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Efficient removal of Pb(II) ions from aqueous solution by novel PVA-sodium alginate immobilized sulfate reducing bacteria

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ABSTRACT

In this study, a novel complex of polyvinyl alcohol (PVA) and sodium alginate impregnated with sulfate-reducing bacteria (SRB) was successfully synthesized and this versatile bio-composite was adopted as a highly efficient biological remover to eliminate toxic Pb(II) in aqueous solution. The immobilized SRB beads were characterized by scanning electronic microscopy-energy dispersive X-ray (EDX), X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FT-IR). The surface of immobilized SRB beads were altered markedly and become much smoother after Pb(II) removal. Both EDX and XPS results confirmed that some Pb(II) accumulated on the surface of immobilized SRB beads. For a reaction to become a feasible treatment technique, the influences of pH, reaction temperature and initial sorbate concentration on the removal rate of Pb(II) were thoroughly investigated. The sorption results elucidated that the solution pH played an important role for the removal of Pb(II) ions. The Pb(II) sorption increased as the initial Pb(II) ion concentration increased and the optimal temperature for the removal of Pb(II) is 35°C. The greatest removal efficiency of Pb(II) was observed at 35°C and pH 8.0 with 50 $mg \cdot L^{-1}$ of Pb(II) in the reactive system. The bio-removal kinetics of Pb(II) by immobilized SRB beads were described well by Lagergren pseudo-second order and Langmuir model, as good kinetic fitting results and isotherms analyses achieved. Maximum adsorption capacity was determined to be 707.3 $mg \cdot g^{-1}$ under optimal conditions. The sorption results of PVA-sodium alginate immobilized SRB was compared with other forms of different adsorbents. The membrane diffusion phase is a rate-control step. FT-IR and XPS analyses implied that biosorption is the main mechanism for removal of Pb. Our findings demonstrated that the immobilized SRB beads had great potentials for the remediation of Pb(II) contaminated wastewater stream.

Keywords: Sulfate-reducing bacteria; Immobilization; Pb(II); Bio-removal; Microorganism

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1. Introduction

The rapid development of industries stimulates the release of heavy metals into the environment, whether directly or indirectly, leading to severe environmental problems in many parts of the world. Heavy metals are toxic to human due to their bioaccumulation and chronic toxicological effects [1]. Heavy metals can be accumulated in planktonic and benthic fish prey items via food chains, posing risks to human because of the consumption of fish. Most of heavy metals (e.g., mercury, cadmium, lead, etc.) are extremely toxic to living organisms even at considerably low concentrations [2,3]. Of them, lead (Pb) is widely used in many fields of industry, such as metallurgy, battery manufacturing, metal finishing and chemical industry, etc. [4]. World Health Organization (WHO) has reported that the deaths caused by lead exposure were estimated to be 143,000 in developing regions in 2015. Various techniques, including ion exchange, membrane filtration, chemical precipitation, co-precipitation and absorption, have been developed for the treatment of Pb(II) containing effluents [5–7]. During a chemical precipitation process, metal-rich sludge will be inevitably generated, and such sludge is generally hazardous to human, thus proper treatment is regulatory required. As to ion-exchange method, it is often restricted in practical use due to the slow sorption kinetics, high operation cost, poor regeneration and reusability, and relatively low thermal and chemical stability [8]. With regard to membrane filtration, it is an energy intensive process because of the need of membrane restoration [9]. Adsorption, utilizing the nature of various adsorbents, is well recognized as an effective alternative for the removal of heavy metals from effluents [10–13].

However, the high cost of adsorbents strongly restrained the practical use of adsorption technique in a large scale [14]. Of note, biosorption has been considered as a promising approach to effectively treat heavy metal contaminated wastewater due to its low costs, environmentally-benign and less secondary pollution [15]. Biological materials commonly include bacteria, fungi, yeast, algae, industrial wastes, agricultural wastes, etc. [15]. Using biosorption process, the concentrations of heavy metal can be reduced to a low concentration, from ppm to ppb level [16].

Sulfate-reducing bacteria (SRB), as a group of microorganisms characterized for its morphologically diverse and anaerobic, have attracted great attentions because of its exceptional biological reduction activity and excellent removal capability of heavy metals [17]. SRB enables to utilize sulfate as terminal electron acceptor to generate hydrogen sulfide, thus Pb(II) can be precipitated in the form of PbS of low solubility. SRB may provide an excellent choice for removing toxic Pb from aqueous phase. Nevertheless, conventional biosorption methods are often with sorbents suspending in the solution and thus the sorbents are difficult to be recovered [18].

Biological immobilization approach is an alternative technique since it shows more stability, higher reactivity and greater processing efficiency [19]. Biological immobilization refers to enzymes or free microbial cells being defined or retained in particular sites by chemical or physical methods. Biological immobilization material has been widely applied in many fields, especially in commercial bioreactor fermentation, compounds production through immobilized organelles and cells, and heavy metals removal by immobilization technology [20]. Furthermore, biological immobilization approaches involved with gel-like medium which retains the SRB in a particular space, have attracted more and more attention for their high efficiency, strong reactivity and stability. In this sense, theoretically, Pb toxicity in the aqueous solution could be reduced, and hence improving the Pb(II) removal capacity. Therefore, the application of immobilized SRB in Pb(II) removal is of great interest.

In this work, immobilized SRB beads were fabricated and employed to eliminate Pb(II) from mimic Pb(II) contaminated wastewater. The immobilized SRB beads before and after Pb(II) adsorption were characterized by scanning electronic microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR) and X-ray photoelectron spectroscopy (XPS). The effects of pH, temperature and initial sorbate concentration in aqueous solution on the removal rate of Pb(II) were thoroughly investigated. The kinetics of Pb(II) removal in immobilized SRB beads was discussed in detail as well.

2. Experimental setup

2.1. Materials

The SRB strain used in this study was prepared as described previously [21,22]. The SRB strain had been successfully identified to be *Citrobacter freundii* and preserved in Guangdong Microbial Culture Collection Center (Guangzhou, China) with the accession number of GDMCC1.1031. Analytical grade chemicals and reagents were used in all the experiments unless otherwise specified. 1.0 L of culture medium of bacteria was made of 2.0 g magnesium sulfate ($MgSO_4$), 0.5 g sodium sulfate (Na_2SO_4), 1.5 g potassium dihydrogen phosphate (KH_2PO_4), 0.1 g ammonium chloride (NH_4Cl), 0.5 g cysteine hydrochloride, 1.0 g yeast extract, 0.1 g calcium chloride ($CaCl_2$), 0.3 g sodium citrate ($Na_3C_6H_5O_7$), 0.1 g ascorbic acid ($C_6H_8O_6$) and 2.0 g sodium lactate ($C_3H_5NaO_3$). All inorganic chemicals used in this study unless otherwise are analytical grade (AR) without further purification. The mimic Pb(II) contaminated wastewater was prepared by serial dilution of 1,000 ppm $Pb(NO_3)_2$ filter-sterilized solution (AR Grade, Guangdong Analysis and Testing Center, China). The separation of solid from aqueous solution using microporous membrane filters (0.45 μm, Laboratory Equipment Co., Ltd.). Polyvinyl alcohol (PVA), with 99.4%–99.8% saponification, was purchased from Damao Chemical Reagent Factory (China), and other chemicals used in this study were from Zhiyuan Chemical Reagent Factory (China). Deionized (DI) water was used throughout this study.

