Front. Environ. Sci. Eng. 2019, 13(5): 68
https://doi.org/10.1007/s11783-019-1154-z

RESEARCH ARTICLE

Biological removal of selenate in saline wastewater by activated sludge under alternating anoxic/oxic conditions

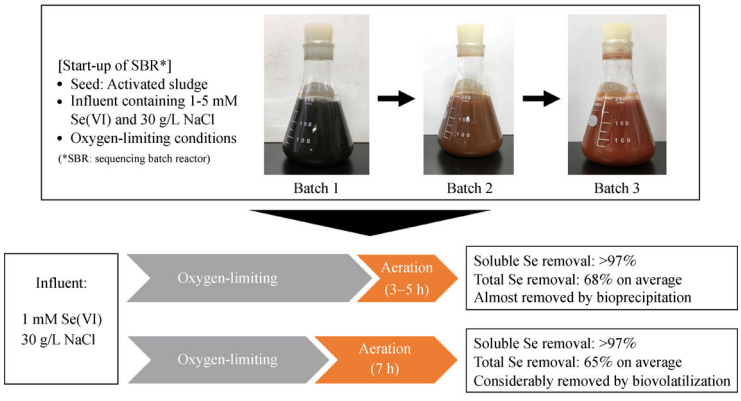
Yuanyuan Zhang[1], Masashi Kuroda[1], Shunsuke Arai[2], Fumitaka Kato[2], Daisuke Inoue[1], Michihiko Ike (✉)[1]

1 Division of Sustainable Energy and Environmental Engineering, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
2 Nippon Steel & Sumitomo Metal Corporation, 20-1 Shintomi, Futtsu, Chiba 293-8511, Japan

HIGHLIGHTS

- Removal of selenate in saline wastewater by activated sludge was examined.
- Sequencing batch reactor was operated under alternating anoxic/oxic conditions.
- Above 97% removal of soluble selenium (Se) was achieved continuously.
- Major Se removal mechanism varied depending on the length of aeration period.
- Various Se-reducing bacteria likely contributed to coordinately to Se removal.

GRAPHIC ABSTRACT



ARTICLE INFO

Article history:
Received 30 January 2019
Revised 23 April 2019
Accepted 12 June 2019
Available online 30 July 2019

Keywords:
Activated sludge
Selenate reduction
Saline wastewater
Sequencing batch reactor
Alternating anoxic/oxic conditions
Selenium biovolatilization

ABSTRACT

Selenium (Se)-containing industrial wastewater is often coupled with notable salinity. However, limited studies have examined biological treatment of Se-containing wastewater under high salinity conditions. In this study, a sequencing batch reactor (SBR) inoculated with activated sludge was applied to treat selenate in synthetic saline wastewater (3% w/v NaCl) supplemented with lactate as the carbon source. Start-up of the SBR was performed with addition of 1–5 mM of selenate under oxygen-limiting conditions, which succeeded in removing more than 99% of the soluble Se. Then, the treatment of 1 mM Se with cycle duration of 3 days was carried out under alternating anoxic/oxic conditions by adding aeration period after oxygen-limiting period. Although the SBR maintained soluble Se removal of above 97%, considerable amount of solid Se remained in the effluent as suspended solids and total Se removal fluctuated between about 40 and 80%. Surprisingly, the mass balance calculation found a considerable decrease of Se accumulated in the SBR when the aeration period was prolonged to 7 h, indicating very efficient Se biovolatilization. Furthermore, microbial community analysis suggested that various Se-reducing bacteria coordinately contributed to the removal of Se in the SBR and main contributors varied depending on the operational conditions. This study will offer implications for practical biological treatment of selenium in saline wastewater.

1 Introduction

Selenium (Se) is an essential minor element with pivotal functions in human beings and animals. It plays a significant role in cellular metabolism at relatively low levels. However, when ingested at levels up to 400 μg/d, it has been identified to be highly toxic (Lenz and Lens, 2009). Se-containing wastewaters are generated from agriculture (Kharaka et al., 1996) and various industrial activities including mining (Muscatello and Janz, 2009), metal and oil refineries (Soda et al., 2011), and coal combustion (Yan et al., 2001). In these wastewaters, Se existed as selenate (SeO_4^{2-}) and/or selenite oxyanions (SeO_3^{2-}) at typical concentrations of 0.4 to 53 mg-Se/L

✉ Corresponding author
E-mail: ike@see.eng.osaka-u.ac.jp

(Mal et al., 2017), which were proved to be highly toxic to many organisms.

Se-containing wastewaters should be well treated before discharging into the environment to avoid its accumulation in aqueous ecosystem. Biological treatment of Se-containing wastewater was an attractive alternative due to its low cost and eco-friendly characteristics compared with existing physico-chemical treatment technologies (Santos et al., 2015). In the biological treatment, selenate was reduced to selenite, and further to less-toxic elemental Se, which is removable from the aqueous solution (Nancharaiah and Lens, 2015). Selenite can be also reduced to volatile methylated Se compounds such as dimethyl selenide (DMSe), dimethyl diselenide (DMDSe) and dimethyl selenenyl sulfide (DMSeS), which can be volatilized into a gaseous phase (Zhang and Frankenberger Jr., 1999; Kagami et al., 2013). To our knowledge, selenate reduction proceeds mainly through dissimilatory metabolism under anoxic conditions, in which selenate is used as the terminal electron acceptor for anaerobic respiration (Macy and Lawson, 1993; Fujita et al., 1997). Furthermore, assimilatory metabolism by both aerobes and anaerobes contributes to selenate reduction (Van Hullebusch, 2017). Selenite can be reduced to elemental Se by anaerobic respiration. On the other hand, *Pseudomonas stutzeri* NT-I, a versatile Se-metabolizing bacterium has been reported to efficiently reduce selenite into elemental Se only under aerobic conditions (Kuroda et al., 2011). In addition, Se biovolatilization from not only soluble Se (selenate and selenite) but also elemental Se to volatile Se compounds occurs under aerobic conditions (Doran and Alexander, 1977; Kagami et al., 2013). Thus, various kinds of Se metabolisms which occur both under anaerobic and aerobic conditions can be utilized for the removal of Se from wastewater. Therefore, alternating anoxic/oxic conditions may possess a considerable potential for biological treatment of selenate-containing wastewater via selenite.

