Bacterial community analysis of sulfate-reducing granular sludge exposed to high concentrations of uranium

Taotao Zeng, Shiqi Zhang, Wei Liao, Hualong Ma, Piet N. L. Lens and Shuibo Xie

ABSTRACT

Sulfate-reducing granular sludge was used for uranium-contaminated wastewater treatment and the responsible microbial community was analyzed. Four feedings, with 6 days for every feeding and 20 mg/L initial uranium, were provided at 27.5 (±2.5) °C. After the four feedings, a uranium removal efficiency of 94% was obtained. Environmental scanning electron microscopy (ESEM) showed that short rod bacteria were the dominant microorganisms in the granular sludge. X-ray energy dispersive spectroscopy (EDS) confirmed the presence of uranium on the granular surface. High-throughput sequencing was carried out for analyzing the bacterial diversity and community structure. The total data set comprised 8,290 high quality sequences, which could be divided into 605 operational taxonomic units (OTUs). The library coverage was 0.96 and the alpha diversity indices of ACE, Chao1, Shannon and Simpson were 2,255.40, 1,346.12, 4.03 and 0.05, respectively. There were 13 bacterial genera present with a ratio of more than 1% of the total 124 genera, among which *Desulfovibrio* (16.48%), *Clostridium* IV (9.29%), *Bacteroides* (3.46%) and *Citrobacter* (1.41%) were assumed as the functional bacteria, with a cumulative proportion of 30.64% of the total bacterial population. The results provide insights into the bacterial community of sulfate-reducing granular sludge exposed to high concentrations of uranium (20 mg/L).

Key words | bacterial population, community structure, high-throughput sequencing, sulfate-reducing granular sludge, uranium exposure

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INTRODUCTION

Nuclear research, nuclear fuel production, mining activities, and weapon manufacturing result in pervasive environmental contamination by uranium (Suriya *et al.* 2017). One of the major sources of uranium contamination is uranium tailings and mining greatly affects the natural groundwater flow (Hu *et al.* 2016; Lipansky 2017). The most common valence states of uranium in nature are hexavalent [U(VI)] and tetravalent [U(IV)] uranium. The reduced form of U(IV) is insoluble and therefore less toxic to microbial cells, while U(VI) exerts great toxicity due to its solubility and mobility (Yan & Luo 2015). The U(VI) can also cause displacement and/or substitution of essential ions from cellular sites or blocking of enzymes and essential nutrient transport systems (Cason *et al.* 2012). Uranium accumulation in the human body is hazardous, as it is toxic to renal cells and osteoblasts (Gouget 2018) and can even be fatal (Sankhla *et al.* 2016). The radioactive compounds in water should be treated to avoid potential health impacts (Rodriguez *et al.* 2009).

Physical-chemical methods are conventionally used in uranium wastewater treatment, which include ion-exchange, coagulation, precipitation, extraction, and reverse osmosis.

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However, physicochemical methods are environmentally intrusive and fairly expensive. Additionally, chemical methods create large quantities of toxic sludge and chemical by-products, which also pollute the environment (Mtimunye & Chirwa 2014). Given these limitations, developing safe, effective and environmentally friendly methods to treat uranium-contaminated wastewater are required. Some microorganisms are found to be uranium resistant, which is an alternative approach for uranium remediation.

Sulfate-reducing bacteria (SRB) can use sulfate as the terminal electron acceptor for the degradation of organic compounds and hydrogen, converting SO_4^{2-} to H_2S, which converts soluble U(VI) to insoluble U(IV) under anaerobic conditions (Zhang *et al.* 2017b). *Desulfococcus*, *Desulfobacterium* and *Desulfovibrio* are the common SRB genera. U(VI) immobilization by SRB occurs by two processes, enzymatically or chemically, by reacting with microbially generated H_2S (Beyenal *et al.* 2004). Uranium concentrations in the contaminated groundwater are usually below 1 mg/L (Hu *et al.* 2016). Aida *et al.* (2010) used batch experiments inoculated with methanogenic granule sludge to investigate their U(VI) removal capacity. The anaerobic removal activity could be sustained after several re-spikes of U(VI). However, there is little knowledge about the removal capacity of high uranium concentrations by sulfate-reducing granular sludge, let alone the bacterial community involved.

