



Characterization of biological iron sulfide composites and its application in the treatment of cadmium-contaminated wastewater

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Abstract

A strain of sulfate reducing bacteria, which could generate biological iron sulfide composites, was applied for Cd (II) removal from wastewater. The influence of biological iron sulfide composites dosage, initial pH and temperature on the rate of Cd (II) removal from wastewater by biological iron sulfide composites were investigated. The microscopic morphological characteristics and elemental composition changes of the biological iron sulfide before and after treatment was compared, using scanning electron microscopy and energy-dispersive spectrometry, then the mechanism of Cd (II) removal was explored. The results showed that Cd (II) reduction rate increased with increase in dosage of biological iron sulfide composites and initial temperature. At 25°C, pH 4.0, with dosage of sulfide and cadmium ions molar equal to 99.93% Cd (II) was removed from cadmium wastewater (100 mg l⁻¹), and the residual concentration of cadmium reached Chinese Integrated Wastewater Discharge Standard (GB8978-1996). The present study revealed that bio-precipitation of FeS, the main component of biological iron sulfide composites, played leading role in the process of Cd (II) reduction. Therefore, it is prospective to apply biological iron sulfide composites in the emergency treatment of cadmium-contaminated wastewater.

Key words

Biological iron sulfide composites, Bio-precipitation, Bio-sorption, Cadmium-contaminated wastewater, Energy-dispersive spectrometry

Introduction

Cadmium had been listed as one of the twenty priority pollutants by the United States Environmental Protection Agency. Several studies have demonstrated its carcinogenic, mutagenic and teratogenic effects on organisms (Dias *et al.*, 2013; Singh *et al.*, 2013). If cadmium is released into groundwater, it will produce long-term toxic effects on humans and environment (Kamashwaran and Crawford, 2001). Cd relatively occurs at low concentration in the natural environment. However, Cd emission due to human activities, mostly mining activities, burning of fossil fuels, increase of metallurgical industries and intensive use of fertilizers, made Cd pollution in water an urgent problem needing to be dealt with immediately (Singh and Agrawal, 2007). Some chemicals, such as lime, sodium hydroxide are used to remove heavy metals. But cadmium ions starts precipitating only when pH is above 9 (Burnett

et al., 2002). Adjusting pH after removal of cadmium increases the treatment cost. Since hydroxide precipitation is affected by pH value of water, pH value directly affects the release of heavy metals in sediment re-suspension. Atkinson *et al.* (2007) study indicated that under high (8.1±0.2) and mid pH (7.2±0.2) no metals are released from sediments. But at low-pH (6±1) more metals are released. Other methods, ion exchange or electrochemical, are more expensive and unfriendly to environment. Besides, removal rates of these methods depend on the concentration of metals and may produce waste sludge which needs further treatment (Costa *et al.*, 2008; Crini, 2006). Therefore, environmentally compatible and cost effective biological methods are employed for the treatment of effluents (Pagnanelli *et al.*, 2010).

Sulphate reducing bacteria (SRB) are diverse and presented in various habitats including sediments (Bert *et al.*,

2009). Compared with other microorganisms which may promote immobilization of heavy metals, SRB is a better candidate. SRB typically oxidize organic compounds, using sulphate as their terminal electron acceptor (Long *et al.*, 2010). Hydrogen sulfide produced by sulphate-reducing bacteria can precipitate metal ions, which has been proposed as an alternative process to dispose wastewater containing heavy metals (Foucher *et al.*, 2001). It is known that hydrogen sulfide can have adverse effect on humans and environment. However, it will be less harmful in the form of sulfur iron. Watson *et al.* (2000) found that SRB could produce *in-situ* nano-sized sulfur iron particles. It is able to stably precipitate heavy metals at low concentration (2.2 mg l^{-1}) in sulfide form due to its low solubility and thus biological iron sulfide composites is considered as an excellent treating product (Blowes *et al.*, 2000). Xie *et al.* (2013) found that biological iron sulfide composites can tolerate and process 600 mg l^{-1} of Cr (VI) after treatment with 1650 mg l^{-1} Cr (VI). Furthermore, biological iron sulfide composites can be regenerated in a suitable condition.

