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Performance and dynamic characteristics of microbial communities in multi-stage anaerobic reactors treating gibberellin wastewater

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To treat gibberellin (GA) wastewater, a full-scale, multi-stage combined contact process was developed. This whole process employs three anaerobic reactors followed by micro-aerobic, anoxic/aeration and biological oxidation treatment. Pollutant removal results showed that the combined process could remove more than 98% of the chemical oxygen demand (COD), NH_3–N, and SO_4^{2-} pollutants because of the different microbial communities in each reactor. 16S rRNA gene sequencing was used to examine the microbial communities in the internal circulation (IC) and in the two up flow anaerobic sludge blanket (UASB) reactors, as well as to investigate the effect of sampling elevation on the microbial community. The results showed that *Firmicutes* and *Euryarchaeota* were the most dominant phyla at the bacterial and archaeal levels, respectively. High levels of *Synergistaceae_uncultured* were detected in IC and UASB1. *Chloroflexi_uncultured* was the dominant genus of bacterial communities within UASB2, and *Methanosaeta* was the dominant genus of archaeal communities. Principal coordinates analysis (PCoA) revealed variations among the microbial communities in 9 samples, and Venn analysis showed different operational taxonomic units (OTUs) among samples collected at various elevations within the three anaerobic reactors. However, partial Mantel tests indicated no significant correlation between the microbial community structure and elevation in the three anaerobic reactors.

[**Key words:** Gibberellin wastewater; Illumina MiSeq sequencing; Bacterial community; Archaeal community; Anaerobic reactor]

Gibberellins (GAs) are highly efficient plant hormones used to promote internode elongation and cambial activity in stems (1) which also participate in the regulation of plant growth and development (2). However, large-scale production of GAs generates a large volume of wastewater which has poor biodegradability owing to its high sulfate concentration, organic content, and NH_3–N concentration as well as its associated strong bacterial toxicity. Therefore, they cannot completely decompose in sewage treatment plants, and discharged directly into water bodies would cause catastrophic harm to the environment. To address with this problem, a new combined, full-scale process combining three anaerobic reactors (IC + UASB1 + UASB2) followed by micro-aerobic, anoxic/aeration (A/O) and biological contact oxidation is used to treat GA wastewater (3).

The main procedure used to treat GA wastewater employs upflow anaerobic sludge blankets (UASB) and internal circulation (IC) anaerobic reactors which contain anaerobic microorganisms to decompose various types of organic matter (OM) until a stable state is reached. Both reactors have been newly developed and operate at low energy consumption with a high organic load. They also produce lower volumes of surplus sludge than other reactors and have been studied extensively for their ability to treat wastewater. For example, Watari et al. (4) employed a UASB reactor to treat natural rubber processing wastewater and found that 92.7% of the chemical oxygen demand (COD) was removed. In addition, Na et al. (5) used a UASB to degrade phenol; their phenol degradation and CH_4 removal rates were 79.0% and 75.3%, respectively. Moreover, Xu et al. (6) reported that the IC removed 85% of the COD in brewery wastewater treatment, and Deng et al. (7) found that the IC reactor removed approximately 80% of the COD with an organic loading rate of 6–7 kg COD/(m^3 day) when treating raw swine wastewater. IC and UASB reactors enable a high pollutant removal rate owing to the large numbers of microorganisms immobilized within their systems. Although the microbial species and microbial diversity were investigated in the A/O reactor during the treatment of GA wastewater (3), they have not been analysed thus far in the anaerobic reactors. Therefore, the current study analyses the species diversity and community structures at various elevations within three anaerobic reactors including an IC reactor and two UASB reactors.

In this study, high-throughput sequencing technology was used to analyse microbial communities in the three anaerobic reactors. Developed approximately 12 years ago, this technology can sequence millions of DNA molecules simultaneously. Moreover, it demonstrates excellent analytical performance and is convenient to use; as such, this technology is widely used to study microbial communities in wastewater treatment systems (8–11). In addition, Illumina MiSeq sequencing was used to study species diversity and community structures at various elevations within the reactors. This popular, high-throughput method of analytical sequencing has been wildly applied to wastewater treatment analysis in recent

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years. For example, Cui et al. (12) identified dominant members of electrode microbial communities as *Enterobacter*, *Desulfovibrio*, and *Enterococcus* in an up-flow bioelectrochemical system using Illumina MiSeq sequencing to treat azo dye wastewater.

The main objectives of the present study are to investigate the relationship between the nutrition removal effect and microorganisms in the anaerobic reactors and to analyse the microbial community structure sampled at various elevations within the three anaerobic reactors. The pollutant removal effects are observed using three nutrient indices, and the microbial community diversity in the anaerobic reactors are studied using Illumina MiSeq sequencing.

MATERIALS AND METHODS

Raw water GA wastewater was obtained from a biochemical company located in Jiangxi province, China (115.478584°E, 27.900485°N) which produces approximately 110 t of GAs per year, accounting for more than 39% of global GA production. GA wastewater consists of three main types of wastewater: raffinate wastewater, high-concentration wastewater, and comprehensive wastewater. In addition, it contains high concentrations of sulfates and organic compounds and varying amounts of NH_3—N, and it is strongly acidic. The process used to produce GAs is shown in Fig. S1.

