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RESEARCH ARTICLE

Microbial community and extracellular polymeric substances analysis of anaerobic granular sludge exposed to selenate, cadmium and zinc

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Abstract

The microbial community and extracellular polymeric substances composition of anaerobic granular sludge exposed to selenate (~10 mg/L), cadmium (Cd) and zinc (Zn) (~2 and 5 mg/L) were investigated by high-throughput sequencing and fluorescence excitation emission matrix (FEEM) spectra, respectively. As a response to selenate, Cd and/or Zn exposure, significant fluorescence quenching of fulvic-like acids and humic-like substances was observed. With selenate, Cd and/or Zn in the influent with respective concentrations of 10, 5 and 5 mg/L, the abundance of the phyla *Proteobacteria*, *Firmicutes*, *Spirochaetae*, *Cloacimonetes* and *Synergistetes* increased significantly, and the dominant taxa in the anaerobic granular sludge exposed to Se, Cd and/or Zn were *Halothiobacillaceae* (10.2%), *Pseudomonas* (8.8%), *Synergistaceae* (7.7%), *Spirochaetaceae* (7.2%), *Blvii28 wastewater sludge group* (6.7%), *Telmatospirillum* (4.6%), *Veillonellaceae* (4.3%), *Geobacter* (4.0%) and *Enterobacteriaceae* (3.0%). Compared with the inoculum, the abundance of the archaea *Methanobacterium* and *Methanosaeta* decreased to below detection limit in the UASB reactor after 116 days exposure to Se, Cd and Zn.

INTRODUCTION

Selenium (Se) is vital for all living organisms. However, it can cause adverse effects on wildlife at moderate concentrations (like 5 μg/L to fish) (Lenz & Lens, 2009). In acid mine drainage (AMD), Se oxyanion contamination is often accompanied by heavy metals such as Cd and Zn (Tan et al., 2016). Santos et al. (2015) reported that Se concentrations can reach up to 12 mg/L in mining wastewater, while Cd and Zn concentrations can reach up to 44 and 5000 mg/L, respectively (Moreau et al., 2013). Wastewater contaminated with Se and heavy metals must be treated appropriately to prevent toxic effects on the surrounding environment (Rosenfeld et al., 2018). According to China's wastewater discharge standards (SEPAC, 2002), Se, Cd and Zn concentrations should be below 0.1, 0.01 and 1.0 mg/L, respectively, in the effluent of municipal wastewater treatment plants.

Under anaerobic conditions, the soluble and toxic Se oxyanions (SeO_4^{2-} and SeO_3^{2-}) are reduced to the immobile and less toxic form Se(0) (Lenz et al., 2008). Alternatively, they are reduced to Se^{2-}, which precipitates with metal cations, resulting in the formation of metal selenides, such as PbSe, CdSe, ZnSe and FeSe (Mal et al., 2016b). Anaerobic granular sludge is an ideal carrier for microorganisms and used for the treatment of wastewaters containing Se oxyanions and heavy metals (Zeng et al., 2021). In a previous study, Mal et al. (2016a) investigated anaerobic granular sludge in batch experiments and found that selenite reduction was not inhibited by Pb levels of up to 150 mg/L and by

Zn levels of up to 400 mg/L. In contrast, the bioreduction of selenite was negatively influenced by Cd, which inhibited selenite bioreduction at concentrations above 150 mg/L. Similarly, Tan, Papirio, et al. (2018) reported that Ni^{2+} addition decreased SeO_4^{2-} and SO_4^{2-} removal by more than 30% in an upflow anaerobic sludge blanket (UASB) reactor, and Se-S and Ni-S minerals were present in the anaerobic granules, which gave an added value for recovery and reuse of biosynthesized minerals.

As a further step, it is necessary to investigate the dominant taxa in anaerobic granular sludge and to determine their capacity to tolerate exposure to Se oxyanions or heavy metals, which is essential for Se oxyanions containing wastewater treatment. Dessi et al. (2016) investigated the microbial communities in UASB reactors for selenite removal at different temperatures. Their mesophilic and thermophilic UASB reactors contained selenate respirers and denitrifying microorganisms. In another study, Tan, Nancharaiah, et al. (2018) found that the families *Campylobacteraceae* and *Desulfomicrobiaceae* were the dominant phylotypes in a UASB reactor for selenate removal, with a relative abundance of approximately 23% and 10%, respectively. Both *Geobacteraceae* and *Spirochaetaceae* were present at a relative abundance of approximately 10% in the granular sludge fed with 12 mg/L influent selenate at a pH 5.0.

To the best of our knowledge, there is no report about the microbial community dynamics in anaerobic granular sludge during simultaneous removal of selenate and heavy metals. Based on previous observations during the operation of UASB reactors for selenate, Cd and Zn removal for 116 days (Zeng et al., 2019), high-throughput sequencing was conducted to investigate the microbial community dynamics in the anaerobic granular sludge of these reactors. Extracellular polymeric substances (EPS) were extracted during each period and recorded by the fluorescence excitation emission matrix (FEEM) spectroscopy.

