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# Removal of selenite from artificial wastewater with high salinity by activated sludge in aerobic sequencing batch reactors

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**Selenite (Se(IV)) in artificial wastewater under high-salinity conditions of 70 g/L was treated by using sequencing batch reactors (SBRs). Activated sludge derived from a municipal wastewater treatment plant was acclimated to high salinity and was subsequently subjected to Se(IV) treatment. Two SBRs with different operating conditions, SBR-A and -B, were tested; the former was fed with influent containing a higher concentration of Se(IV) at 100 mg Se/L, whereas the Se(IV) concentration of influent for the latter was increased stepwise at 20–100 mg Se/L. Both SBRs showed high removal efficiency of up to 98% from the beginning. The removal efficiency gradually worsened in the middle stage, from the 11th and 16th batches in SBR-A and -B, respectively. After the 18th batch, a batch cycle with twice the duration was employed, and the removal efficiency was recovered from the 20th batch onward in both SBR-A and -B. This indicated that the hydraulic retention time is an important factor in maintaining efficient and stable performance of Se removal. The mass balance analysis revealed that Se was removed mainly through biovolatilization, which was attributed to a carbon source containing proteins or peptides. This study was the first to investigate the possibility of using biotreatment for Se-containing saline wastewater.**



[**Key words:** Selenium; Saline wastewater; Activated sludge; Sequencing batch reactor; Biovolatilization]

Selenium (Se) is a minor metal that has recently received significant attention as a semiconductor material in high-technology products such as copper–indium–gallium–diselenide-type solar cells, quantum dots, and light emitting diodes (1). Although Se is a valuable resource, it is also a contaminant in aquatic environments owing to its high toxicity to humans and aquatic ecosystems (2). Se has been found in varying amounts in several types of wastewater such as 140–1400 μg/L in agricultural drainage from seleniferous land (3), 40–610 μg/L in fly ash leachate from a coal-fired power plant (4), and up to 620 mg/L in industrial wastewater (5,6). In order to protect public health and the environment from Se contamination, Se in the wastewater should be properly removed.

Although physicochemical technologies such as coagulation with ferric salts have been conventionally used for Se removal from wastewater, they are limited by high cost and high energy/resource consumption (1). As cost-effective and eco-friendly alternatives, biological technologies have been proposed to be applied for the treatment of wastewater containing Se (6). In such treatment, certain microbes are used to reductively immobilize selenate ($SeO_4^{2-}$, Se(VI)) and selenite ($SeO_3^{2-}$, Se(IV)), the main forms of Se in the water phase, into insoluble elemental Se ($Se^0$) that can be easily removed by solid/liquid separation (7–9). Soluble Se can also be volatilized to be removed into a gaseous phase thorough methylation by microbes (10).

The aforementioned microbial metabolism of Se has been known to proceed via both anaerobic and aerobic processes. Under anaerobic conditions, soluble Se is generally reduced through anaerobic respiration (11–13). On the contrary, aerobic reduction of Se seems to occur for detoxification of toxic forms of Se or unknown mechanisms (14,15). Although various types of bioreactors for Se-containing wastewater have been intensively studied (5,16,17), most previous research has employed anaerobic conditions for Se removal. Recently, attempts have been made to treat Se-containing wastewater by applying processes of activated sludge (18,19) or aerobic granule sludge (20) under aerobic or alternate aerobic–anoxic conditions, and obtained better Se removal efficiencies comparing with the reports on the Se removal under the anaerobic condition. Such results indicated that the aerobic biological process has a better potential for treating wastewater containing Se, especially Se(IV), than the well-documented anaerobic processes.

Se-containing wastewaters are often characterized as having high salinity (21). For example, wastewater generated from a Se refinery was reported to contain 13.2–74.0 mg/L of Se and 6–7% salinity (21). In addition, chemical leaching from kiln powder included 2–39 mg/L of Se with 4.4–13.2 % salinity (22). Moreover, the pH of Se-containing wastewater tends to be low, which leads to further increases in salinity by neutralization (21,23). To best of our knowledge, however, no reports have been published on the aerobic biological treatment of Se-containing wastewater with high salinity.

Therefore, this study attempts to remove Se from artificial saline wastewater by activated sludge in model sequencing batch reactors

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(SBRs) under aerobic conditions. Model SBRs are constructed in shaking flasks on a rotary shaker using activated sludge obtained from full-scale municipal wastewater treatment plant (WWTP) as a seed, and an artificial wastewater (AWW) containing Se(IV) and a high concentration of NaCl, at 70 g/L, is treated. After the acclimation of activated sludge to high salinity, Se removal experiments are performed to clarify the applicability of aerobic biotreatment to Se-containing wastewater with high salinity.

