Synthesis of a novel alginate-rubber joint immobilization strains H-1 and its application in removal of Pb (II) from aqueous solution

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Abstract. In this study, a novel alginate-rubber-strains immobilized beads (ARSIBs) was synthesized at the optimum conditions that the concentration of sodium alginate was 4%; the volume of bacterial suspension was 75%; the quality of rubber powder was 3.2%; the crosslinking time was 24 h by the orthogonal experiments. The optimum conditions for Pb (II) adsorption were 1.2% ARSIBs, 100 mg L⁻¹ initial concentrations, pH 5 and 3 h contact time. The equilibrium data were well fitted by the Freundlich isotherm model. The biosorption process was nearly consistent with the pseudo-second-order model. Meanwhile, the biosorption mechanism could be that Pb (II) was adsorbed by the hydroxyl and carboxyl, finally precipitated with phosphate in the form of NaPb₄(PO₄)₃, Pb₅(PO₄)₃(OH) and Pb(H₂PO₄)₂ based on the spectra of FTIR and XRD, respectively. In addition, the stability of ARSIBs was enhanced due to the addition to the rubber powder in the process of wastewater treatment.

1. Introduction

Pollution caused by heavy metal was a global problem due to their toxicity and bioaccumulation in the food chain that resulted in a serious health and ecological problems [1]. Among different heavy metal, such as Pb (II), Cu(II), Hg(II), Ni(II) etc., lead was a highly toxic metal even at very low concentration [2]. Since the 1980s, people have been continuously studying biosorption to remove heavy metal. Biosorption is an economically effective technology for purification of wastewater and recovery metals [3]. Biosorption involves the passive uptake of Pb (II) by inactive/dead biological material like bacteria, fungi, and algae. Immobilized microbial cells could provide additional advantages, including effective regeneration of the biomass, easier solid-liquid separation and minimize clogging in continuous flow systems compared to freely suspended absorbents [4]. The use of dead biomass was more attractive than live biomass, because heavy metals have little effect on the biosorption process of dead biomass. In terms of the efficiency of pollutants removal, it had been reported that biosorption of heavy metals on immobilized dead Trametes versicolor was more effective than that on immobilized live one [5]. Thus, immobilized dead cells have a potential applicability for the removal of Pb (II) from aqueous solution.

Sodium alginate was the most popular biopolymer for immobilization [6]. Sodium alginate easily forms sol-gel propagules which has the appropriate acid stability, and the remarkable adsorption properties of metal ion attributed to the presence of carboxylic groups at the surface of sodium alginate beads [7]. The sorption efficacy of alginate is also not affected by the presence of ions such as Ca (II),
Mg (II), and K (I) [8]. To date, sodium alginate has been successfully used to immobilize various metal sorbents. In this study, the main research contents as follow: the optimization of the synthesis conditions of ARSIBs; the description of process of Pb (II) adsorption by the kinetics and adsorption isotherm models; the analysis of adsorption mechanism by FTIR and XRD.

2. Experimental

2.1. Preparation of bacterial suspension
The strain H-1 was inoculated on a culture medium slant for 3 days, then were inoculated and cultured in beef extract peptone liquid medium on a rotary shaker 180 rpm at 30 °C for 48 hours achieved to OD<sub>600</sub>=2.0. The cultures were centrifuged at 8000 rpm for 10 min. Bacterial deposition was washed three times with sterilized water, suspended in a certain volume of sterilized water.

2.2. Immobilization factors that affected performance of ARSIBs
In this study, the experimental strains and rubber powder were immobilized by embedding techniques. There are a lot factors that may influence the ARSIBs, such as the concentration of sodium alginate, the volume of bacterial suspension, the quality of rubber powder, crosslinking time, CaCl<sub>2</sub> concentration, ARSIBs diameter, temperature and pH and so on. The first four factors were examined on the physical properties of ARSIBs in this experiment. The testing method for the physical properties of ARSIBs was as follows:

- Spherical efficient: The forming process of ARSIBs was observed by the naked eye.
- Mechanical strength: Three ARSIBs were taken and then blotted up surface water with filter paper and placed them on an electronic balance. ARSIBs were pressed slowly. When they were broken, the electronic balance was counted as the mechanical strength of ARSIBs.
- Permeability: The uniform ARSIBs were selected to immerse in inert red ink. Remove one and cut it every 4 min. Determine the penetration thickness of red ink. The final time was recorded that ARSIBs were completely penetrated by red ink.
- Breakage: ARSIBs were placed into 250 mL conical flaskes filled with 100 mL sterile water on a rotary shaker at 180 rpm for 30 °C. The numbers of broken beads were recorded every 5 days. Finally, the breaking rate of ARSIBs was calculated according to 35 days.