Se-containing industrial wastewater is often coupled with notable salinity. Wastewater samples collected from a Se refinery plant contained 13.2–74.0 mg/L Se and 6%–7% salinity (Soda et al., 2011). Kiln powder leachate contained up to 42 mg/L Se and 4.4%–13.2% salinity (Soda et al., 2015). Biological removal of selenite (Soda et al., 2011; Jain et al., 2015) and selenate (Mal et al., 2017) in freshwater has been well-documented. However, limited studies have examined the biological treatment of Se-containing wastewater under high salinity conditions. A model sequencing batch reactor (SBR) inoculated with activated sludge to treat artificial selenite-containing saline wastewater under aerobic conditions in our previous study yielded high-efficiency Se removal (Zhang et al., 2019a). In another study, we also tried the bioreduction of selenate in artificial saline wastewater under oxygen-limiting conditions (Zhang et al., 2019b). However, the trials on biological selenate removal for saline wastewater are still very limited; specifically, there is no study on the effectiveness of combination of anoxic and oxic conditions.

Therefore, this study focuses on the treatment of selenate-containing saline wastewater (3% w/v NaCl) through alternating anoxic/oxic conditions. A model SBR inoculated with activated sludge was started up by feeding with synthetic saline wastewater containing high concentrations of selenate (1–5 mM) under oxygen-limiting conditions. After the start-up period, Se treatment experiments were performed under alternating anoxic/oxic conditions with varying aeration durations.

2 Materials and methods

2.1 Activated sludge

Activated sludge used as the inoculum of the SBR was obtained from a coke-oven wastewater treatment process in a steel manufacturing plant. The plant employs the activated sludge process to treat marine water-diluted coke-oven wastewater which contains sulfur compounds such as sulfate, thiosulfate, and thiocyanate. Because it was reported that bacterial Se metabolism is associated with sulfur metabolism (Zannoni et al., 2008), we assumed that halotolerant bacterial populations capable of Se metabolisms exist in the activated sludge. Before inoculation into the SBR, the activated sludge was washed three times with 50 mM potassium phosphate buffer (pH 7.5) and re-suspended into the buffer.

2.2 Synthetic wastewater

Synthetic wastewater used contained 30 g/L NaCl, 1 or 5 mM Na_2SeO_4, 28 mM NH_4Cl, 1.7 mM K_2HPO_4, 0.7 mM KH_2PO_4, 0.2 mM $MgSO_4 \cdot 7H_2O$, 0.2 mM $CaCl_2 \cdot 2H_2O$, 0.1 mL/L trace element stock solution, and 44 mM sodium lactate as the sole carbon source. Trace element stock was prepared as previously reported (Nancharaiah et al., 2008). The pH of synthetic wastewater was adjusted to 7.0–7.1 by addition of 2 M NaOH. Supplementation of lactate at a high concentration aimed to facilitate stable selenate reduction based on our previous findings (Zhang et al., 2019a). Salinity was set at 3% in this study because preliminary trials to remove selenate by activated sludge acclimated to 6%−7% salinity, which are within the salinity range in typical Se-containing industrial wastewater, were failed.

2.3 Operation of SBR

The set-up and operation of the SBR was done using the following protocol. The pretreated activated sludge was inoculated into 160 mL of the synthetic wastewater in a 200-mL Erlenmeyer flask to construct a SBR system. Initial concentration of the mixed liquor suspended solids

(MLSS) was set at approximately 3000 mg/L. An SBR cycle consisted of (1) oxygen-limiting or alternating oxygen-limiting/aeration (i.e., anoxic/oxic) reaction, (2) withdrawal of 1–12 mL excess sludge (ES), (3) sludge settling for 0.5–1.5 h, (4) effluent decantation to give an 80% volumetric exchange ratio, and (5) wastewater refill. During the reaction period, the SBR was incubated on a rotary shaker (120 rpm, 28°C). The oxygen-limiting (anoxic) condition was established by sealing the flask with a rubber plug though the head-space air was not removed, while oxic condition was created by aeration at 15–18 L/min through an air stone. The SBR was operated under oxygen-limiting conditions throughout the start-up period (hydraulic retention time: 3.75–8.75 d), and under alternating oxygen-limiting/aeration conditions for further treatment experiments (hydraulic retention time: 3.75 d). Operational phases of SBR are summarized in Table 1. To clarify the effect of aeration duration on Se removal ability of activated sludge, aeration period was extended from 3 h (in phase IV) to 5 and 7 h (in phases V and VI, respectively) in a stepwise manner. Withdrawal of 1–12 mL ES was for Se measurement (1 mL), bacterial community analysis (1 mL), ES discharge (5 mL from batch 16), and MLSS measurement (10 mL).

Table 1 Operational phases of SBR

Phase (batch)	Cycle duration (d)	Oxygen condition	Inf. Selenate (mM)
Start-up			
I (1–3)	7	Oxygen-limiting	5
II (4–6)	7	Oxygen-limiting	1
III (7–9)	3	Oxygen-limiting	1
Se treatment			
IV (10–15)	3	Alternating anoxic/oxic (67.5 h/3 h)	1
V (16–21)	3	Alternating anoxic/oxic (65.5 h/5 h)	1
VI (22–27)	3	Alternating anoxic/oxic (63.5 h/7 h)	1

2.4 Analytical procedures

Concentrations of the total soluble Se and dissolved organic carbon (DOC) both in the influent and effluent and solid Se concentration in the effluent and excess sludge were determined as described in a previous report (Zhang et al., 2019a). For analyses of soluble Se and DOC, samples were centrifuged (15,000 × *g*, 10 min, 4°C), after which the supernatants were filtered through a polycarbonate membrane filter with the pore-size of 0.45 μm (Toyo Roshi Kaisha Ltd., Tokyo, Japan). MLSS concentrations were measured as described previously (Takada et al., 2018).

2.5 Bacterial community analysis

Genomic DNA in the ES samples was extracted by Fast DNA Spin Kit for Soil (MP Biomedicals, Illkirch, France) according to manufacturer's protocol. Illumina Miseq 16S rRNA gene sequencing was carried out at Bioengineering Laboratory Co. Ltd. (Kanagawa, Japan). A 2-step tailed PCR was performed targeting the V4 region of the bacterial 16S rRNA genes with the 515F and 806R primer set (Peiffer et al., 2013). After processing raw sequence data, the qualified sequence reads obtained were clustered into operational taxonomic units (OTUs) with a 97% similarity threshold, and taxonomic assignments were conducted using the Greengenes database (greengenes.secondgenome.com) on QIIME ver. 1.9.1 (Caporaso et al., 2010). Representative OTUs were further analyzed through NCBI BLAST search (blast.ncbi.nlm.nih.gov/blast.cgi). Shannon diversity index and Chao1 richness estimator were calculated as described in (Takada et al., 2018). The similarity patterns of the samples were evaluated through principal component analysis (PCA) and cluster analysis by PAST ver. 3 (folk.uio.no/ohammer/past/).

