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Enhanced biological stabilization of heavy metals in sediment using immobilized sulfate reducing bacteria beads with inner cohesive nutrient

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HIGHLIGHTS

- Nutrient beads of immobilized SRB were more effective in transforming heavy metals into the more stable bound phases.
- Inner cohesive nutrient effectively promoted the stabilization process of heavy metals.
- The excellent removal efficiencies of Cu, Zn, Pb and Cd were 76.3%, 95.6%, 100% and 91.2%, respectively.
- Easy to recycle and avoid secondary pollution.

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ABSTRACT

A series of experiments were conducted for treating heavy metals contaminated sediments sampled from Xiangjiang River, which combined polyvinyl alcohol (PVA) and immobilized sulfate reducing bacteria (SRB) into beads. The sodium lactate was served as the inner cohesive nutrient. Coupling the activity of the SRB with PVA, along with the porous structure and huge specific surface area, provided a convenient channel for the transmission of matter and protected the cells against the toxicity of metals. This paper systematically investigated the stability of Cu, Zn, Pb and Cd and its mechanisms. The results revealed the performance of leaching toxicity was lower and the removal efficiencies of Cu, Zn, Pb and Cd were 76.3%, 95.6%, 100% and 91.2%, respectively. Recycling experiments showed the beads could be reused 5 times with superbly efficiency. These results were also confirmed by continuous extraction at the optimal conditions. Furthermore, X-ray diffraction (XRD) and energy-dispersive spectra (EDS) analysis indicated the heavy metals could be transformed into stable crystal texture. The stabilization of heavy metals was attributed to the carbonyl and acyl amino groups. Results presented that immobilized bacteria with inner nutrient were potentially and practically applied to multi-heavy-metal-contamination sediment.

1. Introduction

Contaminated sediments have been posing a threat on the aquatic environment in recent years. Particularly, heavy metal contamination is a great obstacle to the rapid economic development and people's life [1–3]. With the intensive industrialization and urbanization, some natural sources and anthropogenic activities, such as mining, agricultural drainage, industrial effluents and atmospheric deposition have led to heavy metal intrusion into the aquatic environment, which caused severe environmental destruction [4–6]. In addition, heavy metals are easily transported from the unsaturated zone into the bottom sediments through adsorption, flocculation and precipitation until the saturation of those adsorption sites [1,7,8]. It may be directly harmful to human health and ecosystem via the food chain, and deteriorate the quality of the environment [3,7]. Moreover, a considerable number of fine particles in the sediment influence the mobility of heavy metals and form stable complexes [9,10]. Therefore, finding an effective and

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thorough method is critical for dealing with heavy metal contamination of sediments.

Up to now, a wide variety of ways have been applied to attenuate the heavy metal contamination [9–11]. Some traditional technologies, e.g. ion exchange, chemical precipitation, electrochemical treatment, adsorption and bio-treatment, have been explored in the past few years, which have made some significant progress [12,13]. However, the existing conventional means failed to meet the strict requirements of environment and living quality due to the shortcomings inherent to these methods, including high-cost, and production of poisonous secondary pollutants [1,8,14–22]. On the other hand, bioremediation, a promising and practicable technology, has been recognized as an alternative favorable choice to remove heavy metals owing to its low operating cost, high efficiency, and environmental friendly [9,12,23].

It is reported that many microorganisms can adsorb and precipitate heavy metals in the literature [1]. Sulfate reducing bacteria (SRB), a group of anaerobic microorganisms, are able to reduce sulfates to hydrogen sulfide that can quickly react with heavy metals to form stable precipitate [3,24–28], and thus have been extensively used [25,28,29]. The relevant process can be expressed in Eqs. (1) and (2) [3]:

$$2CH_2O + SO_4^{2-} + 2H^+ = H_2S + CO_2 + 2H_2O \tag{1}$$

$$H_2S + M = MS(S) + 2H^+ \tag{2}$$

Where CH_2O represents organics; M represents the heavy metal ion. Nevertheless, the drawback is that high metal concentrations can inhibit the activity of SRB especially in the state of free cells [28,31], which may make the bio-precipitation process restricted [32]. Therefore, it encouraged researchers to further explore more valid solutions. Immobilized microorganisms have unique superiority compared with free cells: protecting the microorganism against harmful substances; maintaining high microbial activity; easy to separate; and better stability [33–36]. The choice of polymeric matrixes is vital for immobilization considering the catalytic activity, operational stability, mechanical strength and environmental friendliness [27]. Among those materials recommended for immobilizing SRB, sodium alginate (SA) and PVA are more popular because they are cheaper, less poisonous and better biocompatible [27,35]. PVA-sodium boric beads, an improved integrated technique, have been applied as the institute [33,37]. Nevertheless, to date few comprehensive studies have examined the applications of immobilized SRB with inner nutrients to remediating heavy metal-contaminated sediment.