2.3. Optimal ratios for preparation of ARSIBs
In this study, four factors and three levels were adopted in order to obtain the optimal ratio for preparation of ARSIBs by orthogonal experimental design. Test factors included the concentration of sodium alginate, the volume of bacterial suspension, the quality of rubber powder and the crosslinking time. Three levels were developed for each factor. All the experiments were performed in triplicates, and the average values were employed in the calculations.

2.4. Pb (II) adsorption studies
The influencing factors for the dosage, pH, adsorption time and initial Pb (II) concentration was tested to evaluate the adsorption capacity of ARSIBs for Pb (II). ARSIBs were placed into 50 mL of Pb (II) solution on a rotary shaker at 180 rpm for 30 °C. The concentration of Pb (II) was obtained by atomic adsorption spectrophotometer.

The adsorption of Pb (II) on ARSIBs as a function of the biosorbent dosage was investigated by varying the amount of ARSIBs from 0.6% to 4.2% (w/v) at 100 mg L<sup>-1</sup> Pb (II) initial concentration and pH 5 for 3 h contact time. Similarly, the initial Pb (II) concentration ranged from 40 mg L<sup>-1</sup> to 300 mg L<sup>-1</sup> at 1.2% ARSIBs dosage and pH 5 for 3 h contact time. The pH was tested in the pH 3-8 at 1.2% ARSIBs dosage, 3 h contact time and 100 mg L<sup>-1</sup> Pb (II) initial concentration. The contact time was 10 min -1800 min. The ARSIBs were isolated by filtration after the experiment. Dry weight was obtained by oven-dried (65 °C) to constant. The adsorbent capacity was calculated using the following equation:
\[ q_t = \frac{[C_0 - C_t]V_t}{M} \quad (1) \]

- \( q_t \): adsorption of Pb (II) per g of adsorbent (mg g\(^{-1}\));
- \( C_0 \): initial Pb (II) concentration (mg L\(^{-1}\));
- \( C_t \): Pb (II) concentration at time \( t \) (mg L\(^{-1}\));
- \( V_t \): volume of suspension (L);
- \( M \): dry mass of adsorbent (g).

2.5. Kinetic and equilibrium modeling

Kinetic and equilibrium models were used to describe the adsorption process of the adsorbent on metal and to predict the mechanisms of adsorption. Pseudo-first-order kinetic and pseudo-second-order kinetic models are mostly used to describe the adsorption kinetics. Equilibrium models were utilized to determine the adsorption capacity and to illustrate the type of surface binding mechanism of the adsorbents. Equilibrium models are calculated based on the Langmuir and Freundlich equation.

2.6. Analysis on the mechanism

FTIR spectrum of ARSIBs was obtained by the KBr pellet’s method using Fourier Transform Infrared Spectrometer (EQUINOX 55, Bruker Company, Germany). The sample was scanned under 500 cm\(^{-1}\) to 4000 cm\(^{-1}\) at the ambient temperature. The crystalline structure of ARSIBs was characterized by using an X-ray diffractometer (XRD, D-max 2500/PC, Rigaku, Japan). The diffraction data collection over a 2θ ranges between 10° and 80°.

3. Results and discussion

3.1. Effects of various factors on ARSIBs

3.1.1. Concentration of sodium alginate. This experiment chose sodium alginate as embedding agent. Its concentration range was 2%-8%. And the same volume bacteria suspension and 1.6% (w/v) of the rubber powder was mixed to drop into 3% w/v CaCl\(_2\) solution for 12 h for cross-linking.