3 Results and discussion

3.1 Start-up of SBR

The SBR was started under oxygen-limiting conditions to specifically enrich selenate-reducing bacteria capable of growing by selenate respiration, because selenate reduction is the bottle neck in biological Se removal. The removal performance of Se and DOC is shown in Fig. 1.

Initially, a high concentration of selenate (5 mM) was supplied to accelerate the enrichment of selenate-reducers (Table 1). The cycle duration was controlled at 7 d in the beginning. In the first two batches of phase I, though soluble Se removal was not eminent (around 20%) (Figs. 1(a) and 1(b)), reddish color was first observed in batch 2, which became dense in batch 3 (Fig. 2), suggesting the formation of elemental Se in the SBR. In batch 3 of the phase I, considerable amounts of soluble Se removal (nearly 60%) was confirmed (Figs. 1(a) and 1(b)), indicating enrichment of selenate- and selenite-reducing bacteria at certain levels. The influent selenate concentration was decreased to 1 mM from batch 4 (phase II) for simulating Se concentration of real industrial wastewater. Almost complete removal of soluble Se was achieved in phase II. Though the cycle duration was shortened to 3 d from batch 7 (phase III), more than 99% of soluble Se removal was maintained. Thus, bacterial populations responsible for soluble Se removal under high salinity could be successfully enriched during start-up operation of SBR within a short period. This indicated that the seed

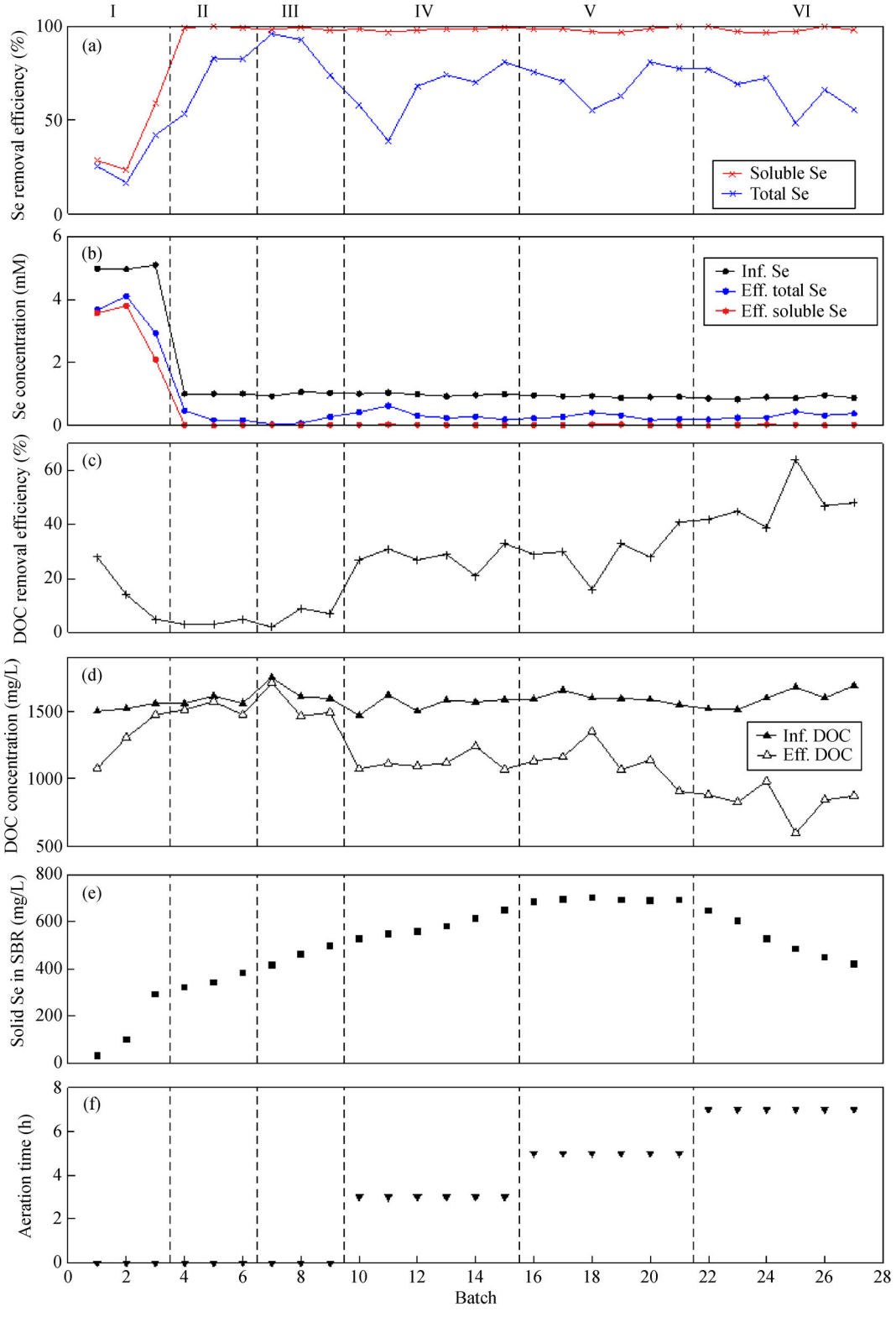


Fig. 1 Se removal efficiencies (a), Se concentrations (b), dissolved organic carbon (DOC) removal efficiencies (c), DOC concentrations (d), solid Se (e) and aeration duration (f) during start-up phase (left) and Se treatment phase (right).

Fig. 2 Sludge color change during the start-up period.

sludge, which had already adapted to high salinity, included halotolerant Se-removing bacteria and they can be easily enriched under oxygen-limiting conditions using lactate as a typically good substrate for anaerobic respiration, including selenate respiration.