Anaerobic system start-up The seed sludge for IC, UASB1, and UASB2 was procured from an anaerobic reactor in a wastewater treatment plant. IC, UASB1, and UASB2 are cylindrical in shape and have diameters of 8 m, 8 m, and 15 m, respectively; heights of 15 m, 12 m, and 12 m, respectively; effective volumes of 376.8 m^3, 301.44 m^3, and 565.2 m^3, respectively; hydraulic retention times of 37.68 h, 21.3 h, and 7.49 h, respectively, and amounts of inoculation sludge within each of 20 t, 20 t, and 40 t, respectively. In the earlier stages of the set-up, low-concentration wastewater was used to domesticate the sludge and maintain an intermittent inflow. Following a period of acclimatisation, the reactors were continuously fed, and the influent concentration was gradually increased. The amount of effluent volatile fatty acid (VFA) determined whether the reactors should be continuously fed. When the VFA concentrations were lower than 180 mg/L, the reactors were continuously fed; if the level remained below this threshold, the concentration of influent wastewater was raised. The reactor set-up was considered to be successful when the effluent index remained stable, the sludge activity was high, and the sludge was dense. The design loadings of IC, UASB1, and UASB2 were 13,000 mg/L, 5000 mg/L, and 3000 mg/L, respectively. The reactors were set up during the spring, when the temperature was suitable for sludge cultivation. Some of the reactor conditions needed to be controlled in the set-up stage; for example, the pH should be 6.5—7.5. A schematic diagram of the treatment system is shown in Fig. S2.

Sample collection All three anaerobic reactors were sampled on April 26, 2016. Samples from the IC, UASB1, and UASB2 reactors were designated as sets A, B, and C, respectively. Three samples per each reactor were taken at elevations of 2 m, 4 m, and 6 m and were designated sets 1, 2, and 3, respectively. Therefore, 9 samples were taken in total. Each sample was dispensed into a 10-mL sterile Eppendorf tube and was centrifuged at 14,000 $\times g$ for 10 min. The supernatant was then decanted, and the pellets were stored at −20°C prior to analysis.

DNA extraction and polymerase chain reaction amplification Microbial DNA was extracted from samples by using the E.Z.N.A. anaerobic sludge DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. The V4—V5 region of the bacteria 16S ribosomal RNA gene was amplified by polymerase chain reaction (PCR) at 95°C for 2 min followed by 25 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min. This process used primers 515F 5′-barcode-GTGCCAGCMGCCGCGG)-3′ and 907R 5′-CCGTCAATTCMTTTRAGTTT-3′, where the barcode is an eight-base sequence unique to each sample. The PCR reactions were performed in triplicate using 20 μL of mixture comprising 4 μL of 5 × FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu polymerase, and 10 ng of template DNA.

Illumina MiSeq sequencing Amplicons were extracted from 2% agarose gels and were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and were then quantified using QuantiFluor™-ST (Promega, Madison, WI, USA). The purified amplicons were pooled in an equimolar and paired-end sequence (2 × 250) on an Illumina MiSeq platform according to standard protocols.

Processing of sequencing data Raw fastq files were demultiplexed and then quality-filtered using QIIME (version 1.17) by employing the following criteria: (i) 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window, and truncated reads shorter than 50 bp were discarded; (ii) exact barcode matching was employed: 2 nucleotide mismatch in primer matching, and reads containing ambiguous characters were removed; (iii) only sequences that overlapped by longer than 10 bp were assembled according to their overlap sequence, and reads that could not be assembled were discarded.

Operational taxonomic units (OTUs) were clustered with a 97% similarity cut-off using UPARSE (version 7.1, http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analysed by an RDP classifier (http://rdp.cme.msu.edu/) against the SILVA (SSU115) 16S rRNA database using a confidence threshold of 70% (13).

Statistical analysis The Shannon—Wiener index was used to assess microbial diversity, and principal coordinates analysis (PCoA) was performed to examine variations among the microbial communities of the 9 samples. Furthermore, OTU Venn analysis was used to present the shared and unique characteristics of the different communities, and partial Mantel tests were applied to identify the relationship between microbial communities and the elevation from which they were sampled within the three anaerobic reactors. These statistical analyses were performed using the VEGAN package in R (v.2.15.1; http://www.r-project.org/).

Nucleotide sequence accession number Raw reads were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the accession number SRP090195.

