Removal of Manganese(II) Ions from Water by *Leptothrix discophora* with Carbon Fiber

Keiko Sasaki1, *, Mai Endo2, Kunihiko Kurosawa3 and Hidetaka Konno2

1Laboratory of Environmental Science, Otaru University of Commerce, Otaru 047-8501, Japan
2Graduate School of Engineering, Hokkaido University, Sapporo 060-8628, Japan
3Geological Survey of Hokkaido, Sapporo 060-0819, Japan

To apply manganese-oxidizing bacteria to waste water treatment, basic performance of the manganese-oxidizing bacterium, *Leptothrix discophora*, distributed by ATCC was examined. In addition, the effect of carbon fiber on the oxidation by the bacterium was also investigated, since carbon fiber was reported to accelerate the growth of activated sludge during sewage treatment. The bacterium was found to be active in the medium containing 24 ppm Mn(II) ions, the concentration being about 8 times higher than the recommended one and practically useful level. The oxidation rate was higher with the static culture than the shaking culture. This was considered to be due to physical damage to the sheath structure of bacteria which is reported to be important to oxidize Mn(II) ions. The carbon fiber did not accelerate the microbial oxidation of Mn(II) ions. This is partly attributed to the lack of contact between the bacterial cell, which floated as thin membranes, and the carbon fiber sunk in the bottom of vessels. However, oxidized Mn species precipitated on the carbon fiber, which resulted in an improvement of the transparency of water. This effect is different from the one caused by activated carbons, since the carbon fiber has very low specific surface area and no pore structure. The effect was more remarkable in the shaking culture than the static culture, indicating that organic substances originated from the bacterium play an important role in the adsorption of oxidized Mn species.

(Received March 18, 2002; Accepted August 19, 2002)

Keywords: *Leptothrix discophora*, carbon fiber, removal of manganese, mine drainage

1. Introduction

There are a number of abandoned manganese mines in west Hokkaido, Japan, where manganese carbonate (rhodochrosite, MnCO3, known as vein type, so called Inakuraishi-type) occurs typically. Manganese carbonate is not very common worldwide because manganese generally occurs in oxide forms (e.g. pyrolusite, MnO2). Rhodochrosite dissolves in aqueous environments more easily than pyrolusite, and spillage of Mn-rich mine drainage is a serious and characteristic problem at abandoned mines in Hokkaido.

Mine water containing high concentration of Mn(II) ions is treated by the following process: first it is alkalized to a pH higher than 8.5, then it is aerated to oxidize Mn(II) ions to MnO2 precipitates, and finally the alkaline water is neutralized by acids to discharge. Accordingly, the treatment of Mn-rich water costs high. It has been reported that Mn-oxidizing bacteria play an important role in the precipitation of manganese oxide minerals at neutral pH environments, such as hydrothermal vents1–5 and in manganese mine environments.5) Application of the bacteria to water treatment is interesting and attractive. *Leptothrix discophora* was isolated from springs rich in Fe and Mn, and well characterized as one of the most typical manganese-oxidizing bacteria.6) This bacterium can maintain sheath-forming ability, which is reported to be important to oxidize Mn(II) ions. Shortcomings of bacterial treatment are a slow reaction rate and low tolerance of bacteria to metal ions. Recommended concentration of Mn(II) ions for culturing *Leptothrix discophora* is about 3 ppm, which is too low to apply to the practical processes. Therefore, it is necessary to examine whether the bacterium can oxidize much higher concentrations of Mn(II) ions by devising some conditions of culture.

Recently, it was reported that carbon fibers accelerate the growth of activated sludge during sewage treatment and are utilized as a "bed" for water treatment with activated sludge both on a laboratory scale and a small plant scale.7, 8) The reported effects are remarkable and many plans of application are now proceeding in the field.9) The carbon fibers are a kind of micro-fiber of several micrometers in diameter, and also known to have bio-philic surfaces. Accordingly, there is a possibility that carbon fibers can accelerate the biological oxidation of manganese.