In this study, anaerobic granular sludge was collected from a citric acid wastewater treatment plant and cultivated under sulfate-reducing conditions. Then, the biomass was used to investigate the removal capacity of high uranium concentrations (20 mg/L) in synthetic wastewater. The microstructure was analyzed by environmental scanning electron microscopy (ESEM) and X-ray energy dispersive spectroscopy (EDS), whereas the microbial diversity and community composition were investigated by high-throughput sequencing.

MATERIALS AND METHODS

Source of biomass

Anaerobic granular sludge, collected from a full-scale upflow anaerobic sludge blanket (UASB) reactor in Yueyang (Hunan, China), was used to enrich sulfate-reducing granular sludge under anaerobic conditions. The microbial community of the inoculum anaerobic granular sludge was already characterized by Zeng *et al.* (2016a).

A batch reactor was used to enrich SRB for three months, which was fed with a culture medium containing (mg/L): $C_6H_{12}O_6 \cdot H_2O$ (3,300), Na_2SO_4 (4,437), NH_4Cl (230), KH_2PO_4 (65), |$NaHCO_3$ (1,250), yeast extract (500), $FeSO_4 \cdot 4H_2O$ (32), $CaCl_2 \cdot 2H_2O$ (38), $MgSO_4 \cdot 7H_2O$ (42), $CuCl_2 \cdot 2H_2O$ (0.5), $ZnCl_2$ (0.5), $MnCl_2 \cdot 4H_2O$ (0.5), $CoCl_2 \cdot 6H_2O$ (10), $NiCl_2 \cdot 6H_2$ O (0.5) (Xie *et al.* 2015). In this culture medium, the chemical oxygen demand (COD): N: P ratio equaled 200:5:1 and the COD:SO_4^{2-} ratio was 1:1, with an initial pH value of 7.2 (±0.2) and temperature of 35 °C. Then, the cultured sulfate-reducing anaerobic granular sludge was used as the inoculum for uranium removal in this experiment.

Batch experiments for uranium removal

Serum bottles, with 100 mL culture medium, were used for batch assays in duplicate. The standard 20 mg/L U(VI) was prepared according to the oxidization of U_3O_8 by nitrohydrochloric acid of GB6768-86 (SEPAC 1986). The chemical reaction was set as follows:

$$U_3O_8 + H_2O_2 + 2HCl + 4HNO_3UO_2Cl_2 + 2UO_2(NO_3)_2 + 4H_2O$$

After thoroughly washing using deionized water thrice, 10% w/v of the granular sludge was added to the bottles. The final volatile suspended solids/total suspended solids (VSS/TSS) of the granular sludge was 0.706. The pH of the medium was adjusted to 6.0, which was previously found to be the optimum pH for uranium removal by the sulfate-reducing granular sludge (Xie *et al.* 2015). The batch bottles were put on an orbital shaker at 150 rpm and 27.5 (±2.5) °C. The control test was set as incubating bottles with only synthetic culture medium and uranium, but without any anaerobic granular sludge. After 6 days' incubation, the new uranium (20 mg/L) containing medium was transferred to the anaerobic granular sludge. A total of four replacements were carried out as feeding I to IV (Table 1).

Table 1 | Operation of uranium removal by sulfate-reducing granular sludge in batch tests

Phase	Influent U(VI) concentration (mg/L)	Time (d)	Temperature (°C)	pH	Rotating speed in shaker (rpm)	Average U(VI) removal (%)	Average residual U(VI) concentration (mg/L)
I	20	6	27.5 ± 2.5	6	150	97.4	0.53
II	20	6	27.5 ± 2.5	6	150	92.4	1.53
III	20	6	27.5 ± 2.5	6	150	94.0	1.20
IV	20	6	27.5 ± 2.5	6	150	93.2	1.37

ESEM and EDS

The sulfate-reducing granular sludge was collected together after the final uranium exposure and ESEM-EDS was used to observe the microstructure and morphology according to Zeng *et al.* (2016b). Briefly, the sludge was washed twice in deionized water for 2 min, and then centrifuged at 8,000 *g* and 4 °C for 10 min. Then, the samples were frozen at −80 °C for 24 h (SANYO, MDF-U32 V). After drying by a vacuum freeze-drying machine (Freeze Dryer, FD5-series) for 24 h, gold sputter coating was carried out on the samples. The micromorphology and elemental composition were measured by an ESEM (FEI QUANTA 200) and X-ray EDS (EDAX Genesis 2000), respectively.