Though the SBR was able to completely remove most of the soluble Se, considerable amount of solid Se was contained in the effluent as suspended solids (SS), which was designated SS-Se. Consequently, the total Se removal of SBR was lowered with an average of 73% and 88% in phases II and III, respectively (Fig. 1(a)). Also, DOC removal of SBR was as low as 2% to 9% during phases II and III (Fig. 1(c)). Therefore, removal of SS-Se and DOC as well as soluble Se removal were further evaluated in the following Se treatment experiments.

3.2 Se treatment under alternating anoxic/oxic conditions

After the start-up under oxygen-limiting conditions, aeration period was added from batch 10 (phase IV) to create alternating anoxic/oxic conditions for Se treatment (Table 1, Fig. 1(f)).

While aeration period was prolonged from 3 h to 7 h stepwise from phase IV to phase VI, the removal efficiency of soluble Se was maintained at above 97% (Fig. 1(a)). Therefore, it is possible to steadily remove soluble Se from selenate-containing saline wastewater by alternating anoxic/oxic treatment. However, considerable concentrations of SS-Se were still found in the effluent during the treatment, and removal of total Se varied between 39% and 81% (Fig. 1(a)). The removal of total Se was a little lower at the beginning of phase IV than that observed during the other periods which may be due to the instability of activated sludge microbial community during transition to new conditions (alternating anoxic/oxic conditions), and was not recovered to high levels throughout the treatment. Therefore, it is necessary to remove SS-Se from the effluent with certain post-treatments such as high-speed centrifugation, filtration, chemical coagulation (Staicu et al., 2015b), and electrocoagulation (Staicu et al., 2015a), which have been proven effective for removal of biogenic elemental Se particles. Previous study reported the entrapment of elemental Se in activated sludge flocs under high dissolved oxygen (DO) conditions (Jain et al., 2015). The settleability and hydrophilicity of the activated sludge could be improved by the entrapment of elemental Se, leading to low SS-Se contents in the effluent. Therefore, some researchers suggested that activated sludge owns the better entrapment ability of elemental Se compared to anaerobic sludge (Mal et al., 2017). However, in this study, aeration leading to high DO levels caused the increase of solid Se in the effluent compared to the start-up period with no aeration. This was probably because soluble Se was reduced to produce elemental Se extracellularly during oxygen-limiting conditions, and the shearing force generated by aeration made the elemental Se particles to remain suspended in the effluent.

Though DOC removal was less than 10% during the start-up phases, average DOC removal improved to 28%, 30% and 48% in phases IV, V and VI, respectively, in accordance with the extension of the aeration period (Figs. 1(c) and 1(d)). This would be resulted from higher biodegradation ability of organics under oxic conditions than under anoxic conditions, as generally known. However, the DOC concentration in the effluent was still too high to be discharged. One reason of the low DOC removal could be the inhibitory effects of high salinity on activated sludge. Unfortunately, lactate concentrations remained after treatment were not determined in this study. However, it was reported that chemical oxygen demand removal of an activated sludge process decreased sharply when NaCl concentration increased from 4 to 28 g/L (Li et al., 2014). In addition, salinity could promote the release of cellular materials of activate sludge microbes, resulting in an increase of soluble organics in the effluent (Kincannon and Gaudy Jr., 1966). In either case, reduction of substrate supplementation to wastewater and/or post-treatment for DOC removal is required for the practical application, even though sufficient organic substrates were essential to facilitate stable Se removal (Zhang et al., 2019a). Especially, the optimization of substrate donation level is a vital issue to be addressed for reducing operational cost.

3.3 Se mass balance

To understand Se removal pathway by the SBR, the mass balance of Se during phases IV, V, and VI was evaluated based on the soluble and the solid Se in the reactor, influent, and effluent and the ES discharged from SBR. In Table 2, the amounts of Se remaining in the SBR at the beginning and the end of each phase are shown to evaluate the Se accumulation/loss in the reactor during the corresponding experimental period. The cumulative Se mass balance in each phase is summarized in Table 2 with input from the influent and discharge as effluent and ES as

Table 2 Cumulative Se mass balance in phases IV, V and VI

Se mass		Phase IV	Phase V	Phase VI
Accumulation/Loss in SBR [mg-Se, (soluble, %)]	Initial	96.10 (0%)	127.03 (0%)	131.94 (0%)
	End	127.03 (0%)	131.94 (0%)	78.60 (0%)
	Loss in SBR	–30.93	–4.91	53.34
Input to/Output from SBR [mg-Se, (soluble, %)]	Input as Inf.	74.08 (100%)	68.90 (100%)	66.05 (100%)
	Output as Eff.	24.38 (5%)	19.56 (6%)	22.57 (5%)
	Output as ES	3.49 (0%)	25.05 (0%)	16.41 (0%)
	Loss	46.21	24.29	27.07
Total loss during in each phase [mg-Se, (total loss Se to input, %)]		15.28 (21%)	19.38 (28%)	80.41 (122%)

output. Most of the Se existed as solid phase in the reactor, effluent and ES; while Se in the influent was selenate as soluble form. The "total loss" in Table 2 should include the volatilized Se in addition to measurement errors. The Se mass balance for phases IV, V and VI summarized in Table 2 is visually depicted in Fig. S1 in Supplementary Material.

During phase IV, Se fed into the SBR as selenate (input = ca. 74 mg) was removed via effluent and the ES from the reactor mostly as solid Se, which accounted for approximately 37.6% of the total input Se. In addition, approximately 31 mg of solid Se, which accounted for 41.7% of the input, was accumulated in the SBR during phase IV. These indicated that approximately 80% of the input soluble Se was transformed to solid Se by the treatment, with only a minute amount of soluble Se left in the mixed liquid. Thus, contribution of biovolatilization to Se removal seems to be low in phase IV (nearly 20%). During phase V, while approximately 69 mg of Se was fed, nearly 18 mg and 25 mg was removed from the SBR as solid Se being contained in the effluent and ES, respectively. Taking into account that 5 mg of Se (7.1%) was accumulated in the reactor, it can be explained that nearly 70% of the input was converted into solid Se and remaining portion (nearly 30%) was volatilized into gaseous forms in phase V. Thus, it appears that prolonged aeration from 3 h (phase IV) to 5 h (phase V) did not dramatically change the Se metabolisms in the SBR.