2.2. Preparation of immobilized SRB beads

The immobilized SRB beads were prepared according to a previously reported method (Fig. S1) [23]. In brief, 6% (w/v) PVA solution, 3% (w/v) silica solution (Damao Chemical, China), and 0.2% (w/v) sodium alginate solution (Damao Chemical, China) were well mixed. The mixture was kept boiling until a colloidal state was formed. The as-received SRB diluted solution in 35% (w/v) was added rapidly into the colloid stock solution. The immobilized bacteria used were described elsewhere [22], and immobilized beads were prepared by an entrapping method [23]. Immobilization of biomass via entrapment was carried out as following: first, the polyvinyl alcohol solution (6%, w/v), silica solution (3%, w/v) and sodium alginate solution (0.5%, w/v) were dissolved in deionized water and mixed thoroughly. Second, activated carbon solution (1%, w/v) and SRB diluted solution (35%, w/v) were added into the colloid and mixed evenly at 40°C.

Sequentially, the mixture was thoroughly blended at 40°C. Then the well-blended suspension was filtered with 0.45 μm pore size filters and dropped into the saturated boric acid solution (100 mL) with 1% (w/v) $CaCl_2$. The size of the bead used in this study is Φ2 mm. Each gram of beads is expected to be loaded with 0.15 mg of as-received SRB. The beads impregnated with SRB were washed for several times using sterile DI water and placed in a flask of anaerobic condition by replacing the head space with nitrogen gas. The as-prepared immobilized SRB beads were kept in a desiccator at 4°C for later use (Fig. S2).

2.3. Characterization

The morphology of immobilized SRB beads before and after Pb(II) removal were characterized on a SEM (JSM7001F, JEOL Ltd., Japan), equipped with energy dispersive X-ray spectroscopy system, at 30 kV to monitor the difference on the surface of immobilized SRB beads. Before SEM, the immobilized SRB beads were dried at room temperature for 4 d. The samples for SEM imaging were sprayed platinum (Pt). FT-IR spectra of Pb(II) absorbed on immobilized SRB beads between 4,000 and 400 cm^{-1} were analyzed using a FT-IR spectrometer (Tensor27, Bruker, Germany) coupled with a KBr beam splitter and a DTGS detector. The background spectrum was also measured on a ZnSe spectral window. The absorbance was normalized with mean value and standard error calculation. Energy dispersive X-ray (EDX) spectra were recorded on JSM7001F (JEOL Ltd., Japan). XPS (Kratos Axis Ultra, Japan) was performed with monochromatic Al Kα radiation (hv = 1,486.6 eV) for the identification of the elements as well as the compounds contained in the adsorbents.

2.4. Removal of Pb(II)

The effects of solution pH, temperature and the initial Pb(II) concentration on the removal of Pb(II) were studied in this work. The sorption experiments were carried out in 250 mL flasks with 1 g immobilized SRB beads in each flask. During each test, 1 g beads (dry weight) containing 0.015 g SRB (dry weight) were added to a flask containing 100 mL of Pb(II)-bearing solution.

The flasks were incubated on a shaker with a speed of 200 ± 10 rpm. 10 mL samples from the reactive system were withdrawn after 24 h shaking. Liquid sample was separated from solid residue using 0.45 μm membrane filters and the filtrate was used to estimate the residual Pb(II) concentration in the aqueous solution. Three parallel sorption experiments were conducted to ensure the repeatability of the results.

The Pb(II) concentration was determined by a flame atomic absorption spectrometry (FAAS, TAS990, Beijing, China) [24]. The removal capacity of metal q ($mg\cdot g^{-1}$) was expressed as:

$$q = \frac{(C_0 - C_i)V}{m} \tag{1}$$

where C_0 is the initial Pb(II) concentration, $mg\cdot L^{-1}$; C_i is the equilibrium concentration of Pb(II), $mg\cdot L^{-1}$; V is the solution volume, L; m represents the weight of immobilized SRB, g.

3. Results and discussion

3.1. Morphology of immobilized SRB beads

Fig. 1 shows the variation of the morphology of immobilized SRB beads before and after Pb(II) adsorption reaction. Prior to the removal of Pb(II), the surface of immobilized SRB beads was composed of many randomly distributed uneven gaps and pores, as shown in Figs. 1(a) and (b). These rough surface and pore structures in immobilized SRB beads offer numerous activity sites to absorb Pb(II) ions, realizing the aim of Pb(II) removal. Of note, the rough surface can lessen the mass transfer and diffusion resistance of Pb(II) ions in immobilized SRB beads [25]. With uptake of Pb(II) ions, the surface of immobilized SRB beads altered to be much smoother. A number of irregular particles were attached on the surface of immobilized SRB beads (Figs. 1(c) and (d)), and most of the micro pores on the immobilized SRB beads were covered with a layer of these particles. EDX results further confirmed that Pb accumulated on the surface of immobilized SRB beads, as shown in Fig. 2.

3.2. XPS analysis of immobilized SRB beads

The original and used beads were further characterized by XPS. As can be seen from Figs. 3 and S3, after adsorption, doublets characteristic of Pb can be observed at 138.7 and 143.4 eV which is assigned to Pb 4f7/2 and Pb 4f5/2, respectively. It indicated that Pb(II) ions were removed and a portion of Pb(II) retained on the surface of immobilized SRB beads. The increases of the peak intensity of N1s and O1s were mainly due to the addition of N source (e.g., NH_4^+) for biological process or the adsorbed NO_3^- on the beads surface. Because C was consumed, a decrease of the peak intensity of C1s was observed. The signal for S2p was detected in the XPS spectra, suggesting certain amount of S remained in the sample.

3.3. FT-IR analysis of immobilized SRB beads

FT-IR analysis was performed for the identification of variations of functional groups after bio-removal of Pb(II) by immobilized SRB beads. FT-IR spectra of immobilized SRB beads are given in Fig. 4. The 3,500–3,100 cm^{-1} spectral region was presented by –O–H stretching vibrations of fatty acid components of membrane [26]. The signal at 3,393 cm^{-1} shifted to 3,421 cm^{-1} after bio-removal of Pb(II) by immobilized SRB beads, which was mainly due to the hydrolysis reaction. It is worthy to note that the networks with a certain amount of hydroxyl groups in immobilized SRB beads were beneficial to bacteria protection because of the formation of hydrophilic micro-environment [27]. The 3,000–2,800 cm^{-1} spectral region is illustrated by C–H stretching vibrations of fatty acid components of membranes. Of note, after adsorption, the peak at 2,920 cm^{-1} shifted to 2,916 cm^{-1}. This is mainly ascribed to the

