A Laboratory Experiment System for Developing Mine Drainage Treatment Technologies Using Constructed Wetlands—Sequencing Batch Treatment of Cd-Containing Neutral Mine Drainage—

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Abstract
A lab-scale experimental system accommodating soil and plants was designed to evaluate the applicability of constructed wetlands (CWs) to mine drainage treatment. Synthetic wastewater containing Cd (0.11 mg/L) and other minerals (pH 6.8) was prepared based on the chemical composition of an actual neutral mine drainage (NMD). In lab-scale CWs consisted of a column (ID 12.5 cm, H 50 cm) filled with pumice stones and loamy soil were planted reed (Reed-CW) or cattail (Cattail-CW) plants. Some were left unplanted (Unplanted CW). The synthetic NMD (2.0 L) was treated in a 1-week cycle sequencing batch mode in the CWs in a greenhouse. The unplanted CW removed cadmium sufficiently to satisfy the effluent standard (0.03 mg/L) from the NMD, mainly by soil adsorption. Presence of the emergent plants, especially cattail, enhanced metal removal possibly by filtration with their elongated roots and metal sulfide precipitation by sulfate-reducing bacteria in the rhizosphere of the Cattail-CW.

Key words: Cadmium, Cattail, Constructed wetland, Mine drainage, Sulfate-reducing bacteria

1. Introduction

Because of increasing labor costs and import liberalization of metal resources, about 5000 mines have been abandoned or closed in Japan since the 1970s1. Among them, about 80 mines must continue treatment of mine drainage, which contains toxic metals that threaten ecosystems and human health. Mine drainage is treated conventionally using physicochemical processes such as neutralization, coagulation, and filtration, with high costs for energy and chemicals.

A cost-effective and low-maintenance alternative technology for mine drainage treatment is constructed wetlands (CWs) incorporating physical, chemical, microbial, and botanical processes2-4. As a passive treatment system, CWs are useful with and without other processes. As a pretreatment for physicochemical processes, the CW can adjust the pH value and reduce the drainage amount through evapotranspiration. As a post-treatment with a low operational cost, a CW can remove remaining toxic metals from effluent of the main process.

The use of CWs for mine drainage treatment has spread rapidly during the last few decades in North America and Europe5,6. In the case of Japan, a CW for treating acid drainage was installed for removing manganese in 2000 at Kaminokuni Dam, Hokkaido Prefecture7. At Motokura Mine in Hokkaido Prefecture, a CW was installed on a small scale in 2006 for removing zinc, lead, and arsenic and has been scaled up for a demonstra-
tion test to replace the existing neutralization process\(^8\). Emergent aquatic plants such as cattails and reeds are generally used in CWs for uptaking metals and for growing microorganisms in the rhizosphere. Reportedly, sulfate-reducing bacteria (SRB) in the soil decomposed organic matter and formed metal precipitates\(^9,10\).

Nevertheless, the practice has not become widespread in Japan because the obtained knowledge is not always applicable to other cases. The chemical composition of mine drainage, soil and vegetation, terrain, and meteorological conditions differ among mines. Systematic knowledge about the design and operation of the CWs should be fully accumulated and systematized for their widespread use. The purpose of the research is to develop an experimental system to systematically evaluate the applicability of CWs according to the composition of mine drainage and the combination of soil, plants, and microorganisms, with the aim of putting it into practical uses. Because mines are often far from urban areas, collecting actual mine drainage is not always easy. Use of synthetic wastewater simulating the chemical composition of actual mine drainage is a promising option for the conduct of basic experiments at research institutes. For screening a suitable combination of soil and plants for mine drainage treatment, it would be convenient to use a small apparatus that can prepare multiple replicas in a small space. For this case study, cadmium (Cd)-containing synthetic mine drainage was designed to simulate the neutral mine drainage (NMD) of a mine in Kyoto Prefecture (A-Mine). Lab-scale CWs with loamy soil and emergent plants were designed for demonstrating their applicability by sequencing bath treatments for synthetic NMD.

