Removal of multi-heavy metals using biogenic manganese oxides generated by a deep-sea sedimentary bacterium – *Brachybacterium* sp. strain Mn32

Wenming Wang,† Zongze Shao,‡ Yanjun Liu† and Gejiao Wang†

†State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China

‡The Third Institute of Oceanography, State Oceanic Administration, Xiamen 361005, PR China

A deep-sea manganese-oxidizing bacterium, *Brachybacterium* sp. strain Mn32, showed high Mn(II) resistance (MIC 55 mM) and Mn(II)-oxidizing/removing abilities. Strain Mn32 removed Mn(II) by two pathways: (1) oxidizing soluble Mn(II) to insoluble biogenic Mn oxides – birnessite (δ-MnO$_2$) group and manganite (γ-MnOOH); (2) the biogenic Mn oxides further adsorb more Mn(II) from the culture. The generated biogenic Mn oxides surround the cell surfaces of strain Mn32 and provide a high capacity to adsorb Zn(II) and Ni(II). Mn(II) oxidation by strain Mn32 was inhibited by both sodium azide and o-phenanthroline, suggesting the involvement of a metalloenzyme which was induced by Mn(II). X-ray diffraction analysis showed that the crystal structures of the biogenic Mn oxides were different from those of commercial pyrolusite (β-MnO$_2$ group) and fresh chemically synthesized vernadite (δ-MnO$_2$ group). The biogenic Mn oxides generated by strain Mn32 showed two to three times higher Zn(II) and Ni(II) adsorption abilities than commercial and fresh synthetic MnO$_2$. The crystal structure and the biogenic MnO$_2$ types may be important factors for the high heavy metal adsorption ability of strain Mn32. This study provides potential applications of a new marine Mn(II)-oxidizing bacterium in heavy metal bioremediation and increases our basic knowledge of microbial manganese oxidation mechanisms.

INTRODUCTION

Manganese (Mn), the fifth most abundant transition metal in the earth’s crust, is an essential micronutrient for most organisms. However, high concentrations of Mn are toxic for cells. Many micro-organisms have been reported to be able to oxidize Mn(II) to insoluble Mn(III, IV) oxides, yet natural Mn(II) oxidation happens very slowly when the environmental pH ranges from 6 to 9 (Tebo *et al.*, 1997).


Microbial oxidation and precipitation of Mn(II) in the deep sea are important oceanic biogeochemical processes. Manganese is an important nutrient element in the marine water column and is also required for photosynthesis. The acquisition of manganese by organisms and the biogeochemistry of manganese in the oceans is therefore an essential part of global carbon fixation processes. It has been reported that Mn oxides are formed in sea water via bacterial catalysis by multicopper oxidases, but the detailed mechanism of Mn(II) oxidation has not been clarified so far (Tebo *et al.*, 2004). Biogenic Mn oxides located on spores, cell surfaces or exopolymers subsequently settle in the marine water column. Such biogenic Mn oxides may help to mediate the trace element and nutrient composition of sea water via sorption and oxidation/reduction reactions (Webb *et al.*, 2005a).

Biogenic Mn oxides have been reported to be able to oxidize a variety of organic and inorganic compounds such

Abbreviations: AAS, atomic absorption spectroscopy; SEM, scanning electron microscopy; XRD, X-ray diffraction.
as humic substances, Cr(III), Fe(II) and As(III), and scavenge many metals such as Cu(II), Co(II), Cd(VI), Zn(II), Ni(II) and Pb(II) (Francis et al., 2001, 2002; Croal et al., 2004; Klewicki & Morgan, 1999). Due to the higher surface area and the higher binding energy per unit area, biogenic Mn oxides have a larger capacity for sorption of metal cations than abiogenic Mn oxides (Nelson et al., 2002). For example, Mn oxides produced by the freshwater bacterium Leptothrix discophora SP-6 are nanosized, todorokite-like porous layer-type MnO₂ that are expected to absorb more heavy metals than other, abiogenic Mn oxides (Kim et al., 2002). Biogenic Mn oxides produced by L. discophora SS-1 had a higher absorption efficiency of Pb(II) than did synthetic Mn oxides. So far, knowledge of the structures and compositions of marine biogenic manganese oxides remains limited.

