Characterization of the Biogenic Manganese Oxides Produced by *Pseudomonas putida* strain MnB1

Shaofeng Jiang, Do-Gun Kim, Jeonghyun Kim, Seok-Oh Ko[†]

Department of Civil Engineering, Kyung Hee University, Yongin, 449-701, Republic of Korea

Abstract

Biogenic Mn oxides are expected to have great potential in the control of water pollution due to their high catalytic activity, although information on biological Mn oxidation is not currently sufficient. In this study, the growth of a Mn oxidizing microorganism, *Pseudomonas putida* MnB1, was examined, with the Mn oxides formed by this strain characterized. The growth of *P. putida* MnB1 was not significantly influenced by Mn(II), but showed a slightly decreased growth rate in the presence of Pb(II) and EE2, indicating their insignificant adsorption onto the cell surface. Mn oxides were formed by *P. putida* MnB1, but the liquid growth medium and resulting biogenic solids were poorly crystalline, nano-sized particles. Biogenic Mn oxidation by *P. putida* MnB1 followed Michaelis-Menten kinetics, with stoichiometric amounts of Mn oxides formed, which corresponded with the initial Mn(II) concentration. However, the formation of Mn oxides was inhibited at high initial Mn(II) concentration, suggesting mass transfer obstruction of Mn(II) due to the accumulation of Mn oxides on the extracellular layer. Mn oxidation by *P. putida* MnB1 was very sensitive to pH and temperature, showing sharp decreases in the Mn oxidation rates outside of the optimum ranges, i.e. pH 7.43-8.22 and around 20-26 °C.

Keywords: Biogenic Mn oxides, *Pseudomonas putida* MnB1, Mn oxidation kinetics

1. Introduction

Manganese oxides (Mn oxides) promote the oxidation of a variety of synthetic and natural organic pollutants, such as humic substances, phenols, endocrine disrupters and chlorinated organic compounds, due to their high redox potential [1-4]. Recently, biologically generated Mn oxides have been of increasing interest due to their high available surface area [5]. Microorganisms (bacteria and fungi) that oxidize Mn(II) to Mn(IV) oxides are known as Mn-oxidizing microorganisms. The Mn-oxidizing bacteria present in raw water can grow and reproduce under appropriate conditions and are able to oxidize Mn(II), with precipitation of the oxidized form, Mn(IV). The biogenic Mn oxides formed by Mn-oxidizing bacteria have many specific characteristics, such as high specific surface areas and reactivities, and can be used for adsorbents, catalysts, and oxidants or reductants [6]. Under circumneutral conditions, the microbial oxidation of Mn(II) proceeds several orders of magnitude faster than the abiotic oxidation, such as in homogenous and mineral surface-catalytic reactions [5]. Therefore, the microbial Mn(II) oxidation process has gained increasing attention with regard to its environmental applications.

Studies on biogenic Mn oxidation have focused on several organisms, including *Bacillus* sp. strain SG-1, which is a gram-positive, rod-shaped bacterium that produces Mn oxidizing

spores [7], *Leptothrix discophora* strains SS-1 and SP-6, which are sheet-forming bacteria, living in fresh water [8-9], and *Pseudomonas putida* strains MnB1 and GB-1, which are gram-negative bacteria that produce Mn oxides on the cell surface, and live in both fresh water and soil [10-11].

The objectives of this study were to characterize the growth of *P. putida* MnB1, investigate the kinetics of Mn(II) oxidation by *P. putida* MnB1 and examine the property of the biogenic Mn oxides produced. Factors affecting the growth of *P. putida* MnB1 and Mn(II) oxidation were also investigated.