DNA extraction and polymerase chain reaction (PCR)

At the end of the incubation (Feeding IV), 5 g granular sludge was collected and homogenized for bacterial DNA extraction. Samples were rinsed twice with phosphate buffer saline (PBS). After centrifugation at 5,000 *g* for 5 min, the pellet was collected for DNA extraction using the E.Z.N.A.® Soil DNA Kit (OMEGA, USA) (Huang *et al.* 2016). The DNA quality was detected using 1% agarose gel electrophoresis. After purification of the DNA, the concentrations were determined using a Qubit 2.0 fluorometer (Invitrogen, USA).

The extracted DNA samples were used as template for 16S rRNA gene amplification, with 341F/805R primers targeting the V3–V4 region (Antwi *et al.* 2017). The PCR reaction system contained 0.5 μL forward primer (50 μM), 0.5 μL reverse primer (50 μM), 0.5 μL Plantium Taq (5 U μL^{-1}, Thermo, USA), 10 ng genomic DNA, 5 μL 10 × PCR buffer and 0.5 μL dNTP (10 mM each), and ultrapure water was added to a final volume of 50 μL. The PCR protocol was as follows: denatured at 94 °C for 3 min; 25 cycles of denatured at 94 °C for 30 s, 50 °C annealed for 20 s, 72 °C extended for 30 s; and a final extension at 72 °C for 5 min. PCR products were detected by 2% agarose gel electrophoresis and purified using a SanPrep DNA Fragment Purification Kit (Sangon Biotech, China).

High-throughput sequencing and bioinformatics analysis

High-throughput sequencing was carried out three times by an Illumina Miseq sequencer at Sangon Biotech Co., Ltd (Shanghai, China) as described in a previous report (Cui *et al.* 2016). For bioinformatics information analysis, Mothur was used for sequences matching the specific barcodes. Quality control was performed using Prinseq software to remove the sequence with an average quality score of less than 20, or with any unknown bases. Operational taxonomic units (OTUs) were assigned by Uclust software with more than 97% sequence similarity. Alpha indices of Coverage, ACE, Chaos, Shannon and Simpson were calculated by Mothur software. Taxonomic classification of OTUs was conducted by Ribosomal Database Project (RDP) Classifier (version 2.6) at a confidence threshold of 80% (Zhu *et al.* 2018). The sequences were submitted to the Sequence Read Archive (SRA) database in the National Coalition Building Institute (NCBI) and obtained the access number PRJNA490812.

Analytical methods

TSS and VSS of the granular sludge were characterized following APHA (2012). The U(VI) concentrations were measured

daily in triplicate using the 2-(5-bromo-2-pyridylazo)-5-(diethylamino) phenol (5-Br-PADAP) spectrophotometric method at a wavelength of 578 nm (Ganesh *et al.* 2014), which had a detection limit of 50 μg/L for U(VI).

RESULTS AND DISCUSSION

Uranium removal in batch experiments

The U(VI) removal efficiency of the sulfate-reducing granular sludge is shown in Figure 1. During the first feeding, the U(VI) removal efficiency reached 95.6% on the first day, and further increased to 98% on the sixth day, which indicated that the sulfate-reducing granular sludge performed well upon uranium exposure. On the first day of feeding II, the U(VI) removal efficiency decreased to 83.1% with a residual uranium concentration of 3.38 mg/L after adding fresh culture medium containing 20 mg/L uranium. Two days later, the U(VI) removal efficiency increased to above 95.1% and the residual uranium concentration decreased to less than 0.98 mg/L in the last four days of feeding II. During feedings III and IV, a similar phenomenon was observed with higher uranium removal efficiencies achieved on the third day after fresh uranium containing medium was added. When four feedings were given, 94% U(VI) removal efficiency was retained at the end of feeding IV.

