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Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat



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.

ARTICLE INFO

Article history:

Received 1 July 2016

Received in revised form 5 October 2016

Accepted 27 October 2016

Available online xxx

Keywords:

Sediment

Heavy metal contamination

Inner nutrient

Stability

Toxicity

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.

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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 activi-

ties, 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

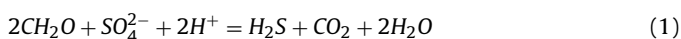
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thorough method is critical for dealing with heavy metal contamination of sediments.

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]:



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_2HPO_4 , 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.

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