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Citation	Annual Report of FY 2006, The Core University Program between Japan Society for the Promotion of Science (JSPS) and Vietnamese Academy of Science and Technology (VAST). 2007, p. 389-393
Version Type	VoR
URL	<a href="https://hdl.handle.net/11094/13226">https://hdl.handle.net/11094/13226</a>
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# REMOVAL OF DISSOLVED METAL IONS BY BIOGENIC MANGANESE OXIDES PRODUCED BY ENRICHMENT CULTURES OF MANGANESE-OXIDIZING BACTERIA

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## ABSTRACT

We examined the removal of dissolved metal ions by biogenic Mn oxides produced by Mn(II)-oxidizing microbial cultures enriched from a streambed biofilm. The cultures converted dissolved Mn(II) to insoluble Mn(IV) oxides that were present as Mn microconcretions in the suspended solids. Transmission electron microscopy demonstrated that a range of bacteria (encapsulated and sheathed types) contribute to the Mn oxide formation. Pb(II) sorption by the suspended solids (pH 5.8; 25°C) followed a two-term Langmuir model with a Pb sorption capacity of 1.4  $\mu\text{mol/mg}$  of solids. This corresponded to a Pb/Mn molar ratio of 0.41. Such high Pb(II) sorption is consistent with the properties of known bacteriogenic Mn oxides. During repeated batch culture, sorption of trace Zn(II) and Ni(II) by the suspended solids concurred with the removal of dissolved Mn(II). The results indicate that microbial Mn(II)-oxidizing activity would be applicable to treatment of water contaminated with Mn(II) and other toxic metals.

## KEYWORDS

Biogenic manganese oxides, bioreactor, Mn-oxidizing bacteria, trace metal sorption.

## INTRODUCTION

Microbial activity that converts Mn(II) to insoluble Mn(III, IV) oxides has been practically used for removal of dissolved Mn(II) from groundwater. This microbial process serves as a promising tool for the treatment of dissolved Mn as well as Fe. Some water purification plants in Japan and EU countries have sand filter facilities wherein microbially mediated precipitation of Fe and Mn occurs (Mouchet, 1992).

Diverse microorganisms (bacteria and fungi) living in freshwaters oxidize Mn(II) to produce Mn oxides, resembling a layer-type Mn mineral, vernadite ( $\delta\text{-MnO}_2$ ) or birnessite (Tebo *et al.*, 2004; Miyata *et al.*, 2006). These Mn minerals are common in freshwater environments. The biogenic Mn oxides exhibit high sorption affinity and capacity of divalent metal cations such as Zn, Co, Ni, and Pb (Nelson *et al.*, 1999; Tani *et al.*, 2004a; Villalobos *et al.*, 2005), owing to their high specific surface area (100–250  $\text{m}^2/\text{g}$ ) and the negatively charged layer structures. These observations could explain the fact that naturally occurring Mn oxides serve as primary adsorbents for trace or contaminated metals in lakes and streambed sediments (e.g., Tani *et al.*, 2003). Moreover, a biogenic Mn oxide oxidizes As(III) to As(V) extensively, and in the presence of metal cations, it incorporates As(V) effectively (Tani *et al.*, 2004b). These studies indicate that the microbial Mn(II) oxidation may be useful for the removal of trace toxic metals as well as dissolved Mn(II) from contaminated groundwater.

Our objective in this study was to examine the removal of dissolved metals by Mn(II)-oxidizing microbial cultures enriched from a streambed biofilm. We show the characteristic features with regard to ultrastructures and metal sorption ability, of Mn oxides occurring in the cultures.

## MATERIALS AND METHODS

Mn(II)-oxidizing microorganisms were enriched from a streambed biofilm (Tani *et al.*, 2003) using a 3-L acrylic cylindrical reactor (12.5 × 28 cm) with conic bottom at ambient temperature. At the beginning of experiment, a reactor was filled with 1 L of unsterilized culture medium (per liter: 50 mg sodium acetate trihydrate, 20 mg peptone, 5 mg KH<sub>2</sub>PO<sub>4</sub>, and 5–6 mg of Mn(II) (pH 7.0) and inoculated with the biofilm sample. The reactor was aerated at a flow rate of 2–3 L/min. At the end of one batch, suspended solids were sedimented by allowing the culture to stand for 10 min, and the spent medium was removed by decantation. The reactor was then filled with fresh medium, and the batch culture was repeated. Except during the start-up period, spent culture medium was replaced by fresh one after confirming that >95% of the initial dissolved Mn(II) was removed. The concentration of dissolved Mn(II) was routinely monitored by a colorimetric method with formaldoxime.