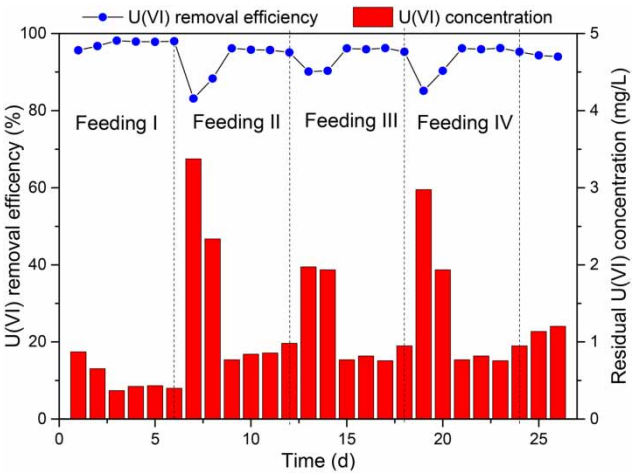

Figure 1 | Batch feeding of experiment on U(VI) removal by sulfate-reducing granular sludge exposed to U(VI) concentration of 20 mg/L.

Aida *et al.* (2010) showed that methanogenic granular sludge had a stable U(VI) removal capacity exceeding 99.8% using batch experiments. However, little information was given about the bacterial community of the methanogenic granular sludge. Beyenal *et al.* (2004) reported that U(VI) was immobilized by *Desulfovibrio desulfuricans* G20 (SRB). Hence, SRB were assumed to contribute to U(VI) removal in the present study. Our previous study (Chen *et al.* 2014) found that 93.72% of 10 mg/L U(VI) could be adsorbed by dried powder of crushed anaerobic granular sludge (2 g/L) within 60 min at pH 6.0 and 30 °C, wherein the equilibrium data fitted well with the pseudo-second-order model and Freundlich model ($R^2 > 0.99$). Hydroxy, amino and carbonyl groups were the major functional groups involved in the adsorption process. However, long-term performance studies are still lacking. In previous reports, the uranium concentrations were often less than 1 mg/L in the groundwater of contaminated sites (Hu *et al.* 2016; Wu *et al.* 2018). In this study, 92.4–97.4% of the supplied 20 mg/L U(VI) was removed during four feedings, which confirmed granular sludge can be used for the treatment of high concentrations of uranium-contaminated wastewater.

Microscopic observations of sulfate-reducing granular sludge

ESEM-EDS was used to reveal the morphology and elemental composition of the sulfate-reducing granular sludge upon uranium exposure for four successive feedings (Figure 2). Under 500-times magnification (Figure 2(a)), a honeycomb structure was observed. At 5,000-times magnification (Figure 2(b)), the major proportion of microorganisms had a short, rod shape. These bacteria retained a complete cell morphology, and the morphology was different from Zeng *et al.* (2016b), who observed cells with a spherical shape. The probable reason is the different microbial composition of the inoculum anaerobic granular sludge used in both studies. EDS showed that the major elements on the cell surface were O (19.45%), P (4.63%), and S (3.91%) (Figure S1(a) and (b) in Supplementary Materials). The U element ratio was 1.85%, which confirmed uranium was precipitated on the sulfate-reducing granular surface.

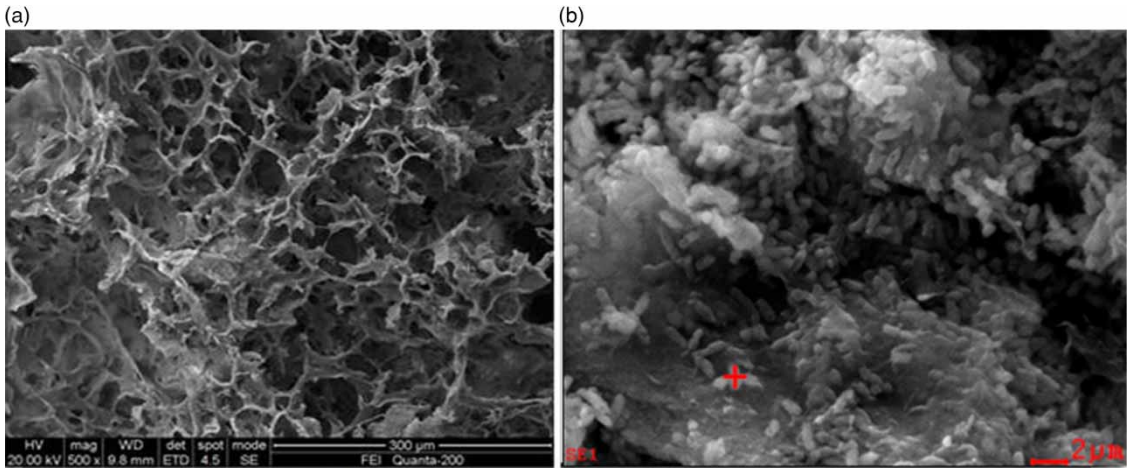

Figure 2 | SEM observation of granular sludge at (a) 500 and (b) 5,000 times magnification after exposure to U(VI) at a concentration of 20 mg/L with temperature of 27.5 (±2.5) °C.