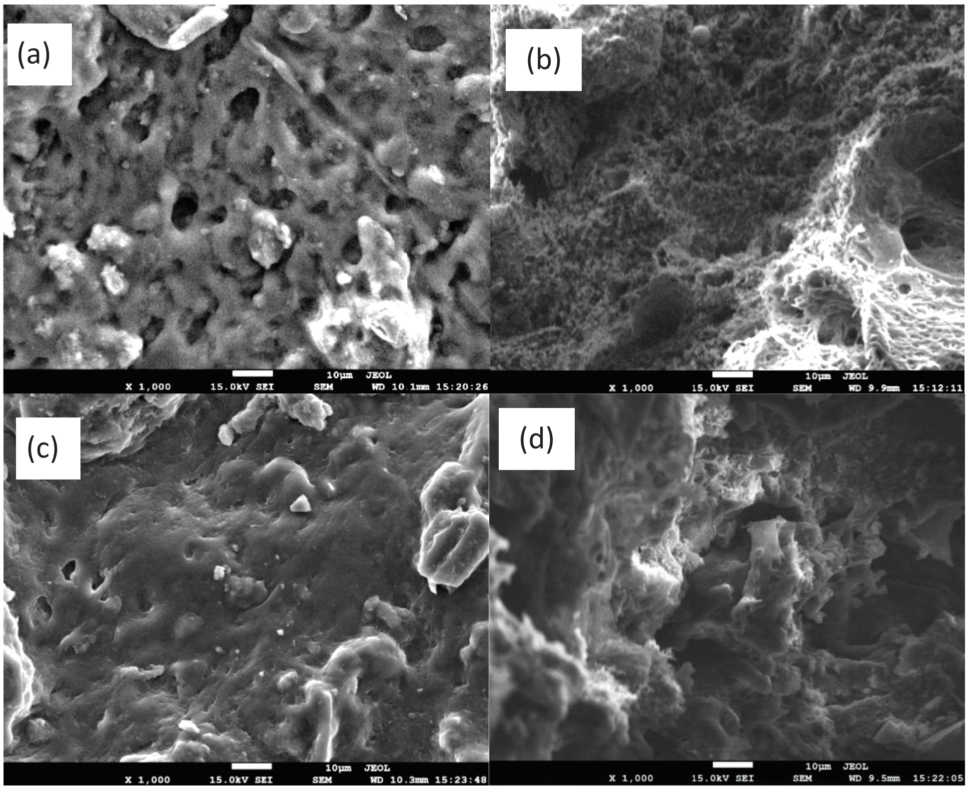

Fig. 1. SEM photographs of immobilized SRB beads. (a) and (b) Surface and cross-section photographs of immobilized SRB beads without bio-removal. (c) and (d) Surface and cross-section photographs of immobilized globule after bio-removal.

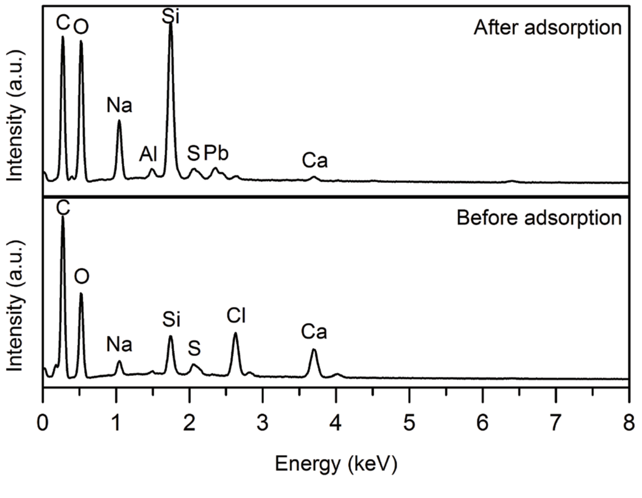

Fig. 2. EDX spectra of the immobilized SRB beads before and after Pb(II) absorption.

asymmetric and symmetric stretching vibrations of hydrocarbons of immobilized SRB beads. The intensity of the peak after bio-removal of Pb(II) shifted from 1,800 to 1,879 cm^{-1} because of the –C=O stretch of polymeric (Fig. 4). These variations can be interpreted by carboxyl groups being transformed into carboxylate, which is markedly beneficial to the removal of Pb from the aqueous solution via adsorption process. The groups of carboxylate radical in PVA-immobilized SRB were involved in the Pb adsorption and the variations are happened in the reaction among carboxyl and Pb(II) ions [25]. The peak between 1,082 and 1,084 cm^{-1} can be interpreted as –C–O–C stretching vibrations of hydrocarbons. Strong and broad adsorption bands at 1,000 and 1,100 cm^{-1} can be observed. These bands represent the asymmetric and symmetric stretching vibration absorption band of sulfate groups. The peaks at 461 and 694 cm^{-1}, corresponding to bending vibration of sulfate groups, underwent shifts to 459 and 693 cm^{-1}, respectively (Fig. 4) [28].

3.4. Effect of solution pH

pH is considered as one of the most crucial processing variables in the bio-process of the removal of heavy metals from wastewater [29]. To examine the effect of solution pH, the pH values were varied in the range of 3.0–9.0. The reaction conditions of Pb(II) removal were devised as follows: aqueous solution of pH 3.0–9.0 and 50 mg·L^{-1} Pb(II), 1 g of immobilized SRB beads of each flask, reaction temperature and time of 30°C and 24 h, respectively. As shown in Fig. 5, when the solution pH elevated from 3.0 to 7.0, the amount of Pb(II) removed by immobilized SRB was promoted remarkably from 262.67 to 540 mg·g^{-1}. However, the removal efficiency of Pb(II) did not enhance significantly under the conditions with pH above 7.0, suggesting the Pb(II) removal from aqueous solution is dominated by adsorption. The removal amount of Pb(II) was only 562.67 mg·g^{-1} at pH 8.0. Alone with the pH values increased to 9.0, the removal amount of Pb(II) was only 566.67 mg·g^{-1} which is close to the value obtained at pH 8.0. According to Hao et al. [30],

