A Biological Sulfate-Reducing Process for Zinc-Containing AMD in Japan: Comparison of Annual Treatment Performances Among Nutrient Conditions of Ethanol, Rice Bran and Their Hybrid for Sulfate-Reducing Bacteria

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Abstract

The performance of anaerobic sulfate-reducing processes was compared in a large-scale test with water flow rate of 100 L/min in winter period under different nutrient conditions: ethanol addition, rice bran addition, and their hybrid. Soluble Zn concentrations were efficiently decreased to < 0.06 mg/L on average under all conditions even in winter, whereas differences were found in sulfate reduction capacity and total Zn removal efficiency among the conditions. Total Zn removal was affected by ethanol feed concentration, the timing of rice bran feeding rather than rice bran feed weight, and the microbial community structure in the media.

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

Introduction

A large amount of money is spent for continuous mine water treatment to meet domestic discharge standards at approximately 100 abandoned metal mines in Japan (Ministry of Economy, Trade and Industry, Japan 2023). JOGMEC (Japan Organization for Metals and Energy Security) has been conducting research on passive mine water treatment processes in order to reduce treatment costs. Since a limited area is available for mine water treatment at domestic mine sites, passive systems with relatively short hydraulic retention time were developed; approximately 2 h for Fe oxidation and precipitation and 25 h for anaerobic sulfate reduction (Hayashi *et al*. 2021). A multi-step passive mine water treatment test was started with a water flow rate of 100 L/min in 2020 (Figure 1). After the Fe oxidation using Fe-oxidizing bacteria and its removal, the AMD was introduced into the anaerobic process in a vertical down-flow biochemical reactor (BCR) containing a mixture of rice husk (an agricultural waste) and limestone. Ethanol and rice bran were used as direct and indirect nutrients for the sulfate-reducing bacteria, respectively. The treatment capability of the passive process should be lower in winter due to lower microbial activity at relatively low water temperatures. The aim of this study was to investigate treatment performance in winter among three different nutrient conditions, ethanol, rice bran and their hybrid.

Methods

Test Site and Mine Water Chemistry

The passive treatment test was carried out at an abandoned metal (gold, silver, copper, lead and zinc) mine located in northeast Japan. Annual temperatures at the site changed from -10 to +40 °C. A portion of the AMD (Table 1) flow was distributed to the passive treatment system using a pipe without exposure to air. The AMD was first introduced into vertical down-flow BCRs filled with rice husk media to a depth of 0.5 m, in order to remove much of the Fe. In these aerobic BCRs, the Fe^{2+} were oxidized by Fe-oxidizing bacteria; thereafter, the Fe^{3+} precipitated mainly as schwertmannite (Masaki *et al.* 2021). The aerobic BCRs effluent water was introduced into the anaerobic BCRs (Table 1).

Anaerobic BCR Structures

In 2020, two vertical downflow BCRs (Width 5 m × Length 16 m × Depth 3.5 m) were constructed in parallel with concrete walls and floor below the prevailing ground surface to maintain the water temperature above approximately 4 °C. The surface of the anaerobic BCRs was open to facilitate maintenance. At the bottom, perforated pipes and limestone (20 to 40 mm) bed were placed to collect the effluent water. A mixture of rice husk and limestone (20 to 40 mm) was placed in the anaerobic BCRs with different weight ratios in the lower and upper layers: The weight ratios of rice husk/limestone in the lower (1 m thick) and upper (0.5 m thick) layers were 1/4 and 1/8, respectively, anticipating higher limestone consumption rates in the upper layer than in the lower. The total media volume was 120 m^3 in each reactor. Effluent water from the aerobic BCRs (100 L/min) was evenly distributed (50 L/min each) into the two anaerobic BCRs by adjusting the heights of the BCR's water introduction pipe ends. The hydraulic retention time in the media was calculated to 22.5 h. Note that limestone was added to the upper media layers in both anaerobic BCRs in 2022, resulting in a 1/16 weight ratio of rice husk/limestone in the upper layer.

