Annual Treatment Performances of Sulfate-Reducing Process Under Ethanol Addition Conditions with Rice Bran for Zinc-Containing AMD in Japan

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Abstract

This study compares three nutritional conditions – ethanol alone and ethanol supplemented with two different proportions of rice bran – in a large-scale anaerobic sulfate-reducing process. pH neutralization and soluble Zn removal were consistently effective throughout the study period. Sulfate reduction and total Zn removal performance during winter varied depending on the nutrient conditions. The ethanol-only nutrient conditions resulted in insufficient sulfate reduction for total Zn removal during winter, whereas ethanol supplemented with rice bran was effective for sufficient sulfate reduction and subsequent total Zn removal. Supplementing ethanol with rice bran improved ethanol consumption efficiency and sustained treatment performance during winter.

Keywords: Passive Treatment, Ethanol, Rice Bran, Sulfate-Reducing Process, Total Zinc

Introduction

In Japan, active treatment is used to continuously treat mine water at approximately 100 abandoned mine sites. The Japan Organization for Metals and Energy Security (JOGMEC) has been researching and developing passive treatment systems to reduce treatment costs, focusing on processes that offer a short hydraulic retention time (HRT) and a small footprint for application in Japan. A large-scale, multi-step passive treatment test, consisting of a 2-hour aerobic iron (Fe) oxidizing/removal process followed by a 22.5-hour anaerobic sulfate-reducing process, was initiated in 2020 (Hayashi *et al.* 2021). The water flow rate during this test was set to 100 L/min. After the Fe oxidizing/removal process, acid mine drainage (AMD) was directed into the anaerobic process within vertical-flow biochemical reactors (BCRs) filled with a mixture of rice husk and limestone. Three different nutritional conditions with a continuous ethanol feed have been tested since 2020. Ethanol and rice bran have been used as direct, indirect, and supplemental nutrients for sulfate-reducing bacteria (SRB) (Masaki *et al.* 2023, Sato *et al.* 2024). The objective of this study is to investigate the annual treatment performance among three nutritional conditions based on ethanol feed, with or without the addition of rice bran.

Methods

Test site and chemistry of acid mine drainage

A large-scale passive treatment test was conducted at an abandoned metal mine in northern Japan (Fig. 1). The annual temperature at the test site varies from −10 to +40 °C. During winter, the site experiences heavy snowfall. AMD containing Fe, zinc (Zn), copper (Cu), and cadmium (Cd) was partially discharged from an adit into the passive treatment system via a pipe (Table 1). The AMD was first introduced into an aerobic vertical flow BCR filled with a mixture of rice husks and limestone for Fe removal. In this

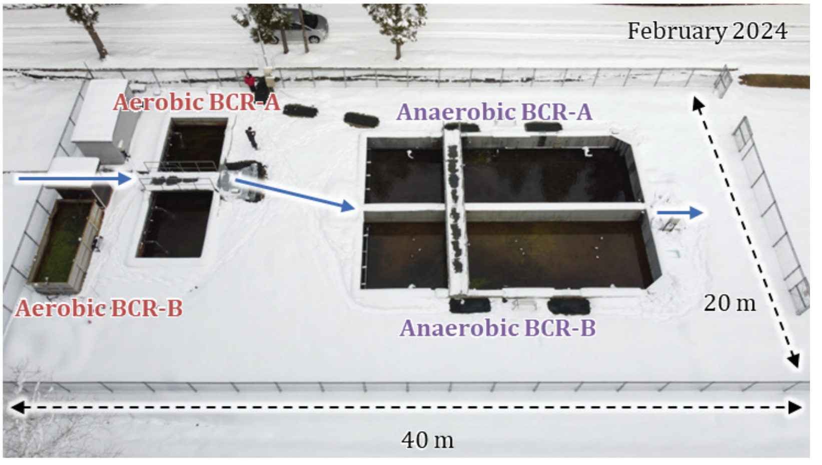

Figure 1 Large-scale, multi-step passive treatment test (image courtesy of Masataka Kondo). The blue arrows indicate the direction of flow. BCR, biochemical reactor.

process, ferrous iron (Fe^{2+}) was oxidized to ferric iron (Fe^{3+}) by Fe-oxidizing bacteria and precipitated primarily as schwertmannite (Masaki *et al.* 2021). The effluent from this process was then introduced into anaerobic BCRs for sulfate reduction. Fifty L/min of water, following Fe removal, was introduced into each anaerobic reactor, and the HRT in the reactor media was calculated to be 22.5 h. This study focused on the treatment results of the anaerobic BCR-A.

