Short Communication

Cr(VI) reduction by *Pseudomonas aeruginosa* immobilized in a polyvinyl alcohol/sodium alginate matrix containing multi-walled carbon nanotubes

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**Abstract**

*Pseudomonas aeruginosa* (P. aeruginosa) was immobilized with polyvinyl alcohol (PVA), sodium alginate and multiwalled carbon nanotubes (MCNTs). After immobilization, the beads were subjected to freeze-thawing to enhance mechanical strength. When exposed to 80 mg/L Cr(VI), the immobilized bacteria were able to reduce 50% of them in 84 h, however the free cells were deactivated at this concentration. The beads were used to reduce 50 mg/L Cr(VI) for nine times, with the reduction efficiency above 90% in the first five times and 65% in the end.

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**1. Introduction**

Hexavalent chromium Cr(VI) is highly toxic, but can be reduced to less toxic Cr(III) by bacteria such as *Desulfovibrio vulgaris* (Mabbett et al., 2002), *Pseudomonas aeruginosa* (Song et al., 2009), and *Escherichia coli* (Liu et al., 2010). The tolerance of these bacteria to the toxic effects of Cr(VI) can be increased by immobilization. For instance, Konovalova et al. (2003) observed that *Pseudomonas* sp. immobilized in agar–agar films on the surface of synthetic membrane exhibited higher tolerance towards Cr(VI) and higher Cr(VI) reduction activity than free cells, and Camargo et al. (2004) demonstrated the feasibility of large scale Cr(VI) detoxification using Calginate immobilized *Bacillus* sp. in a bioreactor.

Cr(VI) reduction by microorganisms involves a series of reductases (Puzon et al., 2005; Xu et al., 2005; Viamajala et al., 2007). Entrapment of the enzymes in the immobilization matrix would also be desirable. Multiwalled carbon nanotubes (MCNTs), which have a large specific surface area and a high affinity for protein, have been used as an immobilization material for enzymes (Banks and Compton, 2005; Jeykumari and Narayanan, 2008). This material in conjunction with PVA and sodium alginate was explored as an immobilization matrix for *P. aeruginosa* in this work, and the Cr(VI) reduction activity of the immobilized cells was examined.

Abbreviations: ATCC American Type Culture Collection

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2. Methods

2.1. Preparation of immobilized *P. aeruginosa*

*P. aeruginosa* was supplied by the China Center for Type Culture Collection in lyophilized form (the storage number is CCTCC AB93066). The strain was grown in basic medium (beef extract 3.0 g, peptone 5.0 g, agar 20.0 g, NaCl 5.0 g in 1 L deionized water, pH 7.0 ± 0.2) at 37 °C for 48 h, and then a loop of cells was transferred to 100 mL of enrichment medium (its composition was the same with basic medium except excluding agar) and incubated for 20 h with shaking at 150 rpm. The biomass was collected by centrifugation at 10,000 × *g* for 10 min and washed with 0.9% saline.

For immobilization of *P. aeruginosa*, 6 g PVA, 4 g sodium alginate, and 0.4 g MCNTs were mixed and diluted to 100 mL with water. Subsequently, it was stirred and heated to boiling to obtain a homogeneous mixture. After storage at room temperature for 24 h to cool and remove air bubble, 5 g wet weight of *P. aeruginosa* was added into it, and the mixture was extruded dropwise into 0.25 mol/L Ca(NO$_3$)$_2$ solution and immersed for 2 h to obtain the beads with a diameter of 3 mm. The beads were frozen at −20 °C for 24 h, kept at 4 °C for 12 h, and thawed at room temperature for further use. The dilatability of the beads was examined by immersing them in deionized water for 24 h. Diameter before and after immersion was measured by vernier caliper. The dilatability was calculated according to the formula

\[
\text{Dilatability} = \frac{d_s - d_0}{d_0} \times 100\%
\]
where $d_0$ was the initial diameter, $d_a$ was the diameter after immersion.

### 2.2. Reduction and determination of Cr

A 1000 mg/L Cr(VI) stock solution was obtained by dissolving 99.9% purity of potassium dichromate in deionized water. Reduction of Cr(VI) was carried out at 37 °C with shaking at 150 rpm in 250 mL conical flasks, which contained 100 mL of fermentation medium (Song et al., 2009). 10 g immobilized *P. aeruginosa* beads (i.e., 0.476 g wet weight bacteria) and a desirable concentration of Cr(VI). Samples of 1 mL were withdrawn at an interval of every 12 h or 24 h, centrifuged at 10,000×g for 10 min and the concentrations of Cr(VI), Cr(III) and total Cr in the supernatant were determined by UV spectrophotometry, ion chromatography and atomic absorption spectrometry, respectively. The average reduction rate ($v$) was calculated based on the formula

$$v = C_0 - C_t / t$$

where $C_0$ (mg/L) was the initial concentration of Cr(VI), $t$ (h) was the incubation time, and $C_t$ (mg/L) was the Cr(VI) concentration at time $t$.

To investigate the effect of MCNTs, two immobilization matrices, one was composed of PVA, sodium alginate and MCNTs, the other was composed of the same except excluding MCNTs, were used to immobilize *P. aeruginosa*, respectively. Prior to reducing 40 mg/L Cr(VI), the immobilized cells were incubated in pure fermentation medium for 24 h growth.

