28].

The removal of Pb(II) (R_e) by the biopolymer at different system pH values should be refer to biosorption (R_B) and non-biosorption
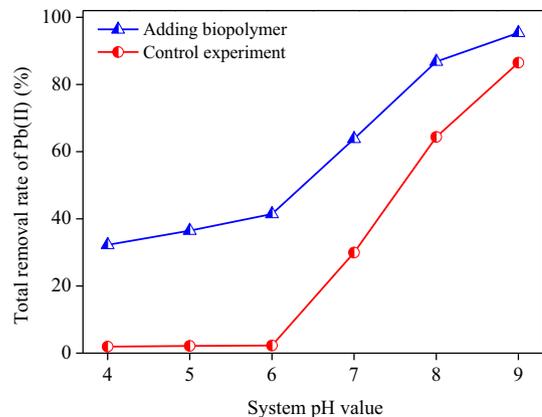

Fig. 1. Influence of system pH value on the Pb(II) removal rate without and with adding the biopolymer at 35 °C with initial $C_{\text{Pb(II)}}$ = 30 mg/L and initial $C_{\text{Biopolymer}}$ = 75 mg/L.

(R_N). Under acid conditions, the non-biosorption should be Erlenmeyer flask adsorption (R_E) as no Pb(OH)$_2$ precipitate formed. But under alkaline conditions, the non-biosorption might contain both Erlenmeyer flasks adsorption (R_E) and Pb(OH)$_2$ precipitation (R_P). Their relationship can be expressed as Eq. (3).

$$R_e = R_B + R_N = R_B + R_E + R_P \qquad (3)$$

Since the Erlenmeyer flask adsorption was very low and almost constant, the biosorption should be the predominant in the removal of Pb(II) as the system pH value lower than 6.0. The precipitates formed as the pH value higher than 6.0, and the increase of the total removal rate of Pb(II) could be mainly attributed to both biosorption and precipitation. However, their interactions and respective contributions in the removal of Pb(II) under alkaline conditions need to be further investigated.

3.2. Influence of system pH value on the major functional groups in the biopolymer

Analyzing the influence of system pH value on the functional groups in biopolymer is helpful to qualitatively understand the contribution change of the removal by the biosorption. The influence of pH value on the major functional groups (hydroxyl, amino, carboxyl and amide) in the biopolymer was investigated at different system pH values. As shown in Fig. 2, a small peak at 1710 cm^{-1} was observed in the biopolymer at pH 4.0, which corresponded to C=O stretching of protonated carboxyl groups [18]. The
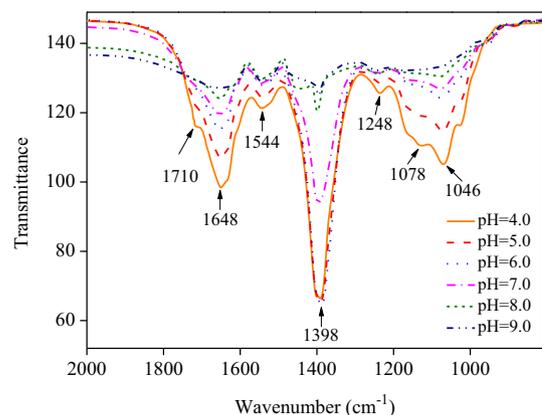

Fig. 2. Influence of system pH value on the functional groups of the biopolymer.

peak at 1648 cm^{-1} (vibrations of C=O in amide groups) considerably decreased with rising pH value. The peak intensity at 1398 cm^{-1} kept unchanged when pH value increased from 4.0 to 6.0, because O—H and N—H could not dissociate hydrogen ions under acid conditions [29]. However, they considerably decreased under alkaline conditions. The peaks disappeared or decrease with rising pH value due to the continuous dissociation of carboxyl and amide groups [18]. In addition, the peak intensities at 1076 and 1046 cm^{-1} decreased with rising pH value because of the structural destruction of polysaccharides and/or nucleic acids [18]. As shown in Fig. S1, the adsorption mechanisms of Pb(II) by the biopolymer in activated sludge should be complexation and ion-exchange between functional groups and metal ions.