Organic Nutrient Conditions

Three different organic nutrient conditions were tested in the two parallel anaerobic BCRs from 2020 to 2023 (Figure 2). One was an ethanol condition where industrial

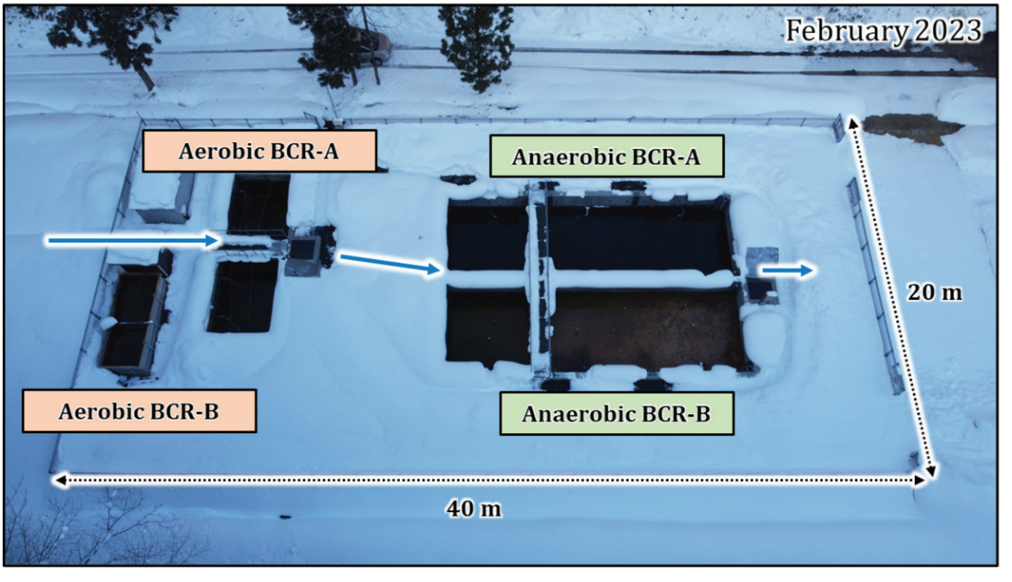

Figure 1 *multi-step passive mine water treatment test in the large-scale (image: Yusei Masaki)*

Table 1 *Water chemistry of the AMD and the influent of the anaerobic BCRs after Fe removal*

	Temp. °C	pH	Total Fe mg/L	Total Zn mg/L	Total Cu mg/L	Sulfate mg/L	DO mg/L
AMD	10-18	3.2-4.0	32-47	13-20	1-10	256-339	0.1
Influent of the anaerobic BCRs	9-16	2.8-3.3	4-14	15-20	2-11	257-328	1-3

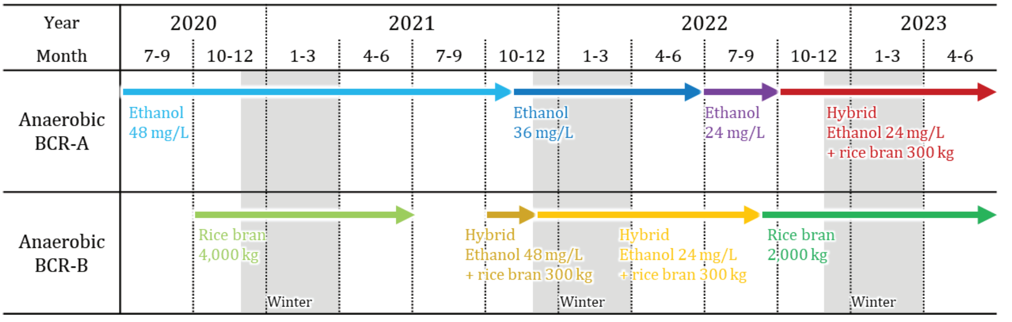

***Figure 2** Changes in the nutrient conditions in the anaerobic BCRs*

ethanol stock solution (50% alcohol) was continuously added using a metering pump into the anaerobic BCRs to set final feed ethanol concentrations of 48 mg/L (July 2020 to November 2021) and 36 mg/L (November 2021 to July 2022). The second condition was an initial addition of rice bran (2,000 or 4,000 kg) on the media of rice husk and limestone, to be indirectly used by the sulfate-reducing bacteria after aerobic bacteria decomposed the high molecular weight organic compounds (extracted from rice bran) to low molecular weight organic compounds. The third was a hybrid condition where ethanol was continuously supplied at a final concentration of 24 mg/L, and 300 kg of rice bran was initially added as a supplemental nutrient to support biodiversity in the rice husk media.