## MATERIALS AND METHODS

**Activated sludge** The activated sludge used in this study as the seed for the SBRs was obtained from a municipal WWTP located in Osaka, Japan. The WWTP employs the conventional activated sludge process to treat sewage discharged from urban areas that does not contain significant levels of Se and salinity. The activated sludge sample collected in the WWTP was transported to the laboratory on ice, washed three times with 50 mM potassium phosphate buffer with pH 7.5, and used for seeding the SBRs.

**Artificial wastewater** The Se and salinity concentrations of AWW used in this study were referred to the wastewater discharged from Se refinery plant (21), as a typical industrial wastewater containing Se. Since the industrial wastewater usually contains no carbon sources for bacterial growth, the supplementation of external carbon source is essential. We chose bonito extract and peptone as the carbon source to simulate sewage (24) because it should be compatible with activated sludge obtained from the municipal WWTP, and be the ideal carbon source from the viewpoint of the cost. For such reasons, the AWW used in this study had the following basic composition per liter: 30 mg NaCl, 800 mg 35% Ehrlich bonito extract (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan), 1200 mg peptone (Kyokuto Pharmaceutical Industrial Co., Ltd.), 200 mg urea, 14 mg KCl, 18.54 mg $CaCl_2 \cdot 2H_2O$, 120.5 mg $MgSO_4 \cdot 7H_2O$, and 167.2 mg $Na_2HPO_4 \cdot 7H_2O$. The dissolved organic carbon (DOC) resulting from the basic composition of the AWW was approximately 780 mg-C/L. When it was necessary to increase the DOC to approximately 1560 mg-C/L, the bonito extract, peptone, and urea were additionally supplemented at twice the concentration of the original AWW (2× AWW). NaCl was added to give a defined salinity concentration up to 70 g/L to make the AWW saline, and $Na_2SeO_3$ was added for Se treatment experiments at approximately 20–100 mg-Se/L.

**Operation of model SBRs** The SBRs were constructed by placing the 200 mL activated sludge sample with 1200 mg/L of mixed liquor suspended solids (MLSS) into 500 mL Erlenmeyer flasks capped with silicone foam plugs. As illustrated in Fig. 1, the SBRs were operated in 24-h or 48-h cycles. The 24-h cycle operation consisted of rotary shaking for 23.5 h at 28°C and 120 rpm to simulate aeration, withdrawal of 20 mL excess sludge, sludge settling for 0.5 h, effluent discharge of 140 mL, and influent refill of 160 mL. Under the operational conditions, hydraulic retention time (HRT) and sludge retention time (SRT) were defined as 1.25 days and 10 days, respectively. For the 48-h cycle, the same operation was performed except that the rotary shaking period was prolonged to 47.5 h with HRT and SRT of 2.5 days and 20 days, respectively. Approximately 780 mg-C/L of AWW with salinity/Se(IV) was fed for the 24-h cycle operation as the influent, whereas about 1560 mg-C/L of 2× AWW was used for 48-h cycle operation to maintain the same organic loading rate.

**Acclimation of activated sludge to high salinity** Prior to the Se treatment experiments, the seed activated sludge was acclimated to AWW with high salinity in two SBRs (SBR-A and -B). In the acclimation, the SBRs were operated in 24-h cycles in principle and occasionally 48-h cycles during the weekends. The SBRs were fed with AWW in which the NaCl was supplemented. The NaCl concentration of the influent was increased from 10 to 20, 30, 50, 60, and 70 g/L stepwise during the 42-day acclimation period (Fig. S1). As a type of control, another SBR, SBR-C, was acclimated to the AWW without addition of NaCl in the same manner as that for SBR-A and B but for 14 days.

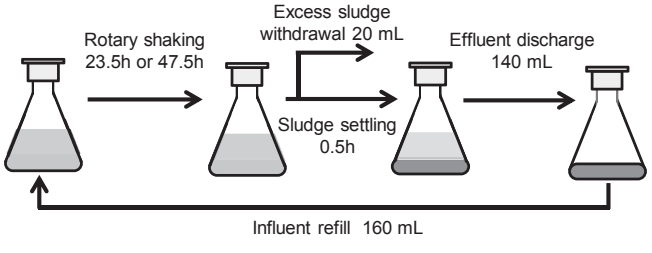


FIG. 1. Schematic representation of SBR operation.