2. Materials and Methods

2.1 Synthetic NMD

Figure 1 presents the chemical composition of NMD of A-Mine in 2016–2017. The average pH value in NMD was 6.8. The Cd concentration always exceeded the effluent standard in Japan (0.03 mg/L). The maximum zinc (Zn) concentration was as high as the effluent standard (2.0 mg/L). Among cations and anions, calcium and sulfate were respectively dominant in the NMD. The total iron concentration was low in the NMD. For reproduction of this composition with limited number of elements and ions, synthetic NMD was prepared using chemical reagents through trial-and-error combinations of cations and anions, as shown in Table 1. The Cd and Zn concentrations were set respectively to the maximum observed values of 0.11 mg/L and 2.0 mg/L. The pH and electrical conductivity (EC) of the synthetic NMD showed similar values to those of the actual NMD.

2.2 Lab-scale CWs

For preventing effects of wind and rain, lab-scale CWs were set up in a greenhouse at Ritsumeikan University in Kusatsua City, Shiga Prefecture, Japan. A schematic diagram of the CW is depicted in Figure 2. Each CW consisted of a plastic column (ID 12.5 cm × H 50 cm) filled from bottom to top with pumice stones (20–30 mm, 3.8 cm depth), medium loamy soil (akadama) (10–15 mm, 14 cm depth; Sowa Recycle Corp., Tokyo, Japan), and fine loamy soil (akadama) (3–4 mm, 13 cm depth; Sowa Recycle Corp.). The CWs were planted with common reeds (Phragmites australis, 40–50 cm shoot, 15–20 cm root, 115–145 g-wet) (Reed-CW), cattails (Typha domingensis, 40–70 cm shoot, 15–15 cm root, 30–50 g-wet) (Cattail-CW), and other species. The CWs were operated for 6 months following the setup of the reagents and plants.
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90–120 g-wet) (Cattail-CW) on 16 Oct 2017, or were left unplanted (Unplanted CW). All CWs were prepared in duplicate. These emergent plants were purchased from Tojaku Engei Co. Ltd. (Kyoto, Japan). A temperature and humidity data logger was installed in the greenhouse. An oxidation–reduction potential (ORP) meter was set at 5 cm soil depth in the CW. All CWs were kept by filling with tap water for a week.

2.3 Sequencing batch treatment of mine drainage

The synthetic NMD was treated in the CWs in the 1-week cycle sequencing batch mode from 2 November 2017. On the first day, the synthetic NMD (influent, 2.0 L) was poured into the CW. After 1 week, the treated water was fully drained (effluent). Then the synthetic NMD was poured again to the CW. This 1-week batch operation was repeated nine times in all CWs every week, except for the 8th batch with 2-week retention time.

2.4 Analytical procedures

Metal concentrations in water samples were measured using inductively coupled plasma spectroscopy (ICP-OES 700 series; Agilent Technologies Japan, Ltd., Tokyo, Japan). After the 9th batch treatment of the synthetic NMD, soil samples (5 g-wet each) were collected from upper (fine loamy soil) and middle (middle loamy soil) layers in the CW. The Cd concentrations in these soil samples were measured by Hiyoshi Corp. (Shiga, Japan) according to the method set out in JIS (Japan Industrial Standards) K0102. The errors in these measurements were less than 10%.

Heterotrophs and sulfate-reducing bacteria (SRB) were enumerated respectively using the plate-count technique using R2A agar medium (Merck KGaA, Germany) and Postgate’s medium F11. Agar plates for heterotrophs were incubated aerobically for 1 week at 28°C. Agar plates for SRB were incubated anaerobically for 2 weeks at 28°C in an anaerobic chamber.

The soil samples were subjected to DNA extraction and Illumina Miseq 16S rRNA gene sequencing at Bioengineering Lab. Co. Ltd. (Kanagawa, Japan). Amplicon libraries were prepared using a two-step tailed PCR method. Briefly, the 1st PCR amplified the V3–V4 region of 16S rRNA gene using primers 341f and 805r12; the 2nd PCR test was performed using the 1st PCR products and index primers. Amplicon sequencing of the 2nd PCR products was conducted with paired-ending (2 × 300 bp) on an Illumina MiSeq platform (San Diego, CA, USA).