Monitored Parameters and Analytical Methods

Monitored parameters on the site were temperature, pH, oxidation-reduction potential (ORP, vs. Ag/AgCl) and dissolved oxygen (DO). Un-filtered and filtered (0.45 µm) water samples were regularly collected to determine the concentrations of Zn and Cu (ICP-OES; Agilent 5110 ICP-OES, Agilent Technologies Inc.). The detection limits were 0.08 µg/L for Zn and 0.18 µg/L for Cu. Sulfate concentrations and total organic carbon (TOC) in the filtrates were determined by ion chromatography (IC, Dionex ICS-6000, Thermo Fisher Scientific Inc.) and TOC analyzer (TOC-L, Shimadzu Corp.), respectively. Concentrations of organic acids and ethanol in the filtrates were quantified by UPLC using an IC-Pak ion exclusion column (7 µm, 7.8 mm × 300 mm; Waters), with photodiode array detection (λ = 210 nm) and refractive index detection, respectively.

Results and Discussion

Treatment Performances of the anaerobic BCRs in Non-Winter Periods

Our previous studies revealed that this biological sulfate-reducing process showed stable and effective treatment performances in non-winter periods (from April to November) in 2020 to 2022, regardless of organic nutrient conditions. Table 2 shows a water quality of the effluents in the anaerobic BCRs under

***Table 2** Water quality of the effluents in the anaerobic BCRs in non-winter periods (April - November)*

	Temp.	pH	Soluble Fe (filtered)	Soluble Zn (filtered)	Total Zn	Total Cu	Sulfate reduced
	°C		mg/L	mg/L	mg/L	mg/L	mg/L
Average	15	7.0	0.8	0.01	0.2	0.01	80
Min-Max	8-24	6.5-7.3	nd-8.0	nd-0.23	nd-2.6	nd-0.22	20-192

"nd" indicates that metal concentrations were less than detection limits of ICP-OES.