Table 2 | Alpha index of microbial diversity in uranium-fed sulfate-reducing granular sludge

Sequencing number	Filtered number	Mean length (bp)	OTU number	Shannon index	ACE index	Chao1 index	Simpson index	Coverage
9,839	8,290	416.6	605	4.03	2,255.40	1,346.12	0.05	0.96

Microbial diversity assessment

After high through-put sequencing 9,839 raw sequences were obtained. Quality control measures were carried out for removing barcode sequences, terminal primers and low-quality sequences. Then 8,290 sequences were available for further analysis, with an average length of 416.6 bp, coverage of 0.96 and OTUs of 605 (Table 2). The abundance-rank curve showed that more than 97% of bacterial OTUs had less than 1% abundance in the total bacterial sequence (Figure 3(a)). Only 2.8% of the OTUs had an abundance >1%, with 17 of the total 605 OTUs; 97.2% of the OTUs had abundances less than 1%, showing an even bacterial distribution in the sulfate-reducing granular sludge.

Alpha diversity is an indicator for evaluating the richness and diversity within communities (Yan *et al.* 2016), which is expressed by indices of ACE, Chao1, Shannon and Simpson. The ACE and Chao1 indices were 2,255.40 and 1,346.12, respectively. The corresponding ACE and Chao1 rarefactions are listed in Figure 3(b). The above curves tendencies were quickly close to saturation, which indicated a high richness available in the sulfate-reducing granular sludge. The Shannon and Simpson indices were 4.03 and 0.05, respectively, and the corresponding curves leveled up quickly (Figure 3(c)), which indicated high community diversity. Rarefaction curves nearly reached a plateau, which also demonstrated appropriate sequencing depths (Suriya *et al.* 2017). Alpha indices and the rarefaction curves revealed high microbial abundance and diversity in granular sludge, which benefits resistance to toxicity in radioactive wastewater (Edberg *et al.* 2012).

Microbial community composition

Based on the taxonomic assignment at phylum level using the RDP database, a total of 20 phyla were identified, which contained 8,290 reads (sequences). The results revealed that seven bacterial phyla had a relative abundance of more than 0.5% (Figure 4(a)), with a cumulative contribution of 97.89%. The total proportions were 1.16% for the bacteria whose ratios were less than 0.5% separately, marked as 'Others' in Figure 4(a). The sulfate-reducing granular sludge comprised 0.93% unassigned sequences derived from bacterial phyla.

The Bacteroidetes phylum had the largest part in the composition, which accounted for 42.25% with 3,504