The results of experiments showed that concentration of 2% sodium alginate hardly formed beads, 6% of sodium alginate formed spherical inhomogeneity. The primary reason was that the concentration of sodium alginate was too thick to drop. Mechanical strength of ARSIBs progressively increased from 142.81 g to 889.60 g when the concentration of sodium alginate ranged from 3% to 8%, respectively. Permeability of ARSIBs progressively decreased from 4 min to 16 min with the increase of the concentration of sodium alginate. ARSIBs had good permeability and can be fully permeated within 12 min. The breakage rate of ARSIBs reached approximately 50% at the concentration of 3% sodium alginate. The breaking rate was less than 20% at 5%. It has been reported that alginate immobilized live cells, 2.5 g fresh weight of biomass was mixed with 50 mL of sterile water, and then the suspension was mixed with 50 mL of 4% sodium alginate[9]. Based on the above results, the concentration of 3%-5% sodium alginate was chosen for the follow-up test in this experiment.

3.1.2. The volume of bacterial suspension. In this experiment, the different volumes of the bacterial suspension were added to the mixed liquor of concentration of 5% sodium alginate and 1.6% (w/v) the rubber powder. Then, the solution was crosslinked with 3% w/v CaCl\(_2\) solution for 12 h of cross-linking.

The results displayed that the mechanical strength of the ARSIBs decreased from 674.61 g to 201.75 g when the volume of bacterial suspension increased from 25% to 125%, respectively. The breakage rate gradually increased from 2.14% to 40.71% with increase of the volume of the bacterial suspension from 25% to 125%, respectively. It was the reduced concentration of sodium alginate in the mixed solution due to the quality of the bacterial suspension that was responsible for the decrease of mechanical strength and the increase of breakage rate. When the volume of suspension was greater than 50%, the ARSIBs had good permeability, and the ARSIBs could be permeated completely in 8 min. The physical properties and the amount of immobilized strains can be better served the requirements of the experiment with the volume of bacterial suspension was 50%-100%.
3.1.3. The quality of rubber powder. The different quality of rubber powder was added to the mixed solution of 100 mL of 5% sodium alginate and 1:1 the volume of bacterial suspension in the experiment. Then the solution was crosslinked with 3% w/v with CaCl₂ solution for 12 h of crosslinking.

The data from the experiment indicated that the mechanical strength of the ARSIBs gradually increased to the maximum value of 417.87 g at 1.6% of rubber powder. However, it slowly declined with the increase of the quality of rubber powder. Conversely, the breakage rate of ARSIBs firstly reduced and then increased. The above results indicated that the appropriate quality of rubber powder would be helpful to mechanics and stability of ARSIBs. When the quality of rubber powder was more than 6.4%, the relatively low mechanical strength (386.11 g), and the high breakage rate (92.86%) may arise from an excessive quality of rubber powder that destroyed the internal structure and the permeability of ARSIBs. The ARSIBs had good permeability, and can be permeated completely from 8 min to 6 min at 1.6% - 4.8% of rubber powder, respectively.

3.1.4. Crosslinking time. Crosslinking time was the reaction time after the mixture into the CaCl₂ solution. The mixed solutions of 100% bacteria suspension, 3.2% rubber powder and 5% sodium alginate were mixed fully and then dropped into 3% CaCl₂ solution. The ARSIBs were cross-linked in 20 °C, then the mechanical strength, permeability and breakage rate of the ARSIBs were measured every 6 h.

The results displayed that the mechanical strength of the ARSIBs gradually increased from 315.11 g to 640.32 g in the period of 6 to 48 h. The breakage rate remarkably decreased from 35.71% to 1.43% in the period of 6 h to 48 h. The penetration time increased from 8 min to 12 min. The mechanical strength of ARSIBs was weaker and the breakage rate was higher when the crosslinking time was less than 6 h. The permeability of the ARSIBs was too low more than 42 h for crosslinking that may be due to the crosslinking time too long and the internal pore diameter too small. Therefore, crosslinking time on the test was chosen to 12 h, 24 h and 36 h.

3.2. Orthogonal test

To summarize, the concentration of sodium alginate, the volume of bacterial suspension, the quality of rubber powder and crosslinking time were chosen for the orthogonal experimental factors, and the four factors were denoted as A, B, C and D, respectively. Each factor had three levels.

