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Enhancement of bioremediation by *Ralstonia* sp. HM-1 in sediment polluted by Cd and Zn

Yong-Jin Park [a], Jae-Jung Ko [a,*], Sang-Leen Yun [a], Eun Young Lee [b], So-Jung Kim [a], Sung-Won Kang [a], Byung-Cheol Lee [a], Seog-Ku Kim [a]

[a] *Department of Environmental Research, Korea Institute of Construction Technology, 2311 Daehwa-dong, Ilsanseo-gu, Goyang-si, Gyeonggi-do 411-712, Republic of Korea*
[b] *Department of Environmental Engineering, University of Suwon, San 2-2, Wau-ri, Bongdam-eup, Hwaseong-si, Gyeonggi-do 445-743, Republic of Korea*

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Abstract

In this study, the potential for the application of the bioaugmentation to Cd and Zn contaminated sediment was investigated. A batch experiment was performed in the lake sediments augmented with *Ralstonia* sp. HM-1. The degradation capacity of 18.7 mg-DOC/l/day in the treatment group was bigger than that of the blank group (4.4 mg-DOC/l/day). It can be regarded as the result of the reduction of the metal concentration in the liquid phase due to adsorption into the sediments, with the increased alkalinity resulting from the reduction of sulfate by sulfate reducing bacteria (SRB). The removal efficiency of cadmium and zinc in the treatment group was both 99.7% after 35 days. Restrain of elution to water phase from sediment in the *Ralstonia* sp. HM-1 added treatment group was also shown. In particular, the observed reduction of the exchangeable fraction and an increase in the bound to organics or sulfide fraction in the treatment group indicate its role in the prevention of metal elution from the sediment. Therefore, for bioremediation and restrain of elution from the sediment polluted by metal, *Ralstonia* sp. augmentation with indigenous microorganism including SRB, sediment stabilization and restrain of elution to surface water is recommended.

Keywords: Cd; Zn; *Ralstonia* sp.; Sediment; Sulfate reducing bacteria

1. Introduction

Paldang-Lake is one of the main water reserves for the city of Seoul, Korea. Recently, the accumulation of phosphorus and metals, as sediment pollutants, has seriously affected the water quality (Yun et al., 2007). Specifically, metal contamination is linked to birth defects, cancer, skin lesions, mental and physical retardation, learning disability, liver and kidney damage, and a host of other maladies (Malik, 2004; Singh and Cameotra, 2004). This is because heavy metal can react with proteins, nucleic acids and phospholipids, and thus arrest cellular proliferation (Fraústo da Silva and Williams, 1993). Various techniques have been reported for the removal of metals from sediments, including soil washing, thermal extraction, ion exchange, electrokinetic treatment, reverse osmosis, membrane technology, evaporation recovery, solidification, plasma vitrification, and bioremediation (Vullo et al., 2008; Mulligan et al., 2001b). Most of these are ineffective or excessively expensive when the metal concentrations are less than 100 mg/l (Ahluwalia and Goyal, 2007). However, bioremediation technology is economically feasible, easy to apply to contaminated sites and causes little secondary pollution compare to other techniques. Since microorganisms have a generic character for survival strategies in heavy metal polluted habitats, their specific microbial detoxifying mechanisms such as bioaugmentation, biotransformation, biomineralization or biosorption can be applied either ex situ or in situ to the design of economical bioremediation

* Corresponding author. Tel.: +82 31 910 0514; fax: +82 31 910 0291.
E-mail address: caryotes@hotmail.co.jp (J.-J. Ko).

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process (Malik, 2004; Muñoz et al., 2006; Umrania, 2006). Unlike organic pollutants, metals are not degraded, and mineralization to CO_2 is impossible. Therefore, for the removal of metals, the goal of bioremediation focuses on the transformation of the metal, and its subsequent biosorption and biomineralization, which is the formation of insoluble metal precipitates due to interactions with microbial metabolic products (Barkay and Schaefer, 2001). Many studies have demonstrated the efficiency of metal removal by microorganism. Biological process can either solubilize or immobilize metals, thereby increasing their bioavailability and potential toxicity, or reduce their bioavailability, respectively (White et al., 1997). To prevent leaching of metals from sediments into the water phase by microorganism, metals should be immobilized by biosorption, the formation of metal-binding molecules, reductive precipitation, sulfide precipitation or phosphate precipitation (Gadd, 2000).