EXPERIMENTAL PROCEDURES

Sources of biomass

Anaerobic granular sludge was collected from a laboratory-scale upflow anaerobic sludge blanket (UASB) reactor, which was used for the continuous biological removal of selenate in the presence of cadmium and zinc under psychrophilic (17.5 ± 0.4°C) conditions for 42 days and mesophilic (30 ± 0.5°C) conditions for 74 days (Zeng et al., 2019). The UASB operational conditions for selenate, Cd and Zn removal are shown in Table 1. At the end of the periods I, III, IV and V, 10 g of biomass was collected from the UASB reactor for EPS and microbial community analysis. The laboratory-scale UASB

TABLE 1 Average Se(VI), Cd(II) and Zn(II) removal efficiencies of the UASB reactor during different operational periods.

Periods	Influent concentration (mg/L)	Carbon source	Hydraulic retention time (HRT)	Influent pH	Operation temperature	Duration (days)	Average Se(VI) removal efficiency (%)	Average Cd(II) and Zn(II) removal efficiency (%)
I	Se(VI): ~10	Sodium lactate 1800 mg/L	12 h	7.1–7.3	17.5 ± 0.4°C	0–21	81.0	–/–
II	Se(VI): ~10; Cd(II): ~2					22–42	98.2	81.2/–
III	Se(VI): ~10; Cd(II): ~5				30 ± 0.5°C	43–63	99.6	85.5/–
IV	Se(VI): ~10; Cd(II): ~5; Zn(II): ~2 for 2 weeks and ~5 for 2 weeks					64–91	99.7	95.0/97.9
V	Se(VI): ~10					92–116	98.0	–/–

Note: '–' means not applicable.

reactor was inoculated with UASB sludge collected from a full-scale pulp and paper wastewater treatment plant, described in detail by Roest et al. (2005).

EPS characterization

Loosely bound EPS (LB-EPS) were extracted from the inoculum and sampled anaerobic granular sludge according to a previously described protocol and modification as follows (D'Abzac et al., 2010): prior to extraction, the anaerobic granular sludge was washed twice with deionized water. Then, it was centrifuged at 10,000 *g* for 20 min at 4°C, and the supernatants were collected as LB-EPS.

The EPS samples were subjected to analysis in a spectrofluorophotometer (Helmholtz Zentrum Dresden-Rossendorf) for FEEM spectra collection at 25°C. The emission spectra were scanned from 290 to 500 nm after excitation in the range of 240–450 nm, using 4-nm increments. The software package Panorama Fluorescence 3.1 (LabCognition) was used for fluorescence data processing.

DNA extraction and PCR amplification

Inoculum and anaerobic granular sludge samples derived from each period (Table 1) were used for microbial community determination. Total genomic DNA was extracted using the E.Z.N.A. Soil DNA Kit (OMEGA Bio-Tek) according to the manufacturer's protocol. The quality and concentration of the DNA were determined using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific).

Primers of 515FmodF and 806RmodR were selected, which target the V4 regions of both bacterial and archaeal 16 S rRNA genes (Walters et al., 2016). The PCR amplification reaction mixtures contained 10 ng of genomic DNA, 0.8 μl of forward and reverse primers (5 μM), 2 μl of dNTPs (5 mM), 0.4 μl of FastPfu Polymerase, 0.2 μl of bovine serum albumin (BSA) and 4 μl of 5× PCR buffer. The solution was brought to a volume of 20 μl with double-distilled H_2O. The thermal program consisted of 95°C for 3 min, 30 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s and a final extension step at 72°C for 10 min. All samples of each period (Table 1) were amplified in triplicate on an ABI GeneAmp®9700 thermal cycler, and 2% agarose gel electrophoresis was used for PCR product determination.

High-throughput sequencing and bioinformatic analysis

The PCR products were purified using the AxyPrepDNA gel extraction kit (Axygen Scientific Inc) and quantified by a fluorescence quantitative system (QuantiFluor™-ST; Promega). High-throughput sequencing was performed on an Illumina MiSeq platform at Shanghai Majorbio Bio-pharm Technology Co., Ltd.

Quality control was conducted using the Trimmomatic software to remove poor-quality sequences and sequences shorter than 50 bp from the raw reads. The qualified data were clustered and classified into operational taxonomic units (OTUs) at a 97% sequence similarity threshold, using Usearch (version 7.0). The alpha index values of Chao and Shannon, and their rarefaction curves, were calculated via the Mothur software (version v.1.30.1). Taxonomic assignment was performed with the Ribosomal Database Project (RDP) Classifier (version 2.6) with a confidence threshold of 80%. The Beta diversity of samples was determined using the R software with the weighted UniFrac distance matrix algorithm (Moroenyane et al., 2016), and the results are shown in the form of a distance heatmap. Bacterial abundance was determined using the R software at the phylum and genus levels.

Analytical methods

After filtering through a 0.45-μm cellulose acetate syringe filter (Sigma Aldrich), the selenate concentration was determined using a Dionex ICS-1000 Ion Chromatograph (Thermo Fisher Scientific), equipped with an AS4A 2 mm Dionex column, with the automated sample injector Dionex ASI-100 (Thermo Fisher Scientific). The retention time of selenate was approximately 8.0 min in the ion chromatograph. The liquid samples were acidified with 0.5% HNO_3 prior to the measurement of Cd and Zn concentrations. The levels of Cd and Zn were measured using a flame AAS (Analyst 200; PerkinElmer) with a detection range of 0.01 to 0.5 mg/L.