| Number | A (%) | B (%) | C (%) | D(h) | Pb (II) removal rate (%) |
|--------|-------|-------|-------|------|--------------------------|
| 1      | 3     | 50    | 1.6   | 12   | 95.93                    |
| 2      | 3     | 75    | 3.2   | 24   | 98.14                    |
| 3      | 3     | 100   | 4.8   | 36   | 95.33                    |
| 4      | 4     | 50    | 3.2   | 36   | 96.59                    |
| 5      | 4     | 75    | 4.8   | 12   | 97.72                    |
| 6      | 4     | 100   | 1.6   | 24   | 98.26                    |
| 7      | 5     | 50    | 4.8   | 24   | 97.19                    |
| 8      | 5     | 75    | 1.6   | 36   | 98.02                    |
| 9      | 5     | 100   | 3.2   | 12   | 96.35                    |

| K₁ 96.47 | K₂ 96.57 | K₃ 97.40 | K₄ 96.67 | the best combination : A₂B₂C₂D₂ |
|-------|---------|---------|---------|--------------------------|
| R 1.05 | 1.39    | 1.38    | 1.21    |

As shown in Table 1. \( K_{A2} > K_{A3} > K_{A1} \), therefore, \( A_2 \) was chosen as the best level of the factor A. Similarly, \( B_3, C_2 \) and \( D_2 \) can be determined as optimal levels of factor B, C and D. The factors of \( A_2, B_2, C_2 \) and \( D_2 \) were chosen as the optimal combination of experiments, that is, the concentration of
sodium alginate was 4%, the volume of bacterial suspension was 75%, the quality of rubber powder was 3.2%, and the crosslinking time was 24 hours.

The data (Table 1) indicated $R_B > R_C > R_D > R_A$. Therefore, the main sequence of factors affecting the test indexes was B C D A. That is, the volume of bacterial suspension has the greatest impact on Pb (Ⅱ) removal rate, followed by the quality of rubber powder, crosslinking time and the concentration of sodium alginate, respectively. Therefore, the ARSIBs were prepared under A₂B₂C₂D₂ conditions as the adsorbent for subsequent adsorption experiments.

3.3. Batch adsorption experimental

![Graphs](image)

Figure 1. (a) Effect of ARSIBs dosage on Pb (Ⅱ) adsorption capacity, (b) initial Pb (Ⅱ) concentration, (c) pH, (d) time; the initial concentration of Pb (Ⅱ) 100 mg L⁻¹, pH 5 and contact time 3h.

3.3.1. Effect of ARSIBs dosage on Pb (Ⅱ) adsorption capacity. As shown in Figure 1(a), the optimum Pb (Ⅱ) adsorption capacity of the adsorbent was 107.04 mg g⁻¹ at 1.2% (w/v) on ARSIBs dosage. But the adsorption capacity of Pb (Ⅱ) would gradually decrease with the increase of ARSIBs dosage. Therefore, the optimum ARSIBs dosage was selected as 1.2% in the next test.

3.3.2. Effect of initial Pb (Ⅱ) concentration on Pb (Ⅱ) adsorption capacity. As shown in Figure 1(b), the adsorption capacity of ARSIBs remarkably improved by the increase of initial concentration up to 300 mg L⁻¹. The reason may be due to that the chance contact of between Pb (Ⅱ) and adsorbents was increased in higher Pb (Ⅱ) concentrations in solution [9]. Furthermore, mass transfer resistance of the adsorbents was also overcome, and then the collision was accelerated between the Pb (Ⅱ) and sorbents, finally the adsorption capacity was improved. The adsorption capacity of Pb (Ⅱ) reached 215 mg g⁻¹ at 300 mg L⁻¹, however, the removal efficiency of Pb (Ⅱ) was only 54.98%. It's exciting that the removal efficiency can reach 86% and the corresponding adsorption capacity was 105.16 mg g⁻¹ when the
initial concentration of Pb (II) was 100 mg L$^{-1}$. Therefore, the initial concentration of Pb (II) was chosen as 100 mg L$^{-1}$.

3.3.3. Effect of pH on Pb (II) adsorption capacity. The Figure 1(c) displayed that the optimum adsorption capacity of Pb (II) by ARSIBs occurred at pH 5, while poor sorption was observed at pH 3 and pH 8. The highest adsorption capacity of Pb (II) was 109.53 mg g$^{-1}$ at the optimum pH. This was attributed to the availability of metal binding sites on the adsorbents at this pH. The lower adsorption capacity of Pb (II) was 60.61 mg g$^{-1}$ at pH 3. The lower adsorption capacity was attributed to the existence of a large number of H$^+$ in low pH solution that resulted in the binding sites on the adsorbents to be protonated, and then leading to a decrease in attractiveness between the adsorbent surface and Pb (II) [10]. Similarly, adsorbents were also less effective at pH 8 with 60.52 mg g$^{-1}$ for ARSIBs. The low adsorption capacity was due to the formation of Pb (II) hydroxide complexes.