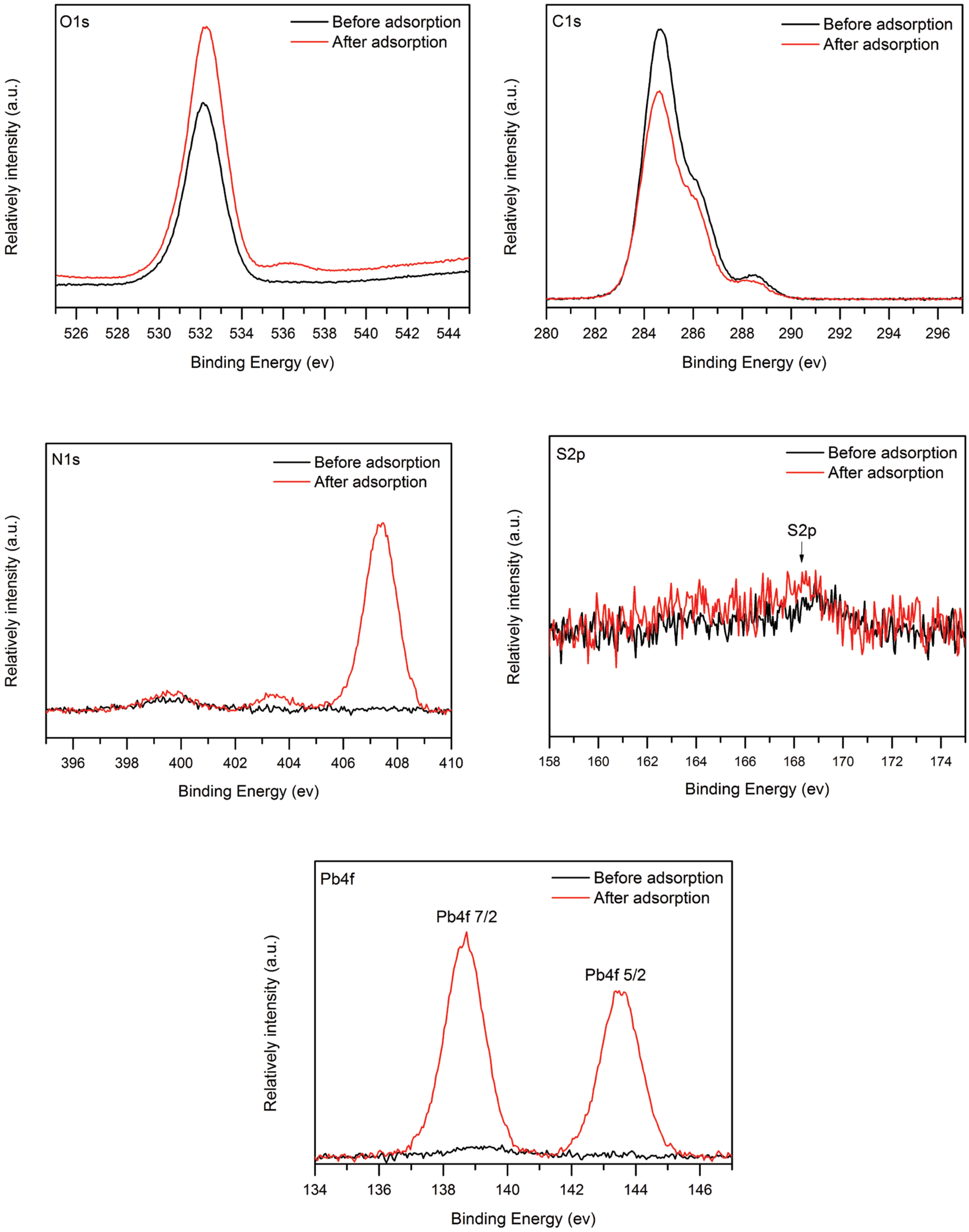

Fig. 3. Variations of O 1s, C 1s, N 1s, S 2p and Pb 4f XPS signals on the tested samples before and after Pb(II) adsorption.

hydrogen ions preferred to vigorously compete with Pb(II) ions during the adsorption process. Furthermore, the activity of bacteria will be inhibited under low pH conditions. A low microbial removal amount of Pb(II) (262.67 mg·g^{-1}) was observed at pH 3.0. Microbial removal amount gradually increased due to the increased activity of bacteria as pH increased. The higher pH values (pH > 7.0) not only lead to formation of Pb(OH)$_2$ precipitates but also inhibit the activity of sulfate reduction of SRB. Therefore, only slightly increase in Pb(II) removal was achieved because the pH values were beyond the optimum range and the role of bio-precipitation was somewhat offset [31]. To achieve high pH values, a considerable input of alkaline reagents is required, leading to high cost of the treatment process. Thus, the suitable pH values for Pb(II) removal by immobilized SRB should be below 7.0.

3.5. Effect of temperature

The effect of temperature on the activities of immobilized SRB beads was investigated thoroughly by varying the temperatures from 10°C to 40°C, with pH value of 7.0, initial Pb(II) concentration of 50 mg·L^{-1}, reaction time of 24 h and 1 g immobilized SRB beads of each flask. As shown in Fig. 6, the removal amount of Pb(II) increased from 318.67 to 565.33 mg·g^{-1} when temperature was elevated from 10°C to 35°C. However, along with the temperature further raised up to 40°C, the removal amount of Pb(II) dropped down to 530.67 mg·g^{-1}. Moreover, if further increasing the temperature to 42°C and 45°C, the removal amount of Pb(II) declined to 466.67 and 386.67 mg·g^{-1}, respectively. It was reported that more active sites will be formed because of the promoted protonation and deprotonation rate of the functional groups when temperature increased [32]. However, the activity of bacteria was inhibited because of protein denaturation and inactivation of bacteria when the temperature was higher than 35°C [33,34]. Due to the protection of PVA-alginate matrix, the SRB of the beads could bear relatively higher temperature than SRB cells freely suspending in the medium aqueous solution. The removal amount of Pb(II) slightly decreased when temperature increased up to 40°C. Nonetheless, when temperature increased up to 42°C and 45°C, the activity of SRB began to decrease markedly. At the temperature of 35°C, the greatest removal rate is able to be achieved.

3.6. Effect of the initial Pb(II) concentration

Fig. 7 shows the influence of initial Pb(II) concentration in the solution on the removal capability of Pb(II) by immobilized SRB beads. The adsorption capability of

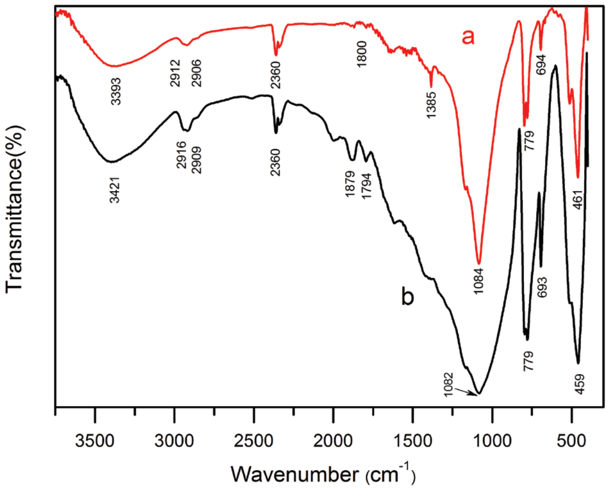

Fig. 4. FT-IR spectra of immobilized SRB beads (a) before bio-removal and (b) after bio-removal.

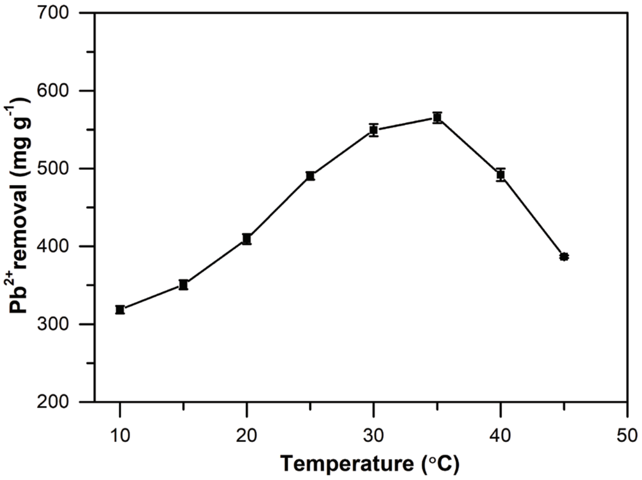

Fig. 6. Effect of temperature on the removal of Pb(II) (The reaction conditions are initial Pb(II) concentrations of 50 mg·L^{-1}, reaction temperature of 30°C, reaction time of 24 h and pH value of 7.0).

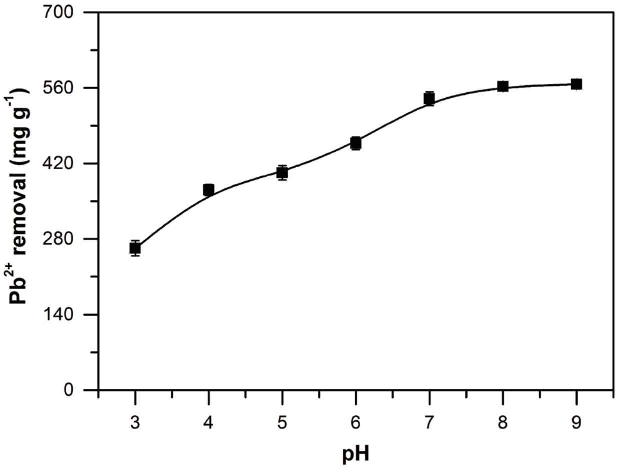

Fig. 5. Effect of pH on the removal of Pb(II) from aqueous solution. (The reaction conditions are initial Pb(II) concentrations of 50 mg·L^{-1}, reaction temperature of 30°C, reaction time of 24 h and pH value of 3.0–9.0).