We isolated a Mn(II)-oxidizing bacterium, Brachybacterium sp. strain Mn32, from the sediment of deep-sea Mn nodules in the Pacific Ocean (Tian & Shao, 2006). The aim of this current study was to investigate the potential manganese oxidation mechanism and heavy metal removing abilities of strain Mn32. The sorption efficiencies of Zn(II) and Ni(II) by the biogenic Mn oxides are presented and compared with those of commercial and fresh chemically synthesized MnO₂ powders. In order to explain the heavy metal adsorption differences between the biogenic Mn oxides and the abiogenic MnO₂, scanning electron microscopy and X-ray diffraction analyses were performed. Results of this study indicate that strain Mn32 is a highly efficient Mn(II)-oxidizing bacterium with potential application in multi-heavy-metal removing systems.

METHODS

Bacterial strains, optimal pH condition and heavy metal resistance levels. Strain Mn32, a marine manganese-oxidizing Brachybacterium, originally isolated from Pacific deep-sea Mn nodule sediment, was grown at 28 °C in liquid A medium (Tian & Shao, 2006) containing 10 g sodium acetate l⁻¹, 0.5 g yeast extract l⁻¹ (Oxoid), 0.5 g peptone l⁻¹ (Oxoid) and 75 % (v/v) artificial sea water (17.53 g NaCl l⁻¹, 0.75 g KCl l⁻¹, 12.32 g MgSO₄·7H₂O l⁻¹, 1.11 g CaCl₂ l⁻¹). When MnCl₂ was added to the medium, black-coloured Mn oxides occurred after several days of incubation (see below).

The optimal pH for growth of strain Mn32 was determined in liquid A medium with pH ranging from 5 to 10, incubated for 12 h at 28 °C with 150 r.p.m. shaking. The OD₆₀₀ was measured with a UV-3000 spectrophotometer (Perkin-Elmer).

The minimal inhibitory concentrations (MICs) of Mn(II), Zn(II) and Ni(II) for strain Mn32 were determined. Triplicate samples of each single colony were inoculated into 3 ml liquid A medium supplemented with increasing concentrations of MnCl₂, ZnSO₄ or NiSO₄, incubated at 28 °C with 150 r.p.m. shaking for one week. The OD₆₀₀ values were determined. The MIC is defined as the lowest heavy metal concentration that completely inhibited the growth of strain Mn32.

Removal of Mn(II) by manganese oxidation/precipitation of strain Mn32. Liquid A medium with an initial concentration of 0.2 mM MnCl₂ was used to determine the Mn(II)-removing ability of strain Mn32. One millilitre of Mn32 culture (OD₆₀₀ 1) was inoculated into triplicate 100 ml volumes of the described medium and incubated at 28 °C with 150 r.p.m. shaking for up to 10 days. The Mn(II) concentration in liquid A medium was monitored by aseptically removing 10 ml culture every 24 h, centrifuging at 12 000 r.p.m. for 10 min, filtering through a 0.2 µm filter and measuring the Mn(II) concentration using an atomic absorption spectroscope (AAS, Beijing Purkinje General Instrument, Beijing, China) as recommended by the manufacturer. Abiotic controls were performed using the same medium without inoculation of strain Mn32. The amount of Mn(II) removed from the solution was calculated as [(Mn(II) initial − Mn(II) final)/Mn(II) initial] × 100.

The black precipitates (biogenic Mn oxides) generated by strain Mn32 may include solid Mn oxides and Mn(II) absorbed on the surface of Mn oxides. In order to measure the amount of Mn(II) absorbed to the Mn oxides, the black precipitates were washed three times with deionized water and subsequently mixed with 10 ml of a 20 mM CuSO₄ solution and incubated at 28 °C with 150 r.p.m. shaking for 12 h. This operation was to release the adsorbed Mn(II) from the solid Mn oxides (Tani et al., 2004). The solution was then centrifuged and filtered, and the Mn(II) concentration in the supernatant was measured using an AAS as described above.

