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Effects of indigenous bacteria on Cr(VI) reduction in Cr-contaminated sediment with industrial wastes

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Abstract

Black, clay-like sediments have been obtained from the area of the pigment manufacturing factories in Dongducheon city, Korea. These sediments were contaminated by heavy metals, especially chromium (700 mg/kg). Indigenous bacteria in the sediments were isolated to investigate their ability to reduce Cr(VI) to Cr(III). The enriched bacterial consortium reduced over 99% of dissolved Cr(VI) in 96 h from the onset of the experiments under anaerobic condition, while there was no change in Cr(VI) concentration until 300 h in abiotic controls. Total amount of dissolved Cr decreased simultaneously when Cr(VI) was reduced, which was likely due to precipitation of $Cr(OH)_3$ after microbial reduction of Cr(VI) to Cr(III). Under aerobic condition, only 30% of dissolved Cr(VI) was reduced by indigenous bacteria until 900 h. The reduction of Cr(VI) did not accompany bacterial growth since the amount of protein did not show a significant change with time both in the presence and absence of O_2. These indigenous bacteria may play a role in the treatment of Cr(VI)-contaminated sediments.

Keywords: Indigenous bacteria; Chromium; Reduction; Cr(VI)

1. Introduction

Two oxidation states of chromium are environmentally important: Cr(III) and Cr(VI) (Fukai, 1967). At neutral pH, Cr(VI) exists as a soluble and highly toxic form and Cr(III) is a relatively insoluble and non-toxic form (Rai et al., 1987). Thus the reduction of aqueous Cr(VI) to Cr(III) can lead to Cr detoxification.

Various industrial practices such as chrome-plating, wood preservation, leather tanning, corrosion inhibition and alloy formation release large quantities of chromium-laden wastewater (Wang and Shen, 1995). Once Cr is released in the subsurface, it undergoes a series of oxidation–reduction reactions with various oxides present in the soil (Eary and Rai, 1987) and ultimately reaches the groundwater in its oxidized form, Cr(VI) (Guha et al., 2001).

In general, the treatment technologies for removing chromium from industrial waste include ion-exchange, electrodepositing and chemical reduction with iron- and sulfur-containing solutions followed by precipitation (Zhao and Duncan, 1997). These methods can be quite costly, requiring high energy input or large quantities of chemical reagents, and can create other forms of waste. The biological reduction of Cr(VI) to Cr(III) by microorganisms may provide a less costly approach to remediation (Marsh et al., 2000).

A number of bacterial species have been isolated and shown to be capable of Cr(VI) reduction. Sulfate- and

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iron-reducing bacteria can indirectly reduce Cr(VI) via their anaerobic metabolic end products, hydrogen sulfide (HS^-) and Fe(II), respectively (Pettine et al., 1994, 1998; Sedlak and Chan, 1997; Patterson et al., 1997). Some bacteria, such as *Desulfotomaculum reducens* and *Pantoea agglomerans* strain SP-1, may use Cr(VI) as an alternate electron acceptor for anaerobic growth (Tebo and Obraztsova, 1998; Francis et al., 2000). *Desulfovibrio vulgaris* can also reduce Cr(VI) enzymatically (Lovley and Phillips, 1994).

Although a number of chemical mechanisms for Cr(VI) reduction have been reported, information on natural microbial effects on Cr(VI) reduction is still limited. Therefore, more information on bacterial Cr(VI) reduction in various environment is necessary. In this study, an indigenous bacterial consortium was isolated in Cr-contaminated sediment from the area of the pigment manufacturing factories and its effects on Cr(VI) reduction with time were investigated under anaerobic and aerobic conditions.

2. Materials and methods

Black sediments that were clay-like in appearance were taken from a stream in Dongducheon city, Korea, in June 2004. The area was contaminated with chromium from nearby pigment manufacturing activities. Total Cr concentration in the sediments was 700 mg/kg after aqua-regia digestion.

The sediment samples were put immediately to Nalgene HDPE bottles, sealed tightly to prevent the direct contact with atmospheric oxygen, and transported to the laboratory, where they were stored at 4 °C until used.

The enrichment of bacterial consortium was prepared in 500 mL serum bottles in which 20 mL sediment slurry was mixed with 200 mL of sterilized Postgate culture medium (Postgate, 1984). Stock solution of $K_2Cr_2O_7$ was used as a Cr(VI) source. The pH of the final slurry was adjusted to 7.3 ± 0.2. The headspace of serum bottles was filled with pure N_2 gas, and capped with butyl rubber stopper and aluminum crimp seal. The above processes were conducted in an anaerobic chamber.

After 168 h incubation, the inoculum of 20 mL was transferred to a fresh 200 mL medium. After three sequential transfers, the inoculum was used in the experiments to investigate microbial reduction of Cr(VI) with time. To prevent the abiotic reduction and precipitation of chromium, the sulfidogenic enrichment consortium was degassed with N_2 for 25 min prior to inoculation to remove HS^- (Arias and Tebo, 2003). Aliquots of solution for Cr(VI), total Cr, Fe(II), total Fe and SO_4^{2-} analysis were periodically taken by using sterile syringes and needles and filtered and assayed immediately.

The non-inoculated medium was prepared following strictly the same procedures and served as a control. To test the effect of oxygen on chromium reduction, the bottles were also prepared under aerobic condition in the same procedures. Each enrichment was performed in duplicate experiments to assess reproducibility.

Cr(VI) was quantified by the colorimetric diphenylcarbazide (DPC) method at 540 nm (Lovley and Phillips, 1994). Fe(II) was measured by the phenanthroline method at 510 nm (American Public Health Association, 1992). The amount of protein was determined by Coomassie blue reaction (Bradford assay) at 595 nm (Daniels et al., 1994). The concentrations of total dissolved Cr and Fe were analyzed by AAS. SO_4^{2-} was measured by IC.

3. Results

3.1. Anaerobic condition

From the onset of the experiment, 99.8% of dissolved Cr(VI) was rapidly reduced in 96 h under anaerobic condition, and then the reduced form was maintained during the rest of experiment. In comparison, non-inoculated control did not show a significant variation in dissolved Cr(VI) concentration (Fig. 1). The amount of total dissolved Cr also decreased simultaneously as Cr(VI) was reduced. Therefore, the dissolved Cr(VI) was presumably reduced to Cr(III) as a precipitate of $Cr(OH)_3$ form. pH was maintained as about 7 until the end of both the biotic and abiotic experiments.

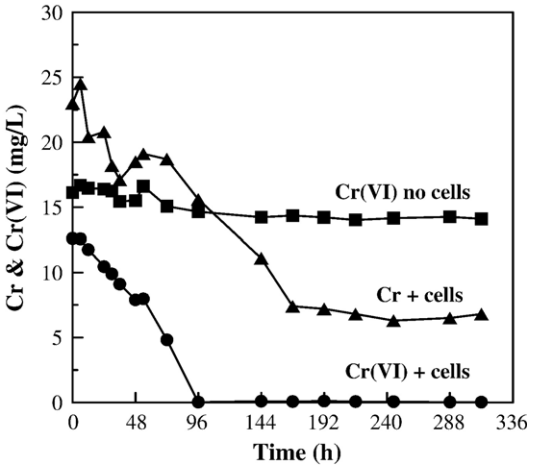

Fig. 1. The variations of Cr and Cr(VI) in enrichment culture under anaerobic condition.