RESULTS AND DISCUSSION

Performance of the UASB reactor

The UASB performance for selenate, Cd and Zn removal is shown in Table 1. The average selenate removal efficiency was 81.0% in period I over 21 days. The average selenate removal efficiency increased to 98.2 and 99.6% for periods II and III, respectively. During these periods, the average cadmium removal efficiencies were 81.2% and 85.5%, respectively, with influent Cd concentrations of 2 and 5 mg/L, respectively. In period IV, the average selenate, Cd and Zn removal efficiencies were 99.7%, 95.0% and 97.9%, respectively. It should be mentioned that for almost the entire duration of period IV of the UASB operation, the Se (IV) concentration was very low in the effluent. In period V, Cd and Zn were not added to the influent of the UASB reactor; the reactor had an overall selenate removal

efficiency of 98.0%. Thus, the presence of 5 mg/L Cd and Zn enhanced both the Se(VI) removal efficiencies compared to feeding only selenate in influent, which was elaborated in a previous report (Zeng et al., 2019).

EPS characterization

The FEEM spectra of the EPS extracted from each period of the anaerobic granular sludge showed three main peaks (Figure 1). The first was identified at excitation/emission wavelengths (Ex/Em) of 240–300/290–380 nm (peak A), while the second and third peak occurred at Ex/Em of 250–290/380–470 nm (peak B) and Ex/Em of 360–420/430–490 nm (Peak C), respectively. Peak A was a combination of several protein-like (PN-like) peaks and soluble microbial products (SMP), which are soluble organic compounds released during normal biomass metabolism (Ni et al., 2011). The compounds contributing to peak A were mainly associated with

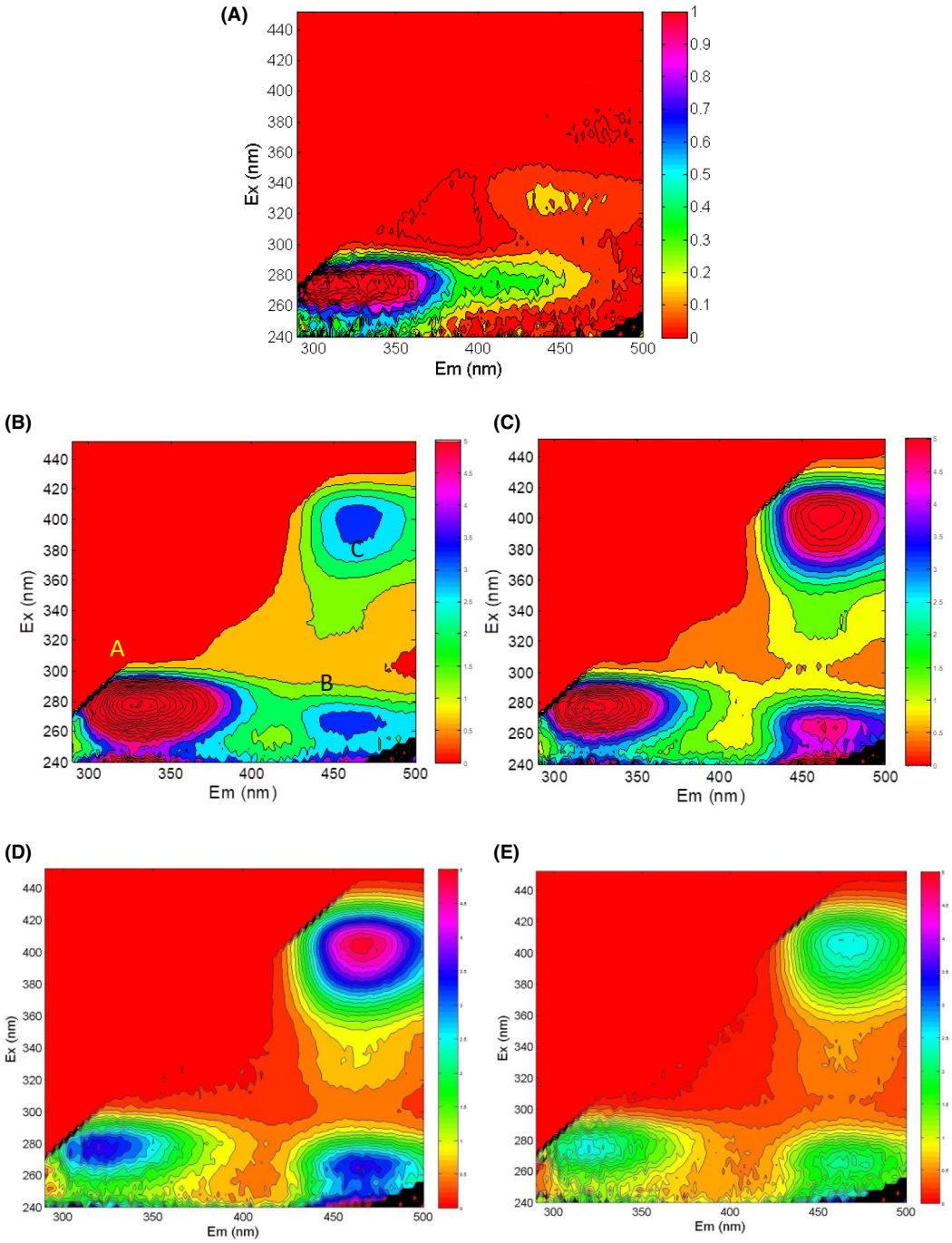

FIGURE 1 Three-dimensional EEM (excitation emission matrix) fluorescence spectra of the extracted loosely bound EPS from the inoculum (A) and the reactor biomass in periods I (B), III (C), IV (D) and V (E).

aromatic tyrosine and/or tryptophan PN-like substances (Mal et al., 2017). Peaks B and C are related to fulvic-like acids and humic-like substances (Li et al., 2008), respectively.