As is known to all, the choice of nutrients is essential for SRB growth [29,30,34]. Sodium lactate was used as inner cohesive nutrient, which can ensure the high efficiency for SRB and avoid secondary organic pollution. In order to enhance the strength of the gel beads and stronger shock load, some auxiliary materials could be added. Active carbon (AC), a common and popular adsorbent, is used due to its large specific surface area, high porosity, porous structure, strong adsorption capacity [34,38–40]. It is believed that an integrated immobilized SRB with inner nutrient + PVA-sodium boric containing silica, calcium carbonate and AC approach is a preferred and promising idea.

In this study, we investigated the effectiveness and related mechanism of integrated approach in sediment treatment systems about stabilization ability of heavy metals and the regeneration of beads. The article was mainly focused on five parts: (1) preparation of immobilized beads under optimal condition; (2) metal speciation analysis under different treatments; (3) leaching toxicity of heavy metals in the batch experiment; (4) reuse of immobilized beads; and (5) underlying mechanisms.

2. Materials and methods

2.1. Cultivation of sulfate-reducing bacteria and preparation of experimental materials

The aquifer sediment samples used in this research were taken from Xiangjiang River sediment in Hunan province, China. Samples were stored in airtight plastic bags and some were passed through a 20-mesh sieve for analyses of physical and chemical properties, others were passed through a 100-mesh sieve for determination of heavy metal contents after removing large particles of matter, and then dried at 105 °C for 12 h.

The bacteria were obtained from State Key Laboratory at Hunan University in this study. SRB was cultivated in an airtight serum bottle at 37 °C on heterotrophic medium containing 0.5 g/L Na_2SO_4, 0.1 g/L $CaCl_2$, 2.0 g/L $MgSO_4$, 1 g/L NH_4Cl, 0.5 g/L K_2HPO4, 3.5 g/L sodium lactate, 1.0 g/L yeast extract, 0.5 g/L ferrous ammonium sulfate, 0.5 g/L ascorbic acid [41]. The initial pH was adjusted to 7.2. First, the solution was autoclaved at 121 °C for 20 min and cooled in the ultraviolet. Then SRB was inoculated in culture medium with the amount of 1%, which was purged for 10–15 min with N_2. The growth of SRB can be directly judged by the generation of black precipitates. The enriched SRB culture was obtained by repeating the process. Following cultivation, all operations were sterile and all chemicals were of analytical grade.

2.2. Immobilization of cells and preparation of beads

PVA and SA were added to deionized water and constant water bath was heated to 90 °C for 30 min till the solution was completely dissolved. Then AC, silicon dioxide and calcium carbonate were added slowly into the mixture with stirring constantly to ensure the homogeneity thoroughly when cooled to room temperature. The mixture was sterilized before adding bacterial suspension. The resulting mixture was injected into the saturated calcium chloride ($CaCl_2$) solution of boric acid drop by drop with a syringe and kept for 24 h to form gel beads. Then some beads obtained were soaked in sterile saline, others were immersed in nutrient-amended solution containing 4 g/L sodium lactate, and the operation can be carried out in constant temperature incubator. Subsequently the beads were washed with sterile saline several times to remove boric acid. Finally these beads were stored at 4 °C for subsequent experiments [14].

2.3. Sequential processing experiments with beads

All the above prepared beads were dehydrated by freeze-drying. The effects of different immobilized beads and biomass dosage were probed in pretreatment tests. To determine the optimal condition of beads, various types of beads were applied to experiments. In general, four different experiments were carried out (1) incubation with bio-beads with high nutrient (2) incubation with bio-beads (3) incubation with free bacteria cells (4) a control. Each experimental condition was done in triplicate to reduce the experimental error. Bio-beads and aquifer sediment samples were added to 150 mL serum bottles containing bacterial culture medium. All the materials (medium, serum bottles, aquifer sediment samples and glass beads) were autoclaved at 121 °C for 20 min. The experiment was carried out in a rotary shaker at 37 °C with a constant speed of 150 rpm. After the equilibrium, the beads were collected by filtering suspension, and the supernatant was separated by centrifugation at 3000 rpm for 20 min, the aquifer sediment samples also were collected in valve bags for further speciation extractions and analysis of heavy metals.

2.4. BCR continuous extraction and analysis of heavy metals

Determination of the total metal content cannot accurately evaluate the effects on biological activity. Consequently, a comprehensive knowledge of metal binding to sediment constituents is required to predict their bioavailability and mobility in the environment [13,17]. Sequential extraction is often used to explore the eco-toxicity and bioavailability of heavy metals by analyzing the relative speciation [42,43]. The samples by freeze-drying after reaction were directly used for the determination of heavy metals. 0.2 g lyophilized sample sieved through a 100 mesh nylon screen was put in a 50 mL PTFE centrifugal tube and successively extracted with different reagents to acquire four fractions: acetic acid fraction with 20 mL of 0.11 mol/L HOAc, reducible fraction with 20 mL of 0.1 mol/L $NH_2OH{\cdot}HCl$, oxidizable fraction with 8.8 mol/L H_2O_2 and 20 mL of 1 mol/L NH_4OAc, and residual fraction with 10 mL HCl, 5 mL HNO_3, 5 mL HF and 3 mL $HClO_4$. Concentrations of the heavy metals retained in the extracting solutions were determined by Flame atomic absorption spectrophotometer (PEAA700). Additionally, we examined the fractions of four heavy metals as a percentage of total concentration under optimal conditions in the present study. The tests were implemented in triplicate.

