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

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(マンガン含有金属製錬廃液処理のためのバイオプロセス開発に関する研究)

区　　分　：甲

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 Mn^{2+} attenuation phenomenon was featured by the robust *in-situ* activity of Mn-oxidizers (including isolate SK3) for continuous generation of Mn^{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 ($Mn^{2+} + Mn^{IV}O_2 + H_2O \rightarrow Mn^{III}{}_2O_3 + 2H^+$; Eq. 2). This was due to surface passivation of NMO with $Mn^{III}{}_2O_3$. 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 $Mn^{IV}O_2$ regeneration. The presence of NMO also likely provided the surface for bacterial colonization to support robust bacterial growth: This allowed isolate SK3 to oxidize Mn^{2+} even under originally inhibitory complex conditions such as at high $MgSO_4$ concentrations (2400 mg/L) and at a higher temperature (35℃).

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 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 ($Mn^{III}{}_2O_3$). 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 Mn^{2+} by bacteria for continuous regeneration of $Mn^{IV}O_2$ (Eq. 1) (ii) chemical synproportionation effect of biogenic $Mn^{IV}O_2$ producing $Mn^{III}{}_2O_3$ from Mn^{2+} (Eq. 2) and (iii) chemical oxidation of Mn^{2+} by the AC surface, producing $Mn^{III}{}_2O_3$ (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 [Mn^{2+}] 2 mg/L, [SO_4^{2-}] 780 mg/L; upstream water [Mn^{2+}] 2-5 mg/L, [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 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 Mn^{2+} and 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 MnO_2 accumulated on the spent column carriers. Since groundwater contamination with As(III) is another significant problem associated with mining activity, biogenic MnO_2 was tested for its As(III) oxidation capability ($H_3As^{III}O_3 + Mn^{IV}O_2 + H^+ \rightarrow Mn^{2+} + H_2As^{V}O_4^- + H_2O$; Eq. 3). When synthetic As(III)-contaminated groundwater (pH 7) was tested, retaining active Mn-oxidizing SK3 cells on the 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 Mn^{2+}; Eq. 3) was made.

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