To further elucidate the changes in functional groups of the biopolymer at different system pH values, 2D-COS was applied to enhance the FTIR spectroscopy resolution. Fig. 3(a) and (b) show the 2D FTIR correlation synchronous and asynchronous maps of the biopolymer at different system pH values, respectively. The synchronous map of 800–2000 cm^{-1} region (Fig. 3(a)) contains one *autopeak* at 1395 cm^{-1} and three negative *crosspeaks* at (995, 1395) cm^{-1}, (850, 1395) cm^{-1}, and (1100, 1395) cm^{-1}. The higher intensity change at 1395 cm^{-1} showed that amide II in proteins was more sensitive than O—H in polysaccharides to the pH variation [30]. Meanwhile, the asynchronous map of 800–2000 cm^{-1} region (Fig. 3(b)) displays two positive *crosspeaks* at (1395, 1640) cm^{-1} and (1370, 1400) cm^{-1}, and two negative *crosspeaks* at (1075, 1395) cm^{-1} and (995, 1645) cm^{-1}. Based on the Noda's rule [30], amide II in proteins decreased more quickly with rising pH value than O—H in polysaccharides.

3.3. Influence of system pH value on the binding site number between the biopolymer and Pb(II)

Ascertaining the influence of system pH value on the binding site number between the biopolymer and Pb(II) can quantificationally disclose the contribution change of the removal of the biosorption. Based on the results of fluorescence quenching titration experiments, the modified Stern-Volmer plots are shown in Fig. 4. The values of binding constant (K_b), binding site number (n) and correlation coefficients at different system pH values were listed in Table 1.

The values of K_b for both peaks A and B decreased with rising pH value, meaning that the biopolymer-Pb(II) complex was unstable under alkaline conditions. Pardon et al. [12] reported that alkaline conditions decreased the electronic charge and solubility of metals by forming hydroxylated complex. The metallic species of Pb exists as soluble divalent cations at pH 4.0, but they will be transformed into insoluble hydroxylates as the pH value higher than 6.0 [14]. Therefore, the biopolymer-Pb(II) complex was unstable at higher pH value due to the decrease of the electronic charge. In addition, the higher K_b value for peak B at different system pH values indicated that the binding between Pb(II) and fluorophore in peak B was more stable than that of peak A.