2. Materials and methods

2.1. Culture and medium

Pseudomonas putida strain MnB1 was supplied by the American Type Culture Collection (ATCC), under number 23483. The oxidation of Mn was demonstrated using #279 Broth, as recommended by the ATCC, the composition of which is shown in Table 1. The culture was transferred to this medium and incubated at 26°C. The formation of black precipitates indicated the presence of Mn oxides. The precipitates usually appeared in approximately 24 hr. The bacteria were cultured in #3 Broth (Difco 0003) and #3 Agar (Difco 0001), as recommended the ATCC, the com-

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received September 10, 2010 Accepted October 28, 2010

[†]Corresponding Author

E-mail: soko@khu.ac.kr

Tel: +82-31-201-2999 Fax: +82-31-202-8854

positions of which are presented in Table 2. The liquid growth medium for *Pseudomonas putida* MnB1 was prepared, as presented in Table 3. Trace element solution in Table 3 includes 6.4 mg/L CuSO_4 , 44 mg/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 20 mg/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and 13 mg/L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$.

2.2. Growth of *Pseudomonas putida* strain MnB1

Batch experiments were conducted in 300 mL Erlenmeyer culture flasks (Duran) with the liquid growth medium. For each batch experiment, one tube of *P. putida* MnB1 was firstly taken out of the ultralow temperature refrigerator and transferred to several Petri dishes containing #3 Agar (Difco™) using a loop. The Petri dishes were then incubated at 30°C for 24 hr. All bacteria were transferred from the Petri dishes to a glass vial containing liquid growth medium using a loop, with 2 mg of bacteria per 1 mL of medium. After mixing, 1 mL of the bacteria suspension was added to a flask containing 100 mL of the liquid growth broth. The bacteria were incubated at 26°C and 150 rpm in a reciprocating shaker. To determine the effects of the initial amount of bacteria and Mn(II) concentration, the growth of *P. putida* MnB1 was investigated with 0.1–0.9 mL of the bacterial suspension and 0–100 mg of Mn/L (MnCl_2). The effects of heavy metals and 17 α -ethinylestradiol (EE2) were also studied. The growth curves of *P. putida* MnB1 in the presence of 10–100 μM Pb(II) ($\text{Pb}(\text{NO}_3)_2$), as a representative heavy metal, and those with 1–3 mg/L EE2 were investigated. All batch experiments were carried out for 72 hr, with three replicates. The growth curves over a 48 hr period were obtained because the stationary phase was attained after 24 hr for all experiments. The optical density at 600 nm (OD600), measured using a spectrophotometer (UV-mini

1240, Simadzu), was used as the indicator of the bacterial concentration [8, 13–14]. All chemicals in this study were purchased from Sigma-Aldrich Inc. and were of HPLC or analytical grade.

2.3. Batch experiments for the oxidation of Mn(II)

The oxidation of Mn(II) by *P. putida* MnB1 was carried out in 300 mL Erlenmeyer culture flasks (Duran). The bacterial suspension was inoculated to the flasks containing the liquid growth medium and Mn(II). To determine the effect of the initial Mn(II) concentration on Mn(II) oxidation rate, a series of Mn(II) oxidation experiments was performed at four different Mn(II) concentrations, ranging from 2 to 20 mg/L, at 26°C and an initial pH of 5.56. The effect of pH on the oxidation of Mn was investigated by varying the pH in 1 pH unit intervals, from 4.5 to 8.5, at 26°C and an initial Mn(II) concentration in the Tebo medium of 12 mg/L. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma) was used as the buffer because it is known to be better at maintaining a physiological pH, despite changes in the carbon dioxide concentration produced by cellular respiration, compared to bicarbonate buffers [15]. HCl and NaOH were used to adjust the initial pH. The rates of Mn(II) oxidation were also determined at different temperatures, ranging from 20 to 30°C, at pH 5.56.

The Mn(II) concentration of the filtrate was analyzed according to Standard Methods [16], using a spectrophotometer (UV-mini 1240, Simadzu), with the amount of Mn oxides in the liquid culture quantified by reaction with the reductive dye, Leucoberbelin blue I (LBB) [17]. Oxidized LBB is blue and the intensity of coloration is a function of the amount of Mn oxides having been reduced. The color intensity was measured for optical density at 618 nm using the spectrophotometer.