In this paper, a strain of sulphate-reducing bacteria, which highly yielded biological iron sulfide composites was used to remove of Cd (II) from wastewater. The influence of dosage, initial pH and temperature were studied. Using scanning electron microscopy and energy-dispersive spectrometry (SEM-EDS), the mechanism of biological iron sulfide composites removing Cd (II) was investigated.

Materials and Methods

Source of strain : A pure strain of *Clostridium sp.* YY (srb4) (KJ190021) was isolated from the sludge of a coal washing plant in Taiyuan, Shanxi Province. This strain belonged to sulphate reducing bacteria and could produce biological iron sulfide composites.

Cultivation and observation : sulphate reducing bacteria were cultured in a modified Starkey medium which contained $0.5\text{g K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, $0.1\text{g CaCl}_2 \cdot 2\text{H}_2\text{O}$, $2.0\text{g MgSO}_4 \cdot 7\text{H}_2\text{O}$, $1.0\text{g NH}_4\text{Cl}$, 25.0ml sodium lactate (70%), 15.0g yeast extract and 1.0g NaCl per liter of water. After being sterilized in 121°C for 20 min, $15\text{g FeSO}_4 \cdot 7\text{H}_2\text{O}$ was added into the culture medium through sterile filter and pH was adjusted to 7.4.

The strain was cultured in an anaerobic bottle in an anaerobic tank (BacBasic, ShelLab) at 34°C for 10 days. At the bottom of the anaerobic bottle, there was a thick layer of black precipitate (biological iron sulfide composites). In the present study effective content of biological iron sulfide composite was determined, using sulfide concentration as marker. The sulfide concentration was $1152.023 \text{ mg l}^{-1}$.

In order to observe the biological iron sulfide composites, 4% glutaraldehyde was used to fix the protein (or lipid) in cells. The precipitate was then re-suspended in 0.1M phosphate buffer

saline (PBS). The composites were washed thrice to remove the culture medium and glutaraldehyde thoroughly. The cells were then dehydrated in a series of ethanol with increasing concentration (40%, 70%, 90% and 100%). After thorough drying, they were analyzed using a Jeol JSM 7500 scanning microscope combined with an Oxford Instruments INCA EDS suite.

The factor of biological iron sulfide composites dosage : The effect of biological iron sulfide composites dosage on Cd (II) treatment by biological iron sulfide composites was determined through a series of batch experiments, with the following molar ratios of sulfide and cadmium: 0.5:1, 0.8:1, 1:1, 1.2:1, 1.5:1, 1.8:1, respectively. Each test was performed in triplicate and average values were recorded. The experiment was performed at 25°C , pH 4.0 with initial metal concentration of 100 mg l^{-1} , 50 ml for 10 min. Cadmium in the supernatant was measured by flame atomic absorption spectroscopy (AAS, HITACHI Z-2300).

Factor of initial pH : Tests used an apparatus which applied sulfide iodometric method (HJ/T60-2000) as a reaction system. The reaction system was thoroughly mixed via nitrogen gas stream. The sulfide was also protected and prevented from being oxidized. A series of batch experiments were performed with initial metal concentration of 100 mg l^{-1} for 10 min at 25°C and molar ratio of sulfide and cadmium 0.8:1. Different pH values each 50 ml wastewater, were initially adjusted to set the pH ranging from 2 to 8. Since there was only one set of apparatus, the experiment was done from pH 8 to 2. Each test was performed in triplicate and average values were recorded. When the test was over, concentration of supernatant sulfide were determined in both absorption bottles and reaction flask, while iron concentration of the supernatant, in the reaction flask, were determined to study the treatment effect of cadmium-containing wastewater by biological iron sulfide composites at different initial pH. Metals in the supernatant were measured by flame atomic absorption spectroscopy (AAS, HITACHI Z-2300). sulfide was determined by methylene blue spectrophotometric method.

Temperature factor : The effect of temperature on treatment of Cd (II) by biological iron sulfide composites was determined through a series of batch experiments, with temperature ranging from 5°C to 35°C . For the test with a temperature of 5°C , both wastewater and biological iron sulfide composites were precooled in 5°C refrigerator, until temperature of the experiment samples was stable. For remaining tests with 15, 25 and 35°C temperature, the temperature of the experiment samples was also adjusted in the incubator with a set temperature. The experiment was done with 50 ml wastewater (pH 4.0). The molar ratio of sulfide and cadmium was 0.8:1, an initial metal concentration at 100 mg l^{-1} for 10 min. Cadmium concentration in the supernatant was measured by flame atomic absorption spectroscopy (AAS, HITACHI Z-2300).