3.3.4. Effect of adsorption time on Pb (II) adsorption capacity. Pb (II) adsorption capacity of ARSIBs increased exponentially in first 180 min, as shown in Figure 1(d) achieved at 106.57 mg g$^{-1}$. The reason may be explained by the availability of metal binding sites at the surface of the sodium alginate-based sorbents. The highest adsorption capacity of Pb (II) was 137.89 mg g$^{-1}$ at equilibrium. The results showed that the adsorption capacity of Pb (II) nearly reached dynamic balance since 180 min later. Therefore, optimum adsorption time was chosen as 180 min.

3.4. Adsorption kinetic and isotherm studies

3.4.1. Kinetic model. Table 2. derived from Figure 2. according to experimental data showed the R$^2$ value of the pseudo-second order kinetic model was better than that of the pseudo-first order kinetic model, 0.999>0.977. Furthermore, the predicted adsorption capacity (q$^{\text{eq}}$) for the pseudo-second order kinetic model was similar to the experiments (q$^{\text{eqex}}$), 138.12 mg g$^{-1}$ and 136.87 mg g$^{-1}$, respectively. However, the difference between q$^{\text{eq}}$ by the pseudo-first order kinetic model and q$^{\text{eqex}}$ was relatively large, 126.27 mg g$^{-1}$ and 136.87 mg g$^{-1}$, respectively. Thus, the pseudo-second order kinetic model was more suitable to describe the adsorption process of Pb (II) by ARSIBs rather than the pseudo-first order kinetic model. The pseudo-second kinetic model suggested that the adsorption process of Pb (II) on ARSIBs was a rate-limiting step and the adsorption of Pb (II) may involved valency interactions between the biosorbents and Pb (II). Similar results for the compliance of pseudo-second order kinetic model were also observed with sodium alginate-immobilized Pycnoporous Sanguineus [11].

3.4.2. Adsorption isotherm. To determine the adsorption capacity of ARSIBs, the equilibrium adsorptions of Pb (II) were studied by a function of the initial concentration and the results are shown in Figure 3. The sorption capacity of ARSIBs gradually increased from 47.47 mg g$^{-1}$ to 214.43 mg g$^{-1}$ with the increase of the initial concentration of Pb (II) from 40 mg L$^{-1}$ to 300 mg L$^{-1}$. It was obvious that the isotherm steeply raised, displayed in Figure 3. that indicated a great number of readily accessible sites were available for adsorption. However, the sorption site of ARSIBs completely were occupied and the adsorption reached a dynamic equilibrium with increase of the initial concentration of Pb (II) [12]. The adsorption equilibrium data were analyzed using two the Freundlich and Langmuir isotherm models to determine the adsorption process of ARSIBs for Pb (II) in Table 3 and Figure 3. The Langmuir and Freundlich constants were shown in Table 3. The correlation coefficient R$^2$ of the Freundlich isotherm model fitted well the experimental data than that of the Langmuir isotherm model on Pb (II) adsorption. Therefore, adsorption process for Pb (II) on the ARSIBs can be interpreted by the Freundlich isotherm model.
Table 2. The pseudo-first-order and pseudo-second-order kinetic constants for adsorption of Pb (II).

| Biosorbent | Experimental qₑₑₑₑₒₒₑₑₑₑ (mg g⁻¹) | pseudo-first-order kinetic | pseudo-second-order kinetic |
|------------|---------------------------------|-----------------------------|-----------------------------|
| ARSIBs     | 136.87                          | 1.15                        | 126.27                      |

Table 3. Langmuir and Freundlich isotherm constants for adsorption of Pb (II) on ARSIBs.

| Biosorbent | Langmuir’s constants | Freundlich’s constant |
|------------|----------------------|-----------------------|
|            | qₑₑₑₑₑₑₑₑₑₑ_max (mg g⁻¹) | Kₑₑₑₑₑ (L mg⁻¹) | R² | Kₑₑₑₑₑₑₑₑₑₑ (mg g⁻¹) | n | R² |
| ARSIBs     | 450                  | 0.0029               | 0.981 | 3.2780 | 1.3579 | 0.994 |

3.5. Analysis on the mechanism

3.5.1. FTIR analysis. FTIR couldn’t only be used to detect the functional groups present on the sorbent surface and their interaction with metal ions, but also it was useful for the characterization of biopolymers. The FTIR spectra of before and after sorption of Pb (II) on ARSIBs were showed in the interval 500 cm⁻¹-4000 cm⁻¹ in Figure 4.