In order to measure the amount of solid Mn oxides produced by strain Mn32, the remaining precipitates were washed three times with deionized water and mixed with 10 ml of 20 mM hydroxylamine hydrochloride (NH₂OH.HCl). The mixture was incubated at 28 °C with 150 r.p.m. shaking for 12 h. This operation reduced the solid Mn oxides to soluble Mn(II) that could be measured using the AAS.

To investigate the primary mechanism of Mn(II) oxidation by strain Mn32, the following experiments were performed. (1) An enzyme inhibitor, sodium azide (1 mM), was added to liquid A medium with 0.2 mM MnCl₂, inoculated with strain Mn32 and incubated as described above. The Mn(II) concentration in the medium was determined throughout the incubation period by AAS. (2) Another Mn(II) oxidase inhibitor, o-phenanthroline (0.1 mM), was used under the same conditions as in the case of sodium azide. (3) Strain Mn32 was pre-cultivated in liquid A medium (without adding MnCl₂) for 4 days, then 0.2 mM MnCl₂ was injected at the 4th day and the culture incubated for another 6 days. The Mn(II) concentration in the medium was determined throughout the incubation period by AAS.

Adsorption of heavy metals using different kinds of Mn oxides. To examine the sorption of Zn(II) and Ni(II) on different Mn oxides, biogenic Mn oxides produced by strain Mn32, commercial MnO₂ and fresh synthetic MnO₂ were used. The biogenic Mn oxides were generated by cultivation of strain Mn32 in liquid A medium containing 0.2 mM MnCl₂ and incubated at 28 °C with 150 r.p.m. shaking for 9 days; commercial MnO₂ (99 % purity) was purchased from Fuchen Chemical Reagent Company, Tianjin, China; fresh synthetic MnO₂ was prepared as described by Villalobos et al. (2003) using KMnO₄ to oxidize MnCl₂ in alkaline conditions (pH 10) to form δ-MnO₂.

To examine the adsorption of heavy metals on the biogenic Mn oxides, ZnSO₄ or NiSO₄ was added to triplicate 100 ml aliquots of 9-day-old Mn32 liquid A culture (30 µM final) containing fine generated biogenic Mn oxides. The pH was fixed at 7.0 by adding 20 mM HEPES buffer. Ten millilitres of culture was sampled each day and centrifuged at 12 000 r.p.m. for 10 min. The concentration of ZnSO₄ or NiSO₄ remaining in the supernatant was determined using AAS as described above. To calculate the amount of biogenic Mn oxides generated by strain Mn32 up to the 9th day, the precipitates were treated with 20 ml of 1 M NH₄OH.HCl to dissolve the solid Mn oxides to Mn(II) and later determined by AAS.

For experiments to study adsorption of biogenic Mn oxides, 1 ml of 10 mM commercial MnO₂ or the fresh synthetic δ-MnO₂ was added
to 9-day-old cultures of strain Mn32 in liquid A medium (without MnCl₂). ZnSO₄ or NiSO₄ was each added to the culture at a final concentration of 30 μM and incubated at 28 °C for another 5 days. The ZnSO₄ or NiSO₄ concentration in the medium was determined each day as described above.

To determine if there was heavy metal adsorption to cells or to the generated Mn oxides of strain Mn32, stock solutions of ZnSO₄ or NiSO₄ were each added at a final concentration of 30 μM to 9-day-old strain Mn32 cultures without adding any Mn oxides. Once a day, the concentration of ZnSO₄ or NiSO₄ was determined by AAS.