2.4. Characterization of the biogenic Mn oxides.

Mn oxides particles should be separated for examination of their properties by XRD and microscopy because organics and cell material accounted for more than 80% of the total resulting solid mass prior to the biological oxidation of Mn. The purification procedure aimed at ensuring the removal of organic materials (and absorbed anions and so on) that may interfere with the subsequent characterization. This involved a relatively harsh treatment to oxidize and dissolve the organic matter. The procedure reported by Mandernack et al. was adopted in this study, as described below [18].

1. Raw oxides in the suspension were collected in 40 mL vials and centrifuged for 20 min at 2000 rpm and room temperature. The supernatant and visible solid cell materials settled on the Mn oxides were discarded, with the solids washed by shaking with deionized (DI) water for 5 min. The above procedure was repeated 10 times.
2. Dissolution of the cell material using a 45 min sonication treatment with phenol, and subsequent 10 min sonication treatments with a phenol:chloroform (50:50, v/v) mixture, chloroform and a methanol:chloroform:water (12:5:3, v/v/v) mixture.
3. A 10-cycle DI water wash, as in step 1.
4. The suspension was acidified to pH 3, shaken for 30 min and centrifuged. The supernatant solution was discarded to eliminate any absorbed Mn (II).
5. A 10-cycle DI water wash, as in step 1.
6. Oxidation of the remaining solid organic material was ac-

Table 1. Composition of #279 Broth (ATCC™)

Component	Concentration in deionized H ₂ O (g/L)
MnCO ₃	1.0
Fe(NH ₄) ₂ (SO ₄) ₂ ·6H ₂ O	0.15
Yeast extract	0.075
Sodium citrate	0.15
Na ₄ P ₂ O ₇ ·10H ₂ O	0.05
Agar	10.0

Table 2. Compositions of Nutrient Broth and Nutrient Agar (Difco™).

Nutrient #3 Broth (per liter H ₂ O)	Nutrient #3 Agar (per liter H ₂ O)
Beef Extract : 3.0 g	Beef Extract: 3.0 g
Peptone : 5.0 g	Peptone : 5.0 g
	Agar : 15.0 g

Table 3. Composition of the liquid growth medium [12].

Component	Concentration in deionized H ₂ O (g/L)
Yeast extract	0.5
Casamino acids	0.5
Glucose	1
HEPES buffer	2.38
CaCl ₂	0.222
MgSO ₄ ·7H ₂ O	0.8118
FeCl ₃ ·6H ₂ O	0.001
MnCl ₂	0.1145
Trace element solution	1 mL/L

complished by shaking for 4 hr in 0.17% NaClO solution. The high pH (>10) of this solution ensured the removal of absorbed anions.

7. A 10-cycle DI water wash, as in step 1.

For XRD analysis, the samples were oven-dried overnight at 60°C, homogenized with a mortar and pestle and subjected to an 18 kW X-Ray diffractometer (M18XHF-SRA, MAC Science). The samples were mounted on a holder and scanned over the range of $2\theta = 5-90^\circ$. A field emission transmission electron microscope (FE-TEM, JEM-2100F, JEOL), operating at 200 kV, was used to acquire TEM images of the biogenic Mn oxides. For the TEM, the Mn oxides were dispersed in DI water, with a grain mount prepared by placing a drop of the suspension on a 3 mm Cu grid, with holey carbon film, and left to dry in the air before its introduction into the TEM. Scanning electron micrographs were obtained using a field emission scanning electron microscope (FE-SEM, LEO SUPRA55, Carl Zeiss) via energy dispersive X-ray spectroscopy (Genesis 2000, EDAX).