2.5. Leaching toxicity test

The leaching toxicity test was considered as an indication of the stable treatment efficiency for the contaminated material. The leaching toxicity test was operated to measure the stability of heavy metals and hazards to the environment. Specific method refers to Solid waste-Extraction procedure for leaching toxicity-Sulphuric acid & nitric acid method (HJ/T299-2007). The test sample was collected from the sequential processing experiments with beads.

2.6. Reuse of immobilized beads

Next, the feasibility of reusing immobilized beads was assayed by multiple treatment cycles. The tests were carried out under optimal conditions. Reuse of beads was carried out in a 50 mL medium containing 5 g sample in a rotary shaker at 37 °C with a constant speed of 150 rpm. Then the beads were separated by filtration and washed with sterile distilled water until the pH of solution was neutral and stored at 4 °C. The remained sample was digested by using the procedure of 2.4.

The repeated cycle tests were conducted in a 50 serum bottles. On the completion of one cycle, the beads were taken out and regenerated after washing with sterile water as previously described and used for the next cycle. The initial heavy metal concentration for each recycle cycle was set as background value of 5 g sample. Other experimental conditions were same as the previously described in Section 2.4. The tests were carried out in triplicate.

2.7. Performance test of immobilized beads

The diameter of beads was measured using venire calipers. A certain pressure with a clean glass was put on the beads to observe the damage of beads and qualitatively describe the mechanical strength. Some beads randomly chose were added to a flask containing distilled water and stirred by shaking at 150 rpm for 24 h, which was to quantitatively describe the mechanical strength. Several immobilized beads were immersed in red ink for 1 h. The beads mass transfer was calculated by observing the red ink into the beads.

Table 1
The level and value range of different factors.

Level	Level of factor A (%)	Level of factor B (%)	Level of factor C (%)	Level of factor D
One	8	1	0	20
Two	9	2	2%	30
three	10	3	4%	50

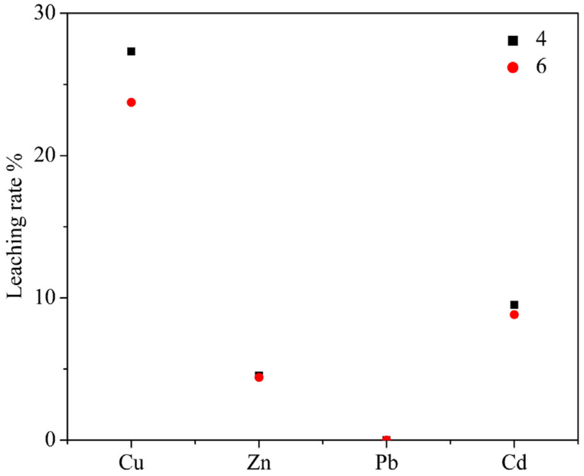

Fig. 1. The leaching rate of heavy metal under the treatment of two kinds of beads.

2.8. Related characterization of beads

The porous structure and surface morphology with loaded microorganism of gel beads were characterized using ESEM (FEI QUANTA 200), and functional group changes before and after treatment were characterized using Fourier transform infrared (FTIR, EQUINOX 55, Bruker, Germany) spectrophotometry. The freeze-dried beads were sputter-coated with gold for 90 s before observation. Surface elemental composition of the beads was determined using energy-dispersive spectroscopy (EDS, JSM-6700F) analysis. The lyophilized sediment samples were analyzed using X-ray diffraction (XRD, SIEMENSD 5005).

3. Results and discussion

3.1. Optimization conditions of immobilized beads

Different immobilized ways were evaluated with the test. The optimized embedding condition included mechanical strength, adhesive, and the mass transfer et al. The key four parameters were concentrations of PVA (A), SA (B), $CaCl_2$ (C), and the volume of bacterial suspension (D). The various beads were made based on the above criteria (Table 1). The features of different immobilized beads were listed in Table 2 after crosslinking coagulation for a period of time. The comparison among these immobilization methods as shown in Table 3 indicates what types of beads can be selected for the subsequent experiment. The optimal level analysis of every factor (Table 3) indicates two types of beads have advantage over others in general. The related components are experiment of 4 and 6. We then targeted the leaching rate of heavy metals as the main factor to further determine the optimal conditions of immobilized beads. The results of leaching rate for the four heavy metals are shown in Fig. 1, and the leach ability of four metals in the experiment of 4 was higher than in the experiment of 6. So the results (Fig. 1) show the optimal composition of immobilized beads is as follows: the mass fraction of PVA, SA and $CaCl_2$ is 9%, 3% and 2%, respectively.