Pb(II) sorption experiment was conducted at pH 5.8 (25°C) using a method adapted from Villalobos *et al.* (2005). Suspended solids (2 mg), collected from the reactor and freeze-dried, were placed in a 55-mL polypropylene tube containing 50 mL of 11 to 220 μM Pb(NO<sub>3</sub>)<sub>2</sub> in 10 mM NaNO<sub>3</sub>/Milli-Q water. Aqueous-phase Pb(II) in the suspensions was determined by ICP-MS. The data for the Pb(II) sorption were fitted to the Langmuir equation (1) or a two-term Langmuir equation (2):

$$q = (b \times K \times c) / (1 + K \times c) \quad (1)$$

$$q = \{(b_1 \times K_1 \times c) / (1 + K_1 \times c)\} + \{(b_2 \times K_2 \times c) / (1 + K_2 \times c)\} \quad (2)$$

where  $q$  is the amount of adsorbed Pb;  $c$  is the equilibrium aqueous-phase concentration of Pb;  $b$ ,  $b_1$ , and  $b_2$  are the maximum sorption capacities; and  $K$ ,  $K_1$ , and  $K_2$  are the equilibrium Langmuir constants.

For examining Zn(II) and Ni(II) sorption, the suspended solids (240–260 mg/L) were cultured with the medium amended with 40 mM HEPES (pH 7.0) and a prescribed concentration of ZnSO<sub>4</sub> or NiCl<sub>2</sub>. Suspended solids were moved to the fresh medium every about one day. During repeated batch culture, the concentrations of Mn(II) and either Zn(II) or Ni(II) in culture supernatants were determined by ICP-MS.

Suspended solids taken from the reactor were observed by transmission electron microscopy (TEM) (Miyata *et al.*, 2006).

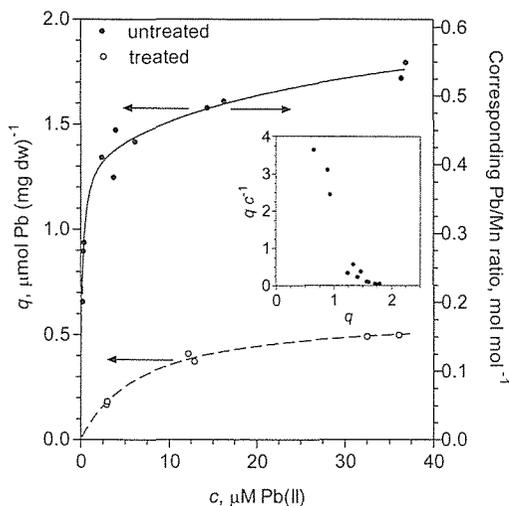
## RESULTS AND DISCUSSION

We constructed a bioreactor using a stream epilithic biofilm, which was cultured in unsterilized medium containing 5–6 mg of Mn(II)/L. After the start-up period of about 50 days, the reactor removed >95% of added Mn(II) usually within 1 or 2 days. The culture medium was routinely exchanged two or three times per week. The suspended solids and biofilm on the wall of the reactor turned dark brown. Diagnostic tests with reductants, hydroxylamine and leucoberberlin blue,

indicated that the dissolved Mn(II) had accumulated in the solids as an oxidized form of Mn. The reactor was operated over approximately 500 days, with removing a portion of grown suspended solids several times by withdrawing 80% of the culture suspension. Throughout the operation, the reactor maintained the ability to remove dissolved Mn(II). Light microscopic observations showed that the suspended solids contained spherical microconcretions (~10  $\mu\text{m}$  in diameter). These morphotypes are similar to those of several species of Mn/Fe-depositing *Siderocapsa*, which are frequently observed in natural metal deposits. TEM study demonstrated that the suspended solids contain different types of bacterial cells, including sheathed and encapsulated cells, that were encrusted with different amounts of filamentous deposits. Thus, a range of Mn-depositing bacteria contributed to the Mn oxide formation in the suspended solids.

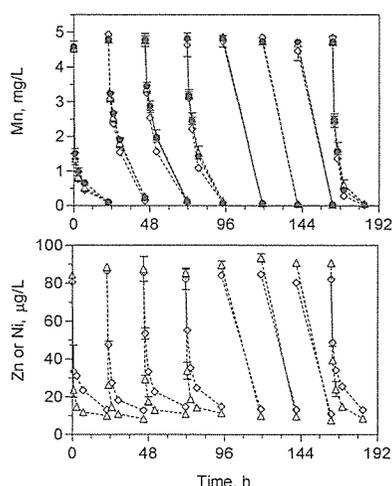
The suspended solids were subjected to Pb(II) sorption experiment. Based on the content of total Mn in this solid sample ( $20 \pm 1\%$ ) and the fraction of oxidized Mn in the total Mn ( $90 \pm 0.1\%$ ), 1 g of solids corresponded to 3.3 mmol of oxidized Mn. The trend of the Pb(II) sorption (Fig. 1) increased sharply at low Pb(II) loadings (~0.9  $\mu\text{mol}/\text{mg}$ ), followed by increasing moderately up to the Pb(II) loading, ~1.7  $\mu\text{mol}/\text{mg}$ . Nonlinear regression analysis revealed that the data followed a two-term Langmuir model ( $r^2 > 0.97$ ) (Fig. 1), consistent with a combined mode of different sorption isotherms with high and low affinities for Pb(II). If Mn oxides in the suspended solids were removed by 0.1 M hydroxylamine hydrochloride, then the Pb(II) sorption was lowered extensively and its isotherm could be well fitted with the single-site Langmuir model (Fig. 1).

