

# Manganese(II) Removal Bioreactor System of Mine Drainage is Dominated by Chemolithotrophic Manganese Oxidizing Microorganisms

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## Abstract

A key environmental issue at mining sites is the unregulated release of hazardous metals, particularly manganese ions (Mn(II)), from mine drainage. Conventional chemical methods for Mn removal are costly and require high pH. Mn-oxidizing bacteria (MnOB) offer a biological alternative, capable of oxidizing Mn(II) at circumneutral pH. This study investigated the use of bioreactors to treat mine drainage in Japan without the addition of organic substrates, achieving over 98% removal efficiency for Mn(II) and zinc. Metagenomic analysis revealed that chemolithotrophic MnOB, including Mn(II)-oxidizing and carbon monoxide-oxidizing bacteria, dominated the bioreactor community. Consistently, six MnOB strains isolated from the bioreactor oxidized Mn(II) under nutrient-limited conditions but not under nutrient-rich conditions, even though some strains did not exhibit cell growth under nutrient-limited conditions. These findings clearly indicate the presence of MnOB adapted to oligotrophic conditions and highlight the potential of an organic-free solution for mine drainage treatment.

**Keywords:** Manganese, manganese-oxidizing microorganisms, chemolithotrophs, oligotrophic

## Introduction

Environmental issues related to mine drainage arise in both active and abandoned mining sites, encompassing tunnels, operational mines, open pits, waste rock heaps, and mine tailings. A key concern is the unregulated discharge of hazardous metals, with manganese ions [Mn(II)] being a major contaminant due to their high solubility and prevalence in the Earth's crust. Conventional treatment methods for Mn(II) removal from mine drainage involve chemical neutralization, where caustic agents are used to oxidize and hydrolyze Mn, requiring a high pH (>9). Subsequent reverse neutralization with acids is also employed to meet effluent standards, which contributes to the overall cost of Mn removal.

Mn-oxidizing bacteria (MnOB) offer a biological alternative, as they can oxidize Mn(II) at circumneutral pH. Microbial treatment strategies for Mn-containing mine wastewater often involve the addition of organic materials. This is because most known MnOB are heterotrophs and rely on organic substrates like carbohydrates and organic acids as sources of carbon and energy for growth. Given that many mine environments harbor poor organic substrates, the exploration of a biological Mn oxidation system that can operate effectively under oligotrophic conditions holds importance in the advancement of Mn-containing mine drainage treatment. However, little is known about microbial lifestyle involved in Mn(II) oxidation in such nutrient-limited



settings. In this study, we deployed in-situ pilot-scale bioreactors to treat mine drainage containing 20 mg/L of Mn(II) and 6 mg/L of zinc (Zn(II)), without the supplementation of organic substrates, in Japan. Physiology and ecology of MnOB in the bioreactors were characterized by cultivation-dependent and -independent experiments.

## Results and Discussion

Two biological contact oxidation systems were installed and operated in an underground tunnel of an abandoned mine site. Limestone was utilized as an inorganic carrier in the system for stabilization and promotion of microbial activity. The fiber filter materials were also utilized as the inorganic carriers to increase the effective volume of the bioreactors. Each system contained three water tanks with a working volume of 600 L. The reactor was filled with mine drainage without pH adjustment, and a suspension of Mn deposit collected from the drainage ditch was added as an inoculant source of MnOB.

After four months of operation, with a hydraulic retention time of 0.5 days, the system achieved more than 98% removal efficiency for both Mn(II) and Zn(II). A total of 6 MnOB strains were isolated from the bioreactor. All of these isolates can oxidize Mn(II) under nutrient-limited conditions, but not nutrient-rich conditions. Metagenomic analysis revealed that chemolithotrophic MnOB dominated the bioreactor community, likely oxidizing Mn(II) via extracellular

electron transfer for energy production and CO<sub>2</sub> fixation. Additionally, other chemolithotrophs, such as hydrogen- and carbon monoxide-oxidizing bacteria, which possessed non-energy-yielding Mn oxidation ability, were also detected in a major proportion.

## Conclusions

These findings highlight the critical role of microorganisms adapted to oligotrophic conditions in facilitating Mn(II) oxidation within bioreactors. This approach indicates potential for mine drainage remediation without the need for added organic materials, offering a cost-effective and environmentally friendly alternative. Future investigations include how costly aeration affects the Mn oxidizing activity of these MnOB.

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## References

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