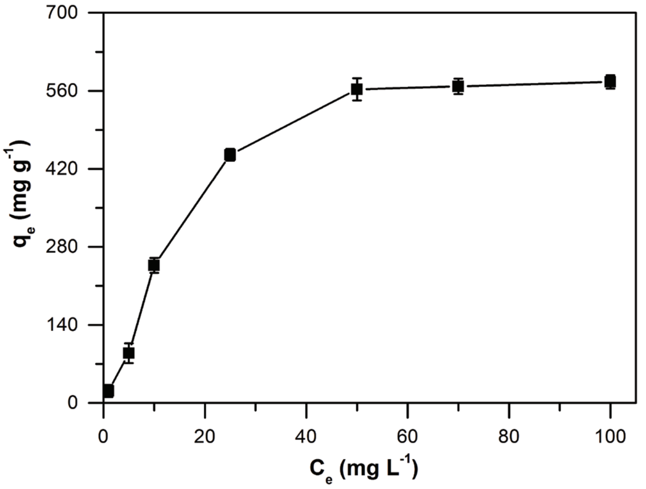

Fig. 7. Effect of the initial Pb(II) concentration on the removal of Pb(II). (The reaction conditions are reaction temperature of 30°C, reaction time of 24 h and pH value of 7.0).

Pb(II) concentrations in the reactive system increased with the increased initial Pb(II) concentration in the range of 0–100 mg·L^{-1} (Fig. 7). It can be found that 50 mg·L^{-1} of Pb(II) as initial concentration can achieve the goal of effectively removing Pb(II) from wastewater stream (Fig. 7).

3.7. Removal kinetic studies

It is well known that the knowledge on the bio-removal kinetics is essential to understand the adsorption properties of heavy metals in adsorption batch experiments. To comparatively depict the Pb(II) sorption data, Lagergren pseudo-first order (LPFO), Lagergren pseudo-second order (LPSO) and intraparticle diffusion model (IPD) [35] were used and given in Eqs. (2)–(4), respectively.

$$\log\left(q_e - q_t\right) = \log q_e - \frac{k_{\text{pt}}}{2.303}t \tag{2}$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^{\ 2}} + \frac{t}{q_e} \tag{3}$$

$$q_t = k_3 t^{1/2} + C \tag{4}$$

where q_e and q_t are the quantity of adsorbed Pb(II) at equilibrium and contact time t, mg·g^{-1}, respectively; k_{pt} is the LPFO rate constant, min^{-1}; k_2 is the LPSO rate constant, mg·g^{-1}·min$^{-1/2}$; k_3 is IPD rate constant, mg·g^{-1}·min$^{-1/2}$; C is a constant for any experiment (mg g^{-1}).

To evaluate the sorption kinetics, correlation coefficient (R^2) and average absolute percentage deviation (D%) were used [36]. D% value was calculated as follows:

$$D\% = \frac{1}{N}\sum\sum_{i=1}^{N}\left|\frac{q_{e.\text{exp}} - q_{e.\text{cal}}}{q_{e.\text{exp}}}\right| \times 100 \tag{5}$$

where $q_{e.\text{cal}}$ represents the removal equilibrium capacity, mg·g^{-1}; $q_{e.\text{exp}}$ represents the actual capacity of removal equilibrium, mg·g^{-1}; N represents the number of removal.

To describe the Pb(II) bio-removal process by immobilized SRB beads, LPFO, LPSO and IPD models were employed and their kinetic fitting curves are given in Figs. 8(a)–(c). It is usually revealed that LPSO is

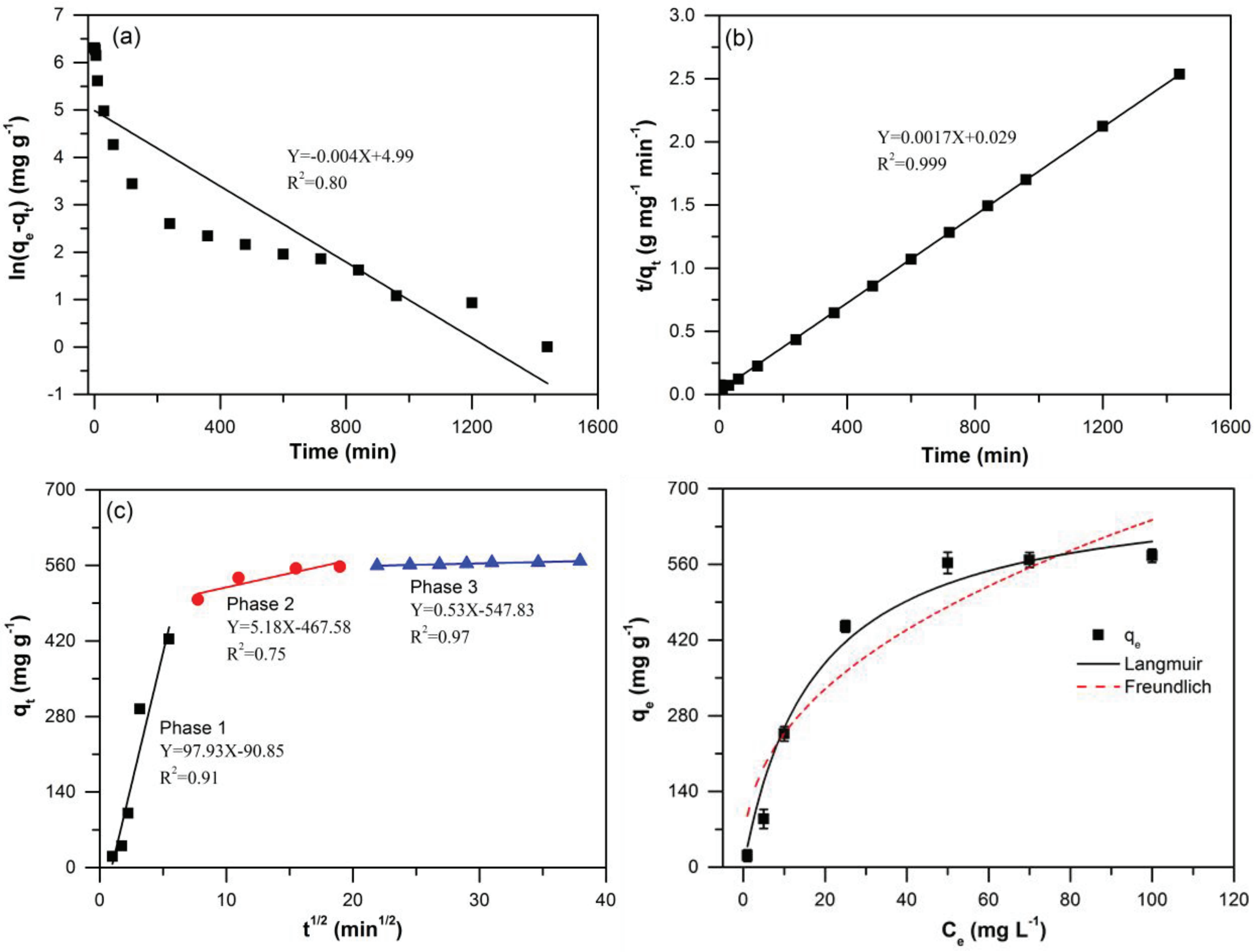

Fig. 8. Kinetic fitting curves of (a) LPFO, (b) LPSO and (c) IPD models for Pb(II) removal by immobilized SRB beads and (d) the fitting results of Langmuir and Freundlich isotherm models.