Compared with the sample from the inoculum (Figure 1A), peak A had a similar intensity during periods I and III (Figure 1B,C). However, its intensity decreased slightly during period IV, with a dramatic decrease in period V. These results lead us to infer that selenate and Cd, at the given concentrations of respectively 10 and 5 mg/L, did not affect the aromatic protein-like substances during periods I and III, which are the key components that determine the structural stability of anaerobic granular sludge (Zhu et al., 2015). Some microorganisms produce higher amounts of SMP due to the strong metal complexing ability of SMP-like materials (Wang et al., 2015), which most likely also contributed to the selenate, Cd and Zn removal in the present study.

The FEEM spectra of EPS extracted from the anaerobic granular sludge in the UASB reactor also showed a slight change in fulvic-like acids (peak B), which appeared to be red shifts to longer wavelengths (~25 nm) compared with the EPS present in the inoculum. Usually, the red shift is a result of the presence of carbonyl, hydroxyl, alkoxyl, amino groups and carboxyl-containing substances (Liu et al., 2015). The intensity of peak B increased during the periods I, III and IV, with a distinct peak in period III. Based on this, the fulvic-like acids in the EPS contained high levels of functional groups, including carboxyl, hydroxyl, alkoxyl and amino groups, which were beneficial for selenate, Cd and Zn removal, with a particularly high cadmium adsorption in period III (Table 1).

The fluorescence of humic-like substances (peak C) was not clearly visible in the inoculum. However, significant fluorescence quenching was evident after the addition of selenate, Cd and Zn during periods I, III and IV, respectively. Thus, humic-like substances probably participated in the removal of these metal(loid)s. Therefore, the fluorescence intensity of peak C decreased slightly in period V, when Cd and Zn were removed from the influent (Figure 1).

Overall, the LB-EPS extracted from anaerobic granular sludge in the UASB reactor contained large amounts of aromatic proteins, soluble microbial products (SMP), fulvic-like acids and humic-like substances. These fluorescent organic ligands with strong binding capacities likely participated in the complexation of EPS with the heavy metals (Mal et al., 2021).

Microbial community diversity

The obtained sequence numbers, OTUs, coverage, Chao and Shannon index values of the UASB sludges are shown in Table S1. The sequence read numbers ranged from 43,005 to 82,370, and each sequence number was normalized by subsampling to an equal sequencing depth (43,005 reads) before further analysis. As illustrated in Table S1, the effective sequences were clustered into 312, 345, 388, 365 and 238 OTUs for the inoculum and the anaerobic granular sludge sampled at the end of period I, III, IV and V, respectively. The coverage of all samples reached 0.999, indicating that the sequence library represented most species present in the samples (Zhou et al., 2018).

Microbial richness is reflected by the Chao index (Huang et al., 2017). Samples taken in the periods I, III and IV had larger Chao index values than the inoculum, indicating that the addition of selenate, Cd and Zn to the UASB reactor influent promoted an increased microbial richness in the granules compared with the inoculum sludge. This was confirmed by the corresponding rarefaction curves (Figure 2A).

The Shannon index is generally used for estimating microbial diversity (Xun et al., 2018). In this study, the Shannon rarefaction curves levelled off quickly (Figure 2B), which suggests a high bacterial diversity in all biomass samples investigated. Compared with the inoculum (3.7), the Shannon index values of the anaerobic granular sludge increased or stabilized in periods I, III, IV and V, suggesting that the addition of selenate, Cd and Zn to the influent promoted the microbial diversity of the UASB reactor sludges slightly.

In contrast to alpha diversity revealing species diversity within a community, beta diversity indicates the microbial diversity of distinct communities (Flores-Rentería et al., 2016). A weighted normalized UniFrac distance matrix was established for the analysis of the microbial composition and the abundance of the communities (Figure 2C). A colour difference between the inoculum and period I suggested a significant difference in the microbial composition and abundance upon selenate exposure. The beta diversity index values between the inoculum and periods III, IV and V were considerably higher (Figure 2C), revealing significant variations in the microbial community composition of these different granular sludge samples exposed to varying Se, Cd and Zn concentrations.