3. Results and Discussion

3.1. Growth of *P. putida* MnB1

The growth of *P. putida* strain MnB1 was initially investigated

to determine the experimental conditions for the oxidation of Mn(II) because the formation of Mn oxides occurs during different growth phases with different microbial strains. For example, the oxidation of Mn(II) takes place after the stationary phase with *P. putida* strains and *Brachybacterium* sp. Mn32 [19-20], but Mn oxides start to form from the log growth phase with *Bacillus* sp. WH4 [13]. Furthermore, the growth at different Mn(II) concentrations and the effects of Pb(II) and EE2 were also investigated.

Growth curves for *P. putida* MnB1, with different initial amounts of bacterial inoculation and Mn(II) concentrations are shown in Fig 1. The acclimation period to the medium was 2-4 hr for the bacteria, and an increase in the volume of initial bacterial suspension led to faster acclimation (Fig 1(a)). The growth rate during the exponential growth phase was not influenced by the amounts of the inoculant, and the populations during stationary phase, which was reached after approximately 24 hr, were nearly identical. Growth curves for the different initial Mn(II) concentrations are shown in Fig 1(b), which show no significant effect on the growth of *P. putida* MnB1 on varying the Mn(II) concentration. These results indicate that the effects of the initial Mn(II) concentration and amount of bacteria on the growth of *P. putida* MnB1 were negligible. Meanwhile, the growth rate decreased in the presence of Pb(II) and EE2, but not significantly, as shown in Fig 2.

Fig. 1. Growth curves for *P. putida* MnB1 in solutions with different initial amounts of bacteria (a) and those with different initial Mn(II) concentrations (b).

Fig. 2. Growth curves for *P. putida* MnB1 in the presence of Pb(II) (a) and EE2 (b).

The growth of *P. putida* MnB1 was slightly retarded, but not significantly, in presence of 10 to 100 μ M Pb(II) or 1 to 3 mg/L EE2. However, the acclimation period and final population were not influenced, and the stationary phase was attained in approximately 24 hr, regardless of the presence or absence of Pb(II) or EE2. This indicates that the growth of *P. putida* MnB1 was not inhibited by the bulk phase Pb(II) or EE2, but by the amounts adsorbed, which was identical regardless of the initial concentration in the bulk phase. It has also been reported that the biosorption of metals and EE2 is negligible and their removal is dominantly contributed to by the Mn oxides in a system of Mn(II) and Mn oxidizing microorganisms. Sabirova et al. investigated the removal of EE2 using *Leptothrix discophora* and *P. putida* strains and found that the degradation of EE2 started after the generation of Mn oxides [19]. It was also reported by Wang et al. that Zn(II) and Ni(III) were not decreased until the Mn oxides had been generated by *Bacillus* sp. Mn32. Therefore, the negligible effects of Pb(II) and EE2 are suggested as being due to their minor accumulation on the cell surface [20].

3.2. The oxidation of Mn(II) by *P. putida* MnB1 and the effects of pH and temperature

The Mn(II) concentrations during 108 hr of incubation with varying initial Mn(II) concentrations are shown in Fig 3. The oxidation of Mn(II) started after the stationary growth of *P. putida*

Fig. 3. The oxidation of Mn(II) by *P. putida* MnB1 with varying initial Mn(II) concentrations (a) and the rate of Mn(II) oxidation as a function of the Mn(II) concentration (b).

MnB1, as observed in the generation of Mn oxides by *P. putida* strains and *Brachybacterium* sp. Mn32 [19-20]. The MnO₂ concentrations for 2, 5, 10, 20 mg-Mn/L reached 3.171, 9.512, 19.024 and 31.706 mg/L, respectively, which are close to the theoretical amounts. The increased initial Mn(II) concentration resulted in the generation of more MnO₂, and the color of the medium and bacteria mixture became darker with increasing initial Mn(II) concentration following 108 hr of the reaction, while the growth of *P. putida* MnB1 showed no dependency on the Mn(II) concentration (Fig 1). It has been accepted in many investigations that biogenic Mn(II) oxidation follows Michaelis-Menten enzyme kinetics, as presented in eq. (1) [8, 21], which was further verified by the study on the oxidation of Mn(II) using the purified enzyme from *Acremonium* sp. strain KR21-2 [22].