Table 2
Comparison of immobilized beads' characteristics.

Experiment	Level of factor A (%)	Level of factor B (%)	Level of factor C (%)	Level of factor D	Bead strength	Bead shape	Bead adhesion	Bead mass transfer	The stable fraction of Cu (%)	The stable fraction of Zn (%)	The stable fraction of Pb (%)	The stable fraction of Cd (%)
1	8	2	2%	20	++	++	+	+	30.07	38.89	15.37	7.11
2	8	2	4%	20	++	++	+	++	31.18	39.78	13.98	6.53
3	8	2	4%	30	++	++	+	++	32.72	42.22	28.47	33.92
4	8	3	2%	30	++	+++	+	++	45.53	50.23	34.04	35.01
5	8	3	4%	30	++	+++	+	++	35.32	45.95	31.9	25.61
6	9	3	2%	20	+++	+++	+	+++	43.98	50.43	35.22	29.14
7	9	2	4%	50	+	++	+	+	29.88	37.58	18.17	4.79
8	9	2	2%	50	+	++	+	+	30.56	36.76	20.08	3.78
9	9	3	4%	20	+	++	+	+	31.67	37.9	22.90	6.36
10	10	1	2%	20	+	++	+	+	30.75	39.45	6.39	4.27
11	10	1	4%	20	+	++	+	++	31.85	38.34	10.57	2.68
12	10	2	2%	30	++	++	+	++	32.15	40.83	10.87	4.27
13	10	2	4%	30	++	++	+	++	32.37	41.57	10.41	5.41
14	10	3	2%	30	++	++	+	+	31.37	42.13	13.98	4.62
15	10	3	4%	30	+	++	+	+	30.78	40.31	10.77	4.27

+++, superb; ++, good; +, bad.

3.2. *Speciation analysis of heavy metals through sequential extraction*

Previous research has indicated the potential of immobilized beads to stabilize the heavy metals. In the untreated samples as a background control, concentrations of Cu and Pb were in the order of reducible fractions > residual fractions > acid-soluble fractions > oxidizable fractions, while Cd was mainly bound in acid-soluble fractions, and Zn was predominantly associated with the acid-soluble and the residual fractions. Speciation of these heavy metals in samples was significantly changed after different treatments. Compared with control, the acid-soluble fractions decreased (e.g. from 62% to 6.8% for Cd), while oxidizable fractions increased (e.g. from 10%-17% for Cd with inner nutrient beads). In addition, reducible fractions were approximately 50% in some metals such as copper, lead and cadmium before and after treatment (Fig. 2). Similarly, it was evident that there was an increase in the reducible fraction and a decline in the acid-soluble fraction compared with the control group.

In general, the stability of metal speciation is in the order of acid-soluble fraction < reducible fraction < oxidizable fraction < residual fraction [13]. What is more, the speciation of heavy metals is closely related to the eco-toxicity and bioavailability [44]. In other words, acid-soluble and reducible fractions represent direct eco-toxicity and mobility, the oxidizable concentration possess potential toxicity, while the residual fraction relates to no toxicity [44,45]. The decline of reducible fraction and the increase of oxidizable fraction were observed comparing the inner nutrient beads with the free cells in terms of Cu, Zn and Cd in this study. Taking Zn as an example, treatment with inner nutrient beads decreased the reducible fraction to 17% (33% for free cells), and increased the oxidizable fraction to 27% (11% for free cells). The oxidizable fractions of all