The binding site number n between biopolymer and Pb(II) for both peaks A and B increased first and then decreased with rising system pH value. The binding site number was close to 1.0 for peak A, but higher than 1.0 for peak B, indicating that only one class of
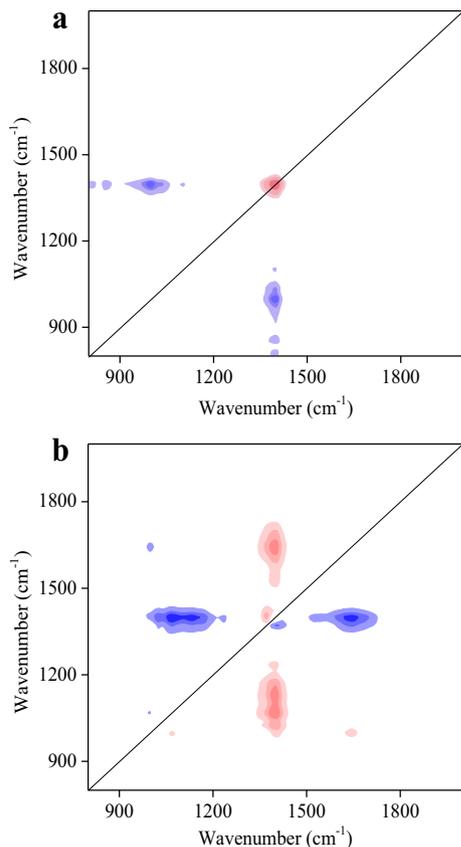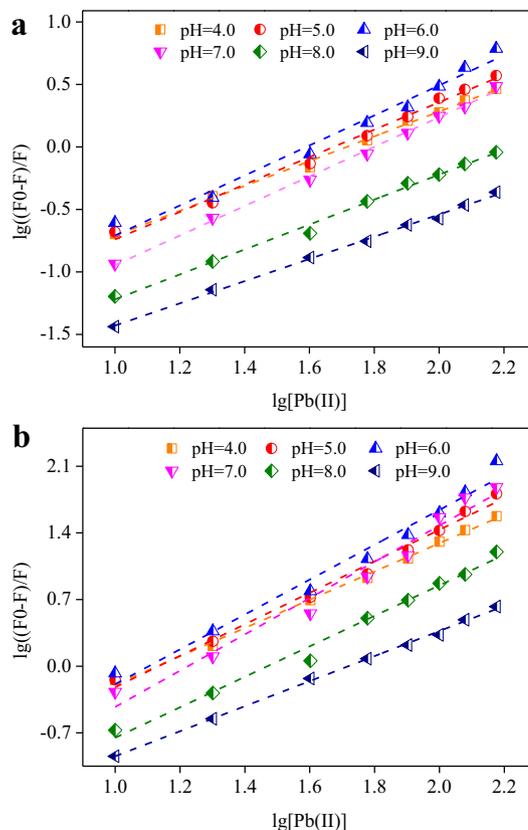

Fig. 3. pH-dependent 2D FTIR correlation (a) synchronous maps and (b) asynchronous maps generated from 800 to 2000 cm^{-1} region of the biopolymer. Red represents positive correlations and blue represents negative correlations; higher color intensity indicates a stronger positive or negative correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. Modified Hill plots of (a) peak A and (b) peak B for the quenching of the biopolymer titrated with increasing Pb(II) concentration at different system pH values.

Table 1
The binding constant (K_b) and binding site number n for biopolymer-Pb(II) complex.

	System pH value	K_b ($\times 10^{-3}$)	n	R^2
Peak A	4.0	19.95	0.992	0.9967
	5.0	14.61	1.09	0.9926
	6.0	12.31	1.20	0.9808
	7.0	7.42	1.18	0.9974
	8.0	6.06	1.00	0.9942
	9.0	4.81	0.889	0.9973
Peak B	4.0	21.08	1.48	0.9972
	5.0	17.84	1.65	0.9899
	6.0	13.38	1.83	0.9781
	7.0	9.68	1.91	0.9732
	8.0	5.67	1.60	0.9879
	9.0	4.29	1.32	0.9983

binding sites was present in peak A and more than one class of binding sites in the fluorophore of peak B for the binding of Pb (II). As the increasing of system pH value from 4.0 to 6.0, the binding site number increased from 0.992 to 1.20 and 1.48 to 1.83 for peaks A and B, respectively, and even reached 1.91 for peak B at pH 7.0, suggesting that the increasing of system pH value in the acid range could increase the binding site number in biopolymer, resulted in the improving of the removal rate of Pb(II). However, as the system pH value increased to 9.0, the binding site number for peaks A and B decreased to 0.889 and 1.32, respectively. It indicated that alkaline conditions caused the decrease of binding site number between the biopolymer and Pb(II).