$$-\frac{d[Mn(II)]}{dt} \frac{1}{X} = \frac{k[Mn(II)]}{K_s + [Mn(II)]} \quad (1)$$

In eq. (1), k is the maximum Mn(II) oxidation rate (mg-Mn/mg-cell·min), K_s the half velocity constant (mg-Mn/L) and X the cell concentration (mg-cell/L), which was 391 mg/L, by measuring the suspended solids in the bacterial suspension, which were assumed to be constant during the oxidation of Mn(II). The k and K_s values were estimated to be 1.33×10^{-3} mg-Mn(II)/mg-cell·h and 8.81 mg-Mn/L, respectively. Based on these parameters, *P. putida* MnB1 was deemed less effective for the oxidation of Mn(II) than either *Leptothrix discophora* SS1 [8] or *Pedomicrobium* sp. ACM 3067 [21]. However, the oxidation of Mn(II) was inhibited at 20 mg-Mn(II)/L, although the growth of *P. putida* MnB1 was not influenced at 100 mg-Mn(II)/L. Tani et al. also reported the inhibition of the oxidation of Mn(II) at high Mn(II) concentrations in their study with *Acremonium*-like hyphomycete fungus, strain KR21-2 [23]. This infers that the inhibition was due to the obstruction of the mass transfer of Mn(II) from the bulk phase to the cell surface due to the accumulation of Mn oxides particles. Toner et al. presented TEM images of the Mn oxides accumulated on the extracellular organic matrix [24], and Saratovsky et al. observed MnOx nucleation on the exopolysaccharide sheath surrounding *L. discophora* SP6 [9]. MnOx solids aggregations were found not only on cell surfaces, but also around the purified enzyme [22]. However, the growth of *P. putida* MnB1 was not affected, even at high Mn(II) concentrations, because the oxidation of Mn(II) and; therefore, Mn oxides accumulation started after the stationary phase (Fig 1(b) and 3). Meanwhile, with an initial Mn(II) concentration of 20 mg/L, all the Mn(II) was consumed to form a stoichiometric amount of MnO₂, indicating the abundance of Mn oxidizing enzymes in the experimental system.

The MnO₂ concentrations with respect to reaction time at different initial pH are illustrated in Fig 4 (a). The maximum rate of MnO₂ generation was observed at pH 7.43, but this was slightly lower at pH 8.22. However, when the initial pH was 5.56, the rate of biogenic Mn oxides generation was decreased significantly and lag phase increased dramatically. No Mn oxides were formed at an initial pH of 4.59. These results show that the Mn(II)-oxidizing enzyme was active at pH higher than 5.56. The rates of Mn(II) oxidation at various initial pH are shown in Fig 4 (b). As the pH decreased from 8.22 to 5.56, the rate of Mn(II) oxidation for *P. putida* MnB1 decreased, with a linear relationship obtained. The optimum pH was between 7.43 and 8.22, which was in agreement with the findings in many studies, in that the biologic oxidation of Mn(II) was strongly inhibited at pH values

less than 6. *Leptothrix discophora* SS1 showed a maximum rate of Mn(II) oxidation at pH 7.5, but this was significantly decreased below pH 6.5 and over pH 8.5 [8]. In addition, Larsen et al. also reported the maximum Mn(II) oxidation activity of *Pedomicrobium* sp. ACM 3067 at pH 7, which decreased sharply at below pH 5.5 and above pH 7 [21]. Fig 4 (c) shows the pH changes with different initial pH values during microbial growth and Mn(II) oxidation over a 72 hr period. Before the microbial population had attained the stationary phase, the pH decreased, started to recover, approached the initial value and slowly increased

Fig. 4. The generation of Mn oxides by *P. putida* MnB1 at different initial pH values (a), the rate of Mn(II) oxidation as a function of the initial pH (b) and pH changes during the oxidation of Mn at different initial pH values (c).

to the original pH after the completion of Mn(II) oxidation. No pH recovery was observed at pH 4.59, when no Mn oxides were formed at all.