Table 1
Kinetic parameters for the removal of Pb(II)

Coefficients	LPFO	LPSO	IPD		
			Phase 1	Phase 2	Phase 3
k_1 (min^{-1})	4.0×10^{-3}	–	–	–	
q_e (mg·g^{-1})	146.94	–	–	–	
k_2 (mg·g^{-1}·min$^{-1/2}$)	–	9.97×10^{-5}	–	–	
q_e (mg·g^{-1})	–	588.24	–	–	–
k_3 (mg·g^{-1}·min$^{-1/2}$)	–	–	97.93	5.18	0.53
C (mg g^{-1})	–	–	90.85	467.58	547.83
R^2	0.80	0.99	0.91	0.75	0.97

Parameters were derived from LPFO, LPSO and IPD models.

most appropriate to describe the biosorption [37,38] and physic-chemical adsorption [39] kinetics for heavy metals removal. The Pb(II) removal capacity (588.24 mg·g^{-1}, Table 1) calculated via LPSO fitting, which the calculated Pb(II) removal capacity accounted for 102% of the maximum experimental one, which is close to the actual experimental outcome. However, the calculated Pb(II) removal capacity via LPFO fitting was far away from the maximum experimental value. The results of kinetic studies suggested that LPSO model can properly describe the Pb adsorption by immobilized SRB.

Generally, the biosorption process can be divided into membrane diffusion phase, internal diffusion phase and biosorption reaction stage for IPD kinetic model [40–43]. It had been demonstrated that the results were closely in line with this three-stage adsorption hypothesis (Fig. 8(c)). The rate-limited step of bio-removal might be due to a chemical adsorption which involves valence forces by exchanging and/or sharing of electrons and total biosorption rate was thus mainly determined by membrane diffusion, internal diffusion, or both [44]. To further investigate the biosorption mechanism of Pb(II) removal, the kinetic data was fitted with IPD model (Fig. 8(c)). In our experiments, the rate parameter (k_3) in Phase 1 is greater than that in the latter two phases, which implies that the membrane diffusion phase is rate-control step for Pb(II) removal by immobilized SRB (Table 1).

3.8. Biosorption isotherms

The Langmuir model was found to be better fitting rather than the Freundlich model for the description on the isotherms of Pb(II) biosorption by immobilized SRB beads (Fig. 8(d) and Table 2), as the correlation coefficient of Langmuir was much higher than that of Freundlich. The obtained Pb(II) removal capacity ($Q_{e\ \max}$ = 707.3 mg·g^{-1}) in our study was found to be much higher than those materials reported by others (Table 3), for example, Pan et al. [45] in which *Pleurotus ostreatus* was fixed in calcium alginate gel presented as an adsorbent with a maximum value of 121.21 mg·g^{-1} Pb(II) removal. Such high Pb(II) removal capacity was achieved in this study, signifying the great potential of this technique for Pb(II) removal.

Table 2
Isotherm parameters of Langmuir and Freundlich models

Coefficients	Langmuir	Freundlich
Q_e (mg·g^{-1})	707.3	–
K_L (L·mg^{-1})	0.057	–
K_f	–	94.99
n	–	2.41
R^2	0.98	0.89

Table 3
Comparisons between our results and other latest work

No.	Materials	$Q_{\max}$ (mg·g^{-1})	Reference
1	*Pleurotus ostreatus* immobilized in calcium alginate	121.21	[45]
2	Large-pore diameter nano-adsorbent	169.34	[46]
3	Fig sawdust activated carbon	80.65	[47]
4	Ash and Fe-nanoparticles loaded ash (nFe-A)	25 and 30, respectively	[48]
5	Nano-composite cation exchanger	18.38 (298 K)	[49]
6	Lignin-based biosorbent	130.20	[50]
7	*Landoltia punctata*	250.00	[51]
8	Hydroxyapatite/yeast biomass composites	195.97	[52]
9	Ti(IV) iodovanadate cation exchanger	18.8	[53]
10	PVA-sodium alginate immobilized SBR	707.30	This study

4. Conclusions

The outcomes in this study demonstrate that immobilized SRB beads of impregnating SRB are capable for the removal Pb(II) from aqueous solution. Results further show that pH, temperature and initial Pb(II) concentration have significant impacts on Pb(II) removal. The optimum bio-removal conditions were determined to be 35°C and pH 8.0 with 50 mg·L^{-1} of initial Pb(II) concentrations. Based on the adsorption kinetic analysis, LPSO is a suitable model to address the bio-removal mechanism of Pb(II) by immobilized SRB beads and the membrane diffusion phase is the rate-control step for Pb(II) removal by immobilized SRB. Biosorption isotherms analyses show that the Langmuir model is better fitted than Freundlich to reveal the isotherms of Pb(II) biosorption by immobilized SRB beads. The surface charge and elemental mapping of PVA-sodium alginate immobilized SBR might help to unravel the bio-adsorption measurement and thus such measurements should be conducted in the future work.

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Conflict of interest

The authors declare no competing financial interest.

References

[1] Q. Yang, Z. Li, X. Lu, Q. Duan, L. Huang, J. Bi, A review of soil heavy metal pollution from industrial and agricultural regions in China: pollution and risk assessment, Sci. Total Environ., 642 (2018) 690–700.

[2] W.B. Huang, Heavy metal concentrations in the common benthic fishes caught from the coastal waters of eastern Taiwan, J. Food Drug Anal., 11 (2003) 324–330.

[3] A. Duran, M. Tuzen, M. Soylak, Assessment of trace metal concentrations in muscle tissue of certain commercially available fish species from Kayseri, Turkey, Environ. Monit. Assess., 186 (2014) 4619–4628.

[4] P. Lodeiro, J.L. Barriada, R. Herrero, M.E.S.D. Vicente, The marine macroalga *Cystoseira baccata* as biosorbent for cadmium(II) and lead(II) removal: kinetic and equilibrium studies, Environ. Pollut., 142 (2006) 264–273.

[5] V.D.B. Bart, V. Carlo, Removal of pollutants from surface water and groundwater by nanofiltration: overview of possible applications in the drinking water industry, Environ. Pollut., 122 (2003) 435–445.

[6] G. Sharma, B. Thakur, M. Naushad, A.A.H. Al-Muhtaseb, A. Kumar, M. Sillanpaa, G.T. Mola, Fabrication and characterization of sodium dodecyl sulphate@ironsilicophosphate nanocomposite: ion exchange properties and selectivity for binary metal ions, Mater. Chem. Phys., 193 (2017) 129–139.

[7] G. Sharma, D. Pathania, M. Naushad, Preparation, characterization and antimicrobial activity of biopolymer based nanocomposite ion exchanger pectin zirconium(IV) selenotungstophosphate: application for removal of toxic metals, J. Ind. Eng. Chem., 20 (2014) 4482–4490.