3. Results

3.1 Environmental conditions in the greenhouse storing CWs

Figure 3 presents temperatures inside the greenhouse in which the CWs were set up. The maximum and minimum temperatures for the 1st batch treatment of the synthetic NMD (2–9 Nov
2017) were, respectively, approximately 30°C and 10°C. During batch treatments 5 and 6, the leaves of the emergent plants in the CWs began to turn brown with decreasing temperature. In the 7th batch treatment (14–21 December 2017), the maximum temperature exceeded 25°C, but the minimum temperature fell below 0°C. In the final 9th treatment (5–12 January 2018), the maximum temperature was below 15°C. Although the above-ground part of the plant withered, the remaining roots extended to the lower soil layers. Solar radiation in daytime during the experimental period was 400–600 W/m².

3.2 Metal removal in CWs

Figure 4 shows the amount of the treated water retained in the CWs for one week (effluent). The difference between the amount of influent (synthetic NMD, 2.0 L) and effluent represents the amount of evapotranspiration. The evapotranspiration of the 4th to 6th batch treatments in the Cattail-CW was larger than that of the unplanted CW and the Reed-CW. In the Cattail-CW, about one-fourth of the synthetic NMD of 2.0 L was sometimes evapotranspired in a week. The ORP in the soil in all CWs decreased to −120–−300 mV during the one-week treatment. The effluent showed neutral pH values of 6.8–7.4.

Figure 4 presents the metal concentration in the effluent of the CWs. The effluent Cd concentration in the 1st batch treatment of the unplanted CW exceeded the effluent standard (0.03 mg/L). However, the Reed-CW and the Cattail-CW sufficiently removed Cd in the synthetic NMD (0.11 mg/L) in all batch treatments. Even the unplanted CW well removed Cd after the 2nd batch treatment. After the 6th batch treatment, the Cattail-CW particularly well removed Cd in the NMD, although the above-ground part of the emergent plants withered. Zinc in the synthetic NMD (2.0 mg/L) was also well removed by all CWs. Furthermore, the Cattail-CW reduced the Zn concentration below to 0.03 mg/L, which is the environmental standard for aquatic organisms in Japan. Copper in the NMD (1.2 mg/L lower than the effluent standard 3.0 mg/L) was also well removed in the CWs below to 0.025 mg/L, especially in the Cattail-CW.

Figure 5 shows the Cd concentration in the soil samples in the CWs after the 9th batch treatment. The original loamy soil contained Cd about 0.1 mg/kg. The Cd concentration increased several times in the middle soil layer and 12–23 times in the upper soil layer in all CWs.

3.3 Soil microorganisms in the CWs

Figure 6 presents the viable cell count of soil microorganisms in the CWs after the 9th batch treatment. The population of aerobic heterotrophs was on the order of 10⁶ colony-forming unit (CFU)/g-dry in the unplanted CW and the Reed-
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CW, but on the order of $10^7$ CFU/g-dry in the Cattail-CW. SRBs were not detected in the unplanted CW and the Reed-CW. However, SRB were present in the soil samples of the Cattail-CW on the order of $10^3$ to $10^4$ CFU/g-dry, although they have not been identified.

Figure 7 presents the composition of the bacterial community in the soil samples. About 31,000–39,000 operational taxonomic units (OTUs) were obtained from soil samples of the CWs. Also, 28,000–32,000 OTUs were obtained from the unused loamy soil sample. At the class level, Alphaproteobacteria and Betaproteobacteria dominated more than 80% of all soil samples. The Alphaproteobacteria were mostly the family Bradyrhizobiaceae. The Betaproteobacteria were mostly of the genus Ralstonia. Gammaproteobacteria occupied 10–20% only in the middle soil layer of the Reed-CW and the Cattail-CW. Among them, the Thiotrichales family and the genus Moraxella were dominant, respectively, in the Reed-CW and the Cattail-CW. Gram-positive Actinobacteria also accounted for 20% in the unused fine loamy soil and the middle soil layer of the unplanted CW. The middle soil layer in the Cattail-CW was characterized by the presence of the class Bacilli (mainly facultative anaerobic Lactobacillus) and the class Saprospirae. Typical SRB such as Desulfovibrio and Desulfobacter were not detected from any soil sample, probably because of their small populations and non-uniform distribution.

