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A Search for Effective Mn(II)-Oxidizing Microorganisms for Mn-contaminating Wastewater Treatment Application

Santisak Kitjanukit[1], Kenji Takeda[2] and Naoko Okibe[1]
[1]*Department of Earth Resources Engineering, Kyushu University*
[2]*Hydrometallurgical process development group, Niihama Research Laboratories, Sumitomo Metal Mining Co.,Ltd*

Abstract
Since manganese (Mn) which contaminated in wastewater is stable at wide-range of pH unlike other heavy metals, strong oxidants, and abundant alkaline substances are required for its treatment (oxidation and precipitation). The present study is aiming to isolate new Mn(II)-oxidizing microbe and study for its application to be used to treat Mn-contaminating wastewater.
The isolated bacterium strain SK3 could completely oxidize and precipitate 100 ppm of Mn(II) within 140 hours. The precipitates were characterized as poorly-crystalline birnessite. The results suggest the potential of this bacteria to treat Mn-contaminating wastewater using an environmental friendly approach.

1. Introduction

Manganese (Mn) is one of the most abundant elements in the Earth's crust and one of the common heavy metals contaminants in wastewater. Problems associated with it include undesirable metallic taste and color water, scale problem in a water pipe. According to Japanese water quality standard, Mn(II) concentration must not exceed 10 mg/L (industrial wastewater) and 0.05 mg/L (drinking water). Conventional technology for Mn-contamination wastewater treatment relies on physicochemical reaction [1] involves the uses of strong oxidant and abundant of the alkaline compound to oxidize and precipitate Mn(II) as Mn oxides (eq.1).

$$Mn^{2+} + 0.5O_2 + H_2O \rightarrow MnO_2 + 2H^+ \quad \text{(eq. 1)}$$

Some microorganisms are capable of oxidizing Mn(II) to Mn(III) and Mn(IV); for example, *Pseudomonas* sp., *Bacillus* sp., *Pedomicrobium* sp., and *Leptothrix* sp. based on their metabolism [2]. Microbial Mn(II)-oxidation is 50,000 times faster than chemical oxidation at same pH [3] and it could be useful for Mn contaminated wastewater treatment. However, knowledge about the maximum Mn concentration, effects of various parameters and mechanism of microbial Mn(II)-oxidation are limited.

This study aims to isolate new Mn(II)-oxidizing microbe and study for its application to treat Mn-contaminating wastewater as well as the oxidation mechanism.

2. Material and Methods

2.1 Screening, isolation, and storage

Sludge sample was heated at 80°C for 10 min and re-suspended in 0.85% (w/v) NaCl solution before spreading on Mn(II)-containing Yu medium. Single brown colony was re-streak several time to completely isolate single bacterium. Finally, the colony was inoculated in Luria-Bertani medium (LB, pH 7.0).

2.2 Culture maintenance and cell suspension preparation

Isolated culture of strain SK3 was routinely sub-cultured in LB medium (pH 7.0). When cell suspension is needed, cells were collected and washed with 0.85% NaCl (10,000 rpm, 4°C).

2.3 Manganese removal test

Both strains were tested for their Mn(II)-oxidization ability in different Mn(II)-containing medium, MY (0.05% (w/v) yeast extract, 2.4 mM $MgSO_4 \cdot 7H_2O$, 0.48 mM $CaCl_2 \cdot 2H_2O$) and PYG (0.025% (w/v) yeast extract 0.025% (w/v) peptone, 1 mM glucose, 2.02 mM $MgSO_4 \cdot 7H_2O$, 0.068 mM $CaCl_2 \cdot 2H_2O$). pH of medium (7.0) was buffered by 15 mM PIPES. Samples were routinely withdrawn to analyze pH, Eh, and dissolve Mn concentration.

2.4 Characterization of residue

Residues from Mn(II)-oxidization experiments were collected and freeze-dried overnight and analyzed with x-ray diffraction (XRD: Rigaku Ultra IV).