RESULTS AND DISCUSSION

System performance Table 1 shows the COD, NH_3—N, and SS removal rate within the three anaerobic reactors and presents results related to the combined process. The average removal rates of COD in IC, UASB1, and UASB2 were 42.93%, 46.89%, and 44.41%, respectively. The effluent concentrations of NH_3—N in the three reactors were higher than those within the influent. This result could have occurred because the nitrogenous organic compound was transformed into ammonia nitrogen by ammonifiers in the three anaerobic reactors, and the ammonia nitrogen produced by the organics thus exceeded the ammonia nitrogen demand required for anaerobic bacteria growth. Additionally, the average removal rates of SO_4^{2-} in IC, UASB1, and UASB2 were 49.05%, 36.86%, and 65.11%, respectively, and the average removal rates of COD, NH_3—N, and SO_4^{2-} for the total combined process were 99.51%, 98.61%, and 99.28%, respectively.

Biodiversity comparison As shown in Table 2, there were 15,343 effective bacteria reads and 21,459 effective archaea reads

TABLE 1. Removal effect of three indices.

Index	COD	NH_3—N	SO_4^{2-}
Influent of raffinate wastewater (mg/L)	17199.21 ± 5178.76	194.56 ± 83.51	11019.7 ± 1763.9
Influent of IC (mg/L)	6354.67 ± 1650.67	290.23 ± 24.23	4394 ± 1179
Effluent of IC (mg/L) (influent of UASB1)	3660 ± 1308	318 ± 11	2196 ± 376
Removal rate of IC (%)	42.93 ± 7.48	—	49.05 ± 5.66
Effluent of UASB1 (mg/L)	1909.33 ± 533.33	361.13 ± 23.13	1389.67 ± 391.67
Removal rate of UASB1 (%)	46.89 ± 5.4	—	36.86 ± 16.67
Influent of UASB2 (mg/L)	835.07 ± 207.87	132.33 ± 15.33	208 ± 38
Effluent of UASB2 (mg/L)	460.73 ± 78.53	146 ± 21	67.40 ± 15.40
Removal rate of UASB2 (%)	44.41 ± 5.35	—	65.11 ± 18.48
Effluent of final effluent (mg/L)	81.64 ± 9.64	2.47 ± 0.95	77.98 ± 9.16
Final effluent removal rate (%)	99.51	98.61	99.28

COD, chemical oxygen demand; IC, internal circulation reactor; UASB, upflow anaerobic sludge blanket reactor.

TABLE 2. Diversity indices of the 9 samples.

Diversity indices	Sample	Sequence	OTUs	Shannon–Wiener
Bacteria diversity indices	A1	15343	598	4.41
	A2	15343	615	4.61
	A3	15343	634	4.75
	B1	15343	682	4.73
	B2	15343	706	4.52
	B3	15343	648	4.5
	C1	15343	690	5.23
	C2	15343	669	5.21
	C3	15343	660	5.22
Archaea diversity indices	A1	21459	26	0.6
	A2	21459	27	0.69
	A3	21459	21	0.51
	B1	21459	31	0.38
	B2	21459	32	0.47
	B3	21459	29	0.32
	C1	21459	24	1.16
	C2	21459	25	1.31
	C3	21459	23	0.93

for the nine anaerobic samples. The average bacteria and archaea OTUs of the samples were 655.8 and 26.44, respectively. The diversity of the bacterial community was identified using the Shannon–Wiener index (14): the bacteria and archaea Shannon–Wiener of all samples were 4.41–5.23 and 0.32–1.31 with average indices of 4.80 and 0.71, respectively. These results indicate that the microbial communities in the UASB2 reactor were more diverse than those in the IC and UASB1 reactors at bacterial and archaeal levels. This may be related to the higher concentrations of SO_4^{2-} in IC and UASB1, which can inhibit microorganism growth.

Bacteria community analysis As shown in Fig. 1A, Firmicutes was the most predominant phylum, accounting for 15.1%–43.9% of the bacterial sequences. The other dominant phyla were Bacteroidetes (17.3%–22.8%), Synergistetes (1.8%–20.7%), Proteobacteria (3.7%–14.2%), Chloroflexi (0.68%–21.1%), and Spirochaetae (2.2%–8.4%). Slight differences in the abundance of some of the dominant phyla were noted among the reactors. For example Synergistetes dominated in IC and UASB1 but was relatively lower in abundance in the UASB2 reactor. However, Firmicutes, which is related to hydrolytic processes for anthraquinone reactive dye degradation (15), was found to be the dominant species in all samples. This result is consistent with those of previous studies that found Firmicutes to be the dominant phylum in anaerobic reactors (16–18). Bacteroidetes has been reported as the dominant phylum in sludge samples (19–23) and is responsible for the degradation of complex OM in continuous azo dye degradation systems (24). Notably, Chloroflexi was dominant only in the UASB2 reactor, although a previous study found it to be the most predominant phylum (25,26). Proteobacteria was one of the dominant phyla in this study, which is in agreement with the results of previous studies (20,22). Although the phylum Synergistetes dominated only in the IC and UASB1 reactors, it was found to be a dominant phylum in anaerobic sludge samples in previous research (17,27). Furthermore, the evidence obtained by Novak et al. (28) and Lefebvre et al. (29) showed that Synergistetes is generally found to dominate in UASB reactors treating brewery wastewater (28) and tannery wastewater (29). Synergistetes can use amino acids made available from the breakdown of proteins and peptides by other organisms to provide short-chain fatty acids and sulfates such as methanogens and sulfate-reducing bacteria for terminal degraders (30). In addition, Firmicutes and Synergistetes can degrade carbohydrates and proteins via hydrolytic enzymes (31).