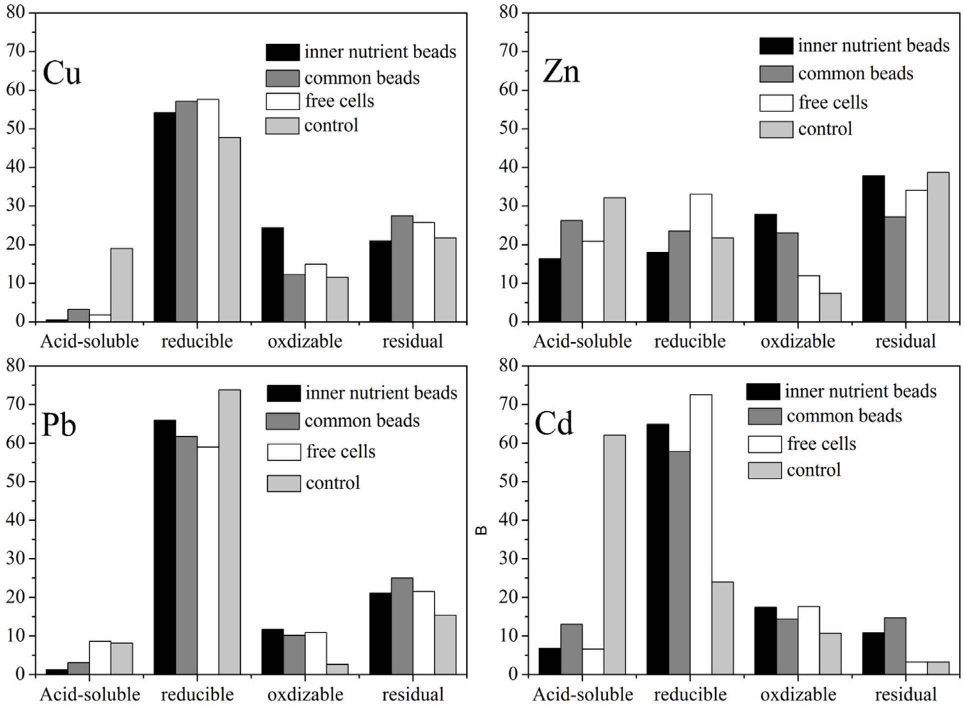

Fig. 2. Speciation of heavy metals in the treated sediment samples with different processing method.

Table 3
The analysis of optimal level of every factor.

	A (%)	B (%)	C (%)	D
Stable fraction of Cu (%)	8	3	2	30
Stable fraction of Zn (%)	9	3	2	20
Stable fraction of Pb (%)	9	3	2	20
Stable fraction of Cd (%)	8	3	2	30
Bead strength	9	3	2	20
Bead shape	9	3	2	20
Mass transfer	9	3	2	20

Table 4
FTIR peaks and assignments.

Peak position(cm^{-1})		Bond
1	2	
3741.285	3737.428	free O—H
3423.73	3416.92	O—H and N—H stretch
2934.55	2927.74	Asymmetric CH_2 stretch
2358.29	2351.47	C—C stretch vibration
1654.65	1635.37	C=O stretch
1426.87	1420.06	COO- and B—O stretch and CH bending vibration
1361.519	–	C=O stretch
1105.04	1085.35	C—O stretch
787.34	781.04	CH_2 wagging vibration and B-O stretch
690.07	682.23	CH_2 rocking vibration

four metals in inner nutrient beads were higher than the common beads, indicating the nutrient played a significant role in transforming heavy metal to more stable speciation and higher treatment efficiency. It was mainly due to the beads with inner nutrient provided good environment where protected SRB against heavy metal toxicity to a certain extent. In short, immobilization can form more stable precipitates in contrast with other controlled trials. It can be speculated immobilization promotes the production of stable metal precipitates and accelerates the process by the cohesion of nutrient.

3.3. *Characteristics of immobilized beads*

3.3.1. *Morphology analysis of immobilized beads using SEM and EDS*

The bio-beads were approximately 5 mm in diameter and almost were spherical. The scanning electron micrographs of the immobilized beads were depicted in Fig. 3(a–c). The beads had a rough and relative dense surface (Fig. 3a), improving stability and avoiding the violation from high heavy metal. And the beads formed a mesh-like porous with large and small pores (Fig. 3b), providing a convenient channel for matter transmission. In addition, bacteria were attached to the pore walls (Fig. 3c), meaning that sulfate-reducing enrichment culture could be embedded in the gel carrier successfully. On the other hand, the reticular structure had a huge specific surface area (Fig. 3b) providing more reaction sites, which promoted high efficiency between heavy metal and beads.

EDS analyses (Fig. 4) confirm copper, zinc, lead, cadmium and sulfur were present on the surface of the beads and hint the possible existence of sulfide precipitation. The presence of carbon, oxygen and silicon may be due to the organic matter itself or added [46].

3.3.2. *FTIR analysis*

Fig. 5 presents the FTIR spectra of beads before and after treatment. Table 4 revealed the complex changes of multiple functional groups. The characteristic broad peaks at 3741–3423 cm^{-1} were assigned to the O—H and N—H stretching vibrations [12]. The asymmetric stretching vibration and rocking vibration of CH_2 was observed at 2927 cm^{-1} and 680 cm^{-1}, respectively, which corresponded to the alkyl groups in proteins and aliphatic acid or CH_2