3.4. Influence of system pH value on the removal distribution of Pb(II) by the biopolymer

When the system pH value was lower than 6.0, no precipitate was observed and the metallic species of Pb should exist as soluble divalent cations [14]. Thus, the removal of Pb(II) should be biosorption by the biopolymer and adsorption by Erlenmeyer flask ($R_B = R_e - R_E$). As shown in Fig. 5, the biosorption removal rate correlated well with the binding site number of biopolymer-Pb(II) complex for both peaks A ($R_{B-A} = 29.40\,n_A$, $R^2 = 0.9995$, $P = 0.00$) and B ($R_{B-B} = 19.46n_B$, $R^2 = 0.9996$, $P = 0.00$), which further confirmed that the biosorption removal of Pb(II) should be the interaction result between Pb(II) and the binding sites in the biopolymer. However, Pb(OH)$_2$ precipitates were observed at system pH value over 6.0 during the titration process. Pb(OH)$_2$ precipitation impaired the biosorption. Which resulted in the decrease of binding site number for both peaks A and B. It was unclear whether the biosorption affected Pb(OH)$_2$ precipitation.

In order to further investigate the interactions and respective contributions of biosorption and precipitation in the removal of Pb(II) under alkaline conditions, we assumed that biosorption would not affect the formation of Pb(OH)$_2$ precipitates under alkaline conditions, and $R_B = R_e - R_E - R_P$. So the calculated biosorption removal rates of Pb(II) at pH 7.0, 8.0 and 9.0 should be 33.9%, 22.5% and 8.84%, respectively. However, based on the binding site number and its relationship with the biosorption removal rate, the theoretical values of the biosorption removal rate at pH 7.0, 8.0 and 9.0 should be 35.3%, 28.7% and 24.5% for peak A, and 38.0%, 30.9% and 23.9% for peak B, respectively (Fig. 5), which were higher than the calculated values for both peaks A and B based on the assumption. This suggested that the hypothesis was inaccurate. The biosorption indeed impaired the formation of Pb(OH)$_2$ precipitates under alkaline conditions, and high system pH value could enhance the impairment.

As the theoretical values of the biosorption removal rate were similar for peak A and B ($R_{B-A} \approx R_{B-B}$), we used the mean value of
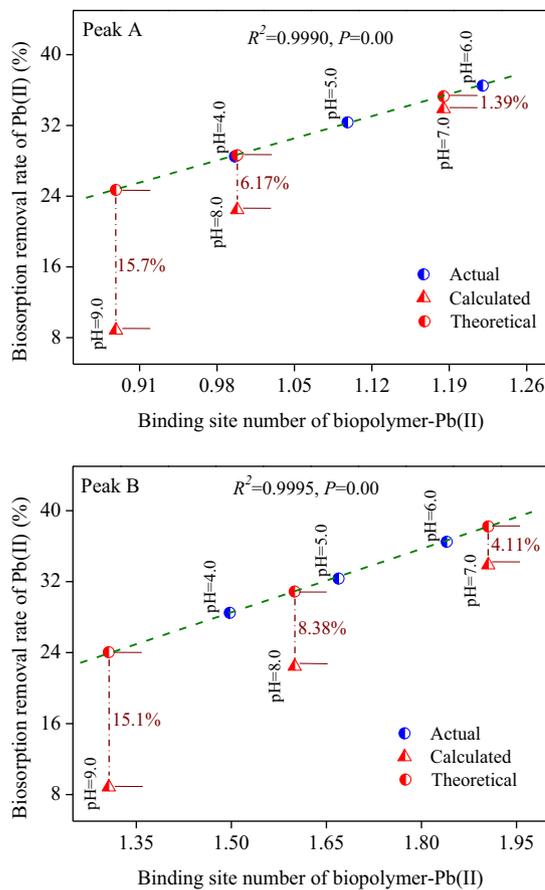

Fig. 5. Linear relationships between the biosorption removal rate and binding site number between Pb(II) and the biopolymer for peak A and B. "Actual" means the biosorption removal rate of Pb(II) under acid conditions as no Pb(OH)$_2$ precipitate formed; "Calculated" means the biosorption removal rate of Pb(II) based on the hypothesis that biosorption would not affect the formation of Pb(OH)$_2$ precipitates under alkaline conditions; "Theoretical" means the biosorption removal rate based on the binding site number and its relationship with the biosorption removal rate under acid conditions.