On the other hand, when aeration time was extended to 7 h in phase VI, 34% and 25% of Se input as the influent (66 mg) was removed from the SBR as solid Se in effluent and ES, respectively, and Se accumulated in the SBR decreased by 53 mg during the operation. Based on the mass balance calculation, Se loss fraction accounted for 122% of the total input Se, surprisingly. The considerable increase in the Se loss fraction during phase VI could be explained by the efficient generation of volatile Se through bio-methylation. Volatile Se such as DMSe, DMDSe and DMSeS could be generated via assimilatory selenate reduction. A previous study showed that *P. stutzeri* NT-I is capable of reducing elemental selenium to volatile organic selenocompounds (DMSe, DMDSe and DMSeS) efficiently under aerobic conditions, and demonstrated the feasibility of bio-methylation as an advantageous Se resource recovery with few impurities (Kagami et al., 2013). Although H_2Se, a highly toxic Se species, might also occur through Se reduction, it is the least stable oxidation state and easily oxidized (Cupp-Sutton and Ashby, 2016), indicating that its occurrence in the gaseous phase should be ignorable. In this study, the prolongation of the aeration period to 7 h seems to have critically contributed to the efficient biovolatilization of Se. Generally, the generation of volatile Se requires proteins and peptides as effective substrates (Zhang and Frankenberger Jr., 1999). For example, effective Se biovolatilization by *P. stutzeri* NT-I was observed when cultivated in trypticase soy broth (Kagami et al., 2013). Activated sludge fed with artificial saline wastewater containing bonito extract and peptone as carbon sources achieved efficient selenite removal through biovolatilization (Zhang et al., 2019a). Although proteins and peptides were not supplemented to the influent in this study, they may be provided through the excretion from the cells lysed by the long-time cultivation under high-salinity conditions, as mentioned above, which might promote biovolatilization of Se.

3.4 Variations of the bacterial community

16S rRNA gene amplicon sequencing of 6 sludge samples, which consisted of seed sludge sample and 5 sludge samples from phases I, II, III, V and VI, obtained 25,936 to 47,648 qualified reads per sample (223,883 qualified reads in total), which were further classified into 3,043 OTUs (Table 3). The OTU numbers and diversity indices showed a declining tendency along with the cultivation, which indicated the progress of enrichment under the given cultivation conditions.

Figure 3 shows the bacterial community compositions at the phylum level. *Proteobacteria* and *Actinobacteria* were the most abundant phyla in the seed sludge, with relative abundance of 49% and 20%, respectively. *Proteobacteria* also dominated during the whole experiment with relative

Table 3 Summary of 16S rRNA gene amplicon sequencing of sludge samples

Sample (phase)	Qualified sequence reads	OTUs	Shannon diversity	Chao1 richness
Seed	41,449	1,456	4.52	3,794
Batch 2 (I)	37,184	1,044	3.85	2,690
Batch 6 (II)	37,445	832	3.36	1,938
Batch 8 (III)	25,936	646	3.50	1,620
Batch 21 (V)	47,648	601	3.22	1,392
Batch 27 (VI)	34,221	347	2.67	850

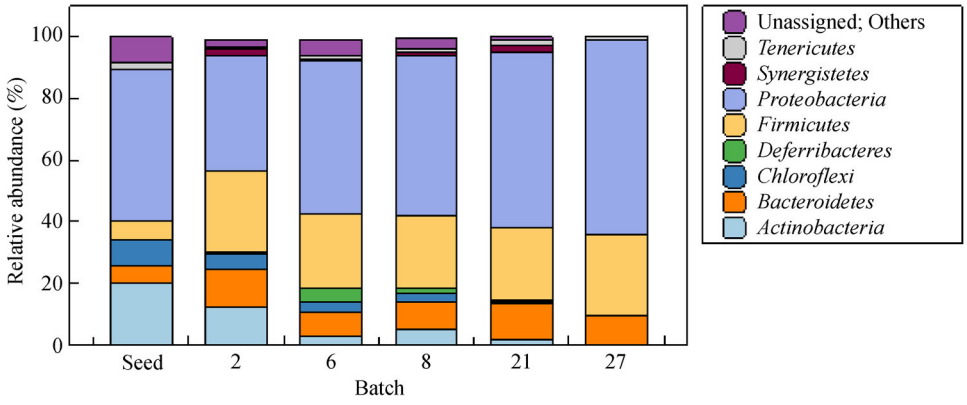


Fig. 3 Bacterial community structure at phylum level. Rare phyla (relative abundance < 1.0%) were classified as "unassigned; Other."

abundance of 37% to 63%, while *Actinobacteria* declined largely during the start-up period and almost disappeared during Se treatment period. Alternatively, *Firmicutes* became predominant from the beginning of the SBR operation, with relative abundance of over 20%.

The results of PCA and cluster analysis based on the total OTUs are shown in Fig. 4. In PCA analysis, principal components 1 and 2 contributed 65.0% and 29.7% of the total variables, respectively. Six sludge samples were clustered into 3 groups: (1) seed sludge and batch 2, (2) batches 6 and 8 under oxygen-limiting conditions, and (3) batches 21 and 27 under alternating anoxic/oxic conditions. These results indicated that the bacterial community in the SBR was changed dynamically depending on the operational conditions, and that the oxygen condition (oxygen-limiting or alternating anoxic/oxic conditions) in addition to high Se and salinity conditions exerts significant impact on the bacterial community structures.

3.5 Identification of potential selenate-reducing bacteria

A variety of selenate-reducing bacteria belonging to different species have previously been reported, such as *Bacillus* (Fujita et al., 1997), and *Pseudomonas* (Narasingarao and Häggblom, 2007; Kuroda et al., 2011), all of which are basically sensitive to the saline conditions. Limited halotolerant/halophilic selenate-reducing bacteria have been reported. Although a marine selenate-reducing strain was isolated from estuarine sediments by Oremland et al. (1989), the strain was subsequently lost and unfortunately its selenate reducing ability was not definitely characterized (Oremland et al., 1994). Recently, a moderately halotolerant selenate- and tellurite-reducing *Shewanella* strain was isolated from brackish areas in Osaka by our group (Soda et al., 2018). However, its selenate removal ability was also unstable. Consequently, there is currently scarce information about halotolerant bacteria capable of reducing selenate steadily.