Venn analysis

Figure 3 shows the Venn image of the inoculum and anaerobic granular sludge samples in the different periods based on bacterial genera, reflecting the microbial similarity and dissimilarity upon selenate, Cd and Zn exposure (Li et al., 2018). All five samples shared 63 genera, which accounted for 23.2% of the total observed genera (272). The numbers of unique genera were 24, 8, 11, 6 and 5 for the inoculum, period I, III, IV

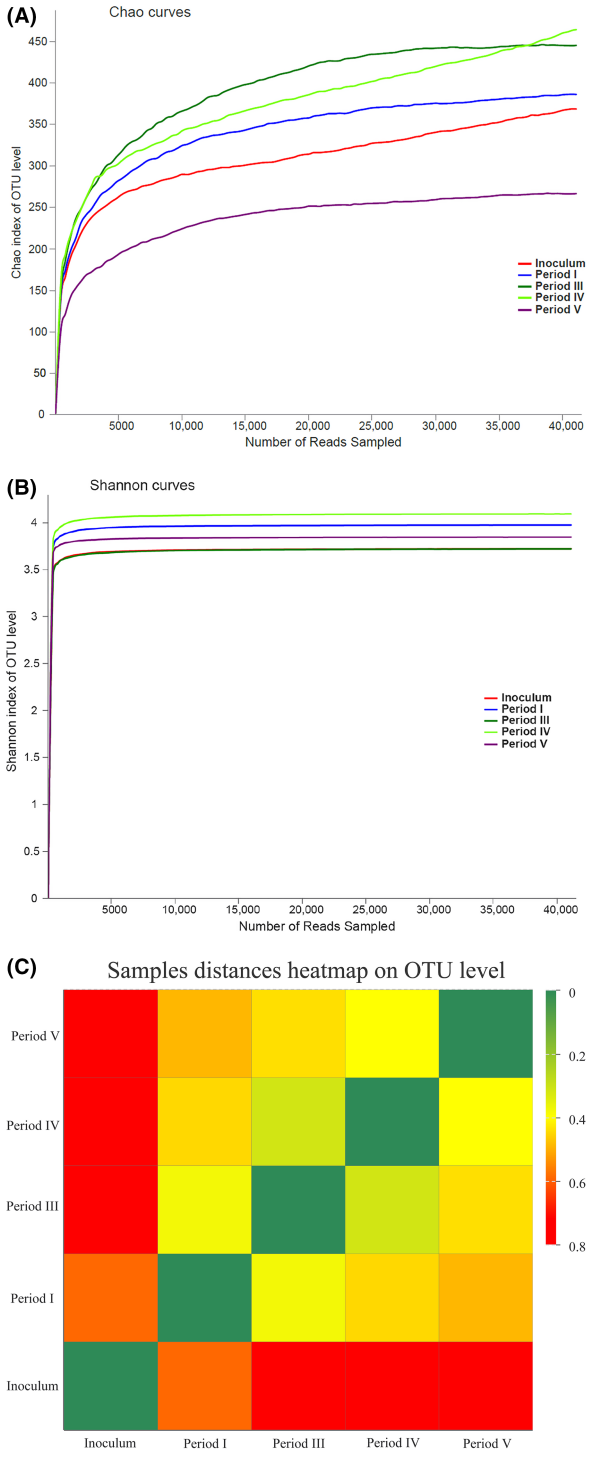

FIGURE 2 Chao (A), Shannon (B) rarefaction curves of alpha diversity and colour-scale heatmap of beta diversity (C) based on OTU levels of anaerobic granular sludge.

and V, respectively. In addition, the inoculum and period I sludge shared 115 genera, the inoculum and period III sludge shared 111 genera, the inoculum and period IV sludge shared 99 genera, whereas the inoculum and period V sludge shared 73 genera. These results indicate the shared genera account for a small proportion of the microbial communities along with the long-term

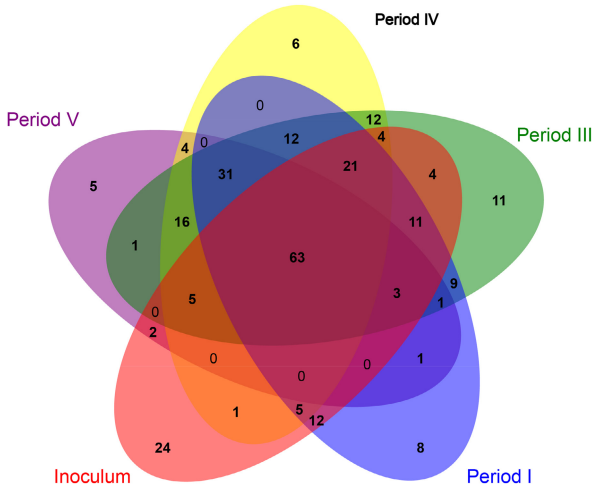

FIGURE 3 Venn analysis of inoculum and reactor biomass in different periods of the UASB reactor, based on the genus level.

exposure to selenate, Cd and/or Zn, which caused the shift of microbial community composition of the UASB reactor.

Microbial community dynamics at the phylum level

The altered microbial community, in response to changes in the operating condition (Table 1), was evaluated at the phylum level (Figure 4) with a relative abundance higher than 1%. *Euryarchaeota* was the most abundant phylum in the inoculum, accounting for 31.7%, and its proportions decreased dramatically to 2.8% and 0.7% in periods I and IV, respectively; in periods III and V, the proportions were even below the detection limit. The phylum *Euryarchaeota* was thus gradually eliminated from the UASB reactor granules. The phyla of *Bacteroidetes* and *Chloroflexi* were the second and third dominant phyla in the inoculum and accounted for 21.9% and 11.0%, respectively (Figure 4). The relative abundance of *Bacteroidetes* decreased to 20.7%, 9.2%, 13.4% and 13.9%, respectively, in periods I, III, IV and V, while that of *Chloroflexi* decreased to 5.1%, 1.4%, 1.9% and 1.3%, respectively, in these periods. The relative abundance of *Actinobacteria* decreased from 7.0% in the inoculum to 1.4%, 2.5%, 0.5% and 0.3%, respectively, in periods I, III, IV and V. *Bathyarchaeota* and *Fibrobacteres* accounted for 2.1% and 1.8%, respectively, in the inoculum. Subsequently, the abundance of both phyla decreased to values below the detection limit. The overall abundances of *Euryarchaeota*, *Chloroflexi*, *Actinobacteria*, *Bathyarchaeota* and *Fibrobacteres* were considerably affected by the selenate, Cd and/or Zn fed to the UASB reactor, while that of *Bacteroidetes* was only slightly impacted (Figure 4).