Fig. 1B shows relative bacterial community abundances at a genus level. *Synergistaceae_uncultured*, *Selenomonadales_uncultured*, *vadinBC27_wastewater-sludge_group*, *Macellibacteroides*, and an unclassified genus were found to be the predominant genera in the IC and UASB1 reactors. The other dominant genera were *Christensenellaceae_R-7_group*, *Prevotella_7*, and *Fastidiosipila* in the IC reactor and *Sphaerochaeta* and *Desulfomicrobium* in UASB1. *Macellibacteroides* can metabolise monosaccharides and disaccharides, where the main fermentation products involved are acetate, butyrate, and isobutyrate (32). The genus *Fastidiosipila* of the phylum Firmicutes was previously found to be responsible for the fermentation process in an anaerobic dynamic membrane bioreactor (33) and it also found to be the predominant genus in a digested municipal solid waste sample 4 (16). *Sphaerochaeta* sp. was detected in hydrogen-producing microbial electrolysis cells (34), and some *Sphaerochaeta* species have been found to ferment xylose to formate, acetate, and ethanol (35). It should be mentioned that sulfate-reducing bacteria *Desulfomicrobium* was found to dominate only in the UASB1 reactor. *Desulfomicrobium* can use hydrogen or simple organic compounds such as formate, ethanol, lactate, pyruvate, malate, and fumarate as electron donors for sulfate respiration (36). Although it was not the dominant genus in the IC reactor, its relative abundance therein was greater than that in UASB2. Therefore, the proportion of *Desulfomicrobium* in the UASB2 reactor was far lower than that within IC and UASB1, which could be the result of low SO_4^{2-} concentration in the UASB2 reactor. The dominant genus in UASB2 also differed from that within the other two anaerobic reactors. The dominant genera in UASB2 were *Christensenellaceae_R-7_group*, *Anaerolineaceae_uncultured*, *Candidate_division_WS6_norank*, *Chloroflexi_uncultured*, *Bacteroidetes_vadinHA17_norank*, *Smithella*, *Hyd24-12_norank*, and an

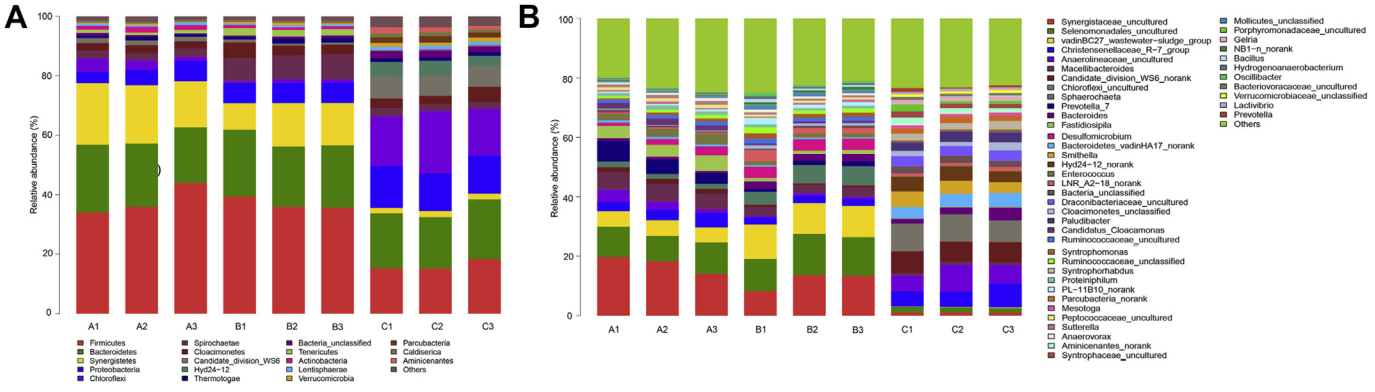

FIG. 1. Abundances of different bacterial-level phyla in the nine anaerobic sludge samples in terms of percentage of total effective bacterial sequences within a sample. The taxonomic compositions of bacterial communities are shown at the (A) phyla and (B) genus levels in each sample.