Removal of cadmium by biological iron sulfide composites: In order to explore the mechanism of Cd (II) removal by biological

iron sulfide composites, the morphological characteristics and elemental composition changes of the biological iron sulfide composites were studied before and after the treatment by SEM-EDS.

Data analysis : One-way analysis of variance (ANOVA) was carried out, using SPSS 16 Software. $P=0.05$ was selected as significance level.

Results and Discussion

Scanning electron micrographs showed that the sulphate reducing bacteria was found to be rod-shaped and slightly curved, with both ends rounded. The size was approximately $0.2\text{--}0.3 \times 1\text{--}3 \mu\text{m}$, and the cell was wrapped by a thick layer of iron-sulfur compounds. The biological iron sulfide composites looked like a beautiful flower-shaped crystal, under scanning electron microscope (Fig. 1).

Watson *et al.* (2000) found the bacterial surface was wrapped by a layer of about $0.1\mu\text{m}$ -thick sulfur iron precipitation. The sulfide concentration was approximately 100 mg l^{-1} (no more than tenth of the biological iron sulfide composites) and the morphology looked similar to flakes, due to limitation of TEM. In scanning electron micrographs, the surface of biological iron sulfide composite could be clearly observed. Using energy-dispersive spectrometry (EDS) composition analysis (Fig. 2), it was found that substances were mainly Fe and S. The iron-sulfur ratio was 0.97, which further confirmed that this crystal was biological iron sulfide composites produced during the growth of SRB. During formation of sulfide ores, pyrrhotite was present. The number of iron atoms in its molecule was usually less than sulfur atom, and therefore the formula was written as Fe_{1-x}S , with x between 0 and 0.2 (Klein and Hurlbut, 1993). In the present study, x was 0.03, indicating that the main component of biological iron sulfide composites was FeS .

The effect of biological iron sulfide composites dosage on Cd (II) removal is shown in Fig. 3 ($P < 0.001$). At constant pH and initial metal concentration, increasing the amount of material, led to more effective removal of Cd (II). With the dosage that the molar ratio of sulfide in the biological iron sulfide composites and cadmium is 1:1, 99.93% Cd (II) was removed from wastewater (100 mg l^{-1}). Furthermore, the residual concentration of cadmium was reached Integrated Wastewater Discharge Standard (GB8978-1996). When the dosage was greater than 1:1, the removal rate reached 100%.

It might be, because, at a fixed initial metal concentration, increasing biological iron sulfide composite dosage provides more surface area or number of available sites for metal ions to attach to (Jong *et al.*, 2004). Sekomo *et al.* (2012) assessed Cd removal in an up-flow anaerobic packed bed reactor filled with porous volcanic rock as an adsorbent and an attachment surface for sulphate reducing bacterial growth. Two different feeding