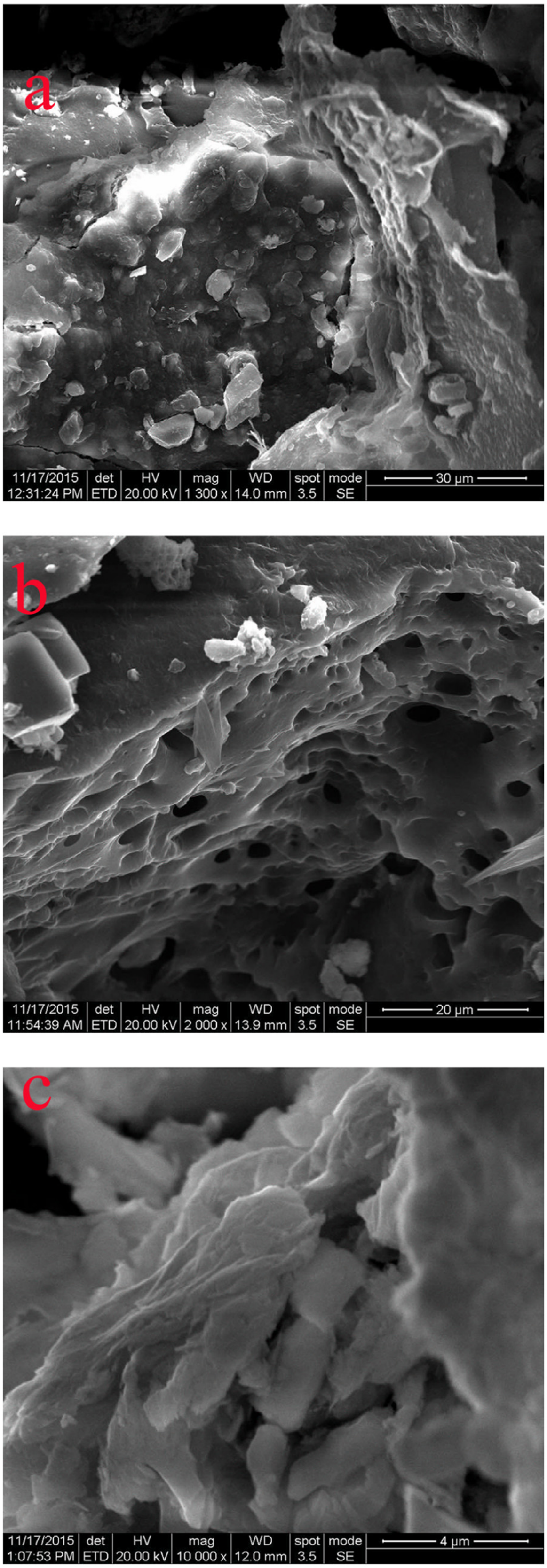

Fig. 3. SEM image of immobilized beads with inner nutrient. (a) surface of bead (b) inner structure of bead (c) bacteria.

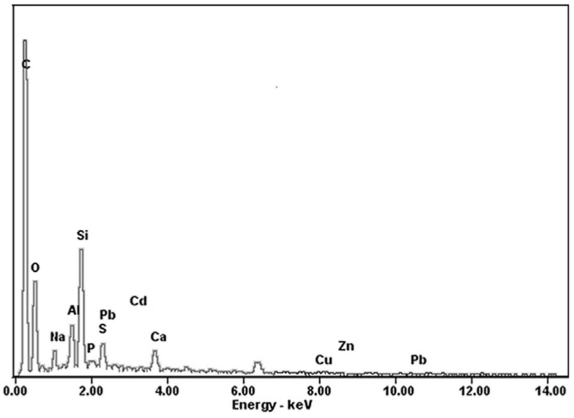

Fig. 4. EDS spectra of the immobilized beads after treatment.

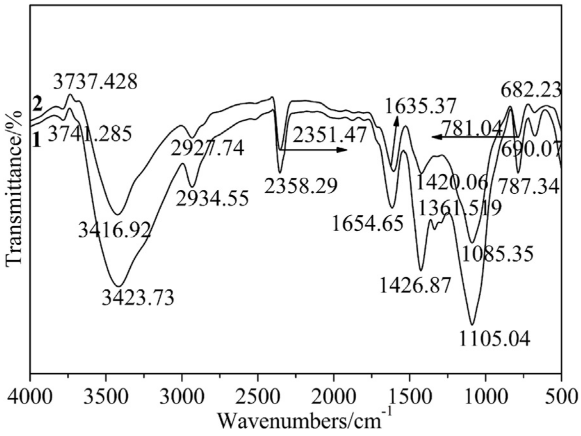

Fig. 5. FTIR spectrum of the immobilized beads before (1) and after (2) equilibrating with sediment samples containing heavy metals.

groups of PVA [7]. The bands were attributed to C—C stretching vibrations at 2351 cm^{-1}. A strong peak at 1635 cm^{-1} may indicate the C=O stretching vibration of the protein or amine [7]. The peak around 1420 cm^{-1} means the presence of carboxyl and the bending vibration consistent with C—H group, while that at 1085 cm^{-1} belonged to the C—O stretching vibration of polysaccharides. It was noticed that the characteristic B-O stretching was near 1420 cm^{-1} and 780 cm^{-1}. After the beads were exposed to the sediment containing heavy metals ions, the corresponding peaks shift in the FTIR spectral pattern was observed. The FTIR spectrum showed a significant change of the peak from 1085 cm^{-1} to 1105 cm^{-1}, marking involvement of C—O of the carboxyl groups in the metal ions binding. The peak shift from 1635 cm^{-1} to 1654 cm^{-1} indicates the involvement of carboxyl groups and amide bonds in the binding of the metal ions [7]. The peak shifts from 3737 cm^{-1} to 3741 cm^{-1}, and 3416 cm^{-1} to 3423 cm^{-1} also imply the involvement of the hydroxyl groups in metal binding [1,7,47]. As can be seen, the peak at 1361 cm^{-1} disappeared after treatment, probably due to the involvement of the C=O group of amino acids and the amine [7,47]. Thus, it is reasonable to assume the carboxyl, hydroxyl, acyl amino groups were the main functional groups in binding of metal ions.