**4. Discussion**

With the aim of developing an experimental system for evaluating the applicability of the CW to mine drainage treatment, we designed a lab-scale system that readily accommodates a combination of soil and plants. Synthetic NMD was designed based on the A-Mine chemical composition. Cadmium and zinc in the synthetic NMD were removed sufficiently from the CWs, especially from the Cattail-CW. Even in the unplanted CW, except for some batch treatments, cadmium was well removed from the NMD, resulting in its accumulation especially in upper soil layers. Therefore, the main mechanism for metal removal in the CW in this study is expected to be soil adsorption. Reportedly, loamy soil (akadama) has high permeability and water retention properties, and metal adsorption capability13,14. Loamy soil (akadama) has a metal sorption property governed by its specific chemical constituents by synergistic effects of physicochemical sorption and ion exchange mechanisms. In the chemical constituents of akadama, SiO$_2$, Al$_2$O$_3$, FeO, CuO, CaO, and K$_2$O, especially CaO has a possible promising ability for removing cadmium14.

Planting the cattails and reeds slightly enhanced metal removal in the CWs. High evapotranspiration can concentrate the nonvolatile pollutants in the CWs. Therefore, the metal concentrations in effluent were assumed to be higher than those in the influent if the CWs had not actively removed the metals. Although the metal concentration in the plant biomass was not measured in this study, some researchers reported that metal amounts that can be assimilated by plants are not meaningful compared to the total amounts removed from CWs5,6,13. Actually, the above-ground part of the plant withered in the latter experimental period. Enhanced metal removal
in the Cattail-CW and Reed-CW is expected to be attributable to phytofiltration by their elongated roots and metabolisms of microorganisms in the rhizosphere. The soil bacteria community composition was influenced by the plants. The SRB were detected from a soil sample from the Cattail-CW. These results suggest that the selection of plants in the CW can indirectly control rhizosphere microorganisms suitable for metal removal. The rhizospheres of the plants generally become aerobic if photosynthesis occurs actively and if oxygen is secreted sufficiently. However, oxygen consumption because of root respiration becomes remarkable during nighttime or when the above-ground part withered. Under such conditions, an anaerobic environment can be formed in the rhizosphere in which sulfate is reduced by SRB. Actually, SRB can use organic matter derived from root exudates as an electron donor and sulfate in mine drainage as an electron acceptor, resulting in production of sulfide. Although the chemical forms of the metals accumulated in the CWs were not determined in this study, precipitation of insoluble sulfides such as CdS and ZnS can enhance metal removal in the CW. New shoots of reeds and cattails in the CWs grew in the following spring. Subsequently, the CWs exhibited stable metal removal in spring and summer in additional sequencing batch treatments.

This study proposes a standard method of designing synthetic mine drainage and of developing lab-scale CW systems that will be necessary for spreading CWs for mine drainage treatment in Japan. In about a dozen of the 80 mines, the pH of the drainage is around neutral (5.8–8.6). The toxic metal concentration slightly exceeds the effluent standard in Japan. Installation of the CW is expected to be prioritized for treating such mine drainage. Additional studies of design and operational conditions of the CW such as hydraulic retention time, plant density, and water depth will be necessary throughout a year. However, the pH of mine drainage is often quite low. Treating acidic mine drainage by the CW requires its neutralization in advance or filling of the CW with alkaline media such as limestone. A method must be developed to prevent clogging by discharging large amounts of sludge generated by neutralization. If the oxidative condition can be maintained by shallow depth and short retention time of the drainage, then soluble iron and manganese can be oxidized to insoluble forms and can be precipitated, resulting in coprecipitation of arsenic. Examining the applicability of CW systematically must be done depending on the mine drainage characteristics.

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