The present work was carried out to investigate if the application of *Leptothrix discophora* distributed by ATCC (American Type Culture Collection) and carbon fibers can be a way out of the shortcomings of bacterial treatment, as the first step to the practical biological treatment of manganese drainage. To begin with, the tolerance of the bacterium to Mn(II) ion was evaluated, then the effect of carbon fiber on the bacterial oxidation was examined.

2. Experimental

2.1 Microorganisms

Manganese-oxidizing bacterium, *Leptothrix discophora* SP-6, was obtained from ATCC (51168).10) The recommended composition of liquid medium is as follows: (NH4)2SO4 0.24 g, MgSO4·7H2O 0.06 g, CaCl2·2H2O 0.06 g, KH2PO4 0.02 g, Na2HPO4 0.03 g, HEPES ([4-[(2-hydroxyethyl)-1-piperaziny]ethanesulfonic acid) 2.383 g, distilled water 984.0 mL. After autoclaving at 121°C for 20 min, the solution pH was adjusted to 7.2 by NaOH. Then, after cooling to approximately 50°C, the following filter-sterilized solution was aseptically added: 10 mmol·L−1 FeSO4·7H2O 1.0 mL, vitamin solution (biotin, 20.0 mg; folic acid, 20.0 mg; thiamine HCl, 50.0 mg; D-(-)-calcium pan-
tothenate, 50.0 mg; vitamin B12; riboflavin, 50.0 mg; nicotinic acid, 50.0 mg; pyridoxine HCl, 100.0 mg; \( p \)-aminobenzoic acid, 50.0 mg; distilled water, 1 L). 1.0 mL, 20% sodium pyruvate 5.0 mL, and necessary amounts of \( \text{MnSO}_4 \cdot 7\text{H}_2\text{O} \). The cells were first grown up in 10 mL-medium without Mn-source contained in 100 mL flasks, according to the recommended procedure by ATCC. After 2–4 days the cells grew to white membrane shapes, and then more broth was added. Many sheaths including chained cells were observed in the culture by a microscope. The cells were mainly cultured in 150 mL medium contained in 500 mL-Erlenmeyer flasks with porous-plugs without shaking in an incubator (EYELA, KCL-1000, Japan) at 20°C. When the colonies on white membrane shapes fully spread the surface of medium, the cell suspension was used for the following experiments. Shaking culture was also carried out at 100 rpm using a TB-16 shaker (Takasaki Co. Ltd., Japan).

2.2 Carbon fiber

Commercially available carbon fiber H-20ST (Showa Denko Co. Ltd., Japan) was used. It is a yarn-tape of about 2–3 mm wide and the diameter of single fiber is 7–10 μm. The tape was cut into about 30 cm long and tied to a loop to avoid threads loosing during experiments. Sizing agents on the fibers were removed by heating at 500°C for 1 h in air. This treatment makes the surface become hydrophilic.

2.3 Laboratory experiments for microbial removal of dissolved Mn(II) ions

First, 500 mL Erlenmeyer flasks were filled with 100 mL of the above medium with 15 ppm Mn(II) at the initial pH 7.2. Each 2 mL of the cell suspension was inoculated to the flask. Control experiments with 2.00 mL of 5 mass%NaNO\(_3\) (sterilizing agent) were also carried out. Parallel experiments were carried out with and without adding the sterilized carbon fiber including control experiments. All flasks were sealed with porous-plugs and installed in an incubator or in a rotary shaking culture-apparatus TB-16 (Takasaki Kagaku Co. Ltd., Japan) at 20 ± 2°C for about 100 h. At intervals, the supernatants were sampled, and filtered by membrane filter with 0.2 μm pore size, then diluted with a hydrochloric acid solution. Dissolved Mn species were determined by atomic absorption spectrometry (Hitachi, Z-6100).