Anaerobic biochemical reactor structures

Two anaerobic BCRs were constructed with concrete walls and floors below the ground surface to maintain a minimum water temperature of 4 °C (Fig. 2). Each reactor was 5 m wide, 16 m long, and 3.5 m deep (total volume of 280 m3). At the bottom of the reactors, perforated pipes and limestone (20–40 mm) with a thickness of 0.15 m were installed to correct effluent water. A mixture of rice husk and limestone (20–40 mm) was then added to the reactors, divided into a lower layer (thickness of 1 m) and an upper layer (thickness of 0.5 m), with varying mixture weight ratios. Starting in 2020, the rice husk/limestone weight ratios in the lower and upper layers were set to 1/4 and 1/8, respectively. In 2022, limestone was added to the upper layers, and the rice husk/limestone weight ratio was changed to 1/16. The total media volume in each reactor was 120 m^3.

Carbon source nutrition conditions

Three different nutrient conditions with continuous ethanol feed were tested in the anaerobic BCR-A from 2021 to 2024. The first condition (Condition I) involved continuous ethanol feed using a metering pump, which introduced ethanol into the reactor from the surface, setting final feed concentrations of 36 mg/L (November 2021 to July 2022) and 24 mg/L (July 2022 to September 2022). The second condition (Condition II) involved continuous ethanol feed (final concentration of 24 mg/L) with an initial addition of 300 kg (0.3 wt% of the mixture media) of rice bran as supplemental nutrition (for approximately 26000 m^3 of annual water flow). The rice bran

Table 1 Average water quality of acid mine drainage (AMD) and the influent to the anaerobic BCRs from November 2021 to September 2024. Fe, iron; Zn, zinc; Cu, copper; Cd, cadmium; DO, dissolved oxygen.

	Temp. C°	pH	Fe mg/L	Zn mg/L	Cu mg/L	Cd mg/L	Sulfate mg/L	DO mg/L
AMD	13.2	3.6	38	17	4.9	0.1	296	–
Influent to the anaerobic BCRs	12.2	3.2	5.2	17	4.6	0.1	284	2.2

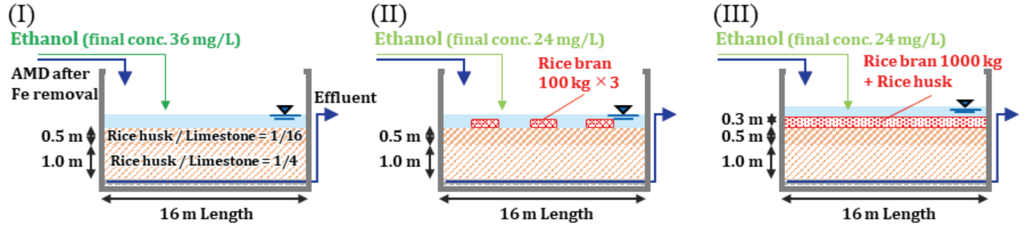

Figure 2 Cross-sectional structure of anaerobic BCR-A under three conditions: (I) 36 mg/L of ethanol, (II) 24 mg/L of ethanol supplemented with 300 kg of rice bran, and (III) 24 mg/L of ethanol supplemented with 1000 kg of rice bran.

was divided into three lines across the surface of the media (September 2022 to September 2023). In September 2023, the old rice bran was collected on one side, and 800 kg of new rice bran was added to the other side. Each batch of rice bran was then mixed with rice husk to form a 0.3-meter nutrition layer. Considering the remaining nutrients in the old rice bran, the third condition (Condition III) involved continuous ethanol feed (final concentration of 24 mg/L) with the addition of approximately 1000 kg (0.8 wt% of media) of rice bran addition (September 2023 to September 2024).