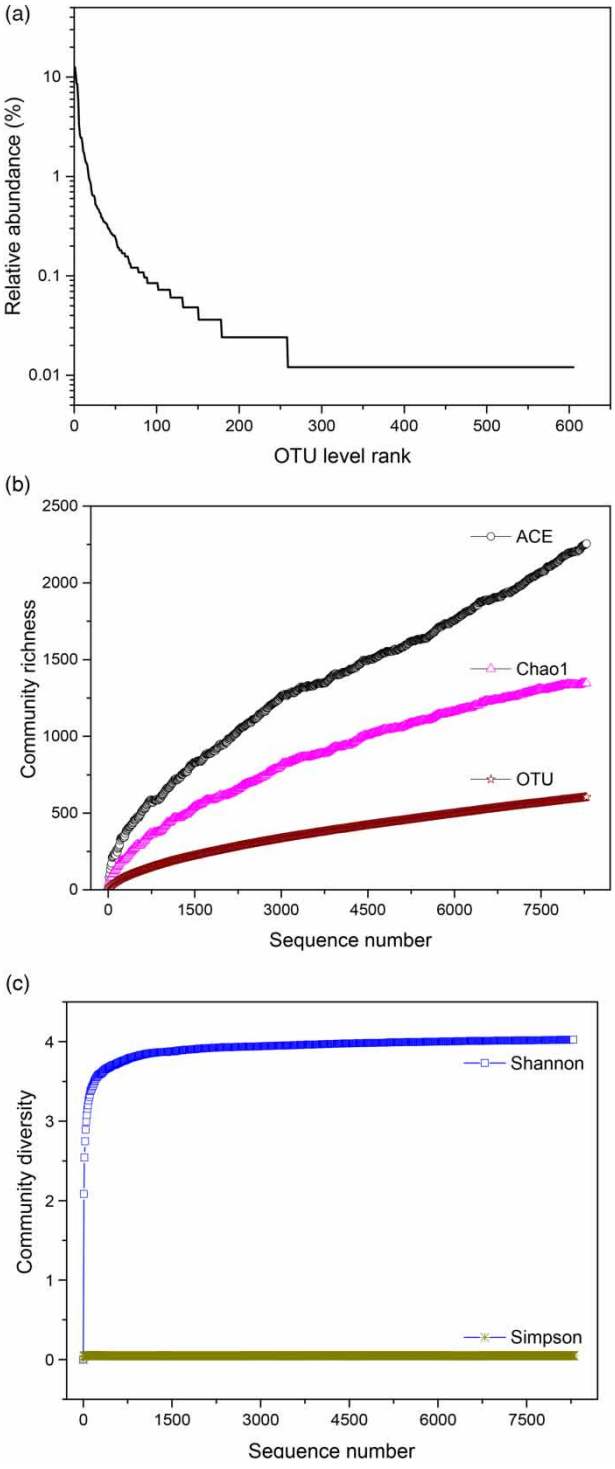

Figure 3 | (a) Abundance-rank, (b) community richness, and (c) diversity charts of bacterial community in sulfate-reducing granular sludge after exposure to 20 mg/L uranium.

reads. The second most dominant phylum was Firmicutes (26.55%) followed by Proteobacteria (20.66%). The top

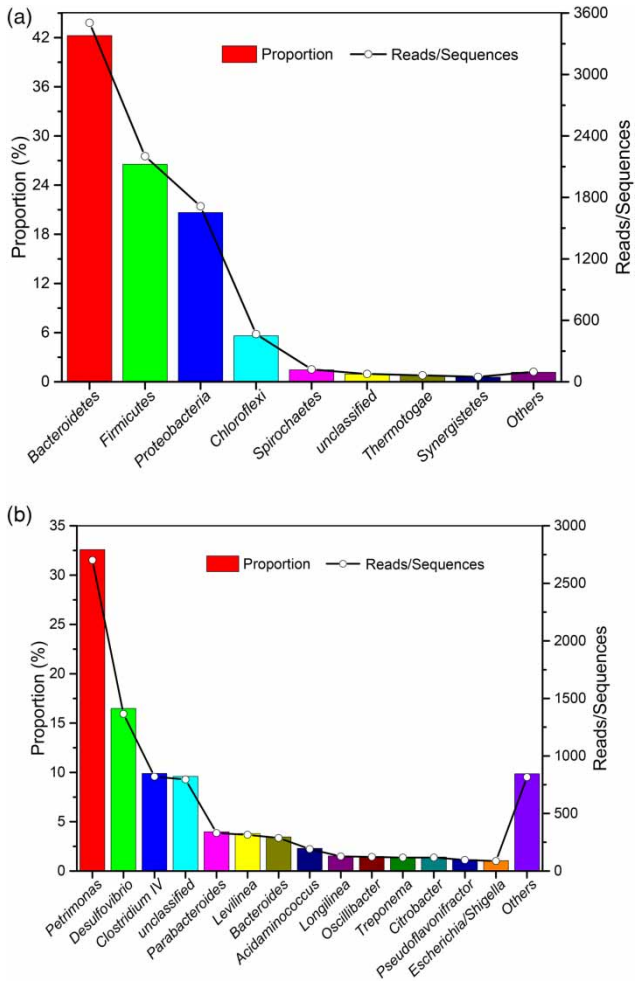

Figure 4 | Bacterial composition of anaerobic granular sludge after exposure to U(VI) at a concentration of 20 mg/L based on (a) phylum and (b) genus level.

three dominant phyla were the same as those present in a uranium-contaminated sediment derived from the sacred Cauvery River, India: Proteobacteria (47.49%), Bacteroidetes (22.36%) and Firmicutes (14.58%) (Suriya *et al.* 2017). Furthermore, a relatively high proportion of Chloroflexi (5.62%) was observed with 466 reads. Phyla of Spirochaetes, Thermotogae and Synergistetes also emerged, with a ratio of 1.46%, 0.77% and 0.58%, respectively.