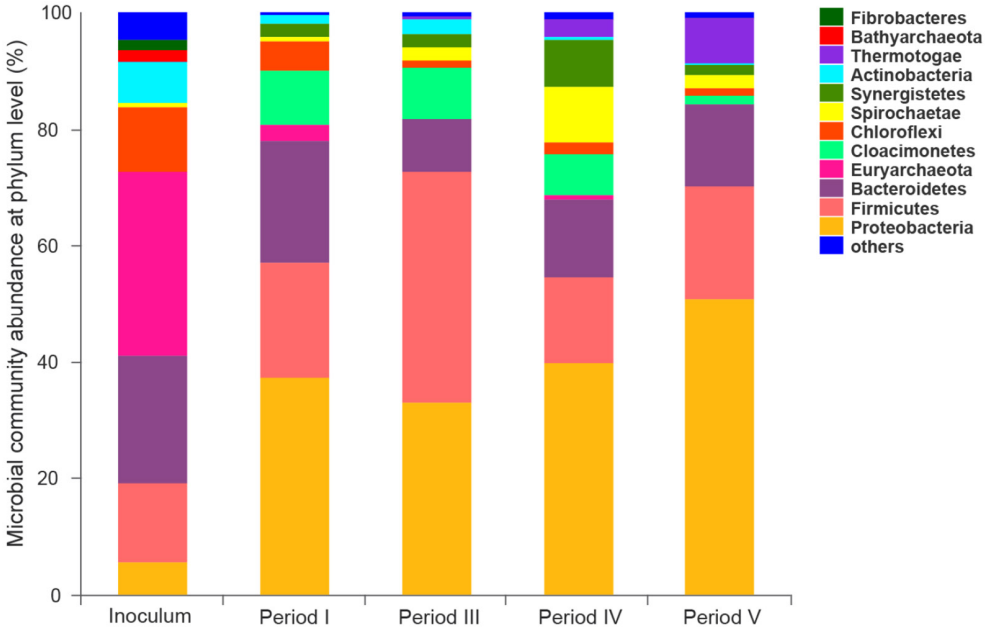

FIGURE 4 Composition of bacterial communities at the phylum level with an average abundance above 1% for different samples. Different bacteria are represented by different colours.

In contrast, the phylum of *Proteobacteria* increased significantly from 5.9% in the inoculum to 37.4%, 33.3%, 39.9% and 51.0% for the periods I, III, IV and V, respectively. The percentage of *Firmicutes* also increased from 13.5% in the inoculum to 19.9%, 39.7%, 14.7% and 19.5% in granular sludge sampled in periods I, III, IV and V, respectively. The abundance of *Spirochaetae* was 0.7% in the inoculum, increasing to 2.3%, 9.5% and 2.4% in periods III, IV and V, respectively. The abundances of both *Cloacimonetes* and *Synergistetes* in the inoculum were below the detection limit, but increased to 9.4% and 2.3%, respectively, in period I, 8.6% and 2.1% in period III, 7.2% and 8.1% in period IV and 1.6% and 1.6% in period V, respectively.

The relative abundances of the above-mentioned five phyla (*Proteobacteria*, *Firmicutes*, *Spirochaetae*, *Cloacimonetes* and *Synergistetes*) increased under selenate, Cd and Zn exposure. In a similar study, the phylum *Proteobacteria* was the dominant phylum in a hydrogen-based biofilm reactor for selenate removal (Lai et al., 2014), while *Proteobacteria* and *Firmicutes* were the dominant phyla in landfills upon Cd and Zn stress (Liu et al., 2019). In another study, *Spirochaetae* could tolerate Cd levels of up to 112 μg/L (Mu et al., 2018). In this study, the phyla *Proteobacteria*, *Firmicutes*, *Spirochaetae*, *Cloacimonetes* and *Synergistetes* were the dominant phyla in the UASB reactor, accounting for 39.9%, 14.7%, 13.4%, 9.5%, 8.1% and 7.2% of the total phyla (Figure 4), respectively. They were supposed to significantly contribute to the removal of selenate, Cd and Zn in the UASB reactor operation.

Microbial community dynamics at the genus level

Figure 5 shows a heatmap of the 15 most abundant genera. Table S2 shows the dominant genera in the UASB reactor during 116 days of operation, accounting for more than 3% of the total genera. *Methanobacterium*, norank *Bacteroides vadinHA17*, *Methanosaeta* and *Anaerolinea* were the four most abundant genera in the inoculum, with relative abundance of 18.6%, 14.0%, 12.6% and 8.0%, respectively (Figure 5). However, their abundances decreased to values below the detection limit after 116 days of operation, suggesting that the selenate, Cd and Zn exposure was toxic or/and inhibited the growth of these genera.