Monitoring parameters and analytical conditions

Temperature, pH, and oxidation–reduction potential (ORP; vs. Ag/AgCl) were measured on-site using a portable multi-water quality meter (MM-42DP, DKK-TOA Corp., Japan), and dissolved oxygen (DO) was measured using a portable DO meter (HQ1130, Hach Company, CO., USA). Filtered (0.45 µm) and unfiltered water samples were collected in high-density polyethylene bottles. The concentrations of Zn, Cu, and Cd were determined through inductively coupled plasma optical emission spectroscopy (Agilent 5110 ICP-OES, Agilent Technologies Inc., CA, USA). The sulfate concentrations in the filtrates were determined via ion chromatography (Dionex ICS-6000, Thermo Fisher Scientific, Waltham, MA, USA). The concentrations of organic acids and ethanol in the filtrates were determined by ultra-performance liquid chromatography using an ion exclusion column (ICPak Ion Exclusion Column: 7 µm, 7.8 mm × 300 mm; Waters Corp., USA), equipped with photodiode array detection (λ=210 nm) and refractive index detection, respectively.

Results and discussion

Treatment performance of the anaerobic process under three nutrition conditions

The water temperature of the effluent from the anaerobic BCRs ranged between 4.6 and 21.0 °C from 2021 to 2024, varying with changes in ambient temperature (Fig. 3). The pH in the effluents increased steadily due to the dissolution of limestone, and ranged between 6.2 and 7.4, with no remarkable differences observed across the nutrient conditions. Anaerobic conditions were maintained, with the ORP remaining at approximately −250 to −300 mV throughout the entire period under all nutrition conditions. During winter, the maximum ORP values were −193 mV under Condition (I), −208 mV under Condition (II), and −255 mV under Condition (III), respectively. The minimum sulfate reduction concentrations for each nutrient condition during winter were 24, 26, and 61 mg/L, respectively.

The stoichiometric requirement for sulfate reduction to remove metals as sulfides from the influent water was approximately 30 mg/L. Under conditions (I) and (II), the sulfate reduction concentration occasionally fell below 30 mg/L. For Zn removal performance, soluble Zn concentrations in the filtered effluent water remained below 0.2 mg/L across all seasons, regardless of nutrient conditions. The total Zn concentration sharply increased to 7.4 mg/L under Condition (I) and gradually increased to 1.9 mg/L under Condition (II). Under Condition (III), the maximum total Zn concentration remained below 0.1 mg/L, even during winter.

The concentration of ethanol in the effluent showed a trend similar to that of the total Zn concentration in the winter season, reaching 31 mg/L (86% of feed) under Condition (I) and 11 mg/L (46% of feed) under Condition (II). The results indicated that the activity of SRB decreased owing to the effect of lower water temperatures, and ethanol, as a carbon source, was no longer consumed. However, the presence of rice bran as a supplemental nutrient source increased the efficiency of ethanol consumption and slowed the increase in total zinc concentration in the effluent. Under Condition (III), the ethanol concentration in the effluent was below the detection limit in nearly all seasons, except during the start-up period.

Table 2 lists the differences in treatment performance for Zn and other parameters during winter across the three nutrient conditions. The total Zn concentration had not exceeded 2 mg/L (the domestic discharge standard) under conditions (II) and (III) throughout the study period, whereas exceeded under Condition (I). Under Condition (II), total Zn concentration