[8] P. Kumar, A. Pournara, K.-H. Kim, V. Bansal, S. Rapti, M.J. Manos, Metal-organic frameworks: challenges and opportunities for ion-exchange/sorption applications, Prog. Mater. Sci., 86 (2017) 25–74.

[9] F. Fu, Q. Wang, Removal of heavy metal ions from wastewaters: a review, J. Environ. Manage., 92 (2011) 407–418.

[10] S.E. Bailey, T.J. Olin, R.M. Bricka, D.D. Adrian, A review of potentially low-cost sorbents for heavy metals, Water Res., 33 (1999) 2469–2479.

[11] Z.J. Yi, J. Yao, Y.F. Kuang, H.L. Chen, F. Wang, Z.M. Yuan, Removal of Pb(II) by adsorption onto Chinese walnut shell activated carbon, Water Sci. Technol., 72 (2015) 983–989.

[12] M. Naushad, S. Vasudevan, G. Sharma, A. Kumar, Z.A. Alothman, Adsorption kinetics, isotherms, and thermodynamic studies for Hg^{2+} adsorption from aqueous medium using alizarin red-S-loaded amberlite IRA-400 resin, Desal. Wat. Treat., 57 (2016) 18551–18559.

[13] A. Mittal, M. Naushad, G. Sharma, Z.A. Alothman, S.M. Wabaidur, M. Alam, Fabrication of MWCNTs/ThO_2 nanocomposite and its adsorption behavior for the removal of Pb(II) metal from aqueous medium, Desal. Wat. Treat., 57 (2016) 21863–21869.

[14] B. Volesky, Detoxification of metal-bearing effluents: biosorption for the next century, Hydrometallurgy, 59 (2001) 203–216.

[15] K. Vijayaraghavan, R. Balasubramanian, Is biosorption suitable for decontamination of metal-bearing wastewaters? A critical review on the state-of-the-art of biosorption processes and future directions, J. Environ. Manage., 160 (2015) 283–296.

[16] J. Wang, Biosorption of copper(II) by chemically modified biomass of *Saccharomyces cerevisiae*, Process Biochem., 37 (2002) 847–850.

[17] A.M. Jiménez-Rodríguez, M.M. Durán-Barrantes, R. Borja, E. Sánchez, M.F. Colmenarejo, F. Raposo, Heavy metals removal from acid mine drainage water using biogenic hydrogen sulphide and effluent from anaerobic treatment: effect of pH, J. Hazard. Mater., 165 (2009) 759–765.

[18] R.J. Valo, M.M. Häggblom, M.S. Salkinoja-Salonen, Bioremediation of chlorophenol containing simulated ground water by immobilized bacteria, Water Res., 24 (1990) 253–258.

[19] R.S. Prakasham, J.S. Merrie, R. Sheela, N. Saswathi, S.V. Ramakrishna, Biosorption of chromium VI by free and immobilized *Rhizopus arrhizus*, Environ. Pollut., 104 (1999) 421–427.

[20] M.B. Cassidy, H. Lee, J.T. Trevors, Environmental applications of immobilized microbial cells: a review, J. Ind. Microbiol., 16 (1996) 79–101.

[21] W. Chen, H. Zhang, Y. Chen, D. Luo, Q. Wu, Effect of pH, temperature and initial concentration on thallium removal by sulfate-reducing bacteria, Chin. J. Environ. Eng., 8 (2014) 4105–4109.

[22] H. Zhang, M. Li, Z. Yang, Y. Sun, J. Yan, D. Chen, Y. Chen, Isolation of a non-traditional sulfate reducing-bacteria *Citrobacter freundii* sp. and bioremoval of thallium and sulfate, Ecol. Eng., 102 (2017) 397–403.

[23] J. Sun, J. Liu, Y. Liu, Z. Li, J. Nan, Optimization of entrapping conditions of nitrifying bacteria and selection of entrapping agent, Procedia Environ. Sci., 8 (2011) 166–172.

[24] E.W. Rice, R.B. Baird, A.D. Eaton, L. Clesceri, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, 2012.

[25] Z. Huang, G. Chen, G. Zeng, A. Chen, Y. Zuo, G. Zhi, Q. Tan, Z. Song, Q. Niu, Polyvinyl alcohol-immobilized *Phanerochaete chrysosporium* and its application in the bioremediation of composite-polluted wastewater, J. Hazard. Mater., 289 (2015) 174–183.

[26] P. Lasch, D. Naumann, Infrared Spectroscopy in Microbiology, in: Encyclopedia of Analytical Chemistry, John Wiley & Sons, Ltd., Chichester, UK, 2006.

[27] X. Bai, Z.F. Ye, Y.F. Li, L.C. Zhou, L.Q. Yang, Preparation of crosslinked macroporous PVA foam carrier for immobilization of microorganisms, Process Biochem., 45 (2010) 60–66.

[28] J.J. Cao, Z.T. Jiang, Z.H. Xiong, Y.N. Yang, Study on infrared spectra characteristics of fault particles of the sulfide deposit, Spectrosc. Spect. Anal., 29 (2009) 956–959.

[29] M.A. Willow, R.R. Cohen, pH, dissolved oxygen, and adsorption effects on metal removal in anaerobic bioreactors, J. Environ. Qual., 32 (2003) 1212–1221.

[30] O.J. Hao, J.M. Chen, L. Huang, R.L. Buglass, Sulfate-reducing bacteria, Crit. Rev. Environ. Sci. Technol., 26 (1996) 155–187.

[31] Y.J. Park, J.J. Ko, S.L. Yun, E.Y. Lee, S.J. Kim, S.W. Kang, B.C. Lee, S.K. Kim, Enhancement of bioremediation by *Ralstonia* sp. HM-1 in sediment polluted by Cd and Zn, Bioresour. Technol., 99 (2008) 7458–7463.

[32] J. Bajpai, R. Shrivastava, A.K. Bajpai, Dynamic and equilibrium studies on adsorption of Cr(VI) ions onto binary bio-polymeric beads of cross linked alginate and gelatin, Colloids Surf., A, 236 (2004) 81–90.

[33] W. Chen, H. Zhang, Y. Chen, D. Luo, Q. Wu, Effect of pH, temperature and initial concentration on thallium removal by sulfate-reducing bacteria, Chin. J. Environ. Eng., 8 (2014) 4105–4109.

[34] T.W. Hao, P.Y. Xiang, H.R. Mackey, K. Chi, H. Lu, H.K. Chui, M.C. van Loosdrecht, G.H. Chen, A review of biological sulfate conversions in wastewater treatment, Water Res., 65 (2014) 1–21.

[35] X. Pan, J.Z.D. Wang, Biosorption of Pb(II) by Pleurotus ostreatus immobilized in calcium alginate gel, Process Biochem., 40 (2005) 2799–2803.

[36] R. Laus, T.G. Costa, B. Szpoganicz, V.T. Fávere, Adsorption and desorption of Cu(II), Cd(II) and Pb(II) ions using chitosan crosslinked with epichlorohydrin-triphosphate as the adsorbent, J. Hazard. Mater., 183 (2010) 233–241.

[37] J. Long, H. Li, D. Jiang, D. Luo, Y. Chen, J. Xia, D. Chen, Biosorption of strontium(II) from aqueous solutions by *Bacillus*

cereus isolated from strontium hyperaccumulator *Andropogon gayanus*, Process. Saf. Environ., 111 (2017) 23–30.