the biosorption removal rates for peaks A and B as the biosorption removal rate of Pb(II) under alkaline conditions (Eq.(4)).

$$R_B = (R_{B-A} + R_{B-B})/2 \tag{4}$$

As the removal rates of Pb(II) by the Erlenmeyer flask at different system pH values almost kept stable, we could calculate the removal rates of Pb(II) by Pb(OH)$_2$ precipitation using Eq. (5).

$$R_P = R_e - R_B - R_N \tag{5}$$

Fig. 6 shows the respective and total contributions of flask adsorption, biosorption and precipitation in the removal of Pb(II) at different system pH values. Therefore, biosorption should be predominant for Pb(II) removal under acid conditions, while biosorption and Pb(OH)$_2$ precipitation co-existed under neutral and alkaline conditions.

3.5. Influences of system pH value on removal mechanisms of Pb(II) by the biopolymer

Under acid conditions, biosorption removal of Pb(II) was dominant, and the biosorption removal rate increased with rising system pH value. Increasing the system pH value was found to promote the dissociation and deprotonation of functional groups
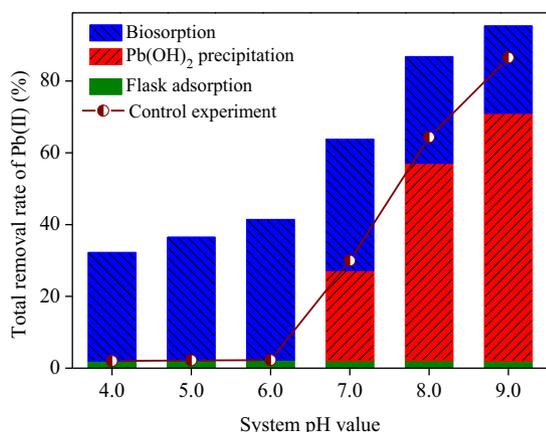
Fig. 6. Removal distributions of Pb(II) via flask adsorption, biosorption and Pb(OH)$_2$ precipitation at different various system pH values and 35 °C with initial $C_{Pb(II)}$ = 30 mg/L and initial $C_{Biopolymer}$ = 75 mg/L; and the removal rates of Pb(II) without adding the biopolymer at different system pH values at 35 °C.

in the biopolymer, which could improve the biosorption removal of Pb(II) via complexation and ion exchange [18].

Under neutral and alkaline conditions, biosorption and Pb(OH)$_2$ precipitation co-existed in the system, and the total removal rate also increased with rising system pH value [3]. However, the biosorption and the Pb(OH)$_2$ precipitation were interestingly found to interfere with each other under alkaline conditions. The decreases in both binding site number and biosorption removal rate indicated that Pb(OH)$_2$ precipitation excelled the biosorption in the competition of capturing Pb(II).

The significance of this study lies in the following two points. On the one hand, the results of this study would help to deepen our understanding in the removal of metal ions by activated sludge system under different pH conditions. On the other hand, the results of this study would provide important theoretical support for the biopolymer extracted as biosorbent for heavy metals in industrial wastewater.

4. Conclusions

The total removal rate of Pb(II) after the addition of the biopolymer increased with system pH value rising from 4.0 to 9.0. The continuous dissociation and deprotonation of functional groups in the biopolymer with rising system pH value could promote the biosorption removal of Pb(II) via complexation and ion exchange. The binding site numbers between the biopolymer and Pb(II) increased first and then decreased with rising system pH value for both fluorescence peaks A and B. Biosorption removal of Pb(II) was dominant under acid conditions, but biosorption and Pb(OH)$_2$ precipitation co-existed under neutral and alkaline conditions. Under alkaline conditions, the biosorption and the Pb(OH)$_2$ precipitation interfered with each other, but Pb(OH)$_2$ precipitation excelled the biosorption in the competition of capturing Pb(II).

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2016.09.141.

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