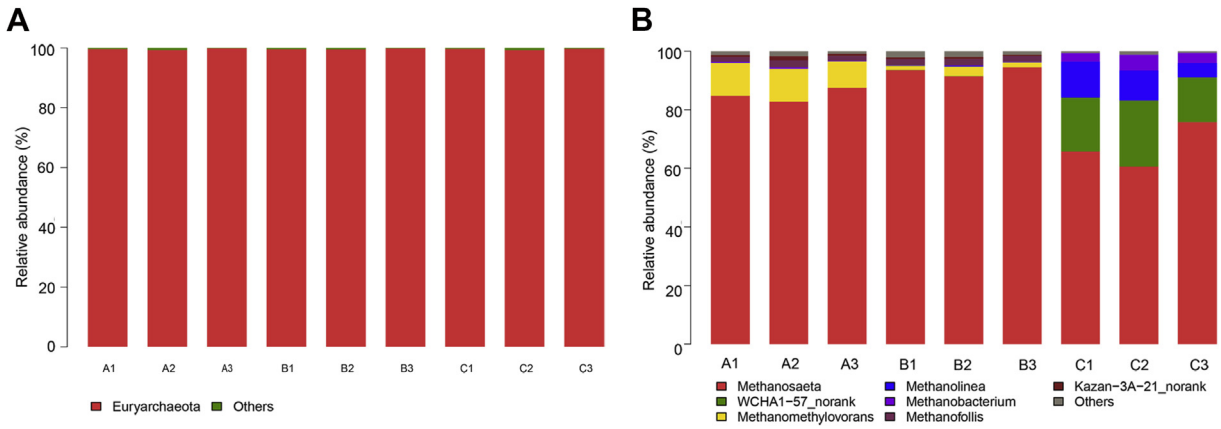

FIG. 2. Relative abundances of different archaeal (A) phyla and (B) genera in the nine anaerobic sludge samples.

unclassified genus. Genus *Smithella* from the phylum Proteobacteria was previously determined to be the dominant genus in submerged anaerobic membrane bioreactors operating at a mean solid residence time of 300 days (37). It has also been found that the propionic acid oxidizing bacteria *Smithella* likely play an important role in sulfur oxidation in UASB reactors (38) and in secondary fermentation steps within methanogenic bioreactors (39).

The 15 most abundant genera in each sample were selected for a total of 48 genera for all 9 samples, and their abundances were compared with those in other samples (Fig. 3A). *Synergistaceae_uncultured* and *Selenomonadales_uncultured* were the genera with the largest abundance in the IC and UASB reactors. Genus *vadinBC27_wastewater-sludge_group* was enriched in the UASB1 reactor, and *Christensenellaceae_R-7_group*, *Anaerolineaceae_uncultured*, *Candidate_division_WS6_norank*, and *Chloroflexi_uncultured* were also present in high proportions within the UASB2 system. A significant decrease was noted in genera *Prevotella_7* and *Smithella* in the UASB2 reactor, and *Christensenellaceae_R-7_group* and *Desulfomicrobium* showed an increasing trend in the IC reactor with respect to an increase in elevation.