**Se treatment experiments** After the aforementioned acclimation period, the SBRs began to be fed with a 70 g/L NaCl saline AWW containing Se(IV), and the treatment performance was evaluated. From the beginning of the treatment experiments, SBR-A and -C were fed with saline AWW containing a very high concentration of Se(IV), at approximately 100 mg-Se/L. On the contrary, the influent to SBR-B was saline AWW with 40 mg-Se/L of Se(IV) at the beginning. From the second batch, AWW with 20 mg-Se/L of Se(IV) was fed (2–6 batches), then the Se(IV) concentration was increased to 30 (7–11batch), 50 (12–17 batches), 80 (18–21 batches), and 100 mg-Se/L (22–24 batches) in a stepwise manner. In the first 1–17 batches of the experiments, 24-h cycle operation was performed for all of the SBRs except for 5th and 9th batches, which were operated in a 48-h cycle. Although the operation of SBR-C was stopped after 17 batches, the operation of SBR-A and -B was continued with 48-h operation in batches 18–24.

**Analytical procedures** The Se concentrations were measured by using inductively coupled plasma atomic emission spectroscopy (SPS7800, SII NanoTechnology, Tokyo, Japan). For liquid/solid separation, samples from the SBRs were centrifuged at 20,000 ×*g* at 4°C for 10 min. The supernatant was filtrated through a 0.2-μm pore-size membrane filter and was subjected to soluble Se analysis. The precipitant was washed three times with ultrapure water, digested by 10 min of boiling in a mixed acid solution containing 60% nitric acid and 95% sulfuric acid at a 20:1 volume ratio, and subjected to analysis of Se in the solid. The DOC was measured by using a total organic carbon (TOC) analyzer (TOC-$V_{CSH}$, Shimadzu, Kyoto, Japan) for samples filtered through the 0.2-μm pore-size membrane filters. The MLSS and sludge volume index (SVI) of activated sludge (waste sludge) were measured according to the Wastewater Examination Methods (25) with minor modifications. The microbial communities in the SBRs were analyzed by terminal restriction fragment length polymorphism (T-RFLP) analysis targeting the eubacterial 16S rRNA gene using restriction enzyme *Hha*I, as described in previous study (25) with minor modifications. Principal component analysis (PCA) and calculation of Shannon's diversity index (*H'*) of the T-RFLP results were conducted according to previous research (26) using PAST ver. 3.11 (https://folk.uio.no/ohammer/past/) (27).

## RESULTS

**Treatment performance of the SBRs** In SBR-C, the activated sludge that had not been adapted to high salinity was applied to treat Se(IV)-containing saline AWW in order to evaluate the Se removal potential of activated sludge obtained from the WWTP. Although the treatment experiment was continued for 17 batches, significant removal of soluble Se was not observed (Fig. S2). Further, DOC removal was very low, and a gradual decrease in MLSS was observed throughout the treatment phase. These results indicate that the high salinity and Se(IV) essentially inactivated the microbial metabolism in the activated sludge; hence, the treatment function of SBR-C was mostly corrupted.

Therefore, activated sludge that was well adapted to high salinity was used for Se treatment in SBR-A and -B. Fig. 2 shows the treatment performance of SBR-A, to which a very high concentration of Se(IV), 90–103 mg/L, was fed from the beginning of the experiment. Even in the first batch, about 98% soluble Se removal was achieved, and relatively high removal efficiency of 87–98% was maintained within the ten batches. However, the soluble Se removal significantly dropped and became unstable after the 11th batch. After the 18th batch, the operation mode was changed to a 48-h cycle from the 24-h cycle, doubling the HRT by adding twice the concentration of initial DOC. Then, soluble Se removal was recovered after the 20th batch and was stably maintained at 86–94%. The DOC removal was mostly about 40–70% and was not significantly influenced by the cycle duration throughout the experiment. Although good settleability of the sludge was kept at an SVI of 27–65 mL/g, the MLSS concentration gradually decreased from approximately 3000 to 2000 mg/L during the experimental period.

For SBR-B, the Se(IV) concentration in the saline AWW was increased stepwise during the experiment. The results are shown in Fig. 3. Although relatively high soluble Se removal at 71–96% was shown from batches 1 to 15 for the initial Se(IV) concentration of 10–50 mg/L, it worsened after the 16th batch. After the 18th batch, the cycle duration of SBR-B was prolonged to 48 h; twice the amount of DOC was added; and the Se(IV) concentration in the