Figure 4. revealed that anion functional groups (hydroxyl, amine) exist on the ARSIBs’ surface. A wide strong band at about 3230 cm⁻¹ may be due to the vibration stretching of the O-H bond and suggested that the presence of the hydroxyl groups or amine groups on ARSIBs. It had been reported that 3000 cm⁻¹-3400 cm⁻¹ were the band of -OH or –NH [13]. As showed in Figure 4 (a), the bands at 2920, 1660, 1420 and 1040 cm⁻¹ in the FTIR spectra referred to the bands of -CH, -COOH, -NH₂ and C-O-H, respectively. It has also been reported that the bands at 2946, 1635, 1455 cm⁻¹ had a certain relation with the vibration stretching of -CH, -COOH and -NH₂ stretch of sodium alginate [14]. The characteristic peak range of all known sugar be studied in 1000-1200 cm⁻¹, and 1038 cm⁻¹ were the vibration stretching of the C-O-H bond from sugar, which may contain the contribution of P-O-C vibration stretching.

Figure 2. Pseudo-second-order kinetic model for Pb (II) adsorption on ARSIBs at the initial concentration of Pb (II) 100 mg L⁻¹, pH 5, contact time 3 h and 30 ℃.

Figure 3. Freundlich plot for removal Pb (II) at pH 5, the volume of ARSIBs 1.2%, contact time 3 h and 30 ℃.
Figure 4. FTIR spectra before (a) and after (b) sorption of Pb (II) by ARSIBs.

Figure 4(b) indicated the FTIR spectra after sorption of Pb (II) by ARSIBs. The sorption peak at 2940 cm$^{-1}$ corresponded to stretching of -CH group shifted to 2920 cm$^{-1}$; 1660 cm$^{-1}$ corresponded to stretching of -COOH group shifted to 1630 cm$^{-1}$; 1440 cm$^{-1}$ corresponded to stretching of -NH group shifted to 1420 cm$^{-1}$; 1080 cm$^{-1}$ corresponded to stretching of C-O-H group shifted to 1040 cm$^{-1}$. In addition, the sorption peak at 3230 cm$^{-1}$ corresponds to -OH group or -NH group stretching appeared. The change in vibrational frequency of functional groups after sorption of Pb (II) shows that these groups were involved in the sorption process.

Figure 5. XRD patterns before (a) and after (b) sorption of Pb (II) by ARSIBs.

3.5.2. XRD analysis. The structural characteristics of ARSIBs were observed by XRD analysis. According to XRD analysis and results were matched in the nearest value of the characteristics of the peak by the JCPDS (joint committee on powder diffraction standards). Figure 5. shown XRD patterns of before (a) and after (b) sorption of Pb (II) by ARSIBs. For before sorption of Pb (II), the wide band at 2θ=45º belonged to NaCl structure in XRD spectrum. After sorption of Pb (II), a strong peaks produced at 2θ=22º in XRD spectrum, which can be attributed to lead phosphates (such as (PO$_4$)$_3$(OH)
and Pb(H₂Pb₅PO₄)₂). The result indicated that compounds of lead and phosphorus existed in the ARSIBs after sorption of Pb (II). The compounds might be produced in the sorption process by precipitation [15]. Therefore, the sorption mechanism of Pb (II) might be that Pb (II) was adsorbed on the surface of stains H-1 and ARSIBs by the functional hydroxyl and carboxyl groups, finally precipitated with phosphate based on the spectra of XRD.

4. Conclusions
Through systematic research, the major conclusions were summarized as follows:

1) The synthetic optimum conditions for ARSIBs were 4% sodium alginate, 75% bacterial suspension, 3.2% rubber powder and 24 h crosslinking time.

2) The adsorption optimum conditions for Pb (II) were 1.2% ARSIBs, 100 mg L⁻¹ initial concentrations, pH 5 and 3 h contact time.

3) The adsorption kinetics of Pb (II) could be well fitted by the second-order model.

4) The adsorption isotherm was better described by the Freundlich isotherm model.