A recent study of *Pseudomonas putida* biofilm containing biogenic Mn oxide demonstrated that the Mn oxide exhibit a high affinity to trace Zn(II) ion and, at low Zn(II) loadings, the coexisting biofilm organic components little interfere with the Zn sorption by the Mn oxide (Toner *et al.*, 2006). Therefore, the predicted high-affinity segment with  $b_1 = 1.36 \mu\text{mol}/\text{mg}$  (Fig. 1) may represent the role of a Mn oxide phase in the suspended solids. This maximum sorption capacity, corresponding to a Pb/Mn ratio of 0.41, is similar to the Pb(II) sorption capacities of the biogenic Mn oxides produced by *Leptothrix discophora* (Pb/Mn = 0.55) (Nelson *et al.*, 1999) and *P. putida* (0.27) (Villalobos *et al.*, 2005), likely owing to the structural features of poorly crystallized, birnessite-type Mn(IV) oxides (Villalobos *et al.*, 2005).



**Fig. 1.** Pb(II) sorption isotherms obtained with suspended solids. The solid line represents nonlinear regression fitting of the data for untreated sample to eq. 2. The parameters calculated were  $b_1 = 1.36 \mu\text{mol}/\text{mg}$ ,  $b_2 = 0.81 \mu\text{mol}/\text{mg}$ ,  $K_1 = 5.75 \text{ L}/\mu\text{mol}$ , and  $K_2 = 0.03 \text{ L}/\mu\text{mol}$  ( $r^2 > 0.97$ ). The dashed line represents nonlinear regression fitting of the data for treated sample (without Mn oxide) to eq. 1. The parameters were  $b = 0.60 \mu\text{mol}/\text{mg}$  and  $K = 0.15 \text{ L}/\mu\text{mol}$  ( $r^2 > 0.98$ ). The inset shows the plot of distribution coefficient ( $q/c$ ) versus  $q$  for the untreated sample.

We examined the simultaneous immobilization of dissolved Mn(II) and a second metal cation (Zn or Ni), by the suspended solids derived from the reactor. The result in Fig. 2 demonstrated that these metal ions added at  $\sim 90 \mu\text{g/L}$  did not interfere with the removal (sorption and oxidation) of dissolved Mn(II) by the suspended solids. Through the batch cultivation, Zn(II) and Ni(II) were removed rapidly by  $90.4 \pm 1.4\%$  and  $86.7 \pm 1.2\%$ , respectively ( $\pm$  SD;  $n = 8$ ). These results indicated that the sorption of these trace metals was not hindered by the presence of  $\sim 70$ -fold excess of dissolved Mn(II). The Mn oxides formed in the suspensions would serve as a primary adsorbent for the trace metal ions, as demonstrated elsewhere (Toner *et al.*, 2006).



**Fig. 2.** Removal of dissolved Zn(II) or Ni(II) by suspended solids. Either Zn(II) or Ni(II) was added at  $\sim 90 \mu\text{g/L}$  to the suspensions containing dissolved Mn(II) at  $\sim 5 \text{ mg/L}$ . Open triangles and diamonds represent the remaining concentrations of dissolved metal ions in the suspensions with Zn(II) and Ni(II), respectively. Closed circles represent the remaining concentration of dissolved Mn(II) in the control suspension (no addition of Zn or Ni). Data points are means  $\pm$  SD of duplicate experiments.

## CONCLUSIONS

The Mn-oxidizing culture could be maintained over a long period without a loss of the ability to convert Mn(II) to insoluble Mn oxides. A range of Mn-depositing bacteria (encapsulated and sheathed types) contributed to the Mn oxide formation. The high capacity of Pb(II) sorption and the concurrent removal of dissolved Mn(II) and trace Zn(II) or Ni(II) emphasize the notion that the Mn-oxidizing bacteria play a significant role in the fates of Mn(II) and trace metal ions in freshwater environments. Application of microbial Mn(II) oxidation would be an attractive subject in the research area of biological treatment of water contaminated with Mn(II) and other toxic metals.

## ACKNOWLEDGEMENT

This study was supported by a Grant-in-Aid for Young Scientists (B) (17710065) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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