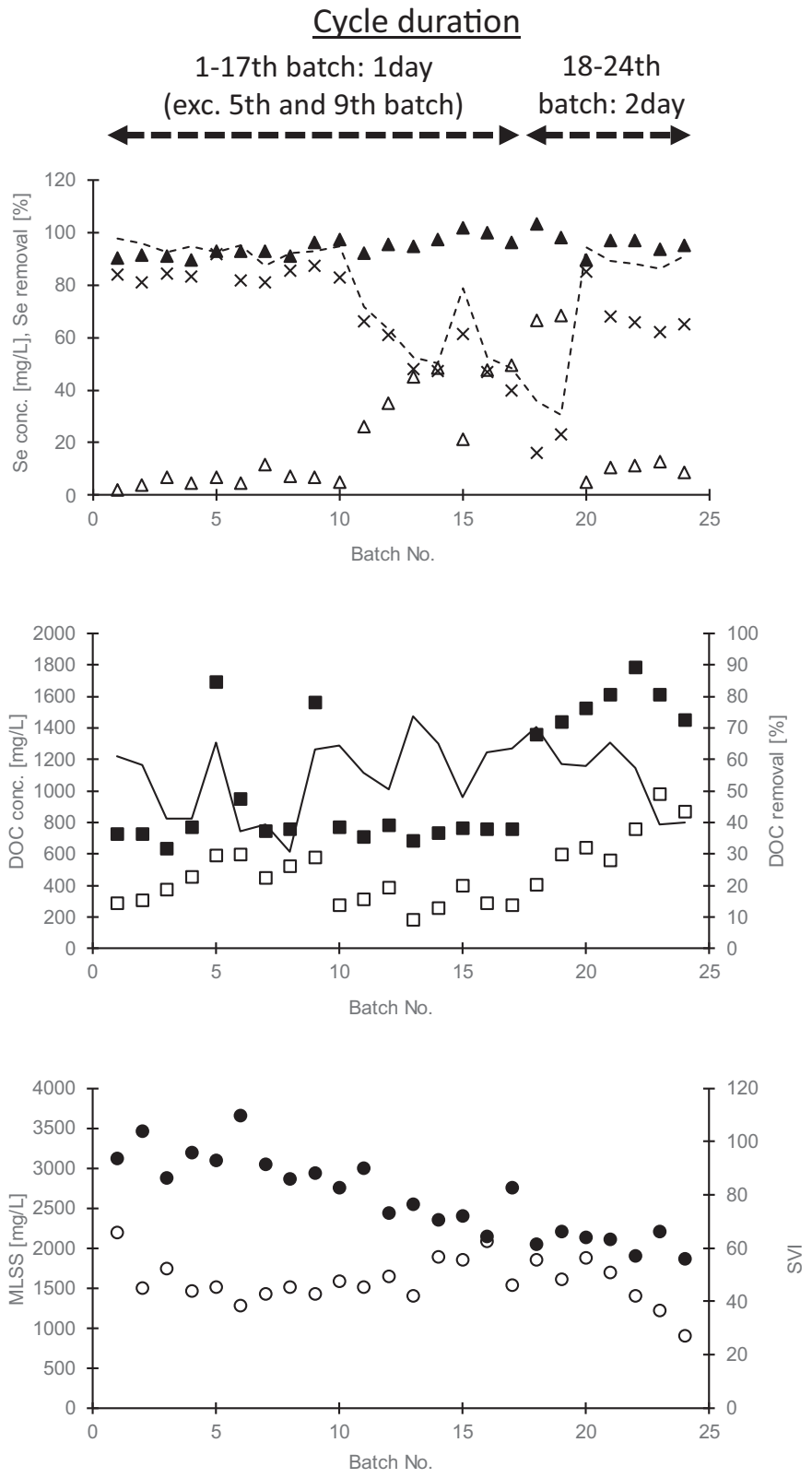


FIG. 2. Treatment performance of SBR-A. Closed triangle, soluble Se concentration in influent; open triangle, soluble Se concentration in effluent; dashed line, soluble Se removal efficiency; cross, concentration of solid-phase Se in the reactor; closed square, DOC concentration in influent; open square, DOC concentration in effluent; line, DOC removal efficiency; closed circle, MLSS; open circle, SVI.