Manganese-oxidizing microorganisms are known to be both pH and temperature sensitive. The production of Mn(II) at different temperatures, ranging from 20 to 30°C, at pH 5.56 and an initial Mn(II) of 12 mg/L is shown in Fig 5 (a). The rate of Mn(II) oxidation as a function of the temperature is also shown in Fig 5 (b). Mn(II) was not oxidized at 28 or 30°C, but Mn oxides formed under 26°C, with an optimum temperature of 24°C, with the shortest lag phase and highest rate of Mn(II) oxidation. In addition, The oxidation of Mn(II) by *P. putida* MnB1 was more sensitive to temperature than that by *Pedomicrobium* sp. ACM 3067, with no great decrease in the rate of Mn(II) oxidation observed between 20 and 30°C [21]. The increase in the rate of Mn(II) oxidation between 20 and 24°C was mainly attributed to the temperature activation of the reaction, while the decrease above 24°C was possibly attributed to the thermal deactivation of the Mn-oxidizing enzyme [8, 25].

3.3. Property of the biogenic Mn oxides

The XRD patterns of the biogenic Mn oxides formed by *P. putida* MnB1 and birnessite are presented in Fig 6. Birnessite showed distinctive peaks at 2θ of 12.3 and 24.8°; whereas, the

Fig. 5. The generation of Mn oxides by *P. putida* MnB1 at different temperatures (a) and the rate of Mn(II) oxidation as a function of temperature (b).

Fig. 6. XRD patterns of abiotic and biogenic Mn oxides.

Fig. 7. TEM image (a) and EDS (b) of the biogenic Mn oxides formed by *P. putida* MnB1.

biogenic Mn oxides exhibited broader peaks of lower amplitude in this region. However, the XRD patterns of both the biogenic and abiotic Mn oxides showed small peaks at 2θ of 35 and 65°. These peaks were typical of varnadite, a fine-grained, poorly crystalline, natural Mn oxide phase found in the oxidized zone of Mn ore deposits, which is considered a major phase in ocean Mn nodules and other Mn oxide crusts and coatings [26]. The TEM image and EDS analysis of the biogenic Mn oxides showed the aggregation of small particles, with irregular shape, confirming the poor crystallinity (Fig 7). The SEM image also shows shape irregularity compared to birnessite (Fig 8).

Biogenic Mn oxides are generally known to be poorly crystalline compared to abiotic Mn oxides. Biogenic Mn oxides have intermediate crystallinity and reactivity, between those of synthetic phylломanganates δ -MnO₂ and acid birnessite, which are formed through the reduction of permanganate with MnCl₂ or HCl, respectively [5, 12]. The X-ray Absorption Near Edge Structure (XANES), Extended X-ray Absorption Fine Structure (EXAFS) spectra and X-ray Diffraction (XRD) intensity data of the biogenic Mn oxides produced by the spores of marine *Bacillus* sp. strain SG-1 indicated that the primary biogenic oxides were fine particulate phylломanganates, with 10Å basal plane spacing [27]. The diameters of the particles vary widely, but most were approximately 200 to 400 nm in diameter [28].