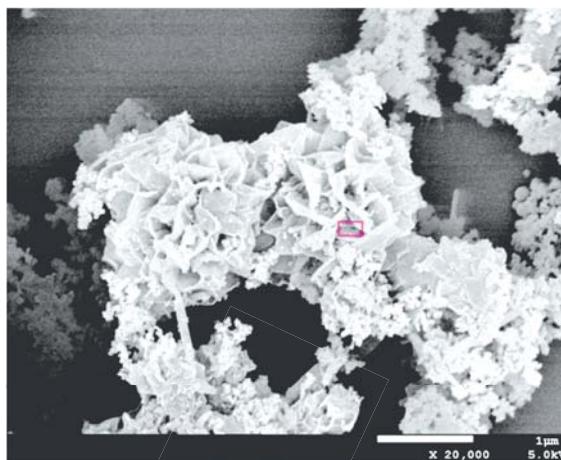


Fig. 1 : Scanning electron micrograph of biological iron sulfide composites

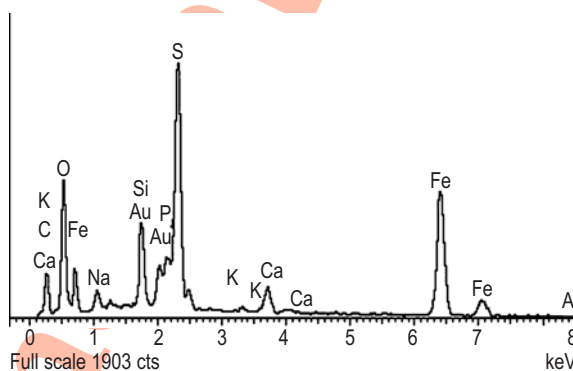


Fig. 2 : EDS spectrum of biological iron sulfide composites

regimes were applied using low (5 mg l^{-1}) and high (10 mg l^{-1}) level of metal wastewater. Overall metal removal efficiency at the outlet of integrated system was 98%. Villa-Gomez *et al.* (2011) explored the location of metal precipitates within sulphate-reducing inversed fluidized bed (IFB) reactors and when 10 mg l^{-1} of Cd was added the Cd removal efficiency reached 97.9%. Malakahmad *et al.* (2011) evaluated the performance of a lab-scale sequencing batch reactor (SBR) to treat synthetic petrochemical wastewater containing mercury and cadmium. The SBR was able to remove 96 ~ 98 % of Cd^{2+} from $15.52 \pm 0.02 \text{ mg l}^{-1}$ Cd water. These studies concluded that biological iron sulfide composites was not only suitable for the treatment of high concentration of cadmium, but also a fast and efficient material for metal removal.

Fig. 4 shows the removal efficiency of Cd (II) by biological iron sulfide composites as a function of initial pH value ($P < 0.001$). Removal efficiency of heavy metals increased rapidly within a narrow range of pH 2-3, reaching peak at pH 4 ($99.55 \pm 0.03 \%$), and then decreased slightly. Both sulfide concentration in absorption bottle and iron concentration in reaction flask decreased when the initial pH increased (Fig. 5 and Fig. 6; $P <$

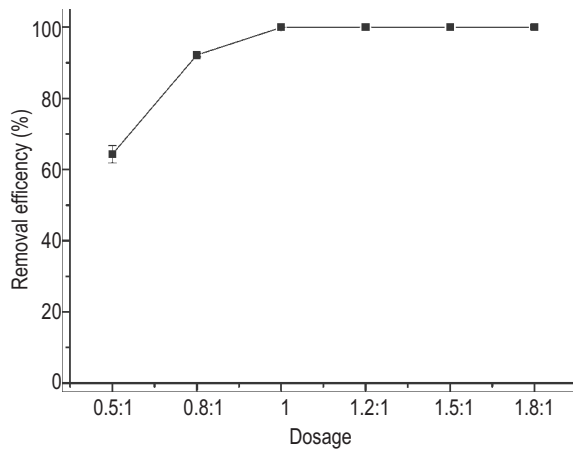


Fig. 3 : Effect of biological iron sulfide composites dosage on Cd (II) removal

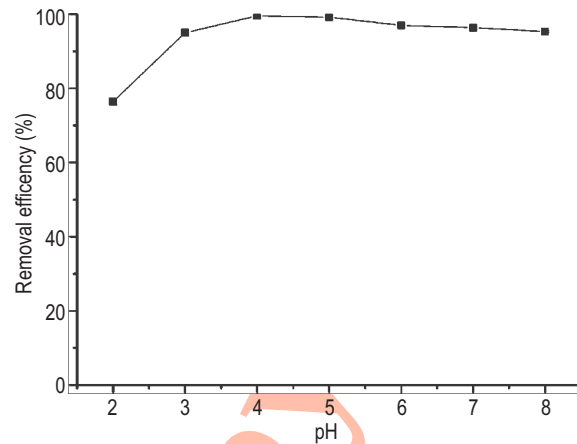


Fig. 4 : Removal of Cd (II) by biological iron sulfide composites as a function of initial pH