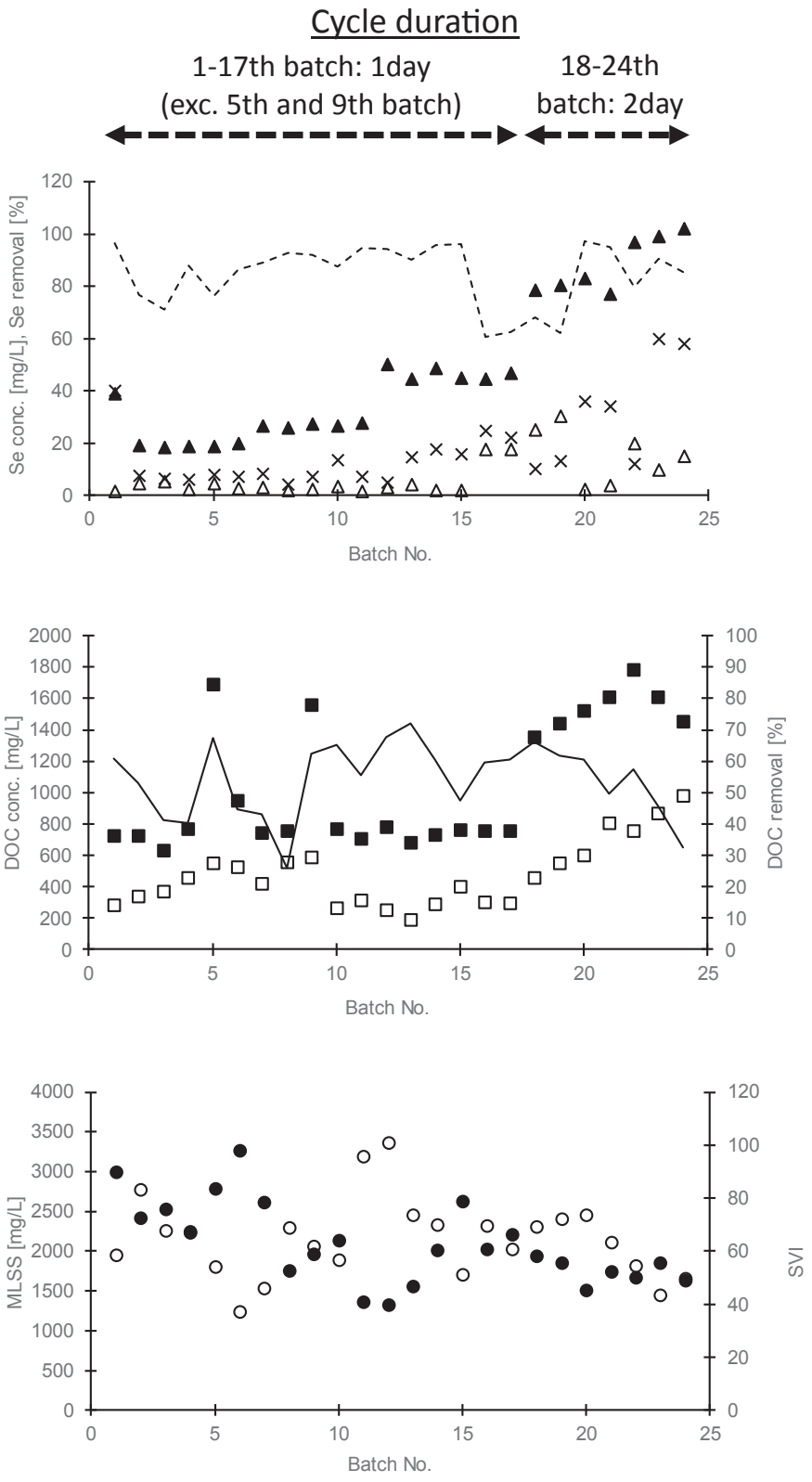


FIG. 3. Treatment performance of SBR-B. Closed triangle, soluble Se concentration in influent; open triangle, soluble Se concentration in effluent; dashed line, soluble Se removal efficiency; cross, concentration of solid-phase Se in the reactor; closed square, DOC concentration in influent; open square, DOC concentration in effluent; line, DOC removal efficiency; closed circle, MLSS; open circle, SVI.