After the experiments, a portion of unfiltered solution was measured by an UV-VIS spectrometer with an integrating sphere (JASCO V-550+ISV-469, Japan) to estimate the turbidity of solution, though this technique is not direct evaluations of turbidity. Precipitates formed on the carbon fiber were collected by filtration using a 0.2 μm pore size membrane filter and stored at 4°C for analysis.

2.4 Analysis of precipitates formed by microbial treatment

The precipitates were analyzed after drying at room temperature by a powder X-ray diffractometer (XRD, RIGAKU Rint-2000 with a monochromator, Cu Kα, 40 kV, 25 mA) and an X-ray fluorescence analyzer (XRF, JEOL JSX-3220Z). The precipitates with carbon fiber were observed by a field emission type scanning electron microscope (FE-SEM, JEOL JSM-6300F) at an acceleration voltage of 2–3 kV after evaporating a thin platinum layer on the sample. The precipitates with the carbon fiber were also analyzed by XPS using an ESCALAB Mk II (VG Scientific) to investigate the chemical states of Mn in sediments. After evacuating to better than \( 10^{-5} \) Pa for 15 min, the sample was transferred into an analyzer chamber of better than \( 5 \times 10^{-8} \) Pa, then irradiated with Al Kα X-ray (15 kV, 10 mA). The binding energies, \( E_B \), were calibrated with \( E_B[\text{Au 4f}_{7/2}] = 84.0 \) eV. Intensity by area was measured after drawing the background by Shirley method.

3. Results and Discussion

Generally, the Mn(II) ion concentrations in Mn-rich mine drainage ranges from 10 to 100 ppm. It has been recommended that the Mn concentration is around 55 μmol L\(^{-1}\) (~3 ppm) for culture of \( \text{L. discophora} \) (ATCC 51168). Therefore, the tolerance of the bacterium to Mn(II) ions was evaluated if the bacterium grow at higher concentrations than the reported one. Figure 1 shows the Mn(II) ion-removal curves for the different initial concentration during the static culture in the absence of the carbon fiber. Hereafter each point is the average of three data and error bars indicate ±1σ. Even at \( [\text{Mn(II)}]_{\text{initial}} = 24 \) ppm, the bacterium was able to oxidize Mn(II) ions perfectly within about 200 h. The Mn(II) concentration was about 8 times higher than the recommended level. With increasing the initial concentration, the induction period became longer but the curve shifted in parallel with the concentration. It indicates that once oxidation started it proceeded in a similar manner irrespective of the concentration, suggesting that the activity of the bacterium did not change by Mn(II) concentration. Changes in the number of bacteria were not measured in the present work because \( \text{L. discophora} \) SP-6 formed thin membrane-shapes and a simple counting method could not be applied.

\( \text{Leptothrix discophora} \) SP-6 belongs to the category of sheath bacteria. Therefore, it is important to examine the effect of shaking on the stability of the bacterial sheath, since

![Fig. 1 Effect of the initial Mn(II) ion concentrations on the oxidation of Mn(II) ions by \( \text{L. discophora} \) without carbon fiber in the static culture. Error bars indicate 1σ (\( n = 3 \)).](image-url)
Removal of Manganese(II) Ions from Water by Leptothrix discophora with Carbon Fiber

Mn oxidizing proteins are in the sheath as reported.\textsuperscript{11}) Effect of shaking on the removal of Mn in the absence of the carbon fiber is shown in Fig. 2. It clearly indicates that shaking results in the adverse effect for the removal. After 100 h in the static culture, the filamentous sheaths of \textit{L. discophora} spread like sheets on the surface of the medium, and brown-color manganese deposits were concentrated in the sheets. It was found by microscopic observation that shaking broke these sheaths, which is one of the reasons of lower oxidation rate.