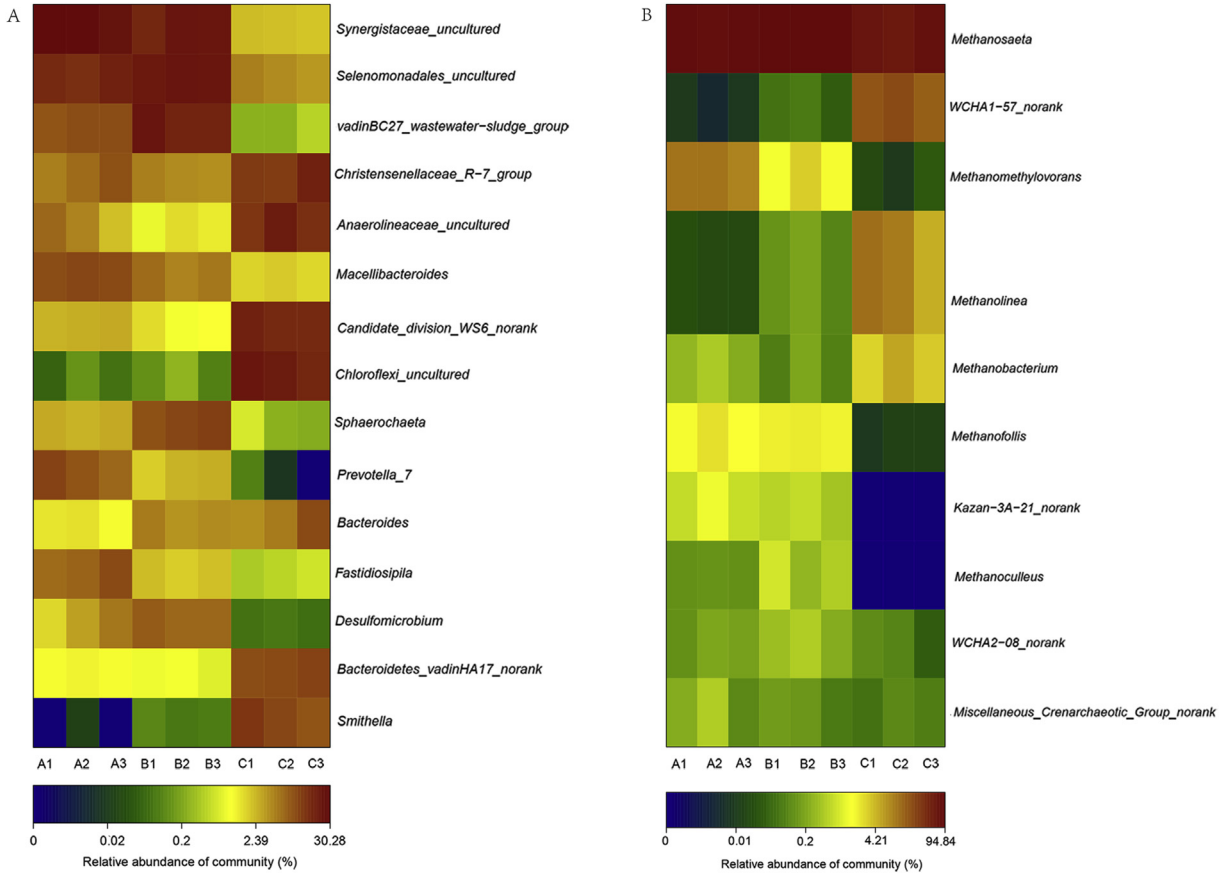

FIG. 3. (A) Heat map of the top 15 bacterial genera in each sample. A total of 48 genera were selected from 9 samples; the colour intensity in each panel shows the genus percentage within the sample, as indicated by the colour key. (B) Heat map of the top 10 archaeal genera in each sample.

A similarity multi-tree was constructed using phylotype dissimilarities and was concatenated with a 97% sequence identity in each sample. Three different clusters were revealed: group 1 with all A samples, group 2 with all B samples, and group 3 with all C samples. The similarities and differences among the nine bacterial community structures are shown in Fig. 4A. The samples collected in the same reactor were normally clustered at the bacterial level.

Archaeal community analysis Euryarchaeota was the dominant archaeal phylum in this study, accounting for 99.23%–99.84% of the total archaeal population (Fig. 2A). No significant changes were detected in its proportion among the three anaerobic reactors.

As shown in Fig. 2B, eight different groups were present at the genus taxonomic rank: *Methanosaeta* (60.6%–94.4%), *WCHA1-57_norank* (0.0047%–22.5%), *Methanomethylovorans* (0.0093%–11.2%), *Methanolinea* (0.0186%–12.3%), *Methanobacterium* (0.089%–5.4%), *Methanofollis* (0.0093%–2.4%), *Kazan-3A-21_norank* (0–1.4%), and an unclassified genus. *Methanosaeta* belongs to the phylum Euryarchaeota and was the most dominant methane-producing archaeal genus within samples. It has also been reported to be dominant in previous research (40,41) and is known to contribute to methanogenesis from acetate and a specialist that uses acetate only (23). In addition, it is distinctly related to biogas production and extracellular polymeric substance (EPS) degradation (42). Of the aforementioned genera, *WCHA1-57_norank* predominated in the UASB2 reactor, whereas its abundance was almost undetected in IC and UASB1. Methanomethylovorans was one of the dominant phyla in the IC reactor. It is not only a key methanogen responsible for converting tetramethylammonium hydroxide and trimethylamine to methane but is also important for methanogenic tetramethylammonium hydroxide biodegradation (43) and can utilise acetate as a substrate to produce CH_4 (44). Of the aforementioned genera, *Methanolinea* dominated only in UASB2, although some studies have reported Methanolinea to be the dominant phylum in anaerobic reactors (45,46). However, the dominance of *Methanosaeta* and *Methanolinea* species in UASB2 indicates that both acetolactic and hydrogenotrophic methanogenesis occurred therein. *Methanolinea* can convert CO_2, CO, or formate to methane using H_2 (47), and it is likely that key members form a syntrophic propionate-degrading consortium (48). Furthermore, *Methanobacterium* of the phylum Euryarchaeota is a hydrogenotrophic methanogen that can utilise formate, carbon dioxide, and hydrogen to produce methane (23).

As shown in Fig. 3B, *Methanosaeta* was the most dominant archaeal genus in all samples, and *WCHA1-57_norank* was the second most dominant genus in the UASB reactor. Other archaeal genera were present in lower proportions in all 9 samples, and *Kazan-3A-21_norank* and *Methanoculleus* were undetected even in the UASB2 samples. *Methanobacterium* was one of the dominant genera in UASB2. It is one of the most often observed hydrogenotrophic methanogens in typical anaerobic digestion processes for treating organic wastes and has been detected also in phenol-degrading enrichment cultures (49,50). However, *Methanolinea* showed a decreasing trend in the UASB2 reactor with an increase in elevation.

Fig. 4B shows the similarities and differences in structure among the nine archaeal communities. The IC samples also clustered with the UASB1 samples at both bacterial and archaeal levels.

