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Bioremediation of hexavalent chromium contaminated soil by a bioleaching system with weak magnetic fields

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ABSTRACT

Strains G1 (*Geotrichum* sp.) and B2 (*Bacillus* sp.) were isolated from a hexavalent chromium (Cr(VI)) contaminated site and they could remove Cr(VI) efficiently. The mixed culture of G1 and B2 (1:1, v/v) was prepared to test remediation of Cr(VI) contaminated soil. The influences of pH, magnetic field, dissolved oxygen, carbon sources and solid/liquid ratio on Cr(VI) removal were investigated, as well as the effects of pH and magnetic field on Cr(VI) desorption. The results indicated that the desorption of Cr(VI) from the contaminated soil was strongly affected by pH of the leaching solution with optimal value of 7.0 based on the growth of both microorganisms and desorption efficiency. Weak magnetic field of 7.0 mT promoted both the Cr(VI) desorption and the growth of G1. Compared with anaerobic conditions, bioreduction rate of Cr(VI) under aerobic conditions was higher. The optimal carbon source and solid/liquid ratio were glucose (5 g l^{-1}) and 1/15 (w/v), respectively. The associated Cr(VI) reduction efficiency was 94.8%, which was 68.8% higher than control. The high bioremediation efficiency of the mixed cultures of G1 and B2 could be attributed to their alternative growth patterns to enhance the performance of bioremediation of the heavy metals contaminated sites.

1. Introduction

Chromium has been widely used in electroplating, printing and dyeing, tanning, metallurgy and other industries, and the improper disposal of wastewater and waste has caused the chromium pollution in the environment (Saha and Orvig, 2010; Narayani and Shetty, 2013; Sandana Mala et al., 2015). Chromium generally exists in two stable oxidation states, Cr(III) and Cr(VI) (Liu et al., 2006; Michailides et al., 2015). Cr(VI) is a priority toxic, mutagenic and carcinogenic chemical, whereas its reduced trivalent form (Cr(III)) is much less toxic and insoluble (Casadevall et al., 1999; Legrand et al., 2004; Krishna and Philip, 2005; Cheung and Gu, 2007). Biological methods such as bioaccumulation, bioreduction and biosorption are cost-effective, environmentally friendly, which is considered as a potential technique in remediation of Cr(VI) contaminated site (Jeyasingh and Philip, 2005; Chai et al., 2009; Gonzalez et al., 2014).

Previous studies have showed that various bacteria naturally present in soil and groundwater, like *Bacillus* sp., *Arthrobacter* sp., *Microbacterium* sp., *Providencia* sp., and *Serratia* sp., can reduce Cr(VI) to Cr(III) by accepting electron via bacterial enzymatic processes, or indirectly via by-products of bacterial activity such as hydrogen sulfide and Fe(II) (Sedlak and Chan, 1997; Kim et al., 2001). Chromium can be removed owing to the readily precipitation of various Cr(III) forms, such as calcium chromium oxides, chromium fluoride phosphate and other organo-Cr(III) crystals, which are a result of biotransformation reducing toxicity greatly (Srivastava and Thakur, 2012).

The effects of magnetic field have been widely studied in the biological treatment of wastewater, and its mechanisms include two parts. Firstly, magnetic field can affect the physical and chemical properties of solution by promoting the ionization of water and changing the movement trajectories of the charged particles (Holysz et al., 2007; Zaidi et al., 2013), which might impact the Cr(VI) desorption from contaminated soil. Secondly, magnetic field can also affect the growth and metabolism of microbes by several mechanisms, such as trans-membrane transportation, genetic expression and cellular enzyme activity. Generally, weak

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magnetic field can promote the growth of microbes, but strong magnetic field may also have negative effects, which depends on the magnetic field types, bacterial species and operating conditions (Potenza et al., 2004; Ji et al., 2009; Santos et al., 2010; Xu et al., 2014). The application of magnetic technology in wastewater treatment has been studied extensively (Chen and Li, 2008; Shen et al., 2009; Zaidi et al., 2013), but its application in soil remediation was rarely reported. On the other hand, many studies focused on Cr(VI) reduction by a single species (Viera et al., 2003; Liu et al., 2006; Chai et al., 2009; Dhal et al., 2010; Narayani and Shetty, 2013; Thatoi et al., 2014), which was more sensitive to the environmental conditions than the mixed culture of microorganisms. Only two yeasts from a textile-dye factory effluent were assessed *in vitro* reduction of hexavalent chromium owing to their similiar habitat (Martorell et al., 2012). However, study about bioremediation using reconstituted microorganisms was also very seldom reported. In this study, Cr(VI) reducing microbes were isolated and enriched for the bioremediation of Cr(VI) contaminated soil. The performance of compound microorganisms consisted of *Geotrichum* sp. and *Bacillus* sp., and conditions including magnetic field for bioremediation of Cr(VI) were also optimized.

2. Materials and methods

2.1. Soil samples

Soil samples were collected from chromium slag contaminated site in Zhongshan City, Guangdong Province, China. The samples were completely mixed after air-dried, mild crushed and then passed through a polyethylene sieve (0.9 mm diameter). The concentration of the total chromium and hexavalent chromium in the soil sample were 1292.4 mg kg^{-1} and 526.6 mg kg^{-1}, with moisture content, organic content and pH value of 1.19%, 5.17% and 6.90, respectively.