In contrast, the relative abundances of *Desulfovibrio*, *Tyzzerella*, *Macellibacteroides*, *Pseudomonas* and *Arcobacter* increased dramatically after 116 days of operation when compared to the inoculum, accounting for 10.7%, 8.2%, 5.2%, 3.4% and 3.1%, respectively, of the total amount of genera present in the sludge (Figure 5). Both *Desulfovibrio* and *Pseudomonas* showed a high-selenium oxyanion removal capacity, as well as a high resistance to Cd and Zn (Ontiveros-Valencia et al., 2016). This study is the first to report a high tolerance of *Tyzzerella*, *Macellibacteroides* and *Arcobacter* to selenate, Cd and/or Zn exposure.

In addition, selenate, Cd and Zn were present in the influent in period IV (Table 1). The dominant taxa were *Halothiobacillaceae* (10.2%), *Pseudomonas* (8.8%), *Synergistaceae* (7.7%), *Spirochaetaceae* (7.2%), *the Blvii28 wastewater sludge group* (6.7%), *Telmatospirillum*

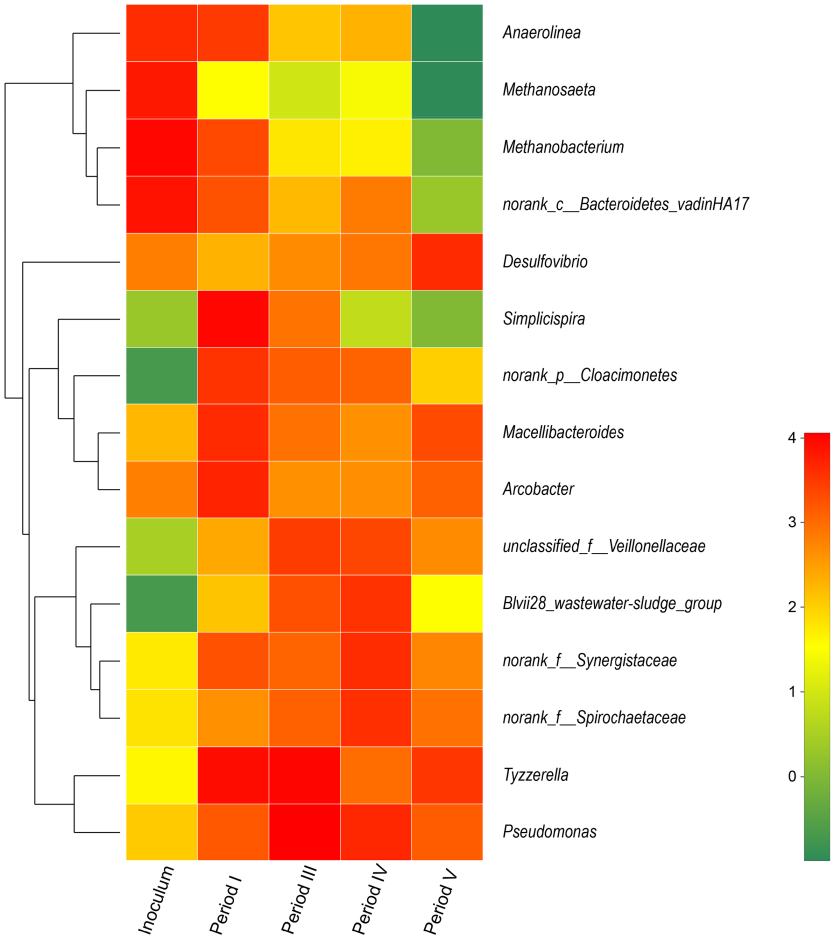

FIGURE 5 Colour-scale heatmap showing the dynamics of microbial communities at the genus level, with the top 15 ratios of inoculum and reactor biomass in different periods of the UASB reactor. Red colour indicates higher abundance; green colour indicates lower abundance.

(4.6%), *Veillonellaceae* (4.3%), *Geobacter* (4.0%) and *Enterobacteriaceae* (3.0%) (Table S2). These genera were assumed to play roles in the process of selenate, Cd and Zn removal.

Comparison of dominant microbial communities upon different heavy metal exposure

To explain the variations in the dominant microbial taxa upon Se, Cd and Zn exposure, Pearson's correlation heatmap of the top 15 genera (ranked by their average abundances from three exposure groups) are listed (Figure 6). The relationships between microbial taxa and environmental factors varied greatly. The taxa *Pseudomonas*, norank_f_*Synergistaceae*, norank_f__*Spirochaetaceae*, *unclassified_f__Veillonellaceae and Blvii28_wastewater-sludge_group* showed a positive correction with the Se, Cd and Zn levels, indicating a tolerance of these taxa to these elements. This result is in agreement with a previous report about *Pseudomonas*, which was resistant against 30 mM of Cd, 30 mM of Zn and 20 mM of Se (Shafique et al., 2017). Exposure to Cd significantly positively impacted the taxa *unclassified_f__Veillonellaceae* and *Blvii28_wastewater-sludge_group*, while Zn exposure had a significant positive influence on norank_f_*Synergistaceae* and norank_f__*Spirochaetaceae*.