3. Results and Discussion

3.1 Isolation of SK3

Figure 1 Brown colony on Yu agar indicated Mn(II)-oxidizing activity.

Mn(II)-oxidizing bacterium was successfully isolated from the sludge and named strain SK3. Brown color indicated Mn(II)-oxidizing activity of the bacterium, since Mn oxide (Mn(IV)) is a brown-black solid.

3.2 Mn(II)-oxidation test

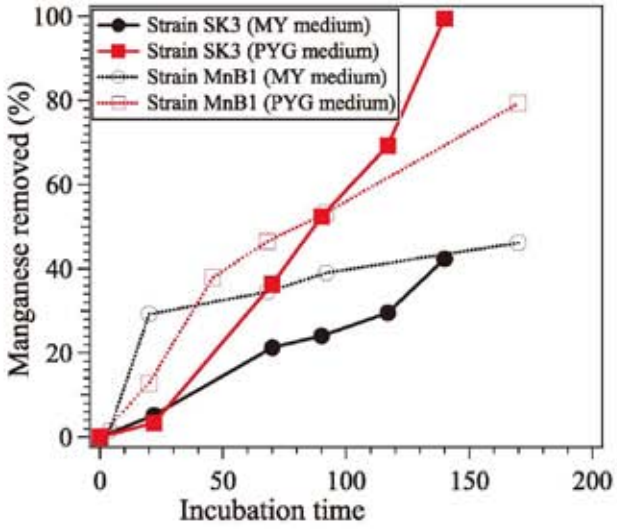

Figure 2 Manganese removal efficiency in culture of strain SK3 and strain MnB1. Red and black color indicate PYG and MY medium, respectively.

At same condition, strain SK3 exhibited stronger Mn(II)-oxidizing ability than *P. putida* strain MnB1. Different Mn(II)-oxidizing behavior is illustrated in figure 2. Strain SK3 took longer time to initiate first stage of the bacterial oxidation.

Glucose is marked as a major different composition between those two medium; therefore, it is interesting to study its effects in the future. Both absorption (by Mn oxide) and bacterial oxidation contributed to Mn removal, the latter played a dominant role to initiate the whole process.

3.3 Characterization of solid residue

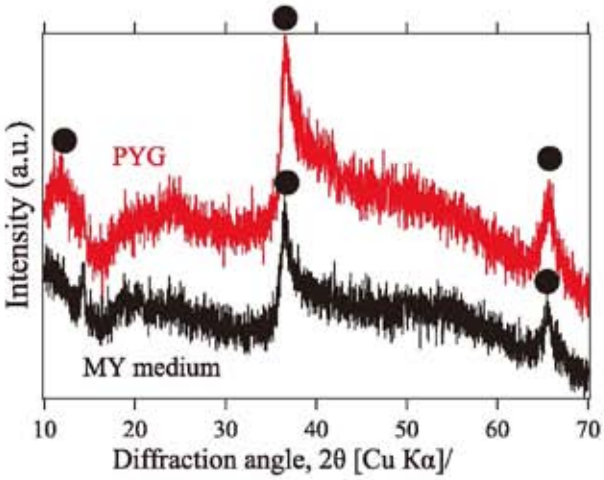

Figure 3 XRD diffraction pattern of immobilized residues from bacterial Mn(II)-oxidation by strain SK3. ● indicates the peak corresponding to birnessite $(Na,Ca)Mn_7O_{14} \cdot 2.8H_2O$ (JCPDS 43-1456).

Immobilized residues from bacterial Mn(II)-oxidation were characterized as birnessite ($(Na,Ca)Mn_7O_{14} \cdot 2.8H_2O$) with poorly-crystalline structure (figure 3).

4. Conclusion

Mn(II)-oxidizing bacterium was successfully isolated from sludge and exhibited strong Mn(II)-oxidizing ability. Further studies are needed in order to better understand the mechanism of bacterial Mn(II)-oxidation and eventually to develop the biological Mn(II) removal processes.

Acknowledgment

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Email: ming@mine.kyushu-u.ac.jp