Relationship between system performance and microbial community As illustrated in Fig. 5, PCoA analysis suggested obvious differences between the bacterial and archaeal communities in the three reactors. The first and second axes explain 90.94% and 7.09% cumulative percentage variances of the species in the bacterial communities, respectively. In total, a 98.03% variance of species can be explained by the two axes at the bacterial level. At the archaeal level, principal coordinates 1 and 2 explain 92.99% and 4.27% of the total community variations, respectively. The contents of IC, UASB1, and UASB2 reactor samples differed significantly from each other with respect to the bacterial and archaeal communities. Samples B2 and B3 were clustered and were strongly distinct from B1 at the bacterial level, which indicates greater similarities in the bacterial communities in B2 and B3. A1 aggregated with A2, and both were distinctly separate from A3. At the archaeal level, B1 and B2 were clustered and distinct from B2.

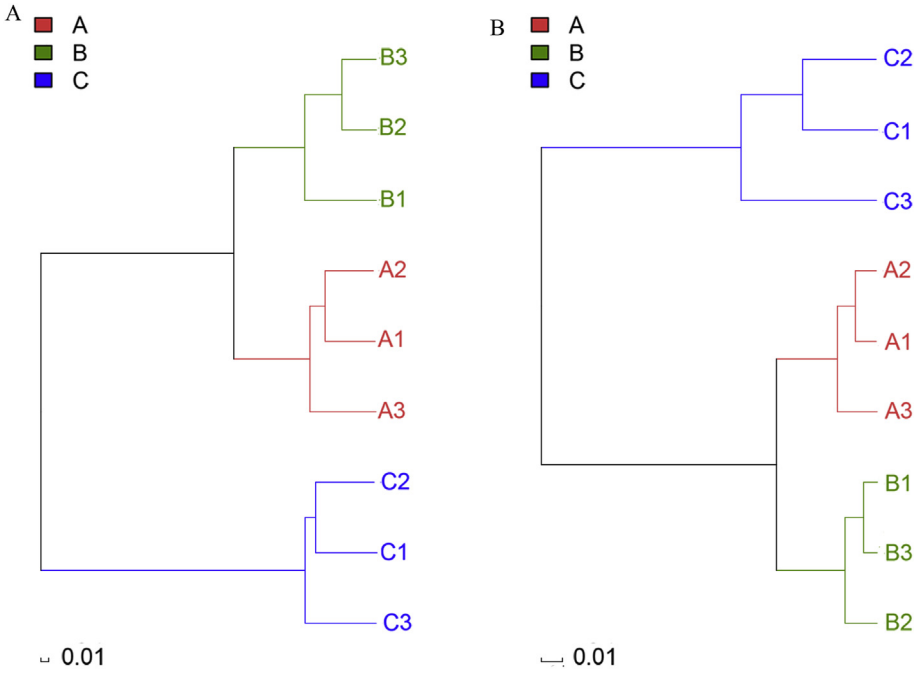

FIG. 4. Similarity multi-tree at the (A) bacterial and (B) archaeal levels.

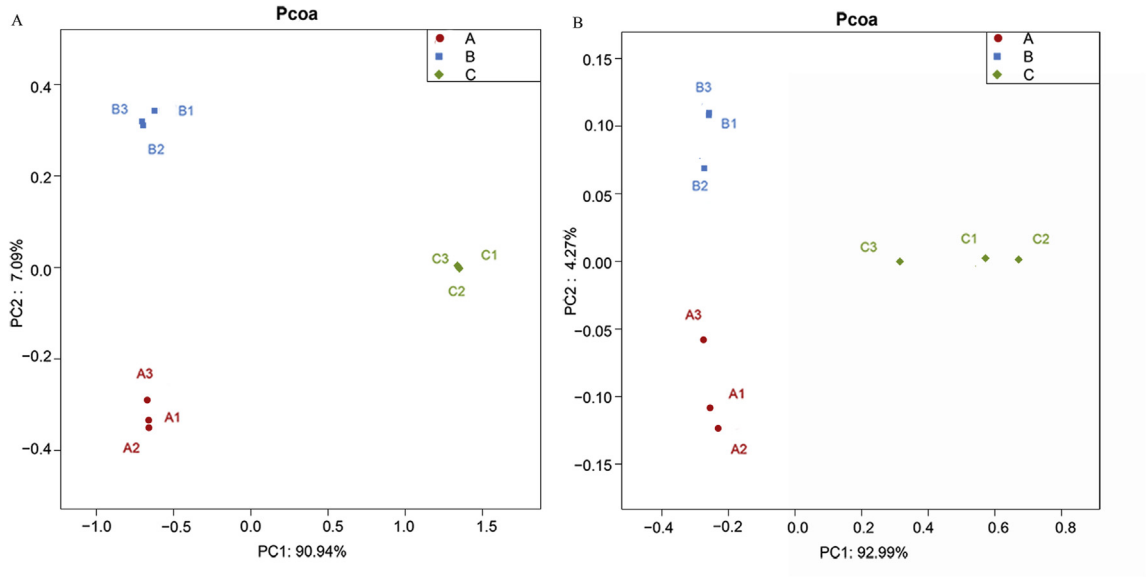

FIG. 5. Principal coordinates analysis (PCoA) of microbial communities from samples based on of Illumina MiSeq sequencing: (A) PCoA of bacterial communities; (B) OTU of archaeal communities.