Observation of Mn oxide structures by scanning electron microscopy (SEM). SEM was used to observe micro-morphologies of biogenic, commercial and fresh synthetic Mn oxides. First, 0.5 ml of strain Mn32 cells grown in liquid A medium for 9 days (with and without adding MnCl₂) were centrifuged at 10 000 r.p.m. for 5 min, and the sedimented material was transferred onto a small coverslip which was fixed with double glue for SEM observation. Second, the powders of the commercial and the fresh synthetic Mn oxides were fixed to a conductive copper tape with double glue and observed directly by SEM. The above samples were examined using a SEM (JSM-6390/LV, JEOL) with 10 or 20 kV accelerating voltage.

Detection of Mn on the cell surfaces of strain Mn32 was performed by energy-dispersive spectroscopy (EDS) (JSM-6390/LV, NTC, Japan). Strain Mn32 was grown at 28 °C in 100 ml A medium containing 0.2 mM MnCl₂ for 4 days. The cells were harvested, centrifuged at 12 000 r.p.m. for 10 min and washed three times with sterile double-distilled water. The washed cells were dried in a vacuum-freeze dryer and subsequently analysed by EDS.

Characterization of the crystal structures of the three kinds of Mn oxides by X-ray diffraction (XRD) analysis. The crystal characteristics of the biogenic, commercial and fresh synthetic Mn oxides were determined using a X-ray diffraction meter (Y-2000, Dandong Aolong Radial Instrumental, China).

For biogenic Mn oxides, 10 ml of 9-day-old strain Mn32 culture (in liquid A medium containing 0.2 mM MnCl₂) was centrifuged at 10 000 r.p.m. for 5 min. The precipitates were air-dried, put in a sample groove and covered with a quartz slice.

For commercial and fresh synthetic Mn oxides, the powder was sifted through a 0.05 mm pore size sieve, loaded in a sample groove and covered with a quartz slice.

The prepared samples were analysed using CuKα radiation (λ=0.1543 nm) at 20 mA and 30 kV over the scanning range 5–90° 2θ, with an interval of 0.08°.

RESULTS

Resistence of strain Mn32 to Mn(II) and other heavy metal ions

Brachybacterium sp. strain Mn32 is a marine Mn(II)-oxidizing, Gram-positive bacterium. The colonies are white, round and translucent, with a moist surface, on plates of A medium (Tian & Shao, 2006). When the bacteria were cultured in liquid A medium, growth approached stationary phase within 3 days (Fig. 1). Strain Mn32 could grow in liquid A medium supplemented with up to 55 mM MnCl₂. When the initial concentration of Mn(II) was below 3 mM, the growth rate was not influenced (data not shown). Strain Mn32 also showed high resistance to some heavy metals. The MICs of Zn(II), Cr(II), Ni(II) and Cu(II) were 1 mM, 1 mM, 0.2 mM and 5 mM respectively. Strain Mn32 grew in liquid A medium with pH ranging from 5 to 10; the optimum growth pH was 7.0.

Mn(II) oxidation and removal by strain Mn32

When strain Mn32 was cultured in liquid A medium containing 0.2 mM MnCl₂, the MnCl₂ concentration began to decrease after 3 days and was almost completely precipitated/removed within 5 days of incubation (>95%) removed: MnCl₂ concentration from 0.197 mM to 0.007 mM; standard deviations (SD) were 0.025 and 0.0058, for 0 and 5 days, respectively. On the third day, solid black Mn oxides were observed and the solution Mn(II) decreased with the incubation time. Meanwhile, the amount of Mn(II) adsorbed on the Mn oxides also increased, reaching a peak on the fifth day, and then began to decrease. After 9 days, the amount of Mn(II)-adsorbed and solid Mn oxides reached equilibrium: in 100 ml Mn32 cultures originally containing 0.2 mM Mn(II), 0.13 mM Mn(II) was converted to solid Mn oxides and 0.07 mM Mn(II) was adsorbed on the solid biogenic Mn oxides (Fig. 1). The total amount of Mn stayed constant during the whole culture time (Fig. 1).