The taxa *Methanobacterium*, norank_c__*Bacteroidets_vadinHA17*, *Anaerolinea*, and *Methanosaeta* were negatively correlated with the Se, Cd and Zn levels, indicating a poor growth or even decay of these taxa in the UASB reactor. In contrast, *Methanobacterium*, norank_c__*Bacteroidets_vadinHA17* and *Methanosaeta* showed a significant negative correlation with the applied Se concentrations. In a previous study, *Methanobacterium* growth was inhibited upon Cd exposure (Mori et al., 2000), which partly explains the poor growth of archaea (*Methanobacterium* and *Methanosaeta*) in the presence of Se, Cd or Zn in this study.

The taxa *Tyzzerella* and *norank_p__Cloacimonetes* were positively correlated with both Se and Cd levels but negatively with Zn levels. Similarly, the taxa *Simplicispira*, *Macellibacteroides*, *Arcobacter* and *Desulfovibrio* were positively correlated with Se, but negatively with Cd and Zn. In a similar study, *Desulfovibrio* was the dominant bacterial genus in a hydrogen-fed biofilm reactor for selenate removal (Ontiveros-Valencia et al., 2016), which is in agreement with the findings of this study.

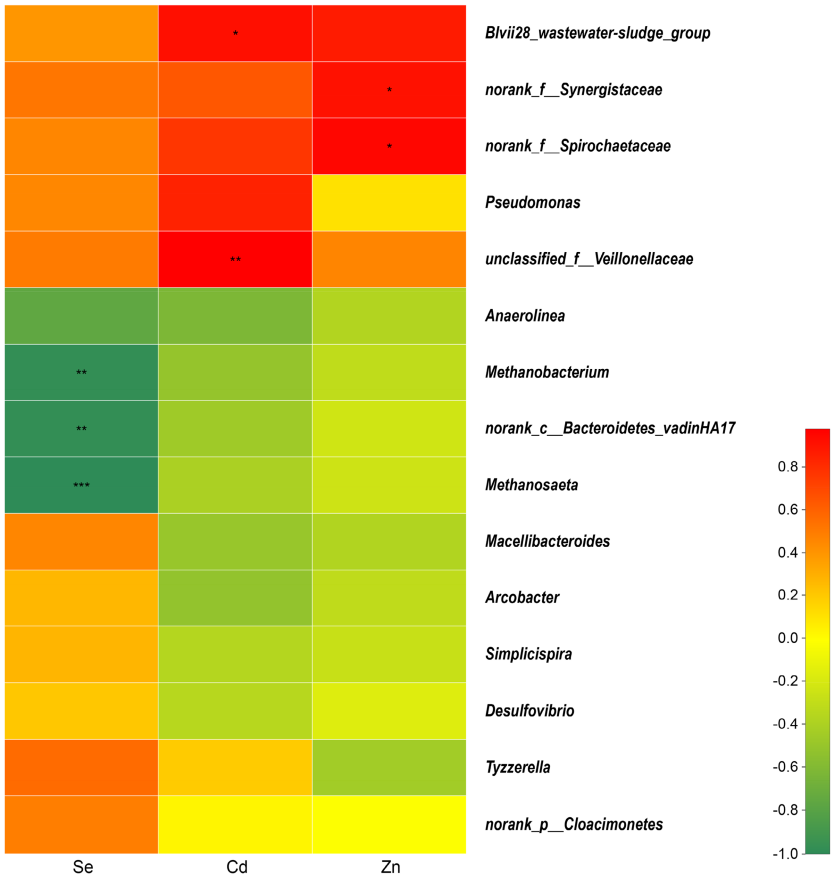

FIGURE 6 Pearson correlation heatmap for the top 15 taxa and the three heavy metals in the influent of the UASB reactor. *X* and *Y* axis represent the environmental factors and genera, respectively. The right side of the legend is the colour range of different *R* values. * indicates $0.01 < p$ value ≤ 0.05, ** indicates $0.001 < p$ value ≤ 0.01, and *** indicates p value ≤ 0.001.

CONCLUSIONS

After exposure to selenate, Cd and/or Zn, large amounts of aromatic proteins, soluble microbial products (SMP), fulvic-like acids and humic-like substances were present in the EPS, which had strong binding capacities for selenate, Cd and Zn. The addition of selenate, Cd and/or Zn enhanced the microbial richness and diversity. As a response to selenate, Cd and/or Zn exposure, the abundance of the phyla of *Proteobacteria*, *Firmicutes*, *Spirochaetae*, *Cloacimonetes* and *Synergistetes* increased evidently, as well as the relative abundances of genera of *Desulfovibrio*, *Tyzzerella*, *Macellibacteroides*, *Pseudomonas* and *Arcobacter*. The taxa *Pseudomonas*, norank_f_*Synergistaceae*, norank_f__*Spirochaetaceae*, *unclassified_f__Veillonellaceae* and *Blvii28_wastewater-sludge_group* showed a tolerance to selenate, Cd and/or Zn based on Pearson's correlation analysis.

AUTHOR CONTRIBUTIONS

Taotao Zeng: Conceptualization (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Qing Hu:** Writing – original draft (equal). **Eldon Raj:** Writing – review and editing (equal). **Piet NL Lens:** Supervision (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All required experimental data of this study are contained within the manuscript. Data that support the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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