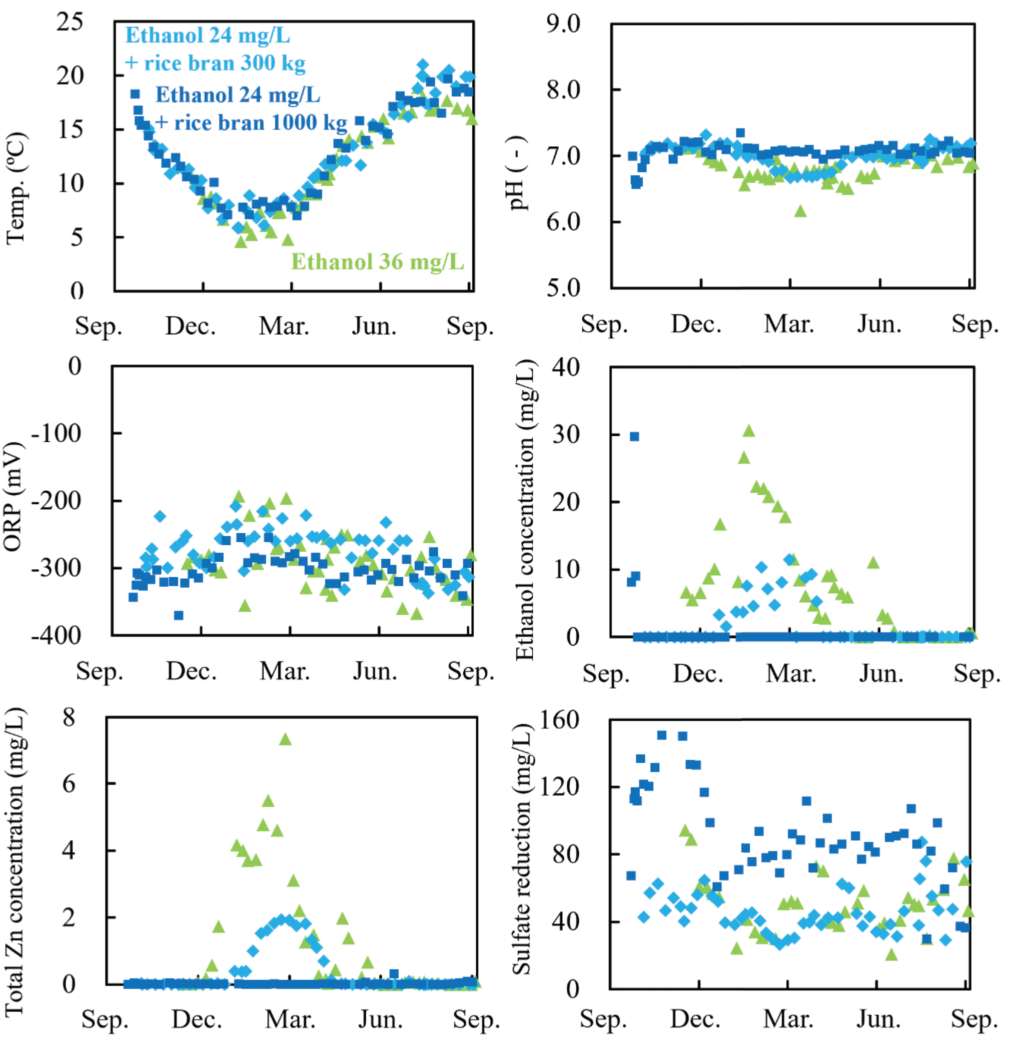

Figure 3 Changes in temperature, pH, oxygen–reduction potential (ORP), concentrations of ethanol and total Zn, sulfate reduction, and effluent characteristics of anaerobic BCR-A. Light green triangle: 36 mg/L of ethanol; light blue square: 24 mg/L of ethanol with 300 kg of rice bran; blue square: 24 mg/L of ethanol with 1000 kg of rice bran.

increased and approached 2 mg/L, suggesting that at least 300 kg of rice bran was required for this test scale, and that a larger amount of rice bran was needed for stable Zn treatment. The total amounts of organic carbon added to the BCRs each year were calculated, assuming an organic carbon content ratio of 45wt% in rice bran based on feed analysis. This amounted to 489 kg-C/y under Condition (I), 460 kg-C/y (325 kg from ethanol and 135 kg from rice bran) under Condition (II), and 775 kg-C/y (325 kg from ethanol and 450 kg from rice bran) under Condition (III).

The total supplied organic carbon was lower under Condition (II) than Condition (I); however, sulfate reduction was higher, and the maximum Zn concentration was lower under Condition (II) compared to Condition (I). This suggested that rice bran supplied not only ethanol but also several types of carbon sources, along with trace elements such as nitrogen (N) and phosphorus (P), which resulted in a diverse community of SRB. As a result, sulfate-reducing activity was maintained even at low water temperatures (Sato *et al.* 2024). It was also assumed that this helped maintain the activity of ethanol-utilizing SRB, resulting in the low concentration of unutilized ethanol at the outlet, as shown in Fig. 3. Therefore, the efficiency of metal removal under Condition (II) surpassed that under Condition (I), which involved ethanol alone, despite the lower amount of organic carbon. Under Condition (III), the high total organic carbon content and the presence of rice bran across the entire surface layer led to a more uniform nutrient supply from the rice bran, resulting in the most stable treatment performance during winter.