An attempt was made to identify the Se reducers among the OTUs detected in this study. Table 4 summarizes predominant OTUs with relative abundance of 10% or larger in at least one sample. The predominant OTUs were divided into three groups: the first group included OTUs that predominated in seed and batch 2 and decreased thereafter (denovo7920, denovo8224, denovo8432 and denovo9521), the second group that became predominant after batch 6 (denovo7350 and denovo13093), and the third group that became predominant during or after batch 21 (denovo1084, denovo2062, denovo7179 and denovo8018). Variations of the type of predominant OTUs would contribute largely to the dynamic changes of the overall bacterial community compositions (Fig. 4). Several OTUs detected predominantly during the SBR operation, such as denovo2062, denovo7920 and

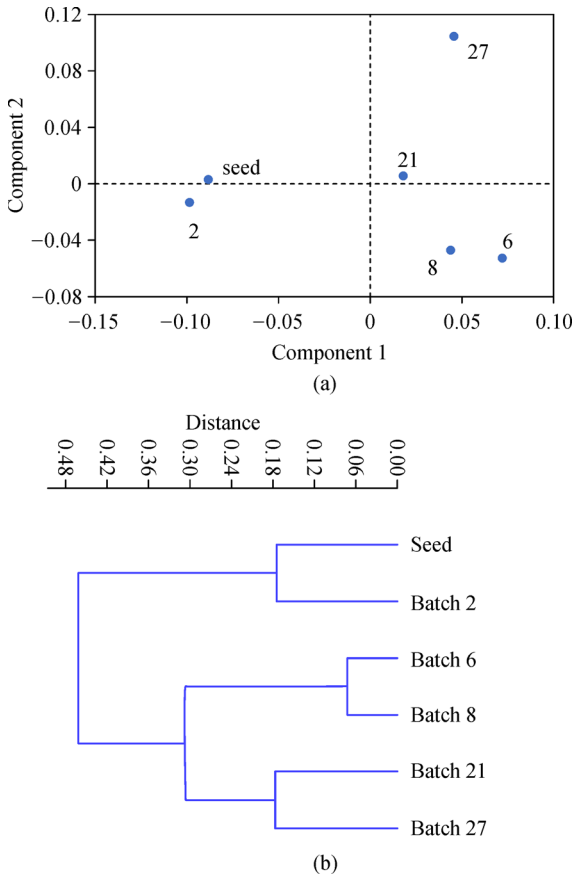


Fig. 4 Scatter plots (a) and dendrogram (b), respectively, of PCA and cluster analysis of bacterial community structures in different batches. The number near the plot is the sampling batch.

denovo8018, were closely related to the widely documented Se-reducing bacteria, like *Pseudomonas* and *Bacillus*. In addition, denovo8432 identified as *Shewanella algae* was detected at a relative abundance of 10% in batch 2 and survived both under the oxygen-limiting and alternating anoxic/oxic conditions. *Shewanella* is of interest because of its ability to reduce the oxidized forms of multiple metals including Se, iron, uranium and plutonium to insoluble forms (Reed et al., 2007; Soda et al., 2018), and probably contributed to Se reduction in the SBR of this study. Two OTUs in the second group (denovo7350 and denovo13093) increased after batch 6 and were predominantly thereafter, with relative abundance of 8 to 17% and 8 to 19%, respectively. Denovo7350 belonged to the family *Tissierellaceae*. Although the identification at the genus level was not possible, all of the currently-known genera in *Tissierellaceae* are anaerobic bacteria, and some members in *Tissierellaceae* were dominantly detected in microbial enrichment capable of simultaneous selenate reduction and denitrification (Subedi et al., 2017). The evidence thus suggests the potential contribution of denovo7350 to Se reduction under oxygen-limiting conditions in the SBR. Also, denovo13093 was identified as *Marinobacterium halophilum*, which is moderately halophilic bacteria that can grow with 3%−12% NaCl (Chang et al., 2007), and has been detected in sulfate-reducing upflow anaerobic sludge blanket reactors (García-Solares et al., 2014; García-Depraect et al., 2017). This species would also contribute Se reduction under high salinity conditions of our SBR. Collectively, it can be indicated that under high salinity and oxygen-limiting or alternate anoxic/oxic conditions, there would not be only one or limited strong Se-reducing bacteria but various bacteria that coordinately contributed to Se reduction and the main contributors varied depending on the operational conditions.

4 Conclusions

Efficient biological reduction of selenate from saline wastewater (3% w/v NaCl) was achieved in SBR inoculated with activated sludge under alternating anoxic/oxic conditions. High amounts of soluble Se removal above 97% were achieved, whereas considerable amount of Se remained in the effluent as SS. Under

Table 4 Predominant OTUs with relative abundance of >10% in at least one sample

OTU ID	Relative abundance						Closest relative (Accession No.)	Identity (%)	Report on Se reduction	Other features	Reference
	Seed	Batch 2	Batch 6	Batch 8	Batch 21	Batch 27					
Denovo1084	0%	0%	0%	0%	0%	15%	*Alcaligenes aquatilis* (AJ937889)	100	+	Denitrification	Van Trappen et al. (2005)
Denovo2062	0%	1%	0%	0%	0%	10%	*Peptoclostridium acidaminophilum* (NR121725)	94		Strict anaerobe; Selenocysteine-containing proteins	Poehlein et al. (2014)
Denovo7179	0%	0%	0%	2%	12%	2%	*Arcobacter bivalviorum* (FJ573217)	100		Facultative anaerobe	Levican et al. (2012)
Denovo7350	1%	2%	16%	17%	13%	8%	*Soehngenia saccharolytica* (GQ461828)	94	+	Denitrification	Subedi et al. (2017)
Denovo7920	0%	16%	0%	1%	0%	0%	*Anaerotignum aminivorans* (AB298756)	94		Strict anaerobe	Ueki et al. (2017)
Denovo8018	0%	0%	0%	0%	11%	18%	*Marinobacterium stanieri* (AB021367)	98		Marine aerobe	Baumann et al. (1983); Satomi et al. (2002)
Denovo8224	16%	10%	2%	4%	1%	0%	*Kineosphaera limosa* (NR113146)	98		Strict aerobe; Growth under up to 3% NaCl	Liu et al. (2002)
Denovo8432	0%	10%	4%	8%	7%	6%	*Shewanella algae* (AB681980)	100	+	Iron reduction; halotolerant facultative anaerobe	Caccavo et al. (1996)
Denovo9521	1%	10%	5%	7%	3%	2%	*Petrimonas mucosa* (KP233808)	98		Thermophilic facultative anaerobe	Hahnke et al. (2016)
Denovo13093	0%	3%	19%	14%	8%	13%	*Marinobacterium halophilum* (AY563030)	100	+	Sulfate reduction; marine bacterium	García-Solares et al. (2014); García-Depraect et al. (2017)

oxygen-limiting conditions, selenate was converted into elemental Se, most of which was accumulated in the sludge. On the other hand, effective biovolatilization of Se appeared to occur by implementation of aeration for 7 h after oxygen-limiting conditions. Multiple bacterial species appeared to coordinately remove soluble Se in saline wastewater in the SBR with varying operational conditions.