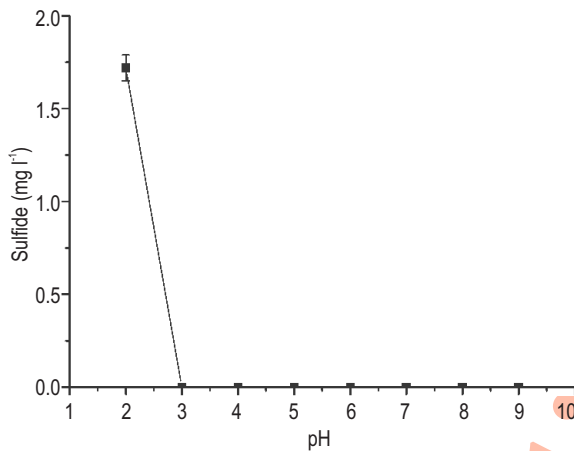


Fig. 5 : Sulfide concentrations in absorption bottle with different initial pH

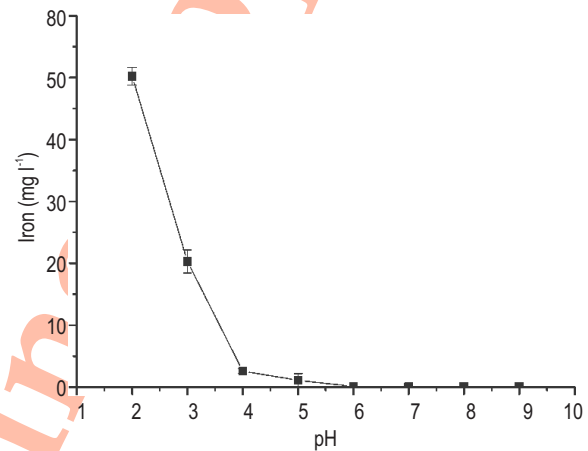


Fig. 6 : Iron concentrations in reaction flask with different initial pH

0.001). As a small amount ($1.72 \pm 0.14 \text{ mg l}^{-1}$) of sulfide escaped from the reaction system at pH 2 (Fig. 5), treatment of metal wastewater was less effective at pH 2 ($76.47 \pm 1.01 \%$). Iron began to precipitate when pH was greater than 2.5 (Montes-Hernandez *et al.*, 2013), whereas iron existed in ion form at pH 2. So a large amount of Fe ions ($50.23 \pm 1.43 \text{ mg l}^{-1}$) were detected in the supernatant (Fig. 6). This suggested that biological iron sulfide composites could remove cadmium by exchange of iron with cadmium. Biological iron sulfide composites could still achieve good removal efficiency under neutral conditions and the pH of reaction system remained neutral after Cd removal, which avoided adjusting the pH. Sulfide in the reaction bottle was not detected at different pH, so it did not cause secondary pollution to water bodies.

Fig. 7 shows the removal efficiency of Cd (II) by biological iron sulfide composites as a function of temperature ($P < 0.001$). The results showed that at pH 4.0, the temperature had a

significant effect on cadmium removal. Higher the temperature, greater was the removal efficiency. At 35°C , the biological sulfur iron composites could remove 95.29% cadmium from wastewater, while at 5°C only 80.38% was removed by the same insufficient dosage. At lower temperature, the metal removal association with exchangeable fraction appeared to be weak. This ion exchange bond was typically considered to be an endothermic reaction, which would support the results reported in this study (Champagne *et al.*, 2008).

As seen from Fig. 8, the surface of biological iron sulfide composites showed no obvious flower-shaped crystals after cadmium treatment. Compared with the extracellular thick layer of iron-sulfur compounds in Fig. 1, the cell surface became relatively smooth after treatment. Comparing Fig. 2 and Fig. 9, the morphological results showed that cadmium significantly increased while was significantly reduced. The ratio of iron cadmium and sulfur was 0.98.

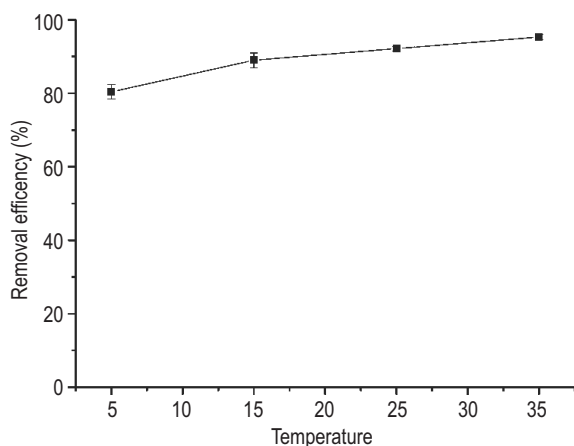


Fig. 7 : Removal of Cd (II) by biological iron sulfide composites as a function of temperature