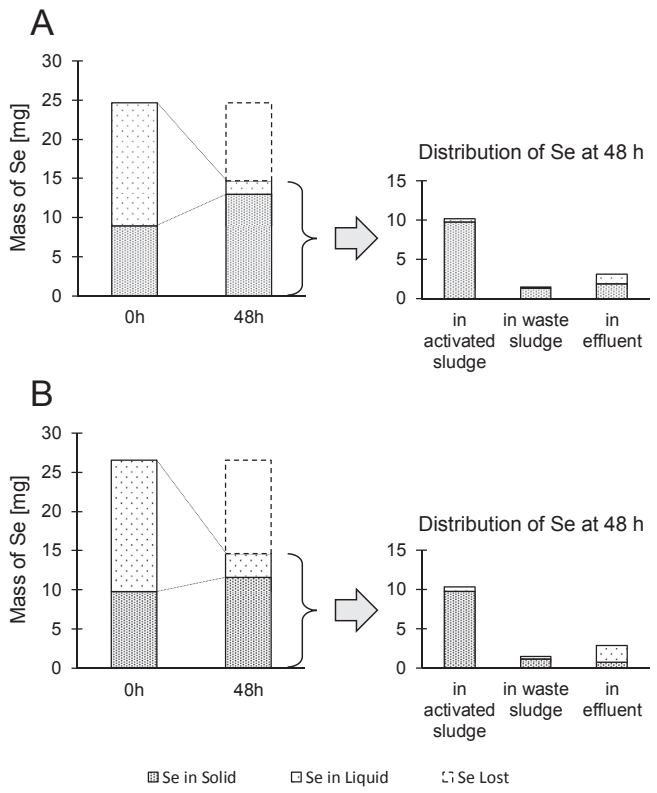


FIG. 4. Mass balance of Se in 24th batch in SBR-A (A) and -B (B). The mass of Se at 0 h and 48 h in the 24th batch are compared. The distribution of Se in the activated sludge, waste sludge, and effluent at 48 h is also indicated.

influent was increased to 80 mg/L. The removal performance of soluble Se recovered after the 20th batch despite the fact that saline AWW with higher Se(IV) concentration was treated. Similar to that for SBR-A, the DOC removal was moderate, at about 26–72%, and the MLSS concentration tended to gradually decrease throughout the experimental period. The activated sludge maintained good settleability, but at SVI 37–101 mL/g, it fluctuated slightly more than that for SBR-A.

**Material balance of Se in the SBRs** In batches 21–24, the Se treatment experiments were successful and stably removed the soluble Se. In these experiments, the mass of Se existing in the liquid or solid phases in the influent, activated sludge, waste sludge, and effluent was analyzed in detail to reveal the fate of Se in the process. Because the mass balance of Se showed similar trends in the batch treatments, Fig. 4 summarizes the mass balance of Se at the beginning and end of the treatment in the 24th batch of SBR-A and -B as typical data.

At the beginning of the batch treatment, the respective Se in SBR-A and -B existed in the liquid phase at 36% and 37% mainly as Se(IV) derived from the influent and in the solid phase at 64% and 63% mainly as $Se^0$ accumulated in the activated sludge carried over from the previous batch. After the treatment, a portion of Se was discharged from the SBRs and was contained in the waste sludge and effluent. The former existed mostly in the solid phase and accounted for 6% of the total amount of Se that existed at the beginning (initial-Se) in both SBRs. However, the latter was present both in liquid and solid phases and accounted for 13% and 11% of initial-Se in SBR-A and -B, respectively. Se in the remaining activated sludge after discharge of excess sludge and effluent, which is mostly in solid phase, represented 41% and 39% of initial-Se in SBR-A and -B, respectively. Assuming that the measurement errors were sufficiently small, a considerable portion of Se of the initial-Se, 40% and 45%, respectively, was lost from the SBRs, which can be explained only by Se removal to the gas phase as methyl selenides. On the basis of these calculation, it can be concluded that Se added to the SBRs as influent was removed mainly by biovolatilization.

**Transition of bacterial community in the SBRs** The bacterial communities at the end of the 7th, 12th, 18th, 21st, and 24th batches of both SBR-A and -B were monitored by T-RFLP analysis (Fig. S3). Then, the data were further analyzed by PCA (Fig. 5) and diversity analysis using Shannon's $H'$ index (Fig. 6).

In SBR-A, the bacterial population with a terminal-restriction fragment (T-RF) of 55–59 base pair (bp), of which the ratio was 21% in the 7th batch, occupied 75% of the activated sludge in the 24th batch. This coincided with the simplification of the bacterial community. In SBR-B, T-RF with 55–59 bp was most dominant similarly to that in SBR-A. However, it accounted for 38% in the 24th batch, and T-RFs with 344–345, 513–514, and 767 bp coexisting at a certain level of abundance. These results are consistent with the diversity analysis of the bacterial communities in SBR-A and -B (Fig. 6), which showed that the diversity index gradually decreased in SBR-A and was maintained in SBR-B.

Fig. 5 shows scatter plots of the scores of bacterial communities calculated by PCA. Loadings on the principal components (PCs) are shown in Table S1. PCs 1–4 retained 73% of the variance in total. For the bacterial communities in SBR-A, only the scores in PC4 changed,