Acknowledgements This work was partially supported by JSPS KAKENHI (Grant No. JP15K16145).

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11783-019-1154-z and is accessible for authorized users.

References

Baumann P, Bowditch R D, Baumann L, Beaman B (1983). Taxonomy of marine *Pseudomonas* species: *P. stanieri* sp. nov.; *P. perfectomarina* sp. nov., nom. rev.; *P. nautica*: and *P. doudoroffii.* International Journal of Systematic Bacteriology, 33(4): 857–865

Caccavo F, Frolund B, Van Ommen K F, Nielsen P H (1996). Deflocculation of activated sludge by the dissimilatory Fe (III)-reducing bacterium *Shewanella alga* BrY. Applied and Environmental Microbiology, 62(4): 1487–1490

Caporaso J G, Kuczynski J, Stombaugh J, Bittinger K, Bushman F D, Costello E K, Fierer N, Peña A G, Goodrich J K, Gordon J I, Huttley G A, Kelley S T, Knights D, Koenig J E, Ley R E, Lozupone C A, McDonald D, Muegge B D, Pirrung M, Reeder J, Sevinsky J R, Turnbaugh P J, Walters W A, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010). QIIME allows analysis of high-throughput community sequencing data. Nature Methods, 7(5): 335–336

Chang H W, Nam Y D, Kwon H Y, Park J R, Lee J S, Yoon J H, An K G, Bae J W (2007). *Marinobacterium halophilum* sp. nov., a marine bacterium isolated from the Yellow Sea. International Journal of Systematic and Evolutionary Microbiology, 57(1): 77–80

Cupp-Sutton K A, Ashby M T (2016). Biological chemistry of hydrogen selenide. Antioxidants (Basel), 5(4): 42

Doran J W, Alexander M (1977). Microbial transformations of selenium. Applied and Environmental Microbiology, 33(1): 31–37

Fujita M, Ike M, Nishimoto S, Takahashi K, Kashiwa M (1997). Isolation and characterization of a novel selenate-reducing bacterium, *Bacillus* sp. SF-1. Journal of Fermentation and Bioengineering, 83 (6): 517–522

García-Depraect O, Guerrero-Barajas C, Jan-Roblero J, Ordaz A (2017). Characterization of a marine microbial community used for enhanced sulfate reduction and copper precipitation in a two-step process. Applied Biochemistry and Biotechnology, 182(2): 452–467

García-Solares S M, Ordaz A, Monroy-Hermosillo O, Jan-Roblero J, Guerrero-Barajas C (2014). High sulfate reduction efficiency in a UASB using an alternative source of sulfidogenic sludge derived from hydrothermal vent sediments. Applied Biochemistry and Biotechnology, 174(8): 2919–2940

Hahnke S, Langer T, Koeck D E, Klocke M (2016). Description of *Proteiniphilum saccharofermentans* sp. nov., *Petrimonas mucosa* sp. nov. and *Fermentimonas caenicola* gen. nov., sp. nov., isolated from mesophilic laboratory-scale biogas reactors, and emended description of the genus *Proteiniphilum.* International Journal of Systematic and Evolutionary Microbiology, 66(3): 1466–1475

Jain R, Seder-Colomina M, Jordan N, Dessi P, Cosmidis J, van Hullebusch E D, Weiss S, Farges F, Lens P N L (2015). Entrapped elemental selenium nanoparticles affect physicochemical properties of selenium fed activated sludge. Journal of Hazardous Materials, 295: 193–200

Kagami T, Narita T, Kuroda M, Notaguchi E, Yamashita M, Sei K, Soda S, Ike M (2013). Effective selenium volatilization under aerobic conditions and recovery from the aqueous phase by *Pseudomonas stutzeri* NT-I. Water Research, 47(3): 1361–1368

Kharaka Y K, Ambats G, Presser T S, Davis R A (1996). Removal of selenium from contaminated agricultural drainage water by nanofiltration membranes. Applied Geochemistry, 11(6): 797–802

Kincannon D F, Gaudy Jr A F (1966). Some effects of high salt concentrations on activated sludge. Journal- Water Pollution Control Federation, 38(7): 1148–1159

Kuroda M, Notaguchi E, Sato A, Yoshioka M, Hasegawa A, Kagami T, Narita T, Yamashita M, Sei K, Soda S, Ike M (2011). Characterization of *Pseudomonas stutzeri* NT-I capable of removing soluble selenium from the aqueous phase under aerobic conditions. Journal of Bioscience and Bioengineering, 112(3): 259–264

Lenz M, Lens P N L (2009). The essential toxin: the changing perception of selenium in environmental sciences. Science of the Total Environment, 407(12): 3620–3633

Levican A, Collado L, Aguilar C, Yustes C, Diéguez A L, Romalde J L, Figueras M J (2012). *Arcobacter bivalviorum* sp. nov. and *Arcobacter venerupis* sp. nov., new species isolated from shellfish. Systematic and Applied Microbiology, 35(3): 133–138

Li J, Yu L, Yu D, Wang D, Zhang P, Ji Z (2014). Performance and granulation in an upflow anaerobic sludge blanket (UASB) reactor treating saline sulfate wastewater. Biodegradation, 25(1): 127–136

Liu W T, Hanada S, Marsh T L, Kamagata Y, Nakamura K (2002). *Kineosphaera limosa* gen. nov., sp. nov., a novel Gram-positive polyhydroxyalkanoate-accumulating coccus isolated from activated sludge. International Journal of Systematic and Evolutionary Microbiology, 52(Pt 5): 1845–1849

Macy J M, Lawson S (1993). Cell yield (Y_M) of *Thauera selenatis* grown anaerobically with acetate plus selenate or nitrate. Archives of Microbiology, 160(4): 295–298

Mal J, Nancharaiah Y V, van Hullebusch E D, Lens P N L (2017). Biological removal of selenate and ammonium by activated sludge in a sequencing batch reactor. Bioresource Technology, 229: 11–19

Muscatello J R, Janz D M (2009). Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. Science of the Total Environment, 407(4): 1318–1325