When two enzyme inhibitors, sodium azide (1 mM) (Fig. 2) and o-phenanthroline (0.1 mM) (data not shown) were added to cultures of strain Mn32 containing 0.2 mM Mn(II), no Mn(II) oxidation/removal occurred. This indicated that the Mn(II) oxidation was catalysed by proteins (Miyata et al., 2004). When the two enzyme inhibitors were added, the amount of soluble Mn(II) did not change, indicating that there was no-biogenic Mn(II) physically adsorbed on the Mn32 cells. Furthermore, when 0.2 mM MnCl₂ was added to
4-day-old Mn32 cultures instead of adding at the beginning of cultivation, no solid Mn oxides were produced after the following 6 days incubation (Fig. 2). This suggests that the protein catalysing Mn(II) oxidation in strain Mn32 may be activated by Mn(II). Since Mn oxides began to accumulate at day 3, when the culture was entering the stationary phase, a Mn(II) quorum-sensing signal transduction mechanism may be involved. The catalytic effect of the protein seems to be evident since the amount of Mn(II) adsorbed on the biogenic Mn oxides decreased after its peak and the amount of solid Mn oxides increased continuously until the two fractions of Mn reached an equilibrium.

**Removal of Zn(II) and Ni(II) by Mn oxides**

The adsorption curves of Zn(II) and Ni(II) on the biogenic Mn oxides produced by strain Mn32, on the commercial MnO₂ and on the fresh synthetic MnO₂ are shown in Fig. 3. When either the biogenic or abiogenic Mn oxides were present in strain Mn32 cultures, the amount of soluble Zn(II) and Ni(II) decreased (Fig. 3a, b) and the amount of Zn(II) and Ni(II) adsorbed to Mn oxides increased (data not shown). For Zn(II), in cultures containing 0.11 mM biogenic Mn oxides, the average concentration decreased from 27.5 μM (SD 0.71) to 4.0 μM (SD 0.17) in 4 days. In another two batches of cultures containing 0.10 mM commercial MnO₂ and 0.11 mM fresh synthetic MnO₂, concentrations of Zn(II) decreased from 30.1 μM (SD 0.98) to 24.5 μM (SD 1.72) and from 32 μM (SD 1.72) to 25.3 μM (SD 1.74), respectively. Both concentrations reached steady state in 1 day. For biogenic Mn oxides, commercial MnO₂ and fresh synthetic MnO₂, 85.5% (SD 0.009), 18.6% (SD 0.039) and 20.9% (SD =0.012) of Zn(II) were adsorbed/removed after 4 days, respectively.

For Ni(II), it took only 2 days to reach the adsorption steady state. However, the removal efficiency for Ni(II) was lower than that for Zn(II): 54.8% (SD 0.019) of Ni(II) was adsorbed on the biogenic Mn oxides, 16.7% (SD 0.035) on commercial MnO₂; 16.7% (SD 0.035) on commercial MnO₂ and 22.7% (SD 0.012) on fresh synthetic MnO₂ (Fig. 3b).

In the control, no significant decrease of Zn(II) and Ni(II) in strain Mn32 cultures occurred during the incubation periods without biogenic Mn oxides or abiogenic Mn oxides, suggesting that no physical metal sorption on strain Mn32 cells occurred (Fig. 3a, b).

The amount of the two metals adsorbed on Mn oxides produced by strain Mn32 was significantly higher than that observed for the two kinds of abiotic MnO₂. The molar ratio of the absorbed metals was defined as the molar amount of absorbed metals/the molar amount of Mn oxides. The average molar ratios of absorbed Zn(II) to biogenic Mn oxides, commercial MnO₂ and fresh synthetic
MnO\(_2\) were 0.206, 0.059 and 0.064, respectively (Fig. 4). These molar ratios for Ni(II) were 0.132, 0.06 and 0.063, respectively (Fig. 4).

**Micro-morphologies of strain Mn32 cells and different Mn oxides**

The micro-morphologies of strain Mn32 cells and the three kinds of Mn oxides observed by SEM are shown in Fig. 5. Cells of strain Mn32 are oval in shape, about 0.7 μm long and 0.5 μm wide (Fig. 5a).

