

## Development of bioprocess for treatment of Mn(II)-contaminated metal refinery wastewaters

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## 論文内容の要旨

Contamination of manganese (Mn) in wastewaters, especially from metal-refinery industries, is a challenging problem. Since  $Mn^{2+}$  is thermodynamically stable over the wide range of pH (0-8) and its chemical oxidation promoted at alkaline pHs, a vast cost is needed for neutralizing-agents in conventional Mn-removal processes. On the other hand, microbiological (enzymatic) reactions enable oxidative precipitation of  $Mn^{2+}$  as biogenic birnessite at circumneutral pHs ( $Mn^{2+} + 1/2O_2 + H_2O \rightarrow Mn^{III,IV}O_2 + 2H^+$ ; Eq. 1), even without the addition of chemical oxidizing-agents. Therefore, the development of bioprocess for  $Mn^{2+}$ -contaminating wastewater could become a more economical and environmentally feasible alternative. This study first analyzed the natural  $Mn^{2+}$  attenuation phenomena in the metal-refinery wastewater pipeline, from which a new Mn-oxidizing bacterium was isolated. The isolate was identified and further evaluated for its Mn-oxidation capability focusing on several metal-refinery water characteristics. Secondly, the individual contribution of biological (by bacteria) and chemical (by Mn-oxides) effect on Mn-oxidation was clarified. Lastly, a continuous biofilter column was constructed to test its feasibility for actual metal-refinery wastewater. Moreover, to find an additional value to the resultant biogenic Mn-oxide product, its potential utility for remediation of toxic arsenite (As(III)) was investigated.

In **Chapter 1**, background information regarding the properties of Mn and its contamination problems were introduced. Previous studies related to the present work were reviewed and discussed in this chapter.

In **Chapter 2**, methodologies used in this work were described.

**Chapter 3** first described the phenomenon of natural  $Mn^{2+}$  attenuation observed in the actual metal-refinery wastewater pipeline, accompanied with extensive dark-brown-colored mineralization on the inner pipe surface ( $Mn^{2+}$  concentration lowered from 1.n to 0.n mg/L after the wastewater traveled through a pipe). The dark-brown deposits taken from the pipe was characterized as mixed phases of crystalline  $Mn^{IV}O_2$ ,  $Mn^{III}_2O_3$ , and  $Fe_2O_3$  (the average oxidation state (AOS) of Mn was 3.75). Due to the high activation energy required for a spontaneous chemical Mn-oxidation, the involvement of microbiological activity was suspected. In fact, the Mn-deposit hosted the bacterial community comprised of *Hyphomicrobium* sp. (22.1%), *Magnetospirillum* sp. (3.2%), *Geobacter* sp. (0.3%), *Bacillus* sp. (0.18%), *Pseudomonas* sp. (0.03%), and non-metal-metabolizing bacteria (74.2%).

In **Chapter 4**, culture enrichments of the Mn-deposit collected in chapter 3 was conducted. After selective screening on the solid agarose media, a Mn-oxidizing colony was isolated and named isolate SK3. Based on the 16S rRNA gene sequence analysis, the closest relative of isolate SK3 was *Pseudomonas (Ps.) resinovorans* (with 98.4% homology; 1398 bp), which is so far unknown as Mn-oxidizer. Next, isolate SK3 was tested for its Mn-oxidation ability under different conditions mimicking actual metal-refinery wastewater characteristics. When compared to the well-studied Mn-oxidizer *Ps. putida* MnB1, the superiority of isolate SK3 became noticeable: i.e. Oxidation of up to 100 mg/L  $Mn^{2+}$  readily progressed and completed by isolate SK3, even in the presence of high contents of  $MgSO_4$  (up to 2400 mg/L; a typical solute in metal-refinery wastewaters). At this  $MgSO_4$  concentration, *Ps. putida* MnB1 completely lost its Mn-oxidizing ability. Additional  $Cu^{2+}$  facilitated Mn-oxidation by isolate SK3 (implying the involvement of multicopper oxidase enzyme), allowing 2-fold greater Mn-removal rate, compared to the case of *Ps. putida* MnB1. Biogenic Mn-oxides formed by isolate SK3 was characterized by XRD as poorly-crystalline birnessite with high  $Mn^{IV}$  fraction of 0.86 and AOS of 3.8. Overall results in chapters 3-4 suggest that

the natural  $\text{Mn}^{2+}$  attenuation phenomenon was featured by the robust *in-situ* activity of Mn-oxidizers (including isolate SK3) for continuous generation of  $\text{Mn}^{\text{IV}}$ .

Mn-oxides produced by Mn-oxidizing bacteria are one of the strong chemical oxidants found in nature. From this point, Mn-oxidation in biological systems includes both direct enzymatic reaction (by microorganisms) and indirect chemical reaction (by Mn-oxides). Therefore, **Chapter 5** aimed to clarify the individual contribution from the two. When only sterilized natural Mn-oxide (NMO) was provided, Mn-oxidation proceeded only to a limited extent by chemical synproportionation ( $\text{Mn}^{2+} + \text{Mn}^{\text{IV}}\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{Mn}^{\text{III}}_2\text{O}_3 + 2\text{H}^+$ ; Eq. 2). This was due to surface passivation of NMO with  $\text{Mn}^{\text{III}}_2\text{O}_3$ . When Mn-oxidizing SK3 cells were inoculated in addition to NMO, Mn-oxidation was significantly promoted, owing to the synergistic effect of chemical synproportionation and microbiological  $\text{Mn}^{\text{IV}}\text{O}_2$  regeneration. The presence of NMO also likely provided the surface for bacterial colonization to support robust bacterial growth: This allowed isolate SK3 to oxidize  $\text{Mn}^{2+}$  even under originally inhibitory complex conditions such as at high  $\text{MgSO}_4$  concentrations (2400 mg/L) and at a higher temperature (35°C).