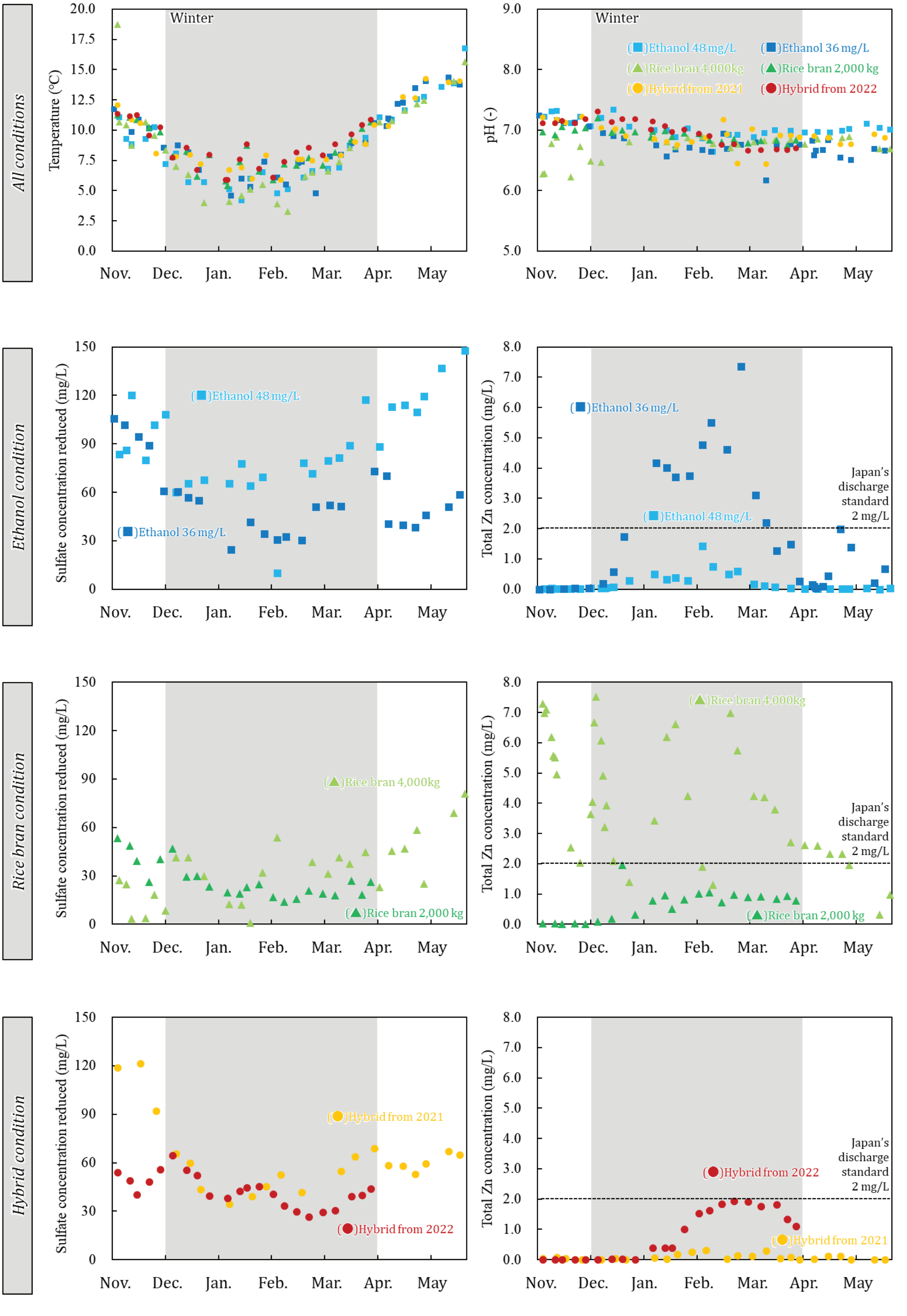

Figure 2 *Changes in temperature, pH, concentrations of sulfate reduced and total Zn under the condition of 48 mg/L ethanol (■ in light blue color), 36 mg/L ethanol (■ in blue color), 4,000 kg rice bran (▲ in light green color), 2,000 kg rice bran (▲ in green color), hybrid began from 2021 (● in yellow color), and hybrid began from 2022 (● in red color), from November to May in years when tested.*

three different nutrient conditions in non-winter periods. Effluent pH was 7.0 on average, and the total Zn concentration on average was 0.2 mg/L, which is much less than Japan's discharge standard. Note that domestic discharge standards are 5.8-8.6 for pH, 10 mg/L for soluble Fe, 2 mg/L for total Zn, and 3 mg/L for total Cu.

Water Temperature and pH in Winter Periods

The temperatures of the influent water into the anaerobic BCRs changed from 8.2 to 11.9 °C from December to March in 2020 to 2023, corresponding to changes in ambient temperatures. Dissolution of limestone mixed with rice husk media and microbial sulfate reduction reaction contributed to an increase in pH in the effluents, compared to the influents (approximately pH 3). The pH value in the effluents was found to be approximately 7.0, even during the winter periods, regardless of the organic nutrients conditions.

Treatment Performance Under the Ethanol Condition in Winter Periods

Water temperatures in the effluent from the anaerobic BCRs changed between 3.3 to 10.9 °C during the winter periods (from December to March) in 2020 to 2023, corresponding to changes in ambient temperatures. Dissolution of limestone mixed with rice husk media and microbial sulfate reduction contributed to an increased pH in the effluents (approximately pH 7). Under the ethanol conditions, ethanol concentrations of 48 and 36 mg/L were tested during 2020-2021 and 2021-2022, respectively. The average effluent pH in winter was found to be 7.0 at 48 mg/L of ethanol and 6.8 at 36 mg/L of ethanol, which implies that higher microbial sulfate reduction capacity corresponded to higher ethanol concentration, which contributed to the higher pH in the effluent. The lowest sulfate reduction capacity was found in February at both ethanol concentrations, which indicates that microbial SRB sulfate reduction was well-influenced by water temperatures. A stoichiometric requirement of sulfate reduction for contaminated metals removal from the influent water was calculated as approximately 30 mg/L. Sulfate reduction capacities in winter were 10-117 and 24-73 mg/L at ethanol concentrations of 48 and 36 mg/L, resulting in redox potential (SHE) of +115 to -106 and +32 to -131 mV, respectively. The results indicate that microbial sulfate reduction capacity was highly influenced by ethanol concentration added into the anaerobic BCRs. As for the Zn concentration in the effluent, Zn ions (filtered water samples) were removed below 0.1 mg/L in winter regardless of the ethanol concentrations, whereas total Zn (un-filtered water samples) were detected up to 1.4 and 7.4 at the ethanol concentrations of 48 and 36 mg/L, respectively. In the anaerobic BCRs, it is suspected that metal precipitates were trapped on the rice husk surface, which is in a reductive layer where sulfate is actively being reduced by SRB. Therefore, the reductive zone likely became relatively thinner due to a weaker SRB microbial activity in winter, also resulting in a thinner metal trapping zone in the following rice husk layer. This might have caused detection of suspended Zn precipitates, but not Zn ions, in the effluent in winter. The total Zn concentrations in the effluent gradually decreased from February to April or May (depending on the ethanol concentration) toward the same concentration level as the rest of the year.