The eubacterial community of the uranium-fed sulfate-reducing granular sludge was further analyzed at the genus level. There were 124 bacterial genera present, among which 13 bacterial genera were present with ratios more than 1% (Figure 4(b)). The total proportions were 9.86% for the bacterium whose ratio was less than 1% separately, marked as 'Others' in Figure 4(b). The sulfate-reducing

granular sludge also comprised 9.6% unclassified sequences derived from the bacterial genus level analysis.

Petrimonas was the most abundant genus in the sulfate-reducing granular sludge, which accounted for 32.59% with 2,702 reads (sequences). *Petrimonas* has been previously found to be an abundant genus in an anaerobic-oxic-sedimentation bioreactor for diazo dyes decolorization and mineralization (Zhu *et al.* 2018). *Desulfovibrio* was the second dominant genus, which occupied 16.48% with 1,366 sequences. In a previous report, Stylo *et al.* (2015) investigated the reduction and immobilization of uranium by *Desulfovibrio vulgaris* biofilms. They concluded that biotic reduction was the most important role for uranium immobilization. Compared with Zeng *et al.* (2016a) who found an abundance of *Desulfovibrio* of below 0.5% in the original anaerobic granular sludge, *Desulfovibrio* was a major population in the sulfate-reducing granular sludge after repeated uranium exposure. Therefore, the *Desulfovibrio* genus was assumed to be one of the functional bacteria in this study. *Clostridium* IV, which belongs to the Firmicutes phylum, accounted for 9.29% and included 820 sequences. *Clostridium* sp. has been reported to be effective in uranium bioreduction and precipitation in a synthesized ionic liquid aqueous solution (Zhang *et al.* 2013). Therefore, *Clostridium* IV could also contribute to the uranium removal within the sulfate-reducing granular sludge.

The *Parabacteroides* genus included 330 sequences and accounted for 3.98% of the total reads. The *Bacteroides* genus was represented by 287 sequences and accounted for 3.46% of the total reads. A small proportion of *Bacteroides* was previously found in a uranium mill tailings at Gittersee/Coschutz, Germany (Akhtar *et al.* 2017). *Bacteroides* sp. and *Parabacteroides* belong to the order Bacteroidales, which are typical fermentative bacteria or cellulose degraders. Maize straw could be degraded by these bacteria, thus providing suitable electron donors to the SRB (Zhang & Wang 2016). Therefore, it can be assumed that *Parabacteroides* and *Bacteroides* might degrade organic matter to provide electron donors for SRB (e.g. *Desulfovibrio*), promoting the uranium removal efficiency.

Levilinea and *Longilinea* accounted for 3.8% and 1.53% of the total bacteria, respectively. They belong to the Chloroflexi phylum (Yamada & Sekiguchi 2009), which were found to play important roles in degrading organic matter. *Acidaminococcus* included 191 sequences and occupied 2.3% of the total reads. *Oscillospira* and *Treponema* included 123 and 117 sequences, respectively, accounting for 1.48% and 1.41% of the total reads. The genera of *Acidaminococcus*, *Oscillospira* and *Treponema* have been previously found in rivers and marine ecosystems (Ming & Zhen 2014).

The *Citrobacter* genus was represented by 117 sequences and occupied 1.41% of the total reads. *Citrobacter* has been previously reported to be present in the Shihongtan, a sandstone-type uranium tailings in Xinjiang, China (Chen *et al.* 2012), which is also possibly a functional genus in the present study. *Pseudoflavonifractor* (1.16%) and *Escherichia/Shigella* (1.05%) were represented by 96 and 87 sequences, respectively. Both genera have not yet been found in uranium rich environments.