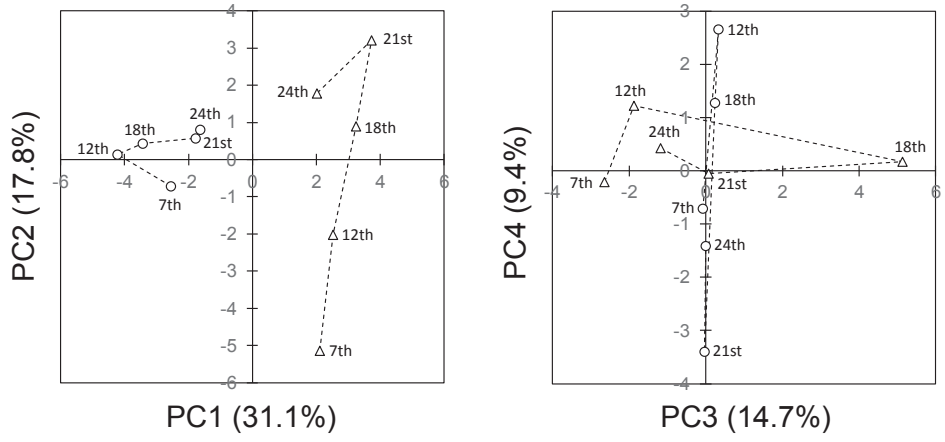


FIG. 5. PCA of bacterial community in SBR-A and -B. Open circles and triangles represent scores of the bacterial community in SBR-A and -B, respectively. The numbers beside the symbols show the batch number. The percentages in parentheses indicate the proportions of the variance.

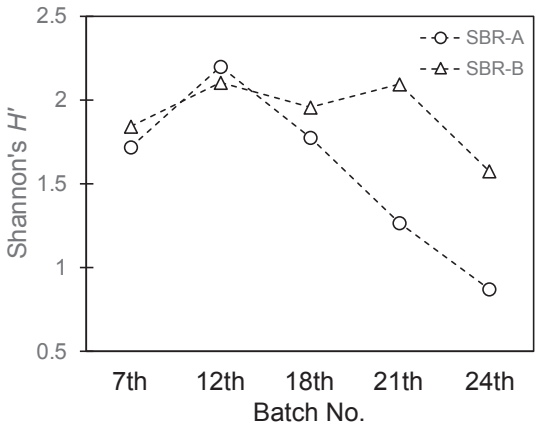


FIG. 6. Diversity analysis of bacterial community in SBR-A and -B.

which is attributed to the prominent increase in T-RF with 55–59 bp over the batches. The change in bacterial community was more dynamic in SBR-B than in SBR-A, which was influenced by the appearance and disappearance of the T-RFs with 344–345, 513–514, and 517–519 bp for PC2 and 136–138, 202, 344–345, and 513–514 bp for PC3.

The results suggest that the bacterial community in SBR-A rapidly adapted to the high concentration of Se(IV), whereas the gradual increase in Se(IV) loading in SBR-B caused a slower transition of the bacterial community.

## DISCUSSION

In this research, the possibility of using biotreatment for Se-containing saline wastewater was investigated for the first time. A model saline wastewater containing Se(IV) as a typical form of Se in industrial wastewater was aerobically treated by activated sludge in SBRs.

The seed sludge for the SBRs was taken from a municipal WWTP that treats domestic sewage without significant Se contamination. Once the activated sludge was acclimated to high salinity, the SBRs removed soluble Se at relatively high efficiency from the beginning of the Se treatment experiments. However, the experiment using activated sludge without acclimation to high salinity failed. These results indicate that halotolerant microbes capable of removing soluble Se certainly exist in the activated sludge; therefore, the treatment of Se-containing saline wastewater by activated sludge has a good potential. Here, from the beginning of the test, SBR-A was applied to treat a very high concentration of Se(IV), at 100 mg/L, whereas the Se(IV) concentration of the influent to SBR-B was elevated stepwise from a low concentration at 20 mg/L from the beginning. Both SBRs showed similar percentages of high Se removal efficiency, suggesting that toxicity of Se(IV) up to 100 mg/L may not cause critical damage to the Se-removing microbes in the activated sludge.

To the best of our knowledge, there is no report on successful Se removal under saline environment so far. Our previous study revealed that 20 g/L of NaCl negatively impacted Se removal by Se-metabolizing bacterium, *Pseudomonas stutzeri* (28). Soda et al. (21) suggested that dilution of wastewater from Se refinery plant to 2% salinity was necessary before biological treatment of Se under anaerobic conditions. On the other hand, few attempts were made to treat saline wastewater by SBR with activated sludge acclimated to high salinity. Park and Marchand (29) evaluated the effect of NaCl concentration on treatment performance of perchlorate by SBR with activated sludge acclimated to saline conditions, and confirmed the degradation of perchlorate under up to 4% NaCl, although the degradation rate became lowered by elevated concentrations of NaCl. Aloui et al. (30) also found that the activated sludge acclimated to 2.5% NaCl achieved favorable removal of COD and ammonium at 4% NaCl, whereas the removal efficiencies were significantly deteriorated at 6% NaCl. Considering the results in these studies, it is noteworthy that this study successfully removed Se from wastewater at 7% of NaCl with acclimated activated sludge.