5) The biosorption mechanism was that Pb (II) was adsorbed by the functional hydroxyl and carboxyl groups, finally precipitated with phosphate based on the spectra of FTIR and XRD, respectively.

This test showed that ARSIBs has not only better adsorption capacity for Pb (II) in wastewater but also the good characteristics of low-cost, preparation-easily, environment-friendly and reutilization. Furthermore, it has displayed a good application prospects in the heavy metal industrial wastewater. This study also provides the basis for other heavy metal wastewater treatment.

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References
[1] Wang F T, Pan Y F, Cai P X, Guo T X and Xiao H N 2017 Single and binary adsorption of heavy metal ions from aqueous solutions using sugarcane cellulose-based adsorbent Bioresource technology 241 482-490

[2] Lalhmunsiaama, Gupta P L, Jung H, Tiwari D, Kong S and Lee S 2016 Insight into the mechanism of Cd(II) and Pb(II) removal by sustainable magnetic biosorbent precursor to Chlorella vulgaris Journal of the Taiwan Institute of Chemical Engineers 000 1-8

[3] Liu S Q, Xu M, Yu T L, Han D, Peng J, Li J Q and Zhai M L 2017 Radiation synthesis and performance of novel cellulose-based microsphere adsorbents for efficient removal of boron (III) Carbohydrate polymers 174 273-281

[4] Wang B E, Hu Y Y, Xie L and Peng K 2008 Biosorption behavior of azo dye by inactive CMC immobilized Aspergillus fumigatus beads Bioreosource technology 99(4) 794-800

[5] Bayrampoglu G, Bektas S and Arica M Y 2003 Biosorption of heavy metal ions on immobilized white-rot fungus Trametes versicolor Journal of hazardous materials 101(3) 285-300

[6] Huang J F, Li Y T, Wu J H, Cao P Y, Liu Y L and Jiang G B 2016 Floatable, macroporous structured alginate sphere supporting iron nanoparticles used for emergent Cr(VI) spill treatment Carbohydrate polymers 146 115-122

[7] Ren H X, Gao Z M, Wu D J, Jiang J H, Sun Y M and Luo C W 2016 Efficient Pb(II) removal using sodium alginate–carboxymethyl cellulose gel beads: Preparation, characterization, and adsorption mechanism Carbohydrate polymers 137 402-409

[8] Bayramoglu G, Denizli A, Bektas S and Arica M Y 2002 Entrapment of Lentinus sajor-caju into Ca-alginate gel beads for removal of Cd(II) ions from aqueous solution: preparation and biosorption kinetics analysis Microchem Journal 72(1) 63-76
[9] Wei S T and Ting A S Y 2012 Efficacy and reusability of alginate-immobilized live and heat-inactivated Trichoderma asperellum cells for Cu (II) removal from aqueous solution. *Bioresource technology* **123**(123) 290-295

[10] Li J X, Hu J, Sheng G D, Zhao, G X and Huang Q 2009 Effect of pH, ionic strength, foreign ions and temperature on the adsorption of Cu(II) from aqueous solution to GMZ bentonite. *Colloids & Surfaces A Physicochemical & Engineering Aspects* **349**(1–3) 195-201

[11] Yahaya Y A, Don M M and Bhatia S 2009 Biosorption of copper (II) onto immobilized cells of Pycnoporus sanguineus from aqueous solution: equilibrium and kinetic studies. *Journal of hazardous materials* **161**(1) 189-195

[12] Jung W, Jeon B H, Cho D W, Roh H S, Cho Y, Kim S J and Lee D S 2015 Sorptive removal of heavy metals with nano-sized carbon immobilized alginate beads. *Journal Of Industrial And Engineering Chemistry* **26** 364-369

[13] Michalak I, Chojnacka K and Witek-Krowiak A 2013 State of the Art for the Biosorption Process—a Review. *Applied biochemistry and biotechnology* **170**(6) 1389-1416

[14] Fu F L and Wang Q 2011 Removal of heavy metal ions from wastewaters: A review. *Journal of environmental management* **92**(3) 407-418

[15] Wang Y Y, Yao W B, Wang Q W, Yang Z H, Liang L F and Chai L Y 2016 Synthesis of phosphate-embedded calcium alginate beads for Pb(II) and Cd(II) sorption and immobilization in aqueous solutions. *Transactions of Nonferrous Metals Society of China* **26**(8) 2230-2237