Fig. 5(b) shows that the biogenic Mn oxides located on the cell surface of strain Mn32 seem to be layer-forming oxides. The particles of the biogenic Mn oxides were regular and much smaller than the abiotic Mn oxides studied here (Fig. 5c, d). Energy-dispersive spectroscopy analysis showed the presence of Mn on the cell surfaces of strain Mn32 (data not shown).

In contrast, the SEM images of the particles of the commercial and fresh synthetic MnO\(_2\) were more irregular (Fig. 5c, d). The particles of the fresh synthetic MnO\(_2\) were smaller than the commercial MnO\(_2\) (Fig. 5c, d). Neither the commercial nor the fresh synthetic MnO\(_2\) formed complex units with Mn32 cells when incubated with cultures of the bacterium (data not shown).

**XRD patterns of Mn oxides**

The XRD pattern of the fresh chemically synthesized MnO\(_2\) in our study closely resembled that of vernadite, which was assigned as δ-MnO\(_2\) (Fig. 6a). This synthetic δ-MnO\(_2\) was poorly crystallized and there was no significant diffraction peak between 5° 2θ to 90° 2θ (Fig. 6a).

The commercial MnO\(_2\) used in this study showed three relatively broadened peaks at 37.3°, 56.6° and 72° 2θ (Fig. 6b, peaks i, ii and iii). Their D values were 0.2412 nm, 0.1625 nm and 0.1310 nm, respectively. This MnO\(_2\) sample resembled pyrolusite, which was assigned as β-MnO\(_2\) (tetragonal system).

The CuK\(_\alpha\) XRD patterns of the biogenic Mn oxides produced by strain Mn32 showed the following features: (1) four narrow diffraction peaks at 36.4°, 38.2°, 43.2° and 53.2° 2θ (Fig. 6c, peaks a, b, c and d) with D values of 0.24682 nm, 0.23574 nm, 0.20955 nm and 0.17203 nm, respectively, that are typical for birnessite, which was also assigned as δ-MnO\(_2\); and (2) three typical diffraction peaks of manganite, which was assigned as γ-MnOOH, at 26.5°,
Microbial manganese oxidation mechanisms have been reported to be either direct or indirect (Greene & Madgwick, 1991). The direct mechanisms have been explained by enzymatic catalysis, while indirect oxidation occurred when the environmental conditions (pH, etc.) were changed by the growth of micro-organisms. In this study, we found that the Mn(II) oxidation in strain Mn32 cultures was enzymatically catalysed since the oxidation was inhibited by sodium azide, an enzyme inhibitor (Francis & Tebo, 2002). The oxidation was also inhibited by o-phenanthroline, which is a Cu(II) chelator, suggesting that Cu(II) is essential for the activity of the proposed metalloenzyme (Francis & Tebo, 2001). This enzyme was most probably activated by Mn(II) and expressed when the culture enters the stationary phase, since adding MnCl₂ to a 4-day-old culture did not result in the production of biogenic Mn oxides. Multicopper oxidases are a diverse family of proteins that utilize multiple copper ions as cofactors to oxidize a wide variety of substrates (Solomon et al., 1996). Genes encoding multicopper oxidases have been cloned from certain Mn(II)-oxidizing bacterial species and shown to be essential for Mn(II) oxidation (van Waasbergen et al., 1996; Corstjens et al., 1997; Brouwers et al., 1999; Ridge et al., 2007).

The Zn(II) and Ni(II) adsorption abilities of the biogenic Mn oxides were two to three times higher than those of commercial or fresh synthetic MnO₂. These adsorption levels were similar to those observed with biogenic Mn oxides produced by a manganese-oxidizing fungus, strain KR21-2 (Tani et al., 2004). The similar adsorption capacities for biogenic Mn oxides produced by strains Mn32 and KR21-2 indicate that biogenic Mn oxides produced by bacteria and fungi may share common physical and chemical properties. In this study, heavy metal adsorption experiments on biogenic Mn oxides were carried out with Mn(II) already absorbed on the biogenic Mn oxides. Tani et al. (2004) reported that there was competition between absorbed Mn(II) and other metal ions on the biogenic Mn oxides produced by fungus KR21-2. Thus, the heavy metal adsorption capacity of biogenic Mn oxides without Mn(II) absorbed previously should be higher than we determined here.