Treatment Performance Under the Rice Bran Condition in Winter Periods

The changes in pH and water temperature in the effluent with the rice bran addition was similar to those with the ethanol condition. Rice bran was externally provided with weights of 4,000 kg in October 2020 and 2,000 kg in September 2022. Those rice bran weights were for a year of operation; therefore, the residues were removed from the BCRs after a year passed from the addition. When 4,000 kg of rice bran was used, the decrease in sulfate concentration was readily observed in the effluent, followed by Zn ion concentrations (filtered water) below 0.2 mg/L after 2 weeks passed from the rice bran addition. However, total Zn removal efficiency was limited and unstable until May 2021; the total Zn concentrations in the effluent changed between 0.7-7.6

mg/L from October 2020 to May 2021, and then decreased to less than 2 mg/L after May 2021. This implies that sufficient microbial community structure in the media layer might have not been established before the winter period in 2020. Therefore, earlier rice bran addition before October would lead to higher total Zn removal performance. When 2,000 kg of rice bran was used, sulfate reduction capacity was 14-47 mg/L and redox potential (SHE) was +44 to -60 mV. The concentrations of Zn ion (filtered water) were found to be stably below 0.02 mg/L. Total Zn concentration was observed to be below 1.0 mg/L from December 2022 to March 2023, meeting the domestic discharge standard for total Zn (2 mg/L).

Anaerobic BCRs Treatment Performance Under the Hybrid Condition in Winter Periods

The hybrid condition was tested twice using 300 kg of rice bran and ethanol addition at 24 mg/L. In 2021, rice bran was added to the anaerobic BCR, and ethanol addition began at series concentrations of 48 mg/L in October, 48-24 mg/L with a gradual decrease in November, and 24 mg/L after December until August 2022. The other anaerobic BCR that had been operated under the ethanol condition was used to test the hybrid condition: In October 2022, rice bran was added in the BCR, and ethanol solvent began to be added at a concentration of 24 mg/L. Sulfate reduction capacity in winter was relatively stable, ranging between 27-69 mg/L in both tests, corresponded to redox potential (SHE) of +48 to -87 mV. Furthermore, Zn ion concentrations (filtered water) were also efficient (below 0.02 mg/L). However, total Zn removal behaviours in both cases were different even though the nutrient condition was the same in winter: The total Zn concentration in the effluent was observed to average 0.08 mg/L in winter in the test that began in 2021, whereas the total Zn concentrations gradually increased from January 2022 and reached 1.9 mg/L in late February 2023 in the latter test. This difference might be due to different structures of microbial community in the media since residual ethanol concentrations in the effluents were found to be 0.3 (1.3% in the feed) and 5.4 mg/L (22.5% in the feed) on average in winter in the test that began from 2021 and 2022, respectively.

Conclusions

The winter treatment performance of anaerobic BCRs was monitored with three different nutrient conditions: ethanol, rice bran and their hybrid. The effluent pH and Zn ion concentrations were similar, while the sulfate reduction capacity and total Zn concentration varied. Under all conditions, the effluent water pH and Zn ion concentrations were found to be 7.0 and 0.06 mg/L on average, respectively, in winter. The test with an ethanol concentration of 48 mg/L had approximately twice the sulfate reduction capacity of that with an ethanol concentration of 36 mg/L. The corresponding effluent total Zn concentrations were 1.4 and 7.4 mg/L, respectively. Under the rice bran condition, the timing of rice bran addition was implied to be more critical than rice bran weight to establish an appropriate microbial community structure in the media, leading to efficient treatment performance. When tested with an addition of 2,000 kg of rice bran from September 2022, sulfate reduction capacity was 14-47 mg/L and total Zn concentration was found to be below 1.0 mg/L in winter. The hybrid conditions where 300 kg rice bran was fed, and 24 mg/L ethanol was continuously added, was tested through the year twice (began from 2021 and 2022), resulted in effluent total Zn concentrations of 0.08 and 0.8 mg/L on average in winter, respectively. In this study, all three conditions were found to show the capability for total Zn removal below its domestic discharge standard of 2 mg/L throughout the year, indicating that an appropriate condition could be chosen depending on AMD chemistry and site situations on mine sites. Other nutrient conditions will be tested in the future in this large-scale BCRs.

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