Nancharaiah Y V, Joshi H M, Hausner M, Venugopalan V P (2008). Bioaugmentation of aerobic microbial granules with *Pseudomonas putida* carrying TOL plasmid. Chemosphere, 71(1): 30–35

Nancharaiah Y V, Lens P N L (2015). Selenium biomineralization for biotechnological applications. Trends in Biotechnology, 33(6): 323–330

Narasingarao P, Häggblom M M (2007). Identification of anaerobic selenate-respiring bacteria from aquatic sediments. Applied and

Environmental Microbiology, 73(11): 3519–3527

Oremland R S, Blum J S, Culbertson C W, Visscher P T, Miller L G, Dowdle P, Strohmaier F E (1994). Isolation, growth, and metabolism of an obligately anaerobic, selenate-respiring bacterium, strain SES-3. Applied and Environmental Microbiology, 60(8): 3011–3019

Oremland R S, Hollibaugh J T, Maest A S, Presser T S, Miller L G, Culbertson C W (1989). Selenate reduction to elemental selenium by anaerobic bacteria in sediments and culture: Biogeochemical significance of a novel, sulfate-independent respiration. Applied and Environmental Microbiology, 55(9): 2333–2343

Peiffer J A, Spor A, Koren O, Jin Z, Tringe S G, Dangl J L, Buckler E S, Ley R E (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proceedings of the National Academy of Sciences of the United States of America, 110(16): 6548–6553

Poehlein A, Andreesen J R, Daniel R (2014). Complete genome sequence of amino acid-utilizing *Eubacterium acidaminophilum* al-2 (DSM 3953). Genome Announcements, 2(3): e00573–14

Reed D T, Pepper S E, Richmann M K, Smith G, Deo R, Rittmann B E (2007). Subsurface bio-mediated reduction of higher-valent uranium and plutonium. Journal of Alloys and Compounds, 444–445: 376–382

Santos S, Ungureanu G, Boaventura R, Botelho C (2015). Selenium contaminated waters: An overview of analytical methods, treatment options and recent advances in sorption methods. Science of the Total Environment, 521-522: 246–260

Satomi M, Kimura B, Hamada T, Harayama S, Fujii T (2002). Phylogenetic study of the genus *Oceanospirillum* based on 16S rRNA and *gyrB* genes: Emended description of the genus *Oceanospirillum*, description of *Pseudospirillum* gen. nov., *Oceanobacter* gen. nov. and *Terasakiella* gen. nov. and transfer of *Oceanospirillum jannaschii* and *Pseudomonas stanieri* to *Marinobacterium* as *Marinobacterium jannaschii* comb. nov. and *Marinobacterium stanieri* comb. nov. International Journal of Systematic and Evolutionary Microbiology, 52(3): 739–747

Soda S, Hasegawa A, Kuroda M, Hanada A, Yamashita M, Ike M (2015). Selenium recovery from kiln powder of cement manufacturing by chemical leaching and bioreduction. Water Science and Technology, 72(8): 1294–1300

Soda S, Kashiwa M, Kagami T, Kuroda M, Yamashita M, Ike M (2011). Laboratory-scale bioreactors for soluble selenium removal from selenium refinery wastewater using anaerobic sludge. Desalination, 279(1–3): 433–438

Soda S, Ma W, Kuroda M, Nishikawa H, Zhang Y, Ike M (2018). Characterization of moderately halotolerant selenate- and tellurite-reducing bacteria isolated from brackish areas in Osaka. Bioscience, Biotechnology, and Biochemistry, 82(1): 173–181

Staicu L C, van Hullebusch E D, Lens P N L, Pilon-Smits E A H, Oturan M A (2015a). Electrocoagulation of colloidal biogenic selenium. Environmental Science and Pollution Research International, 22(4): 3127–3137

Staicu L C, van Hullebusch E D, Oturan M A, Ackerson C J, Lens P N L (2015b). Removal of colloidal biogenic selenium from wastewater. Chemosphere, 125: 130–138

Subedi G, Taylor J, Hatam I, Baldwin S A (2017). Simultaneous selenate reduction and denitrification by a consortium of enriched mine site bacteria. Chemosphere, 183: 536–545

Takada K, Shiba T, Yamaguchi T, Akane Y, Nakayama Y, Soda S, Inoue D, Ike M (2018). Cake layer bacterial communities during different biofouling stages in full-scale membrane bioreactors. Bioresource Technology, 259: 259–267

Ueki A, Goto K, Ohtaki Y, Kaku N, Ueki K (2017). Description of *Anaerotignum aminivorans* gen. nov., sp. nov., a strictly anaerobic, amino-acid-decomposing bacterium isolated from a methanogenic reactor, and reclassification of *Clostridium propionicum*, *Clostridium neopropionicum* and *Clostridium lactatifermentans* as species of the genus *Anaerotignum*. International Journal of Systematic and Evolutionary Microbiology, 67(10): 4146–4153

Van Hullebusch E D (2017). Bioremediation of Selenium Contaminated Wastewater. Cham: Springer Nature,

Van Trappen S, Tan T L, Samyn E, Vandamme P (2005). *Alcaligenes aquatilis* sp. nov., a novel bacterium from sediments of the Weser Estuary, Germany, and a salt marsh on Shem Creek in Charleston Harbor, USA. International Journal of Systematic and Evolutionary Microbiology, 55(6): 2571–2575

Yan R, Gauthier D, Flamant G, Peraudeau G, Lu J, Zheng C (2001). Fate of selenium in coal combustion: Volatilization and speciation in the flue gas. Environmental Science & Technology, 35(7): 1406–1410

Zannoni D, Borsetti F, Harrison J J, Turner R J (2008). The bacterial response to the chalcogen metalloids Se and Te. Advances in Microbial Physiology, 53: 1–72

Zhang Y, Kuroda M, Arai S, Kato F, Inoue D, Ike M (2019b). Biological treatment of selenate-containing saline wastewater by activated sludge under oxygen-limiting conditions. Water Research, 154: 327–335

Zhang Y, Kuroda M, Nakatani Y, Soda S, Ike M (2019a). Removal of selenite from artificial wastewater with high salinity by activated sludge in aerobic sequencing batch reactors. Journal of Bioscience and Bioengineering, 127(5): 618–624

Zhang Y Q, Frankenberger Jr W T (1999). Effects of soil moisture, depth, and organic amendments on selenium volatilization. Journal of Environmental Quality, 28(4): 1321–1326