The morphological results further confirmed the effect of cadmium removal by biological iron sulfide composites. The mechanism underlying was explored, SRB had the ability to reduce sulphate to sulfide and sulfide reacted with certain metals dissolved, such as copper, iron and zinc, forming insoluble precipitates (Benedetto *et al.*, 2005). There was a dissolution equilibrium existing in FeS, which was the main component in the biological iron sulfide composites produced by SRB: $\text{FeS} = \text{Fe}^{2+} + \text{S}^{2-}$, $K_{sp}(\text{FeS}) = 3.2 \times 10^{-18}$. Whereas, the solubility product constant of CdS, that is $K_{sp}(\text{CdS}) = 7.9 \times 10^{-27}$, is nearly 10^9 times smaller. When the solution contained Cd^{2+} , the S^{2-} and Cd^{2+} in the dissolution form the acid precipitation product of CdS. with the hydrolysis conditions ignored. So the biological iron sulfide composites removed cadmium mainly through bio-precipitation by generating more insoluble CdS than FeS through biochemical process by SRB. As iron cadmium ratio was 0.27, this indicated that addition to the role of the main component in the biological iron sulfide composites, FeS, there were other auxiliary ways to remove cadmium. Pagnanelli *et al.* (2010) concluded that removal of cadmium can be done by both bio-precipitation and bio-sorption of SRB. White *et al.* (2003) found that SRB biofilms

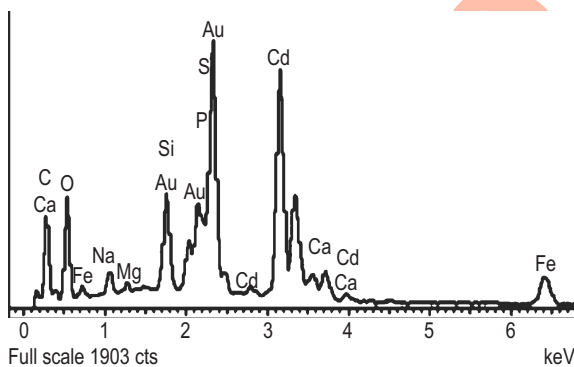


Fig. 9 : EDS spectrum of biological iron sulfide composites after treatment of Cd (II)

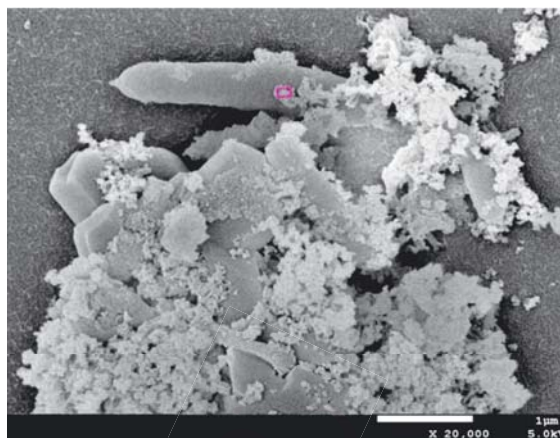


Fig. 8 : Scanning electron micrographs of biological iron sulfide composites after Cd (II) treatment

grown with $200 \mu\text{M}$ Cd in a stirred-tank bioreactor accumulated CdS and their protein and polysaccharide content simultaneously increased, indicating that sulfide precipitation and entrapment by extracellular polymers (EPS) occurred. Liu *et al.* (2011) found that amide groups in the hydrophobic end of the phospholipids and hydrophobic end were damaged and C–H of the fatty-tail structure also changed after SRB cell exposure to Cd (II), using attenuated total reflection fourier transformed infrared spectroscopy (ATR-FTIR). Besides, Jong and Parry (2004) reported that SRB surfaces were hydrated in aqueous solution by water, producing surface hydroxyl (–OH) groups. This was followed by a ligand exchange reaction between the surface hydroxyl groups and metal-absorbing ions, leading to the formation of inner-sphere metal complexes. Many studies have suggested that EPS and other biomolecules produced by free-living or biofilm SRB might bind to soluble toxic metals (Nirmal Kumar and Cini Oommen, 2012; Chhikara *et al.*, 2010; Kiran *et al.*, 2008). Above all, cadmium removal was under the combined function of both bio-precipitation and bio-sorption of SRB.

Acknowledgments

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