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Bioremediation of cadmium by growing *Rhodobacter sphaeroides*: Kinetic characteristic and mechanism studies

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Abstract

The removal kinetic characteristic and mechanism of cadmium by growing *Rhodobacter sphaeroides* were investigated. The removal data were fitted to the second-order equation, with a correlation coefficient, $R^2 = 0.9790$–0.9916. Furthermore, it was found that the removal mechanism of cadmium was predominantly governed by bioprecipitation as cadmium sulfide with biosorption contributing to a minor extent. Also, the results revealed that the activities of cysteine desulfhydrase in strains grown in the presence of 10 and 20 mg/l of cadmium were higher than in the control, while the activities in the presence of 30 and 40 mg/l of cadmium were lower than in the control. Content analysis of subcellular fractionation showed that cadmium was mostly removed and transformed by precipitation on the cell wall.

Keywords: *Rhodobacter sphaeroides*; Cadmium; Kinetic characteristic; Bioremediation mechanism

1. Introduction

Cadmium is one of the metals found in effluents discharged from industries involved in metal plating, metallurgical alloying, mining, ceramics and other industrial operations (Hutton et al., 1987; Nriagu and Pacyna, 1988). Cadmium ions are non biodegradable and tend to accumulate in living organisms, causing various disorders for living organisms (Hardman et al., 1993; Nogaw and Kido, 1996).

The conventional physicochemical technologies such as electrochemical treatment, oxidation–reduction, ion exchange and membrane separation appear to be inadequate or expensive for removing metals at lower concentrations. Bioremediation, which involves the use of microbes to detoxify and degrade environmental contaminants, has received increasing attention in recent times to clean up a polluted environment (Farhadian et al., 2008; Radhika et al., 2006; Malik, 2004; Gadd, 2000). Bioremediation, being in situ treatment, provides a safe and economic alternative to commonly used physicochemical strategies (Eccles, 1995). Especially, application of a judicious consortium of growing metal-resistant cells can ensure better removal through a combination of bioprecipitation, biosorption and continuous metabolic uptake of metals after physical adsorption (Malik, 2004; Sprocati et al., 2006; Yi et al., 2007; Azabou et al., 2007). Furthermore, the recent advances in molecular microbial ecology have provided a further impetus to environmental biotechnological approaches (Ike et al., 2007; Wang et al., 2001; Zhao et al., 2005).

As a bioremediation strategy, the potential advantages of metal sulfide precipitation include production of lower sludge volume and lower solubility products as compared with hydroxide, carbonate and phosphate precipitation (Podda et al., 2000; Allan et al., 2002). In addition, valuable

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metals can be recovered from metal sulfide sludge (Boonstra et al., 1999). Cadmium sulfide is extremely stable and insoluble (solubility product constant, $K_{sp} = 1.4 \times 10^{-28}$). Therefore, sulfide-producing organisms have been used to treat cadmium waste streams in a reactor (White and Gadd, 1996), or the organisms can be utilized at a waste site to detoxify and immobilize heavy metals in situ (Czupyrna et al., 1989).

Although sulfate-reducing bacteria and aerobic bacteria such as *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Treponema denticola* can produce hydrogen sulfide and precipitate cadmium (Wang et al., 1997, 2000; White and Gadd 1996, 1998; Bang et al., 2000), they generate sulfide only under strict anaerobic conditions or strict aerobic conditions. Therefore, they cannot be widely applied. Phototrophic bacteria are ubiquitous in fresh and marine water, soil, wastewater, and activated sludge. They are metabolically the most versatile among all prokaryotes: anaerobically photoautotrophic and photoheterotrophic in the light and aerobically chemoheterotrophic in the dark, so they can use a broad range of organic compounds as carbon and energy sources (Kim et al., 2004).

Very few systematic studies have been carried out to understand the bioremediation kinetic and mechanisms consequent to interaction with toxic heavy metals through growing cells. In this study, phototrophic bacteria *Rhodobacter sphaeroides*, a typical purple non-sulfur bacterium, have been chosen for the bioremediation of synthetic solutions of cadmium sulfate.