As the batch operation was repeated, the MLSS concentration gradually decreased in both SBRs-A and -B. Thus, the Se removal efficiency was lowered and became unstable during prolonged operation in the 24-h cycle mode in batches 1–17, operated at HRT = 1.25 days, SRT = 10 days, and organic loading = 624 mg DOC/L/day. However, after the operation was changed to a 48-h cycle mode for batches 18–24, with HRT = 2.5 days, SRT = 20 days, and organic loading = 624 mg DOC/L/day, the Se removal performance recovered and was stabilized. Considering that the organic loading was same for 24-h and 48-h cycle operations and that the Se removal was immediately recovered after the change in cycle duration, HRT rather than SRT may be the key parameter for recovery of Se removal performance. It may be necessary to operate SBRs with a certain long HRT, i.e., reaction time, to maintain efficient and stable Se removal performance.

Biological soluble Se removal can be achieved via immobilization into the solid phase as $Se^0$ (bioprecipitation) or volatilization into a gas phase as methyl selenides. The calculation of Se mass balance in the SBRs in this study indicated that Se removal depends mostly on biovolatilization. In a previous study on Se wastewater treatment by activated sludge, Jain et al. (31) reported that removal of soluble Se was achieved mainly via bioprecipitation and that less than 15% might be removed via biovolatilization. Nancharaiah et al. (20) also reported that $Se^0$ nanoparticles formed and were removed by entrapment into sludge during treatment of Se(IV)-containing wastewater by aerobic granular sludge SBR. Thus, it is worth noting that Se was removed in this study mainly via biovolatilization by activated sludge, which differs from previous research. Biovolatilization may be more suitable than bioprecipitation for Se recovery from wastewater because gaseous Se can be collected by a gas trap in a recyclable form free from other metals, bacterial cells, and other contaminants (10).

Thus far, little has been published on biovolatilization of Se by microbes, especially by microbial communities such as activated sludge. Thompson–Eagle and Frankenberger Jr. (32) investigated the potential of Se-contaminated environmental water to volatilize Se using various substrates. They clarified that the addition of proteins and peptides such as casein, cottonseed meal, cheese whey, and yeast sludge, enhanced the occurrence of dimethylselenide (DMSe). Zhang and Frankenberger Jr. (33) also reported that amendment of casein or casamino acid to Se-contaminated soil enhanced the DMSe production. Since the AWW used in this study contained bonito extract and peptone as main organic components, it is supposed that these organic substrates promoted the biovolatilization activity in the activated sludge. On the contrary, simple carbon sources such as glucose and lactate that were utilized in previous research on Se removal by activated sludge (19,31) resulted in predominance of Se bioprecipitation over biovolatilization. Therefore, to establish efficient Se removal process via biovolatilization, it may be important to select suitable substrates.

The T-RFLP analysis of SBR-A revealed a rapid decrease in diversity of the bacterial community owing likely to toxicity of a very high concentration of Se(IV), at 100 mg/L. In addition, domination of a specific population, T-RF with 55–59 bp, was observed particularly during the 48-h cycle operation when soluble Se was stably removed. This result suggests that bacteria with T-RF of 55–59 bp were the main contributor to Se removal or volatilization. On the contrary, various bacterial populations existed in SBR-B in

the early phase of Se treatment experiment when lower concentrations of Se(IV) were fed, at 20–40 mg/L. Because Se was significantly removed during this period, it appears that a variety of bacteria related to metabolisms leading to Se removal existed in SBR-B at lower Se loadings. After a high concentration of 80 mg/L Se(IV) began to be fed, T-RFs of 55–59 bp rapidly dominated in SBR-B as well as in SBR-A, suggesting that only limited bacteria like these are capable of removing Se at very high Se loadings. To confirm these inferences on the bacterial population dynamics, it is necessary to isolate dominant bacteria from the SBRs and to examine their ability of Se metabolism in the future.

In summary, this study successfully indicated that activated sludge has a high potential to remove Se(IV) from saline wastewater. Moreover, surprisingly, a proteinous substrate may lead to effective biovolatilization of Se, implying a new mode of Se removal from wastewater that will enable easy recovery of Se. Even though most of the Se was removed through the treatment, further reduction of Se and DOC in the effluent is required to meet quality standards. Design of the total process including reduction of DOC supply, optimization of operating condition, and post-treatment for SS and the remaining DOC are desired to establish this technology as a practical treatment method.

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