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Preparation of metal-resistant immobilized sulfate reducing bacteria beads for acid mine drainage treatment

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HIGHLIGHTS

- Novel immobilized SRB bead was prepared to treat AMD.
- Partially decomposed maize straw as carbon source resulted in sulfate reduction.
- Microbial community included SRB and fermentative bacteria.

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ABSTRACT

Novel immobilized sulfate-reducing bacteria (SRB) beads were prepared for the treatment of synthetic acid mine drainage (AMD) containing high concentrations of Fe, Cu, Cd and Zn using up-flow anaerobic packed-bed bioreactor. The tolerance of immobilized SRB beads to heavy metals was significantly enhanced compared with that of suspended SRB. High removal efficiencies of sulfate (61–88%) and heavy metals (>99.9%) as well as slightly alkaline effluent pH (7.3–7.8) were achieved when the bioreactor was fed with acidic influent (pH 2.7) containing high concentrations of multiple metals (Fe 469 mg/L, Cu 88 mg/L, Cd 92 mg/L and Zn 128 mg/L), which showed that the bioreactor filled with immobilized SRB beads had tolerance to AMD containing high concentrations of heavy metals. Partially decomposed maize straw was a carbon source and stabilizing agent in the initial phase of bioreactor operation but later had to be supplemented by a soluble carbon source such as sodium lactate. The microbial community in the bioreactor was characterized by denaturing gradient gel electrophoresis (DGGE) and sequencing of partial 16S rDNA genes. Synergistic interaction between SRB (*Desulfovibrio desulfuricans*) and co-existing fermentative bacteria could be the key factor for the utilization of complex organic substrate (maize straw) as carbon and nutrients source for sulfate reduction.

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1. Introduction

Microbial treatment of acid mine drainage (AMD) by sulfate reducing bacteria (SRB) is an attractive alternative to traditional precipitation method (Boshoff et al., 2004;Bayrakdar et al., 2009; Kieu et al., 2011; Hao et al., 2014). The SRB method has been used to effectively treat AMD in many studies by using sulfidogenic bioreactor (Kaksonen et al., 2003a; Johnson and Hallberg, 2005; Viggi et al., 2010; Sahinkaya et al., 2011). The effects of various parameters including pH, carbon source, temperature, hydraulic retention time (HRT), COD/SO_4, and the inhibition of heavy metals on treatment efficiency have been intensively studied (Elliott et al., 1998; Jong and Parry, 2003; Kaksonen et al., 2004; Cabrera et al., 2006; Pruden et al., 2007). Among these factors, the toxicity of heavy metals and low pH are the crucial factors affecting the performance of SRB process (Sheoran et al., 2010; Hao et al., 2014). Therefore, it is essential for effective remediation of AMD to enhance the bioreactor tolerance to high concentrations of heavy metals and low pH.

In addition, the effectiveness in AMD treatment using biological sulfate reduction depends on the choice of suitable carbon source (Liamleam and Annachhatre, 2007). The main considerations in selecting carbon source include its effect on microbial activity and economic feasibility (Wakeman et al., 2010). In recent years, organic wastes (e.g. animal manure, wood chip, sawdust, leaf, spent mushroom compost and yard waste) have been used as promising alternatives to traditional carbon sources for SRB in AMD treatment

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because of their low cost (Chang et al., 2000; Gibert et al., 2004; McCauley et al., 2009; Wakeman et al., 2010; Choudhary and Sheoran, 2011; Márquez-Reyes et al., 2013).

Maize straw is an available organic substrate abundant in mining area, which is low cost and degradable under anaerobic condition. The degradation products of complex organic waste can be utilized as carbon and electron sources for SRB (Costa et al., 2009; Wakeman et al., 2010). Moreover, maize straw can provide physical carrier for bacteria and adsorb heavy metal ions, which may be a promising additive to provide suitable environment for SRB growth when AMD influent flowed through the sulfidogenic bioreactor.

In the present study, to enhance tolerance to high concentrations of heavy metals and low pH, novel immobilized SRB sludge beads (the mixture of SRB, maize straw, zero valence iron, silicon sand, polyvinyl alcohol (PVA) and sodium alginate) were prepared for synthetic AMD treatment with high concentrations of heavy metals at low pH. The tolerance of immobilized SRB beads to heavy metals was evaluated and compared with that of suspended SRB. The suitability of maize straw as carbon source for AMD treatment was also evaluated to provide basic data for large scale application. Since the process performance of the bioreactor is highly dependent on microbial community composition and activity, the microbial community in the bioreactor was characterized using denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified bacterial 16S rDNA genes.