Carbon fibers became hydrophilic by the pretreatment and went down to the bottom of flask when added to the medium. The effect of carbon fiber cannot be expected without contact between the fiber and the bacterial cell, so that the shaking culture was used in this experiment at the cost of lowering oxidation rate. Time variations of Mn(II) concentration in the presence and absence of the carbon fiber during the shaking culture of \textit{L. discophora} at 20°C are shown in Fig. 3. Within 100 h, 15 ppm Mn(II) ions were completely oxidized to Mn oxides and hydroxides, and removed from the solutions. Effect of carbon fiber, however, was not clearly observed, showing that the carbon fiber did not accelerate the microbial oxidation of Mn(II) ions by \textit{L. discophora}. The same results were obtained by the static culture additionally carried out.

As mentioned above, the carbon fiber did not affect to the activity (and probably growth rate, too) of the bacterium but the transparency of the solution after the experiments shown in Fig. 3 was much better in the presence of fiber. Light transmittance in a region of 200 to 900 nm was measured for unfiltered solutions, as shown in Fig. 4. The data are not directly related to the turbidity but they confirmed the difference observed with the naked eye. The efficiency was a little better in the shaking culture than the static culture, probably due to better contact between the carbon fiber and oxidized Mn species. Figures 3 and 4 indicate that the carbon fiber functions as a kind of adsorbent of colloidal and fine particles of oxidized Mn species. It should be noted here that the carbon fiber used here has a very low specific surface area and is different from activated carbons. As shown in Fig. 5(a), the diameter of single fiber is about 7 µm and the surface is smooth and has no macro-pore structure except for traces made during spinning. Accordingly, this fiber does not adsorb ions, hydroxides and so on. In Fig. 5(b), precipitates are observed on the surface of fiber after the oxidation experiment shown in Fig. 3. These results imply that the sheath substances or the secretions from bacteria play an important role in the adsorption of precipitates.

No crystalline components were observed on the carbon fiber by XRD. By XRF the clear peaks assigned to Mn K\textalpha{} and Mn K\beta{} were observed on the carbon fibers after the 100 h shaking culture in a 15 ppm Mn(II) ion solution, as shown in Fig. 6. Accordingly, Mn(II) ions were oxidized to poorly crystallized manganese precipitates by \textit{L. discophora}. The products are very similar to those formed from mine drainages.

Fig. 2 Comparison of Mn(II) oxidation rate between the shaking culture and the static culture of \textit{L. discophora} without carbon fiber. Error bars indicate 1\sigma{} (n = 3). ○, shaking; ◦, static; ×, control.

Fig. 3 Time variations of Mn(II) concentration in the presence and absence of carbon fiber in the shaking culture of \textit{L. discophora}. Error bars indicate 1\sigma{} (n = 3).

Fig. 4 Transmittance of the supernatant after the experiment in Fig. 3.
by using the sediments from a hot spring in Hokkaido, which we have reported previously.\textsuperscript{12)}

The XPS spectra of the Mn 3s region for the precipitates with carbon fiber after the shaking and static cultures of \textit{L. discophora} in 15 ppm Mn ion solutions are shown in Fig. 7. Apparently there are two peaks in the Mn 3s region due to the multiplet splitting caused by the two final states, and the oxidation states of Mn species are distinguishable by the value of splitting.\textsuperscript{13)} The splitting was 4.9 eV for the precipitates by the shaking culture, indicating that Mn(III) and Mn(IV) species are mixing in the outermost surface of the precipitates. In case of the static culture, the peak intensities of Mn 3s were too weak to determine the chemical states. Figure 8 shows the Mn 2p spectra for the precipitates on the carbon fiber after shaking and static cultures. The Mn 2p spectrum is not always suitable to determine the chemical states of manganese, but the binding energy, $E_{\text{B}}[\text{Mn 2p}_{3/2}] = 642$ eV, for both samples also supports that manganese is present as a mixture of Mn(III) and Mn(IV). These results and Fig. 5(b) clearly indicate that adsorption of Mn(II) ions on the surface of carbon fiber is negligible. Figure 9 shows the C 1s spectra for the carbon fiber after the shaking and static cultures. Intensity of C 1s peak was much larger than that of Mn 2p and nearly the same for both samples. The peak intensity ratios of Mn 2p to C 1s, $I[\text{Mn 2p}]/I[\text{C 1s}]$, were calculated to

---

\textbf{Fig. 5} SEM images of the carbon fibers (a) before and (b) after the experiment in Fig. 3. Horizontal bars indicate 1 µm.