4. Conclusions

The growth of *P. putida* MnB1 and the generation of Mn ox-

Fig. 8. SEM images of the biogenic Mn oxides formed by *P. putida* MnB1 (a) and abiotic MnO₂ (b).

ides were investigated to characterize the biogenic oxidation of Mn by *P. putida* MnB1. The acclimation period, growth rate in the exponential growth phase and population during the stationary phase of *P. putida* MnB1 were not influenced by the initial Mn(II) concentration, while a higher initial amount of inoculum decreased the lag phase. A slight decrease in the growth rate of *P. putida* MnB1 was observed in the presence of both Pb(II) and EE2, but the growth curves for 10 to 100 μ M Pb(II) and 1 to 3 mg/L EE2 were almost identical. This indicates the adsorption of Pb(II) and EE2 onto the cell surface of *P. putida* MnB1 was insignificant.

Poorly crystalline, nano-sized biogenic Mn oxides were formed in a system containing *P. putida* MnB1, Mn(II) and liquid growth medium. The optimum conditions for the maximum rate of Mn oxidation were 10 mg-Mn/L, pH 7.43-8.22 and 24°C. The biogenic oxidation of Mn by *P. putida* MnB1 followed Michaelis-Menten enzyme kinetics, but was retarded at 20 mg-Mn(II)/L, although the amount of Mn oxides generated was almost stoichiometric. This indicates an abundance of Mn-oxidizing enzyme and the obstruction of mass transfer from the bulk phase Mn(II) to the cell surface due to the accumulation of Mn oxides on the extracellular enzyme layer. The oxidation of Mn by *P. putida* MnB1 was very both pH and temperature sensitive, with optimum ranges of 7.43 to 8.22 and about 24°C, respectively.

Acknowledgement

This work was supported by a Korea Research Foundation Grant, funded by the Korean Government (KRF-2009-0076579).