2. Materials and methods

2.1. Maize straw and SRB sludge

SRB were isolated from excess sludge from one wastewater treatment plant in Jinan, China. Maize straw was collected from a farm in Weifang city, which was partially decomposed under field conditions for over half a year. Maize straw was air dried, smashed into powder and then placed in a sealed plastic bottle with modified Postgate C medium (KH_2PO_4 0.5 g/L, NH_4Cl 1 g/L, Na_2SO_4 4.5 g/L, $CaCl_2 \cdot 6H_2O$ 0.06 g/L, $MgSO_4 \cdot 7H_2O$ 0.06 g/L, sodium lactate 6 g/L, yeast extract 1 g/L, $FeSO_4 \cdot 7H_2O$ 0.25 g/L, sodium citrate$\cdot 2H_2O$ 0.3 g/L) for SRB sludge cultivation with inoculums of SRB under the anaerobic condition (Pagnanelli et al., 2012). The enriched SRB sludge was formed within 14 days suggested by the formation of black precipitate, black lead acetate testing paper and the smell of H_2S. The formed SRB sludge was used for preparation of immobilized SRB beads after centrifugation at 5000 *g* for 5 min.

2.2. Preparation of immobized SRB beads

Immobilized SRB sludge beads were prepared by the mixture of SRB sludge, maize straw powder, zero valence iron (ZVI), silicon sand, polyvinyl alcohol (PVA) and sodium alginate. ZVI powder was used as a reducing agent to enhance SRB activity in AMD treatment (Bai et al., 2013). Maize straw can provide the biomass carrier and carbon source for microbial growth, and protect SRB from the toxicity of heavy metals due to its high adsorption capacity (data not shown). Silicon sand was used to increase the density and compression strength of the formed beads. PVA and sodium alginate were used as gel matrix for the immobilization of SRB sludge (Min et al., 2008; Hsu et al., 2010).

PVA (50 g) and sodium alginate (5 g) was heated in deionized water (500 mL) until dissolved, air cooled (40–50 °C) and then mixed with concentrated SRB sludge (150 g), fine silicon sand (15 g, grain size of 1 mm and purity of 98.3%) and ZVI powder (15 g, purity of 98%). The cell mixture was added drop-wise into crosslinker (saturated boric acid and calcium chloride) using a peristaltic pump and stirred using a magnetic stirrer at 120 rpm for 60 min to form spherical beads. The formed beads were immersed in sodium phosphate solution (0.5 M) for 60 min for hardening, flushed three times with deionized water and then put in the refrigerator for preservation. The diameter of the formed beads was about 3–5 mm.

2.3. Comparsion of tolerance between SRB-beads and suspended-SRB treatments to heavy metals

The tolerance of SRB-beads and suspended-SRB treatments to heavy metals was conducted in 100 mL sealed plastics bottles at 30 °C. SRB beads or suspended SRB inoculum (2% inoculum) were transferred into the bottles with Postgate C medium (containing 50 mg/L Fe(II) and 3354 mg/L sulfate). Two series of batch tests were carried out as follows: (1) the solution contained Cu 10, 20 and 35 mg/L, respectively, to compare the tolerance of the two inoculums to Cu; (2) The solution contained Cd 15, 30, and 49 mg/L, respectively, to compare the tolerance of the two inoculums to Cd. The initial pH of all treatments was adjusted at 5.5. All bottles were sealed to maintain anaerobic conditions at 30 °C. Finally, samples were collected for determination of sulfate and metals concentrations. This batch test was conducted in four replicates.

One-way analysis of variance (ANOVA) based on Fisher LSD test was used to evaluate the differences in sulfate and metals removal between the SRB beads and suspended SRB treatments for each initial concentration using SPSS 16.0. The probability level of $p < 0.05$ was regarded as statistically significant.

2.4. Upflow anaerobic bioreactor test

A glass column (5 cm diameter and 40 cm length) was used as the up-flow anaerobic packed-bed bioreactor, which was filled with immobilized SRB beads in the middle of bioreactor. The schematic diagram of the bioreactor is shown in Fig. 1. 35 g of partially decomposed maize straw was packed on the bottom of SRB beads to provide carbon source. Silicon sand (particle diameter 1–2 mm)

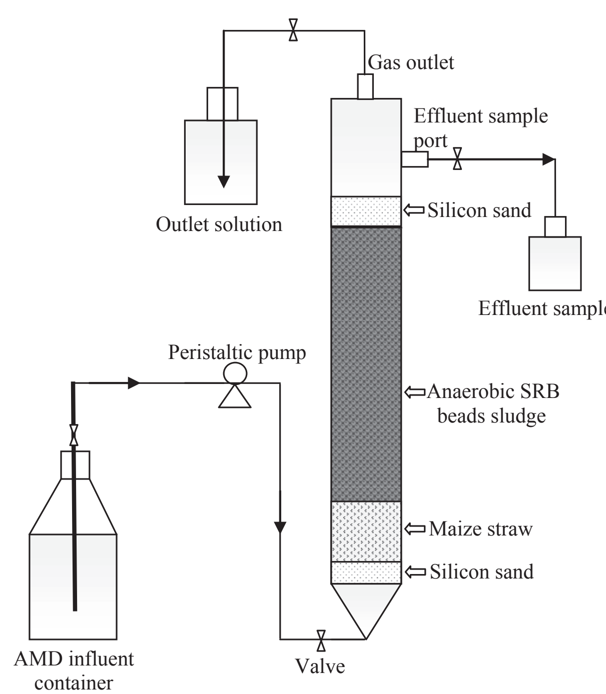

Fig. 1. Schematic diagram of the upflow anaerobic bioreactor.

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