3.3.3. The XRD analysis of metal precipitates

XRD results presented in Fig. 6 show the sediments were primarily composed of SiO_2, $CaCO_3$, CuS, ZnS, PbS, CdS, CuS_2 and PbS_2 (data not shown). On the one hand, the existence of SiO_2 and $CaCO_3$ was likely to the drop of the surface material in the beads; on the other hand it may be due to the incompletely washing in the process of making beads or via the pore into the sediment samples. The findings were in agreement with the results of EDS, which further proved metal ions precipitated with sulfide. As is conveyed by Fig. 6, the XRD patterns of beads with and without nutrients are very similar (the presence of same sulfide), in other words, it appears to expose a truth that both of approaches don't lead to the change of compound composition in the sediment samples but can produce different crystal texture [48]. Taking ZnS as an example, it was mainly in the form of sphalerite crystal structure based on the outcomes of XRD analysis. Approximately 19.4% ZnS content was observed at the samples with inner cohesive nutrient bead, while about 14.2% was observed with common immobilized bead in the form of sphalerite (data not shown), which testified the efficiency of the inner nutrient bead was higher than common bead. It is presumably the nutrient source is made full use [29].

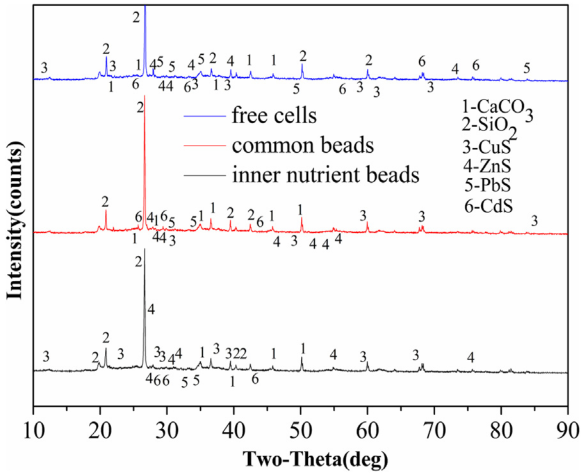

Fig. 6. XRD pattern of the precipitate after treatment by different methods.

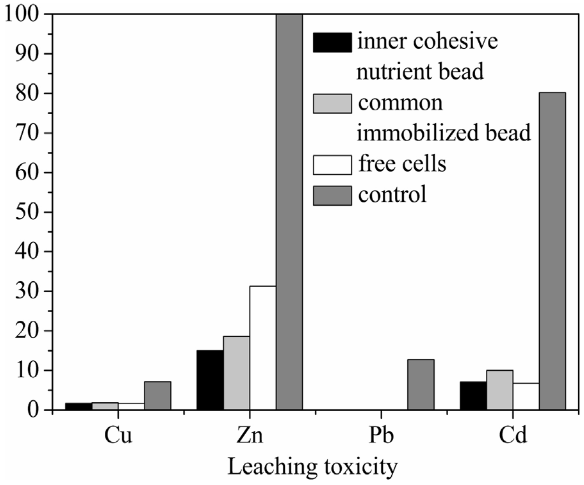

Fig. 7. The concentration of heavy metals leached from the treated sediment samples.

3.4. Leaching toxicity analysis

The leaching toxicities of Cu, Zn, Pb and Cd in treated sediment samples by immobilized beads were determined and the results are shown in Fig. 7. The leaching concentrations of Cu, Zn, Pb and Cd in untreated samples were 7.1 mg/kg, 339.6 mg/kg, 12.7 mg/kg and 80.2 mg/kg, respectively, while the leaching concentration in treated sediment samples by immobilized beads was lower than in