2. Methods

2.1. Analytical reagents

Analytical grade $3CdSO_4 \cdot 8H_2O$ was procured from Tientsin Chemistry Reagents Company, China. All the chemicals used in the each analytical estimation and for the preparation of medium were of analytical reagent grade and deionised water obtained from a Milli-Q system (Millipore, USA) was used in experiment.

2.2. Analytical techniques

The concentration of cadmium was determined using Shimadzu AA-6300 atomic absorption spectrophotometer in an air–acetylene flame at 228.8 nm wavelength (SEPA, 2002). The cell optical density at 650 nm ($OD_{650\ nm}$) was determined by an ultraviolet–visible spectrophotometer (UV-2102PC). The X-ray diffractograms of the biogenic deposit were recorded using a Model Rigaku Dmax-γA X-ray diffractometer system supplied by Jeol Ltd., Tokyo, Japan. The cells were analyzed by transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDXS), using a 100CX scanning transmission electron microscope and a Kevex 8000 EDX system. The cell samples were prepared as previously described (Wang et al., 2001).

2.3. Organism, cultivation and growth conditions

Photosynthetic bacteria *R. sphaeroides* were isolated and cultured by our laboratory and were used in subsequent experiments. The bacteria were grown in purple non-sulfur bacteria-enriched medium: 2 g sodium malate, 0.15 g $MgSO_4 \cdot 7H_2O$, 1.2 g yeast extract and 1.5 g $(NH_4)_2SO_4$, dissolved in 1 l of distilled water; the pH was adjusted to 7.0 with NaOH before autoclaving (Yao and Zhang, 1996). The bacteria were cultured anaerobically at 30 °C under continuous illumination with incandescent lamps at a light intensity of about 2500 lx. After the incubation, the cell pellet was obtained by centrifugation of bacterial culture at 15,000*g* for 25 min. Before the Cd(II) removal experiment, the cell concentration was adjusted to 20 mg wet weight of cell per 10 ml cell suspension.

2.4. Kinetic experiments

In order to determine the kinetics of Cd(II) removal from the medium solution, experiments were conducted at different initial concentrations ranging from 20 to 160 mg/l in 250-ml flasks. Every 10 ml of cell suspension was inoculated, and a series of flasks was incubated on orbital shaker at 30 °C and agitated at 500 rpm. Samples were taken at predefined time intervals (0, 6, 12, 18, 24, 30, 36, 42, 48 and 54 h). Then the mixture was centrifuged at 15,000*g* for 10 min to analyze the cadmium ion concentration. Each experiment was repeated three times (Al-Degs et al., 2006; Radhika et al., 2006).

2.5. Mechanism studies

2.5.1. Removal of cadmium and culture growth studies

The flasks containing 40 mg/l of cadmium were inoculated, then incubated at 30 °C and agitated at 500 rpm. Samples were taken at predefined time intervals (0, 6, 12, 18, 24, 30, 36, 42, 48 and 54 h). Then the mixture was centrifuged at 5000*g* for 15 min. The deposit was termed as biogenic cadmium sulfide. The supernatant were centrifuged at 15,000*g* for 15 min again to analyze the cadmium ion concentration. The pellet containing of culture was resuspended in same volume deionised water, then the optical density at 650 nm ($OD_{650\ nm}$) was determined. Each experiment was repeated three times (Wang et al., 1997).

2.5.2. Biosorption studies

The 10 ml cell suspension so obtained was introduced into 100 ml of 40 mg/l cadmium aqueous solution in a flask at pH 7.0 and equilibrated on orbital shaker for a specified time at 500 rpm and 30 °C. The residual cadmium concentration in the solution obtained after centrifuging at 15,000*g* for 15 min was analyzed using a Shimadzu AA-6300 atomic absorption spectrophotometer. The amount of cadmium biosorbed was obtained by taking the difference between the initial and final concentrations of cadmium (Radhika et al., 2006).