

# Factors Controlling Manganese(II) Removal Efficiency in a Passive Treatment Bioreactor with Mn(II)-Oxidizing Microorganisms

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## Abstract

A pilot-scale bioreactor with Mn(II)-oxidizing microorganisms achieved 98% Mn(II) removal without added organic substrates. To assess its stability and applicability, a lab-scale bioreactor was used to examine the effects of water temperature and pH. Mn(II) removal rate declined from 95% to 88% as temperature decreased from 15 °C to 6 °C. Even from acid mine drainage with a pH2.6, over 90% Mn(II) removal was achieved when pH exceeded 7 due to limestone neutralization. These findings highlight the importance of pH control and temperature stability for maintaining high Mn(II) removal efficiency in this bioreactor.

**Keywords:** Mine drainage, passive treatment, Mn(II)-oxidizing microorganisms, pH, temperature

## Introduction

Removal of manganese (Mn) from mine drainage using active treatment requires the addition of large amounts of caustic chemical reagents to precipitate Mn ions [Mn(II)] (Neculita and Rosa 2019). To reduce the cost associated with chemical reagents, a pilot-scale passive treatment bioreactor system with Mn(II)-oxidizing microorganisms was installed at Legacy Mine X. This system removed 98% Mn(II) from mine drainage including 20 mg/L of Mn(II) without the need for additional organic substrates (Watanabe *et al.* 2024; Tum *et al.* 2024). Given that mine drainage is typically poor in organic matter, this bioreactor is effective for the passive treatment of mine drainage (Miyata *et al.* 2024). However, in the winter of 2023, when the water temperature decreased, the Mn(II) removal rate of the bioreactor declined by nearly 50%, indicating that maintaining stable operation remains a challenge. Moreover, the pilot-scale tests have only been conducted using mine drainage with a neutral pH where Mn(II)-oxidizing microorganisms naturally

occur, so it remains unclear whether the bioreactor is applicable to acidic mine drainage from other mine sites. In this study, to ensure stable operation and assess its applicability to acidic mine drainage sources, a laboratory-scale bioreactor was set up to evaluate the effects of temperature and acid mine drainage.

## Methods

The Lab-scale bioreactor used in this study was divided into 5 lanes, each lane is filled with limestone medium inoculated with Mn(II)-oxidizing microorganisms from the pilot-scale bioreactors at Legacy Mine X (Sunouchi *et al.* 2022) (Fig. 1).

To evaluate the effect of temperature, and establish stable operating conditions for the pilot-scale bioreactor, mine drainage from Legacy Mine X (pH7.4, Mn(II) concentration of 20 mg/L) was used, with water temperature set at 15 °C, 10 °C and 6 °C.

Under each temperature condition, the mine drainage was continuously supplied to 3 lanes of the lab-scale bioreactor. Hydraulic

retention time (HRT) was set to 0.3 days, which corresponds to the HRT during the period when Mn(II) removal rate decreased in the pilot-scale bioreactor.

To investigate the applicability of this bioreactor to acidic mine drainage from other mines, 10 mg/L of Mn(II) containing acid mine drainage sourced from Mine Y (pH2.6) and Mine Z (pH2.8) was used. Each mine drainage was initially supplied at HRT = 4 days and then increasing the flow rate to HRT = 1 day at a temperature of 15 °C.

Mn(II) concentrations and pH were measured at both the inlet and outlet of the bioreactor. Sludge precipitated on the limestone medium was collected for 16S rRNA gene amplicon sequencing at the end of each experimental run.

## Result and discussion

As a result of the temperature evaluation, the average Mn(II) removal rate in the lab-scale bioreactor decreased from 95% to 88% as the temperature decreased from 15 °C to 6 °C. On the other hand, in the pilot-scale passive treatment bioreactor system at Legacy Mine X, Mn(II) removal rate decreased by nearly 50% in winter (December 2022 to February 2023), when the water temperature in the bioreactor dropped from approximately 15 °C to 12 °C. Therefore, the nearly 50% decrease in Mn(II) removal rate in the pilot-scale bioreactor cannot be attributed solely to the effect of water temperature.

The investigation of pH effects revealed that a strong positive correlation ( $r = 0.92$ ) was observed between pH and Mn(II) removal rate, indicating that pH significantly influences Mn(II) removal of the bioreactor. At a low flow rate of HRT = 4 days, the pH of both mine drainages increased to above 7

because of reaction with limestone in the lab-scale bioreactor, and more than 90% Mn(II) removal was achieved. However, when the flow rate was increased to HRT=1 day, the pH in the lab-scale bioreactor dropped to 4.5, and the Mn(II) removal rate also decreased to a minimum of nearly 1%.

Based on Bray-Curtis dissimilarity calculated from amplicon sequencing variants, the decrease in pH from 7 to 4.5 caused more than 2.5 times greater changes in  $\beta$ -Diversity of the microbial community compared to the temperature decrease from 15 °C to 6 °C.

## Conclusions

The lab-scale bioreactor removed over 90% of Mn(II) even from pH2.6 mine drainage due to the neutralization effect of limestone. This suggests that the Mn(II)-oxidizing bioreactors operating without organic substrates, has wide applicability for treatment of mine drainage treatment, which is often acidic and poor in organic matter. On the other hand, water temperature is not considered a major factor contributing to the nearly 50% decrease observed in the pilot-scale passive treatment bioreactor system. Identifying other environmental factors that affect Mn removal rate of the bioreactor remains an important challenge.

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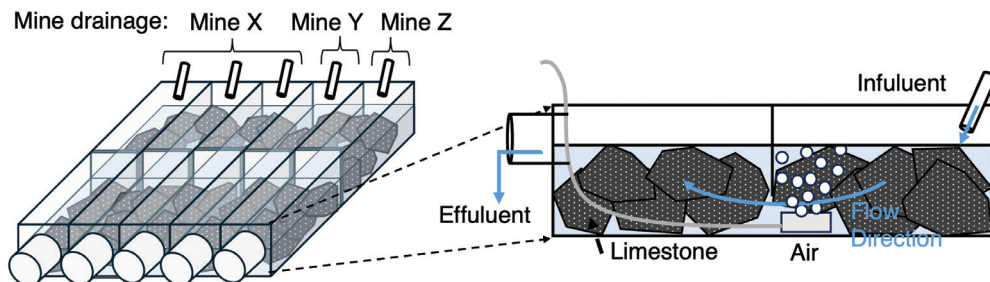


Figure 1 The schematic illustrates of Laboratory-scale bioreactor.



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