Thus, compared with the control anaerobic granular sludge by Zeng *et al.* (2016a), an abundance variation was evident at the genus level (Table 3). The abundance of *Desulfovibrio* and *Parabacteroides* genera increased significantly from below 1% to 16.48% and 3.98%, respectively. The abundance of the *Clostridium* genus also increased from 5.95% to 9.29%. Therefore, the above three genera were thought to contribute to uranium removal in the sulfate-reducing anaerobic granular sludge, which could be

Table 3 | Abundance variation between the uranium exposure granular sludge and the control sludge at genus level

Control	Ratio (%)	Uranium exposure granular sludge	Ratio (%)
Petrimonas	32.14	*Petrimonas*	32.59
Levilinea	11.42	*Desulfovibrio*	16.48
Erysipelotrichaceae	7.68	*Clostridium* IV	9.29
Paludibacter	7.17	*Parabacteroides*	3.98
Clostridium	5.95	*Levilinea*	3.8
Methanosaeta	3.44	*Bacteroides*	3.46
Syntrophomonas	2.75	*Acidaminococcus*	2.3
Longilinea	2.2	*Oscillospira*	1.48
Phascolarctobacterium	1.63	*Treponema*	1.41
Thermovirga	1	*Citrobacter*	1.41
		Pseudoflavonifractor	1.16
		Escherichia/Shigella	1.05

supported by previous reports (Zhang *et al.* 2013; Stylo *et al.* 2015; Zhang & Wang 2016). In the control, the abundance of *Levilinea* decreased dramatically from 11.42% to 3.8%. A dramatic change of abundance also happened for other bacterial genera upon uranium exposure (Table 3). The decrease in abundance of other genera was probably due to their sensitivity to uranium exposure.

The microbial community plays an important role in uranium recovery from contaminated sites, which is also beneficial for the control of uranium contamination. Abhilash & Pandey (2013) reported a 98.3% uranium recovery in 14 days for Turamdih uranium ore (Jharkhand, India) with an enriched culture of *Acidithiobacillus ferrooxidans*, using Fe(II) at pH 2.0, 35 °C and 20% (w/v) pulp density. Coral *et al.* (2018) evaluated microbial communities present in uranium in-situ recovery from acid mine drainage; the dominant bacteria were *Sulfobacillus* sp., *Leptospirillum* sp. and *Acidithiobacillus* sp.

Practical applications

The present study demonstrated that sulfate-reducing granular sludge has a strong uranium-resistant ability. The removal efficiency of 92.4–97.4% from a 20 mg/L U(VI) solution during the batch incubation was most likely due to the natural occurrence of U(VI)-reducing microorganisms in the inoculum sludge (Aida *et al.* 2010). Nancharaiah *et al.* (2006) reported almost complete removal of uranium by aerobic granules in the range of 6–100 mg/L in less than 1 h in an acidic pH range (1 to 6), which indicated the aerobic granular biomass was an effective biosorbent material for recovering/removing uranium from dilute nuclear waste. This study demonstrates the potential of utilizing anaerobic (sulfate-reducing) granular sludge for biological removal of U(VI) from contaminated run-off, groundwater and acid mine drainage.

Desulfovibrio, *Clostridium* IV, *Bacteroides* and *Citrobacter* were the dominant taxa in the present study. As there is little information about uranium removal by anaerobic granular sludge (Aida *et al.* 2010; Zhang *et al.* 2017a), it is necessary to investigate the functional microorganisms by metagenomics in future research, especially to evaluate the roles of Archaea in uranium removal.

CONCLUSIONS

Sulfate-reducing granular sludge exhibited high (92.4–97.4%) removal efficiencies during four feedings for a total of 24 days from synthetic 20 mg/L uranium acid mine drainage, which confirmed granular sludge can be used for the treatment of uranium-contaminated wastewater. Short rod-shaped bacteria occupied a major proportion of the microorganisms in the anaerobic granular sludge. The granular sludge had a high microbial abundance and diversity in spite of the high uranium concentration exposure. Microbial community analysis indicated seven bacterial phyla in the uranium-exposed sulfate-reducing granular sludge, with Bacteroidetes (42.25%), Firmicutes (26.55%) and Proteobacteria (20.66%) accounting for the top three phyla. Thirteen bacterial genera were dominant, wherein the taxa of *Desulfovibrio* (16.48%), *Clostridium* IV (9.29%), *Bacteroides* (3.46%) and *Citrobacter* (1.41%) were the indicators for uranium removal, with a cumulative proportion of 30.64%.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at https://dx.doi.org/10.2166/aqua.2019.027.

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