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# Synergistic Mn(II)-oxidative Removal from a New Mn(II)-oxidizing Bacterial strain, *Pseudomonas* sp. strain SK3 and Refinery Wastewater Deposit

Santisak Kitjanukit[1], Kenji Takeda[2], Satoshi Asano[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 divalent manganese (Mn(II)) is stable at wide-range of pH, loads of alkaline agents and oxidants are required for its treatment (oxidative precipitation). The present study is aiming to improve Mn(II)-oxidative removal efficiency in harsh condition mimicking the actual refinery wastewater. Addition of an aliquot amount of wastewater deposit (identified as Mn oxide) to strain SK3 cell suspension dramatically improved Mn(II)-oxidative removal (100 ppm of Mn(II) was completely removed within 24 hours). The results suggest that the combination of both chemical and bacterial Mn(II) oxidation may be useful for the industrial wastewater treatment process

## 1. Introduction

High stability of Mn(II) at a wide range of pH made it difficult to be removed from wastewater. Conventional technology for Mn(II) treatment relies on physiochemical reaction [1] involves the uses of strong oxidant and abundant of the alkaline compound to oxidize and precipitate Mn(II) as Mn oxides. More economical and environmental benign process is needed.

Previously, a new Mn(II)-oxidizing bacterium, *Pseudomonas* sp. SK3 isolated from refinery wastewater deposit was demonstrated robust Mn(II)-oxidizing activity (Kitjanukit, unpublished data). Mn(II)-oxidizing ability of strain SK3 become deteriorated when subjected to harsher conditions resulted in sluggish Mn(II) oxidative removal speed. This study aims to improve the robustness as well as the speed of Mn removal.

## 2. Materials and methods

### 2.1 Characterization of wastewater deposit

An aliquot amount of refinery wastewater deposit was freeze-dried overnight and digested in aqua regia ($HNO_3$:HCl = 3:1, assisted with microwave irradiation for 60 min), before analyzing its metal composition by ICP-OES. XRD analysis also conducted for identification of metal phase.

### 2.2 Preparation of dried sterile Mn oxide deposit

Refinery wastewater deposit was soaked with DI water and autoclaved (120 ℃, 20 min) for preventing any effect from indigenous microorganisms. The deposit was then freeze-dried overnight prior to being use in further experiments.

### 2.3 Manganese removal test

An aliquot of freeze-dried wastewater deposit (0.5g) was added into SK3 cell suspension in PYG medium (0.025% (w/v) yeast extract 0.025% (w/v) peptone, 1 mM glucose, 2.02 mM (100 ppm) MgSO4・7H2O, 0.068 mM CaCl2・2H2O). pH of the medium (7.0) was buffered by 15 mM PIPES. Samples were routinely withdrawn to analyze pH, Eh, and dissolve Mn concentration.

The solid parts were collected after the experiment for scanning electron microscope (SEM) observation. Both cells and the deposit were fixed (with a mixture of glutaraldehyde and formaldehyde), dehydrated (with ascending concentration of ethanol), and finally sputter coated, prior to observe with SEM.

## 3. Results and Discussion

### 3.1 Characterization of wastewater deposit

The deposit was found to consist mainly of crystalline Mn oxides based on ICP-OES and XRD analysis.

### 3.2 Synergistic Mn(II)-oxidative removal by strain SK3 and Mn oxide

The presence of both strain SK3 cells and Mn oxide improved Mn removal even under originally inhibitory conditions (high temperature (35℃) and high MgSO4 concentration (200 mM)) to planktonic SK3 cells. No synergistic effect was seen when the temperature was raised to 40℃ (fig 1).

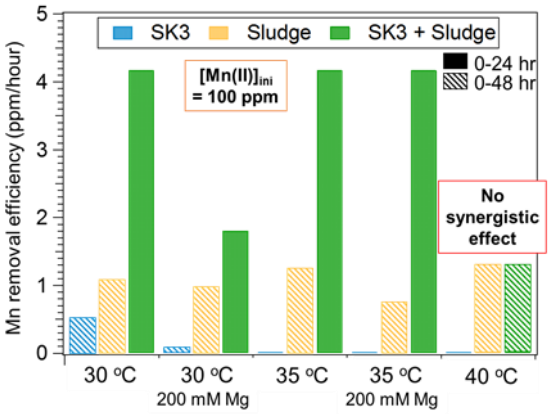


**Figure 1** Mn removal efficiency in the presence of strain SK3 and wastewater deposit.

### 3.3 Biofilm formation on wastewater deposit (Mn oxide) surface

Figure 2 showed desiccated biofilm (resulting from dehydration step during SEM sample preparation) of strain SK3 on wastewater deposit (Mn oxide) surface. Addition of Mn oxide could have provided SK3 cells with a surface to colonize via biofilm forming. This may have enabled cells to be less affected by the inhibitory effect of high temperature and $MgSO_4$ [2]. Moreover, Mn oxide could have facilitated chemical Mn removal through oxidation and adsorption.

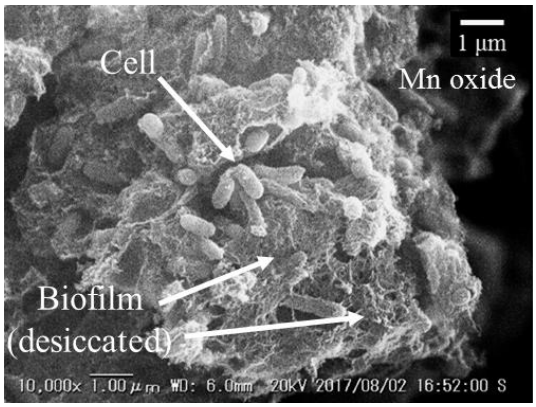


**Figure 2** SEM micrograph of the precipitates collected at the end of experiment showing strain SK3 cell attached to Mn oxide surface with biofilm

### 5. Conclusion

The refinery wastewater deposit was characterized as Mn oxides. Addition of it together with strain SK3 effectively promote Mn(II)-oxidative removal speed. Since it could provide a site for biofilm formation which alleviated the inhibitory factors (ex: high temperature and $MgSO_4$) and also promoted chemical Mn(II)-oxidation. Further studies are ongoing to access the applicability of strain SK3 for bioprocess of Mn(II)-containing wastewater treatment.

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