Based on the fundamental knowledge obtained from the previous chapters, the continuous biofilter column tests were planned. Firstly, screening for the suitable column carrier (bacteria-supporting material) was conducted in **Chapter 6**. Ten different  $\text{SiO}_2$ - or carbon-based materials were tested through the cycle Mn-oxidation test. The difference in the Mn-oxidation rate was noticed, especially in the first cycle. However, once Mn-oxides were attached to the support material, the difference gradually became smaller between the different materials. Nonetheless, generally greater effectiveness was noticed throughout the cycles with carbon-based materials, due to their higher affinity to both bacterial cells and Mn-oxides. Consequently, activated carbon (AC) was chosen for further studies. While AC itself exhibited chemical Mn-oxidizing ability, its effect deteriorated after the second cycle (<40% Mn-removal) due to passivation of the product ( $\text{Mn}^{\text{III}}_2\text{O}_3$ ). Overall, it was suggested that in the following AC-packed column test, the efficient Mn-removal would arise from synergistic interactions between; (i) active oxidation of  $\text{Mn}^{2+}$  by bacteria for continuous regeneration of  $\text{Mn}^{\text{IV}}\text{O}_2$  (Eq. 1) (ii) chemical synproportionation effect of biogenic  $\text{Mn}^{\text{IV}}\text{O}_2$  producing  $\text{Mn}^{\text{III}}_2\text{O}_3$  from  $\text{Mn}^{2+}$  (Eq. 2) and (iii) chemical oxidation of  $\text{Mn}^{2+}$  by the AC surface, producing  $\text{Mn}^{\text{III}}_2\text{O}_3$  (especially at the early-stage).

Finally, the laboratory-scale AC-packed biofilter column test was conducted in **Chapter 7**, using two types of actual metal-refinery wastewaters (downstream water [ $\text{Mn}^{2+}$ ] 2 mg/L, [ $\text{SO}_4^{2-}$ ] 780 mg/L; upstream water [ $\text{Mn}^{2+}$ ] 2-5 mg/L, [ $\text{SO}_4^{2-}$ ] 1500 mg/L). The results obtained from this chapter were expected to offer improvement suggestions for the on-going pilot-scale test column constructed at the metal-refinery site. This *on-site* pilot-scale column was packed with zeolite with the current Mn-removal of around 40%. The advantage of using AC instead of  $\text{SiO}_2$ -based zeolite as column-carrier was reconfirmed in this test, as the contact time required for the complete Mn-removal was shortened with the former. Before starting the water flow (at the hydraulic retention time (HRT) of 20 min), AC granules pre-colonized with actively Mn-oxidizing SK3 cells were packed in the column, in order to kick-start the Mn-removal. The importance of organic supply was clearly indicated, since Mn-oxidation was catalyzed by heterotrophic bacteria: In fact, the addition of the minimum amount of yeast extract (0.01%) was essential to maintain high Mn-removal efficiency (65-90%, compared to 20-40% in control). For the treatment of upstream water with higher  $\text{Mn}^{2+}$  and  $\text{SO}_4^{2-}$  contents, the addition of pulverized AC to granule AC (at 3:7 ratio) promoted Mn-oxidation by 5-10%, resulting in about 85% final Mn-removal at HRT 40 min, even after a harsh backwashing process. Overall results obtained in this chapter suggest that the following factors should be considered to improve performance of the *on-site* pilot-scale column; type of column-carrier, installation of pre-colonization step, the supply of suitable organic nutrient, optimization of HRT.

After the repeated use of the continuous biofilter column, the spent column carriers are to be produced. **Chapter 8** looked for a potential additional value of biogenic  $\text{MnO}_2$  accumulated on the spent column carriers. Since groundwater contamination with As(III) is another significant problem associated with mining activity, biogenic  $\text{MnO}_2$  was tested for its As(III) oxidation capability ( $\text{H}_3\text{As}^{\text{III}}\text{O}_3 + \text{Mn}^{\text{IV}}\text{O}_2 + \text{H}^+ \rightarrow \text{Mn}^{2+} + \text{H}_2\text{As}^{\text{V}}\text{O}_4^- + \text{H}_2\text{O}$ ; Eq. 3). When synthetic As(III)-contaminated groundwater (pH 7) was tested, retaining active Mn-oxidizing SK3 cells on the  $\text{MnO}_2$  surface enabled effective oxidation of As(III) to less toxic and mobile As(V). By so doing, it was possible to complete As(III) oxidation while no loss of Mn (as dissolved  $\text{Mn}^{2+}$ ; Eq. 3) was made.

In **Chapter 9**, conclusions and recommendations for future work were summarized.