Sulfate reduction concentration with depth

A comparison of sulfate reduction across different depths showed that sulfate reduction was higher at depths shallower than 1 m (half the depth of the lower layer) during non-winter seasons under all nutrient conditions (Fig. 4). In contrast, during winter, sulfate reduction decreased at depths shallower than 1 m and increased at depths greater than 1 m under conditions (I) and (II). This indicated that the region of sulfate reduction activity shifted to deeper layers owing to the decrease in water temperature. Under Condition (III), sulfate reduction in areas deeper than 1 m did not decrease substantially during winter, and the total amount of sulfate reduction was the highest among the three nutrient conditions. It was assumed that organic nutrient sources, including ethanol, were consumed at shallower depths due to the addition of rice bran, resulting in more active sulfate reduction. This may have resulted in the precipitation of metal sulfides at shallower depths, trapping them on the rice husk surface over a wider area. Consequently, the total Zn concentrations were lower with rice bran than with ethanol alone (Fig. 3), suggesting that the sulfides were less likely to be released as suspended solids. Considering the capture area of the suspended solids, the reaction by SRBs should occur primarily on the upper side of the contents. To facilitate this, nutrient sources must be present in sufficient quantities during winter.

Conclusions

In this study, the annual treatment performance of the anaerobic BCR under three nutrient conditions with continuous ethanol feeding was investigated. We

***Table 2** Summary of treatment performance under three nutrient conditions during winter (December to February).*

		Condition (I) 36 mg/L of ethanol	Condition (II) 24 mg/L of ethanol + 300 kg of rice bran	Condition (III) 24 mg/L of ethanol + 1000 kg of rice bran
Domestic discharge standard of Zn	–	Over	Under	Under
Average ORP in winter	mV	259	253	281
Average sulfate reduction in winter	mg/L	42	42	81
Total amount of organic carbon	kg/y	489	460	775

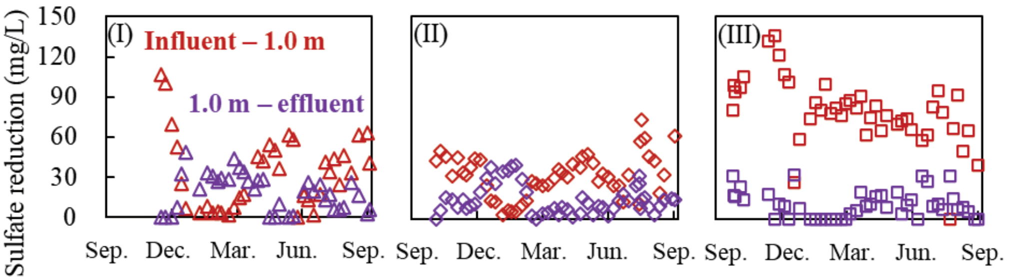

Figure 4 Changes in sulfate reduction with depth: Condition (I) 36 mg/L of ethanol, Condition (II) 24 mg/L of ethanol supplemented with 300 kg of rice bran, and Condition (III) 24 mg/L of ethanol supplemented with 1000 kg of rice bran.

observed no appreciable difference in the treatment performance for metal removal under any of the nutrient conditions during the non-winter period. Sulfate reduction activity, the concentration of unused ethanol, and total Zn concentration exhibited different behaviours during winter. Under 36 mg/L of ethanol (Condition I), sulfate reduction activity decreased to a minimum of 24 mg/L, and the total Zn concentration increased sharply to a maximum of 7.4 mg/L. Under 24 mg/L of ethanol supplemented with 300 kg of rice bran (Condition II), sulfate reduction activity decreased to a minimum of 26 mg/L; however, the total Zn concentration increased gradually to a maximum of 1.9 mg/L meeting Japan domestic discharge standard. Under 24 mg/L of ethanol supplemented with 1000 kg of rice bran (Condition III), sulfate reduction activity was sufficiently high during the winter season, with a minimum of 61 mg/L, and the total Zn concentration increased to only a maximum of 0.1 mg/L. It was found that the addition of rice bran as a supplemental nutrient source, alongside ethanol, improved winter treatment performance without substantial differences in the annual organic carbon supply. Comparing sulfate reduction activity with depth, sulfate reduction in the upper media layer decreased during winter under conditions (I) and (II), with the active sulfate reduction area shifting downward. In contrast, sulfate reduction activity in the upper layer remained relatively high under Condition (III) during winter. It was also found that sulfate reduction activity in the upper layer was maintained during winter, ensuring the preservation of the thickness of the layer where suspended metal sulfide solids were trapped, thereby sustaining effective treatment performance. Other nutrient source conditions, including modifications to the timing of ethanol and rice bran addition, will be tested in future research.

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