References

- Zhang H, Huang CH, Oxidative Transformation of Triclosan and Chlorophene by Manganese Oxides. *Environ. Sci. Technol.* 2003; 37: 2421-2430.
- Tipping E, Heaton MJ. The adsorption of aquatic humic substances by two oxides of manganese. *Geochim. Cosmochim. Ac.* 1983; 47: 1393-1397.
- De Rudder J, Wiele TV, Dhooze W, Comhaire F, Verstraete W, Advanced water treatment with manganese oxide for the removal of 17a-ethynylestradiol (EE2). *Water Res.* 2004; 38: 184-192.
- Zhao L, Yu ZQ, Peng PA, Huang WL, Dong YH, Oxidative transformation of tetrachlorophenols and trichlorophenols by manganese dioxide. *Environ. Toxicol. Chem.* 2009; 28: 1120-1129.
- Tebo BM, Bargar JR, Clement BG, Dick GJ, Murray KJ, Parker D, Verity R, Webb SM, Biogenic manganese oxides: properties and mechanisms of formation. *Annu. Rev. Earth Planet. Sci.* 2004; 32: 287-328.
- Hennebel T, De Gussem B, Boon N, Verstraete W, Biogenic metals in advanced water treatment. *Trends Biotechnol.* 2009; 27: 90-98.
- Van Waasbergen LG, Hildebrand M, Tebo BM, Identification and characterization of a gene cluster involved in manganese oxidation by spores of the marine *Bacillus* sp. strain SG-1. *J. Bacteriol.* 1996; 178: 3517-3530.
- Zhang J, Lion LW, Nelson YM, Shuler ML, Ghiorse WC, Kinetics of Mn(II) oxidation by *Leptothrix discophora* SS1. *Geochim. Cosmochim. Ac.* 2002; 66: 773-781.
- Saratovsky I, Wightman PG, Pasten PA, Gaillard JF, Poeppelmeier KR, Manganese oxides: Parallels between abiotic and biotic structures. *J. Am. Chem. Soc.* 2006; 128: 11188-11198.
- Caspi R, Haygood MG, Tebo BM, Unusual ribulose-1, 5-bisphosphate carboxylase/oxygenase genes from a marine manganese-oxidizing bacterium. *Microbiology* 1996; 142: 2549-2559.
- Caspi R, Tebo BM, Haygood MG, c-Type cytochromes and manganese oxidation in *Pseudomonas putida* MnB1. *Appl. Environ. Microb.* 1998; 64: 3549-3555.
- Villalobos M, Toner B, Bargar J, Sposito G, Characterization of the Mn oxide produced by *Pseudomonas putida* strain MnB1. *Geochim. Cosmochim. Ac.* 2003; 67: 2649-2662.
- Meng YT, Zheng YM, Zhang LM, He JZ. Biogenic Mn oxides for effective adsorption of Cd from aquatic environment. *Environ. Pollut.* 2009; 157: 2577-2583.
- Novak M, Pfeiffer T, Ackermann M, Bonhoeffer S, Bacterial growth properties at low optical densities. *Anton. Leeuw.* 2009; 96: 267-274.
- Baicu SC, Taylor MJ, Acid-base buffering in organ preservation solutions as a function of temperature: new parameters for comparing buffer capacity and efficiency. *Cryobiology* 2002; 45: 33-48.
- APHA. Standard Methods for the Examination of Water and Wastewater. 20th Ed. American Health Association, Washington: DC; 1998.
- Tebo BM, Clement BG, Dick GJ, Biotransformations of manganese. *Manual of Environmental Microbiology*, 3rd ed. 2007; 1223-1238.
- Mandernack KW, Fogel ML, Tebo BM, Usui A, Oxygen isotope analyses of chemically and microbially produced manganese oxides and manganates. *Geochim. Cosmochim. Ac.* 1995; 59: 4409-4425.
- Sabirova JS, Cloetens LFE, Vanhaecke L, Forrez I, Verstraete W, Boon N, Manganese-oxidizing bacteria mediate the degradation of 17a-ethynylestradiol. *Microbial Biotechnology* 2008; 1(6): 507-512.
- Wang W, Shao Z, Liu Y, Wang G, Removal of multi-heavy metals using biogenic manganese oxides generated by a deep-sea sedimentary bacterium - *Brachybacterium* sp. strain Mn32. *Microbiology* 2009; 55: 989-1996.
- Larsen EI, Sly LJ, McEwan AG, Manganese(II) adsorption and oxidation by whole cells and a membrane fraction of *Pedomicrobium* sp. ACM 3067. *Arch. Microbiol.* 1999; 171: 257-264.
- Miyata N, Tani Y, Maruo K, Tsuno H, Sakata M, Iwahori K, Manganese(IV) oxide production by *Acremonium* sp. strain KR21-2 and extracellular Mn(II) oxidase activity. *Appl. Environ. Microb.* 2006; 72(10): 6467-6473.
- Tani Y, Ohashi M, Miyata N, Seyama H, Iwahori K, Soma M, Sorption of Co(II), Ni(II), and Zn(II) on biogenic manganese oxides produced by a Mn-oxidizing fungus, Strain KR21-2. *J. Environ. Sci. Heal.* 2005; 39(10): 2641-2660.
- Toner B, Fakra S, Villalobos M, Warwick T, Sposito G, Spatially resolved characterization of biogenic manganese oxide production within a bacterial biofilm. *Appl. Environ. Microb.* 2005; 71(3): 1300-1310.
- Francis CA, Tebo BM. Enzymatic Manganese(II) Oxidation by Metabolically Dormant Spores of Diverse *Bacillus* Species. *Appl. Environ. Microb.* 2002; 68: 874-880.
- Post JE, Manganese oxide minerals: crystal structures and

- economic and environmental significance. In: National Academy of Sciences of the United States of America; 1999; 96: 3447-3454.
27. Bargar JR, Webb SM, Tebo BM, EXAFS, XANES and in-situ SR-XRD characterization of biogenic manganese oxides produced in sea water. *Phys. Scripta* 2005; 155: 888-890.
28. Krekeler MPS, Transmission electron microscopy (TEM) investigations of Mn-oxide rich cathodic material from spent disposable alkaline batteries. *Waste Manage.* 2008; 28: 2061-2069.