The problem of metal contamination of waters has been under extensive discussion especially during recent years because of its severe adverse effects on human health (Green-Ruiz et al., 2008; Lu and Gibb, 2008; Vullo et al., 2008). However, the majority of the metals are precipitated in the sediments of aquatic environments such as stream, lake, etc. Desorption of metals can be released by the change of pH and reductive mechanism (Vaxevanidou et al., 2008). Thus, the removal of metals should be investigated in both water and sediment. Both Cd and Zn are one of the most toxic heavy metals and can appear either in water or soil of any polluted site because of their high mobility. Biosorption of these metals with the use of indigenous microorganism is efficient and ecofriendly for its immobilization. The application of metalresistant bacteria, including *Ralstonia* sp. for bioremediation offers attractive perspectives (Mergeay et al., 2003). The metal resistance of *Ralstonia* in lake sediments and industrial biotopes has previously been reported (Goris et al., 2001; Konstantinidis et al., 2003). Owing to its capacity of resistance for toxicity by metals, this organism is a very interesting candidate for bioremediation studies (Bonatto et al., 2004).

In this study, the potential for the application of *Ralstonia* sp. HM-1(Lee et al., 2008), which is known to be one of the resistant bacteria to metal, in the bioremediation of sediments polluted by metals of Cd and Zn was investigated. Batch experiments were conducted using the spike of the synthetic Cd and Zn stock solution to investigate the effect of both the indigenous microorganism in sediment and the inoculation of *Ralstonia* sp. HM-1 in the bottle containing sediment and surface water of lake.

2. Methods

2.1. Microorganism

As a part of previous research focused on the removal of heavy metals containing Cr, Ni, Cu, Pb, and Zn by an enriched consortium, a metalresistant microorganism was isolated from the sediments of Paldang-Lake, Korea. Pure culture was obtained by subsequent inoculations to the biosorption medium (Ismail et al., 2005) containing various metals. The *Ralstonia* sp. HM-1 used for the experiment was cultured in bottles containing the biosorption medium supplemented with 100 mg/l of metals at 25 °C for 10 days.

2.2. Batch experiments

The sediment and surface water were obtained from Paldang-Lake, Korea. In the laboratory, sediment and surface water were mixed, and Zn and Cd spiked to the sediment mixed solution during purging with nitrogen gas. Ninety milliliters of the mixed solution containing the sediment and spiked metals was injected into 120 ml serum vials, which were then sealed with teflon coated butyl rubber. The vials were left for 2 days, and then 10 ml of *Ralstonia* sp. HM-1 added. The experimental setup was divided into three portions to investigate the role of *Ralstonia* sp. HM-1 in metal remediation. In the first portion, used as a control, the sediment, surface water, and microorganisms were sterilized twice by autoclaving at 120 °C for 1 h. In the second portion, used as a blank, only the *Ralstonia* sp. HM-1 was sterilized. Finally, in the third portion as a treatment group, no sterilization was performed. The vials were anaerobically incubated at 25 °C for 35 days, without shaking, and samples were collected approximately every 2 days.

2.3. Analytical procedures

During the batch experiments, the general parameters; pH, ORP, and DO were monitored with YSI (MPS 660). The volume and composition of gas released were analyzed. The gas collected at the head space of the vial was measured with a glass syringe before opening the vial, and the gas composition was determined using GC-TCD (GC-8A, Shimadzu) equipped with a PORAPAK Q packed column. Samples of sediment mixtures were filtered through GF/C filter. The filtrates were used for analysis of dissolved organic carbon (DOC) using TOC analyzer (Jena Multi N/C 3000). The sulfate concentration was also measured using ion chromatography (Dionex ICS-3000). The filtrates were acidified and analyzed for the concentrations of Zn and Cd in both the liquid and solid samples using ICP (Thermo IRIS Intrepid II XDL). Solid samples were prepared with centrifugation at 2500 rpm for 10 min, and after discarding the supernatants, the remaining solid dried at room temperature. Since information on merely the total metal concentration is insufficient in assessing the bioremediation effect, a sequential metal extraction method was employed to evaluate the mobilization or immobilization of metals (Silveira et al., 2006; Mulligan et al., 2001a). Sequential extraction was carried out by applying the method of Tessier et al. (1979) (Table 1).