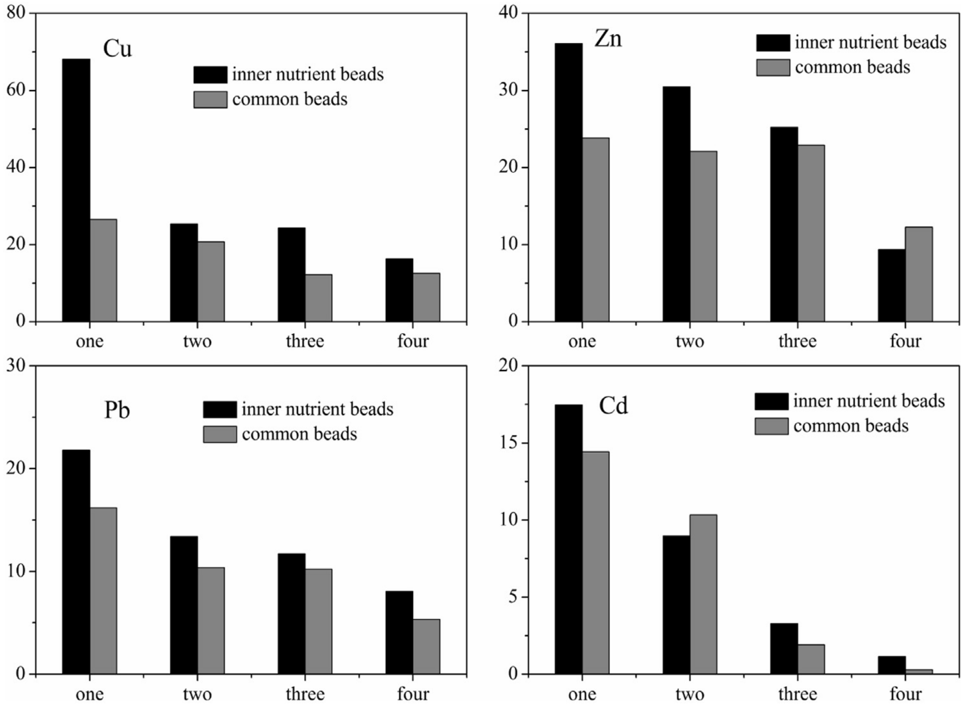

Fig. 8. Repeated experiments by immobilized beads. The beads can be reused for five times, and the content of oxidation states of the sediment samples was as a reference after each cycle.

the control; these results indicate the toxicity and risk to the environment were significantly decreased. A sharp decrease of leaching rate for heavy metals was observed in the treatment of beads, demonstrating excellent heavy metals stability, probably deriving from the formation of metal sulfides (Fig. 6), which are not prone to leaching [45,49]. In particular, the leaching activity of Pb was almost slight, meaning Pb in treated samples posed low toxicity and may be due to the Pb is one of the less mobile heavy metals and its compounds appear to accumulate in sediments [44,45,50]. The elements of Pb, and S were presented in Fig. 4, combining with the XRD pattern (Fig. 6), PbS was formed in the treated sediment samples. It is speculated the PbS does not dissolve in the acidified sediment environment, meaning that some of Pb ions are stabilized into the sediment in the form of PbS compound. The study illustrates Pb ions are easy to accumulate in the sediment, where S^{2-} ions fixes the Pb^{2+} ions as an insoluble matter. The above result is accordance with these obtained by Lao [50].The amount of Zn leached was higher under the same processing condition in comparison with other heavy metals. Compared with different treating system (inner nutrient bead, common bead, free cells), the leaching results from Fig. 7 showed the leaching value of each heavy metal was minimal, which proves the effectiveness of immobilization process.

It is generally accepted that increase in pH leads to the decrease of soluble metal [45,51]. According to the XRD patterns, the metal sulfides were presented in treated samples. These observations are in accordance with the results of heavy metal speciation (Fig. 2) where the acid-soluble fraction was declined and oxidizable fraction was increased after treatment. It is reported that the increase of pH can cause a decrease in the solubility of Pb [52]. It is speculated that the change of pH has a significant influence on the leaching pattern of metals. Additionally, these results may be explained by the fact the mobile and easily available heavy metals fraction were moved into the stable fraction and not prone to leaching [44,45].

3.5. Reuse of beads

Reutilization of the immobilized bead-an important characteristic, is superior to other method for the advantage of low cost, less material and saving time [37]. The results are shown in Fig. 8. It was evident that inner nutrient beads were more efficient than common immobilized beads on the whole. In the first cycle, the reaction time of the system was 5 day and there was no break at 150 rpm. However, the processing capacity of the beads declined gradually with the increase of the cycles no matter what kind of case in the following experiment, which may be caused by the break of the bead and substance leakage. The immobilized beads could be reused for 5 times while some beads were broken in the fourth cycle (data not shown). All these phenomena indicated the immobilized beads processed the proficient reuse property.

4. Conclusions

In this paper, immobilized SRB beads were successfully applied to bio-remediating heavy metal contamination in sediment. The nutrient source-sodium lactate, played an indispensable role in the stabilization of heavy metals. It was evident that the nutrient beads of immobilized SRB had an advantage over common beads and free cells and could achieve excellent remediation efficiency, which testified by the fact-the presence of different crystal texture and lower leaching. The optimal conditions were 9% PVA, 3% SA, 2% $CaCl_2$ and 20% cell suspension. Results showed that acid soluble fractions of Cu, Pb, Zn and Cd were decreased and oxidizable fractions were increased after treatment, which further indicated the immobilization could transform heavy metals into the more stable bound phases such as heavy metal sulfide minerals. The excellent efficiency and stability would provide a promising way to deal with heavy metal pollution in sediments. Further work should be carried out in a continuous test and fully assess regeneration of the beads and the stored heavy metals recover.

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