2.2. Culture medium

The nutrient medium (NM) for the microbial growth consisted of 3 g l^{-1} beef extract, 10 g l^{-1} peptone and 5 g l^{-1} sodium chloride. The agar plate medium (NAM) was the nutrient medium (NM) supplemented with 20 g l^{-1} agar. One liter of mineral salt medium (MSM) for Cr(VI) reduction experiments consisted of 0.5 g K_2HPO_4, 1.0 g KH_2PO_4, 0.5 g $MgSO_4$, 0.01 g $CaCl_2$, 1 g NH_4Cl, 0.01 g $FeSO_4$, 1.0 g NaCl, 0.003 g $MnSO_4$, 5 g carbon sources. The pH of all above mediums was adjusted to about 6.8 with 0.1 M NaOH or 0.1 M HCl. All media were sterilized at 121 °C for 20 min before use.

2.3. Cr(VI) desorption experiment

The schematic diagram of the experimental setup used for the continuous reduction of Cr(VI) is shown in Fig. 1. Four layers of gauze were installed at the bottom and top of a PVC column with the internal diameter of 0.05 m and the length of 0.2 m. The eluent was recycled continuously in the experimental facility at a constant flow rate of 5 ml min^{-1}. Moreover, 200 g of the soil sample (air dried) were filled into the column and the proportion of soil and liquid was controlled by setting different volume of the eluent. The eluate was collected at designed time intervals and the supernatant after centrifugation was analyzed for Cr(VI) concentration. Pure water with various pH (2–10) was used as eluent to study the effects of pH on desorption efficiency of Cr(VI) from soil. And then the effect of the weak magnetic field (0 mT, 7 mT, 17 mT, 33 mT) on the desorption of Cr(VI) from soil were studied with pure water of pH 7.0 as eluent. The experiments were conducted in triplicates.

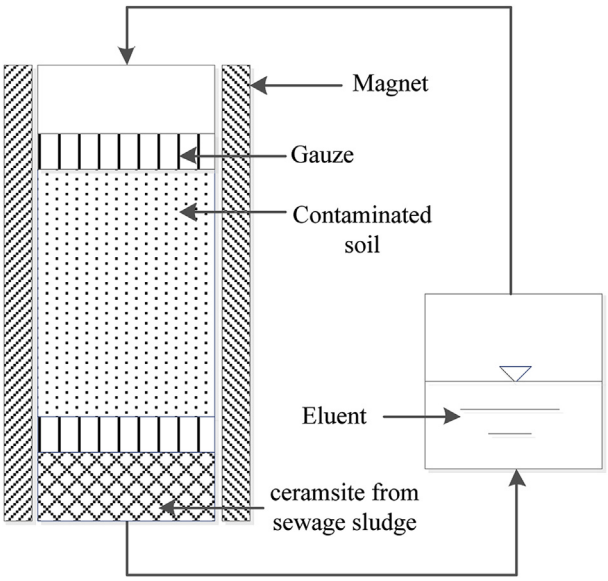

Fig. 1. Schematic diagram of the soil bioreactor.

2.4. Isolation of the Cr(VI) reducing microbes

The Cr(VI) reducing microbes were isolated from the soil samples of an electroplating sludge dumping site in Zhongshan City, Guangdong Province, China. Ten g soil sample (air dried) were added to 100 ml of NM and incubated in a shaker with a speed of 120 rpm at 30 °C for 24 h. Thereafter, 1.0 ml of the cultures was transferred into 100 ml of sterile NM containing 20 mg l^{-1} Cr(VI) and incubated in a shaker with 120 rpm at 30 °C for 24 h. The subculture process was carried out until the Cr(VI) concentration in NM was 50 mg l^{-1}. The predominant strains were isolated by ten-time series dilution and spread onto NAM. After visible colony formation, the single colonies were streaked on agar plates for purification. Preservation on slants of the pure isolates was stored at 4 °C for subsequent experiments.

2.5. Effect of weak magnetic fields on the growth of microorganisms

The suspension of pure culture was prepared by inoculating each isolated strain into 100 ml liquid NM from agar slants and incubating at 30 °C for 24 h on a shaker. Then 1 ml suspension was added into 100 ml NM and then incubated for 24 h at 0 mT, 7 mT, 17 mT and 33 mT, respectively. One ml bacterial suspension of strain B2 was added into 9 ml normal saline and shaken well before using, then, the bacterial density was monitored by measuring optical density (OD) at 600 nm 10 ml cell suspension of strain G1 was filtrated through filter paper which has been dried to constant weight and washed with the sterile saline, then dried to constant weight. The dry weight of G1 was measured by comparing the differences in the weight of filter papers before and after filtration. The pure water (pH 7.0) pretreated by various magnetic fields of 7 mT, 17 mT and 33 mT was used as eluent to explore the effect of magnetic fields on the Cr(VI) desorption from the contaminated soil, with the eluent untreated by magnetic field as the control.

2.6. Effects of aerobic and anaerobic conditions and pH on the Cr(VI) removal

The Cr(VI) reduction capability of compound microorganisms

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