To determine the OTU differences at the bacterial and archaeal levels at different elevations in the IC, UASB1, and UASB2 reactors, Venn analysis of the OTU composition was conducted as shown in Fig. 6. Fig. 6A shows that 486 OTUs were shared by all IC samples. Of the 526 OTUs detected in the UASB1 samples, 552 were found in the UASB2 samples at the bacterial level. There were 103, 134, and 127 unique OTUs in the IC, UASB1, and UASB2 reactors, respectively, which indicates differences in samples collected within the same reactor. As shown in Fig. 6B, the sums of observed OTUs in the IC, UASB1, and UASB2 communities were 31, 37, and 26, respectively, and 19, 25, and 22 OTUs were shared in samples collected in IC, UASB1, and UASB2, respectively. Furthermore, seven unique OTUs were found in the IC and UASB1 reactors and all OTUs in C1 were shared with C2. The unique OTU numbers also indicate differences among samples collected at different elevations within the same reactor. Partial Mantel tests (Table 3) conducted to investigate the correlation between the microbial community structure and elevation revealed that elevation was not significantly corrected ($p > 0.05$) with microbial community structure at either the bacterial or archaeal level.

In conclusion, the combined process of full-scale, multi-stage anaerobic reactors with micro-aerobic + anoxic/aeration (A/O) + biological contact oxidation removed more than 98% of COD, SO_4^{2-}, and NH_3—N, and the final effluent attained the quality demands of

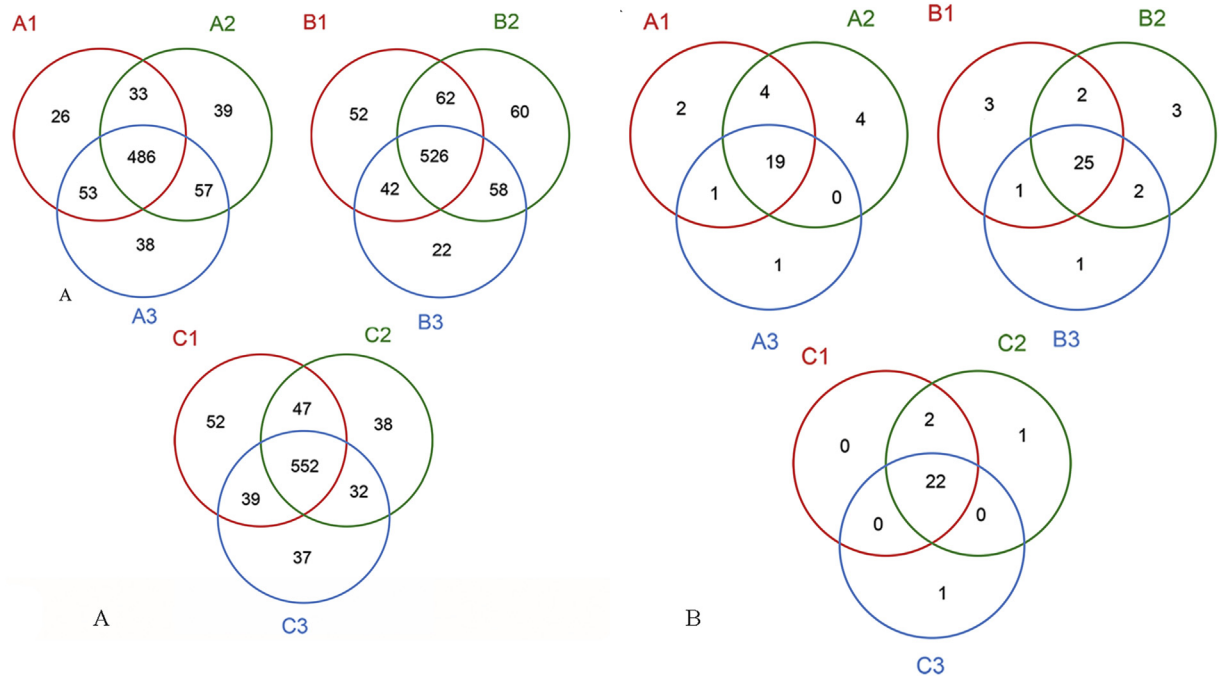

FIG. 6. OTU Venn analysis for the different reactors at the (A) bacterial and (B) archaeal levels.

TABLE 3. Relationship between elevation effect and microbial community in the 9 samples.

Microbial community	Reactor	r^a_M	P value
Bacterial community	A	−0.866	1
	B	0	0.66667
	C	0.866	0.33333
Archaeal community	A	0	0.66667
	B	0	0.66667
	C	0	0.66667

the Integrated Wastewater Discharge Standard in China (GB8978-1996, grade 1). Furthermore, the elevation effect was not significantly corrected with the microbial community structure at either the bacterial or archaeal level. The dominant species within the three anaerobic reactors were determined in this study. Pollutant removal was highly dependent on the function of the microbial communities within these species.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2018.05.017.

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