XRD analysis showed the crystal structures of the biogenic Mn oxides [birnessite (δ-MnO₂ group) and manganite (γ-MnOOH)], while the commercial pyrolusite (β-MnO₂ group) and the fresh synthetic vernadite (δ-MnO₂ group) were poorly crystallized. The crystal structures and the biogenic MnO₂ types might be important reasons for the high heavy metal adsorption ability of strain Mn32.

Birnessite appeared to be the major type of biogenic Mn oxide produced by the marine bacterium strain Mn32. Birnessite is a major manganese-bearing mineral of many soils and an important constituent of ‘desert varnish’ (http://www.mindat.org/min-680.html), and marine manganese nodules (Webb et al., 2005b). Birnessite has high specific surface area of up to 50–300 m² g⁻¹ of all known Mn oxides, it has the most powerful oxidizing capacity, and its cation-exchange capacity also very high (Feng et al., 2003).
2005). For all these characteristics, birnessite plays a very important role in processes of redox, adsorption and desorption in soil, underground water and sea water (Healy et al., 1966; McKenzie, 1981; Golden et al., 1986; Kim et al., 2002; Krauskopf, 1956; Post & Appleman, 1994). This may explain why the Mn oxides produced by strain Mn32 adsorbed more Zn(II) and Ni(II) than the two abiotic Mn oxides in this study.

Interestingly, the XRD pattern of the biogenic Mn oxides showed several diffraction peaks that are typical for manganite (-MnOOH). It has been reported that under specific conditions some bacteria oxidize Mn(II) to manganite (Toner et al., 2005). It is most probable that both Mn(IV) and Mn(III) were produced in our study. Webb et al. (2005a) reported that the Mn(III) form occurred as an intermediate product in the oxidation of Mn(II) by Bacillus sp. SG-1 spores. Sasaki et al. (2004) identified biogenic Mn oxides produced by a Mn-oxidizing fungus as a ramsdellite type.

Manganese-oxidizing micro-organisms are useful for treatment of Mn-contaminated water (Han et al., 2006). Fixed-film bioreactors containing Mn-oxidizing bacteria have been designed to remove heavy metals from wastewater (Han et al., 2006). The next step of our research is to immobilize strain Mn32 with filling materials or matrix to test its ability to remove Mn or other heavy metals in such a system. This study provides valuable information about potential applications of a manganese-oxidizing bacterium in heavy metal bioremediation and increases our basic knowledge of the manganese oxidation mechanisms.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (30570058), The PhD Supervisor Fund of the Ministry of Education, PR China (20060504027), and National Infrastructure of Natural Resources for Science and Technology Program of China (2005DKA21209). The authors would like to express their gratitude to Drs Christopher Rensing and Kris Pruski for their scientific comments.

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</tr>
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<td>5-8</td>
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<td>£231</td>
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<td>£231</td>
<td>£272</td>
<td>£312</td>
<td>£353</td>
<td>£46</td>
</tr>
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</table>

each 8pp extra £18  £23  £29  £35  £40  £46  £53  £58

PAYMENT BY CREDIT CARD  (Note: we cannot accept American Express)

Please charge the sum of £__________ to my credit card account.

My Mastercard/Visa number is (circle appropriate card; no others acceptable):

[ ]  [ ]  [ ]  [ ]  Expiry date  [ ]  [ ]  [ ]  [ ]  Security Number

Signature: ___________________________ Date: ______________

Cardholder’s name and address*: ____________________________________________

*Address to which your credit card statement is sent. Your offprints will be sent to the address shown at the top of the form.

May 2006