[38] F. Huang, Z. Dang, C.-L. Guo, G.-N. Lu, R.R. Gu, H.-J. Liu, H. Zhang, Biosorption of Cd (II) by live and dead cells of *Bacillus cereus* RC-1 isolated from cadmium-contaminated soil, Colloids Surf., B, 107 (2013) 11–18.

[39] H. Li, Y. Chen, J. Long, X. Li, D. Jiang, P. Zhang, J. Qi, X. Huang, J. Liu, R. Xu, J. Gong, Removal of thallium from aqueous solutions using Fe-Mn binary oxides, J. Hazard. Mater., 338 (2017) 296–305.

[40] X. Zhou, J. Wei, K. Liu, N. Liu, B. Zhou, Adsorption of bisphenol A based on synergy between hydrogen bonding and hydrophobic interaction, Langmuir, 30 (2014) 13861–13868.

[41] S. Paul, D. Bera, P. Chattopadhyay, L. Ray, Biosorption of Pb(II) by *Bacillus cereus* M^1_{16} immobilized in calcium alginate gel, J. Hazard. Subst. Res., 5 (2006) 1–13.

[42] P. Xu, G.M. Zeng, D.L. Huang, C. Lai, M.H. Zhao, Z. Wei, N.J. Li, C. Huang, G.X. Xie, Adsorption of Pb(II) by iron oxide nanoparticles immobilized *Phanerochaete chrysosporium*: equilibrium, kinetic, thermodynamic and mechanisms analysis, Chem. Eng. J., 203 (2012) 423–431.

[43] Y.S. Ho, G. Mckay, A kinetic study of dye sorption by biosorbent waste product pith, Resour. Conserv. Recycl., 25 (1999) 171–193.

[44] M. Iram, G. Chen, Y. Guan, A. Ishfaq, H. Liu, Adsorption and magnetic removal of neutral red dye from aqueous solution using Fe_3O_4 hollow nanospheres, J. Hazard. Mater., 181 (2010) 1039–1050.

[45] X. Pan, J. Wang, D. Zhang, Biosorption of Pb(II) by *Pleurotus ostreatus* immobilized in calcium alginate gel, Process Biochem., 40 (2005) 2799–2803.

[46] A. Shahat, M.R. Awual, M.A. Khaleque, M.Z. Alam, M. Naushad, A.M.S. Chowdhury, Large-pore diameter nano-adsorbent and its application for rapid lead(II) detection and removal from aqueous media, Chem. Eng. J., 273 (2015) 286–295.

[47] M. Ghasemi, M. Naushad, N. Ghasemi, Y. Khosravi-Fard, A novel agricultural waste based adsorbent for the removal of Pb(II) from aqueous solution: kinetics, equilibrium and thermodynamic studies, J. Ind. Eng. Chem., 20 (2014) 454–461.

[48] M. Ghasemi, M. Naushad, N. Ghasemi, Y. Khosravi-Fard, Adsorption of Pb(II) from aqueous solution using new adsorbents prepared from agricultural waste: adsorption isotherm and kinetic studies, J. Ind. Eng. Chem., 20 (2014) 2193–2199.

[49] M. Naushad, Surfactant assisted nano-composite cation exchanger: development, characterization and applications for the removal of toxic Pb^{2+} from aqueous medium, Chem. Eng. J., 235 (2014) 100–108.

[50] B. Wang, J.L. Wen, S.L. Sun, H.M. Wang, S.F. Wang, Q.Y. Liu, A. Charlton, R.C. Sun, Chemosynthesis and structural characterization of a novel lignin-based bio-sorbent and its strong adsorption for Pb (II), Ind. Crops Prod., 108 (2017) 72–80.

[51] J. Tang, Y. Li, X. Wang, M. Daroch, Effective adsorption of aqueous Pb^{2+} by dried biomass of *Landoltia punctata* and *Spirodela polyrhiza*, J. Clean. Prod., 145 (2017) 25–34.

[52] Z. Wei, F. Wang, P. Wang, L. Li, Z. Yu, Z. Ping, M. Zhao, C. Hui, L. Yong, Y. Zhang, Facile synthesis of hydroxyapatite/yeast biomass composites and their adsorption behaviors for lead (II), J. Colloid Interface Sci., 477 (2016) 181–190.

[53] M. Naushad, Z.A. Alothman, M.R. Awual, M.M. Alam, G.E. Eldesoky, Adsorption kinetics, isotherms, and thermodynamic studies for the adsorption of Pb^{2+} and Hg^{2+} metal ions from aqueous medium using Ti(IV) iodovanadate cation exchanger, Ionics, 21 (2015) 2237–2245.

Supplementary material

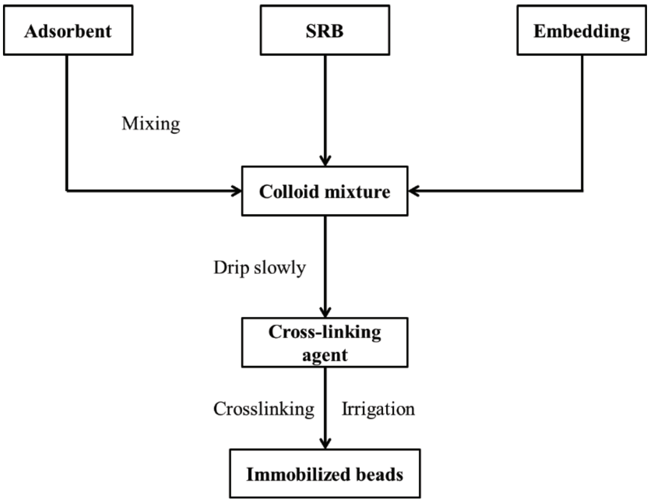

Fig. S1. Preparation process of the immobilized SRB beads.

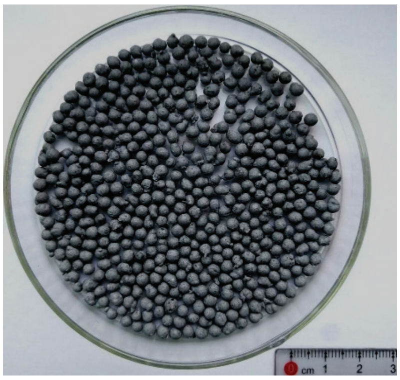

Fig. S2. View of the immobilized SRB beads before Pb(II) adsorption.

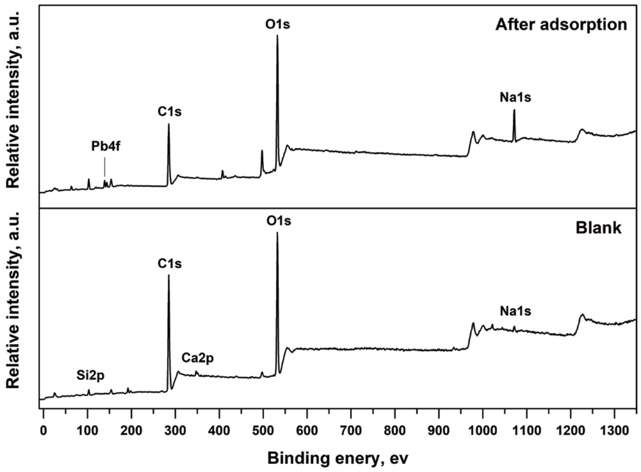

Fig. S3. XPS full scan survey of the tested samples before and after Pb(II) adsorption.