\textbf{Fig. 6} XRF spectrum for the carbon fiber after the experiment in Fig. 3.

\textbf{Fig. 7} XPS spectra of Mn 3s region for precipitates with the carbon fiber after (a) shaking and (b) static culture of \textit{L. discophora} for 100 h in the media of 15 ppm Mn ions. Vertical bars indicate 20 counts s$^{-1}$.

\textbf{Fig. 8} XPS spectra of Mn 2p region for the same samples with Fig. 7. Vertical bars indicate 200 counts s$^{-1}$.

\textbf{Fig. 9} XPS spectra of C 1s region for the same samples with Fig. 7. Vertical bars indicate 2 kcounts s$^{-1}$. 
examine the difference in surface composition between the shaking culture and the static culture. The ratio was 0.49 for the shaking culture, and 0.13 for the static culture. Although there are some contributions of carbonaceous contamination to the intensities of C 1s peak during the measurements, the observed difference is significant, suggesting that the shaking culture enhanced the accumulation of Mn(III, IV) species on the carbon fiber.

The E_B[C 1s] for the carbon fiber and hydrocarbon species are 284.6 and ca. 285 eV, respectively, and higher E_B components consist of the carbon species containing oxygen, such as -COH, -COOH, -C=O, and so on, in this case. Such surface functional groups on the carbon fiber are not large amounts even after the heat treatment, so that the peak intensity of higher E_B region is much lower than that of the carbon fiber peak, which is represented by the C 1s spectrum (b) in Fig. 9. The relative intensity of higher E_B region of the spectrum (a), however, is much larger than (b), indicating that larger amounts of organic components containing the functional groups described above are present on the surface. This suggests that the organic substances originated from the bacteria (possibly the bacteria and their remains also) are contributing the accumulation of manganese species on the fiber, since the experimental conditions are the same except for the shaking. Peak separation of C 1s spectrum by a computer program is possible but it is not appropriate to carry out the separation in this case, because six or more peaks from two components, the carbon fiber and the deposit, must be considered, leading to selfserving results.

4. Conclusion

Application of manganese-oxidizing bacteria to the treatment of Mn-rich waste water is interesting and attractive, considering the cost for conventional water treatment. In the present work, basic performance of the manganese-oxidizing bacterium, Leptothrix discophora, distributed by ATCC was examined. In addition, the effect of carbon fiber on the oxidation by the bacterium was also investigated, since carbon fiber was reported to accelerate the growth of activated sludge during sewage treatment. The following findings were obtained.

(1) *L. discophora* (ATCC 51168) was found to be active in the medium containing 24 ppm Mn(II) ions, the concentration being about 8 times higher than the recommended one and practically useful level.

(2) The oxidation rate was higher with the static culture than the shaking culture of the bacterium. This was considered to be due to physical damage to the sheath structure of bacteria which is reported to be important to oxidize Mn(II) ions.

(3) The carbon fiber did not accelerate the microbial oxidation of Mn(II) ions. This is partly attributed to the lack of contact between the bacterial cell, which floated as thin membranes, and the carbon fiber sunk in the bottom of vessels. However, oxidized Mn species precipitated on the carbon fiber, which resulted in an improvement of the transparency of water.

(4) The effect of carbon fiber is different from the one caused by activated carbons, since the fiber has very low specific surface area and no pore structure. The effect was more remarkable in the shaking culture than the static culture, indicating that organic substances originated from bacteria play an important role in the adsorption of oxidized Mn species.

Acknowledgments

This work was partly supported by a Grant-in-Aid for Scientific Research (No. 13555275) from the Ministry of Education, Science, Sports and Culture in Japan.

REFERENCES