To examine the long-term performance of the immobilized cells, consecutive reduction of 50 mg/L Cr(VI) was conducted. After incubation for 96 h (defined as one time), the beads were easily collected and washed to reduce 50 mg/L Cr(VI) again. The reduction efficiency of each time was calculated using the formula

$$\text{Efficiency} \% = \frac{C_0 - C_t}{C_0} \times 100\%$$

where $C_0$ was 50 mg/L, $C_t$ was the final Cr(VI) concentration of each time.

All batch experiments were conducted in duplicates and the mean values are reported.

### 3. Results and discussion

#### 3.1. Immobilization of *P. aeruginosa*

No agglomeration was observed during the formation of beads. The dilatability test showed that the diameter of beads changed within 5% after immersing in water for 24 h. In addition, the beads, which were subjected to freeze-thawing treatment, remained intact after being shaken at 150 rpm for 10 days, whereas untreated beads were fragmented after one day of shaking. The strong mechanical strength was attributed to the immobilization and treatment methods. In the immobilization process, the Na+ was replaced by Ca2+ to form insoluble Ca-alginate gels when the mixture was added into Ca(NO3)2 solution. Since PVA generally disperses randomly in homogeneous solution, resulting in the intense fluidity and transitory contact among PVA molecules (Pattanapipitpaisal et al., 2001). The formed Ca-alginate gels and insoluble MCNTs were capable to restrict the fluidity of PVA, facilitate the conglomeration among PVA molecules and increase the number of hydrogen bonds between polymer chains, which ultimately resulted in the increase of physical cross linking sites (Lozinsky and Plieva, 1998). In the subsequent freeze-thawing process, a network structure constructed by hydrogen bond, semi-crystallites between PVA molecular and MCNTs was further stabilized (Kobayashi et al., 1998).

### 3.2. Reduction of Cr(VI)

Fig. 1 reveals that the time required for complete reduction of Cr(VI) increases with the increase of initial Cr(VI) concentrations. Cr(VI) reduction was completed in 36 h for an initial Cr(VI) concentration of 20 mg/L. For a high Cr(VI) concentration of 50 mg/L, it took 90 h. Beyond this concentration, complete Cr(VI) reduction was not observed. The average rates of Cr(VI) reduction were 0.277, 0.310, 0.330, 0.305, 0.295 and 0.267 mg/L/h with initial Cr(VI) concentrations of 20, 30, 50, 60, 70 and 80 mg/L, respectively. Rate decrease at concentrations higher than 50 mg/L is likely due to the Cr(VI) toxicity on *P. aeruginosa*.

To demonstrate that the Cr(VI) removal resulted from reduction instead of adsorption by immobilized *P. aeruginosa*, total Cr, Cr(VI) and Cr(III) in the solution were determined. As shown in Fig. 2, the concentration of total Cr remained almost constant during the incubation process, and Cr(VI) decreased with concomitant Cr(III) formation, indicating that bio-reduction of Cr(VI) was completed by forming soluble Cr(III) complexes instead of insoluble Cr(OH)3 or Cr2O3. The speciation was probably due to the interaction between nascent Cr(III) and organic exudates of *P. aeruginosa* (Mabbett et al., 2002; Dogan et al., 2011).

Differences in the Cr(VI) reduction activity using equal amount of (0.476 g) free and immobilized *P. aeruginosa* are illustrated in Fig. 3. The immobilized bacteria showed slightly faster reduction than free cells and reduced half of the Cr(VI) with an initial concentration of 80 mg/L Cr(VI) in 84 h. This concentration was toxic to the free cells.

### 3.3. Effect of multiwalled carbon nanotubes

The presence of MCNTs had a positive influence on Cr(VI) reduction, as the reduction speed of Cr(VI) using MCNTs-containing matrix was faster than the other one (Fig. 4). The extracellular reductase secreted by *P. aeruginosa* was responsible for Cr(VI) reduction (Xu et al., 2005). Despite the two matrices were both able to immobilize the *P. aeruginosa* and its reductase, adding MCNTs should increase the enzyme immobilization. (It was demonstrated that the MCNTs was able to adsorb reductase to reduce 40 mg/L Cr(VI) to 28.6 mg/L in 72 h. See supplementary data.) In addition, the Ca-alginate, PVA and MCNTs interpenetrating network might facilitate the mass transfer and growth of *P. aeruginosa* compared with the other matrix without MCNTs. Because visible colony was formed on some of the MCNTs-contained matrix in

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**Fig. 1.** Time courses of Cr(VI) conversion by immobilized *P. aeruginosa* for different initial Cr(VI) concentrations.
the end of the incubation, however, the phenomenon rarely occurred on the other matrix. In future research, more efforts will be done to further investigate the phenomenon.

3.4. Long-term performance of immobilized *P. aeruginosa*

Immobilized cells were used to consecutively reduce 50 mg/L Cr(VI). It was found that the reduction efficiency of the first six times was above 84%, suggesting that the immobilized bacteria could be used effectively to reduce Cr(VI) for 24 days. When it came into the seventh time, the efficiency declined significantly, and only 65% efficiency was obtained in the end. This might be attributed to the loss of *P. aeruginosa* and reductase in the long-term shaking incubation and repeated wash, as well as the deterioration or mutation of *P. aeruginosa*.

4. Conclusions

In this study, MCNTs, PVA and sodium alginate were used to immobilize *P. aeruginosa* for the purpose of removing Cr(VI). Biological reduction to soluble Cr(III) by the immobilized cells was responsible for Cr(VI) removal. Immobilization increased the chromium tolerance of the bacteria and allowed repeated utilization of the bacteria, indicating that the microbial immobilization technology has potential application in remediation of Cr(VI) pollution in waste water.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.08.078.

References


