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Illumina sequencing reveals bacterial community shift and its role in a full-scale A^2O sewage treatment process at low temperatures

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ABSTRACT

Activated sludge and sewage were sampled from the municipal sewage treatment plant of Lanzhou (Northwest China) between November 2014 and January 2015 to investigate bacterial community composition and shift across the full-scale anaerobic–anoxic–oxic (A^2O) process at low temperatures (≤12°C) using Illumina MiSeq sequencing. Operational taxonomic unit analysis showed that bacterial community richness changed greatly throughout the A^2O process but the bacterial community diversity had no significant difference during the A^2O process. Bacterial community composition was similar between different tanks at the phylum level, although the percentage of dominant phylum had changed significantly. However, at the genus level, the bacterial community composition varied widely across the A^2O tanks. Redundancy analysis (RDA) and hierarchical cluster analysis demonstrated that the bacterial community shifts between different tanks were related to process-based operational conditions and environmental changes, including dissolved oxygen, NH_4^+, bichromate chemical oxygen demand, and total phosphorus concentration. RDA also revealed that nitrogen and phosphorus removal mainly occurred in the anaerobic, anoxic, and oxic tanks and that the efficiency of nutrient removal correlated with bacterial community composition, abundance, and environmental variables, respectively. The results of this study may contribute to an understanding of microbial ecology in wastewater treatment systems of high taxonomic rank and explore the environmental parameters which are involved in the selection of the different bacterial taxa at low temperatures.

Keywords: Municipal sewage treatment; A^2O process; Bacterial community composition; Low temperature; Illumina sequencing

1. Introduction

The anaerobic/anoxic/oxic (A^2O) process is characterized by the simultaneous removal of nitrogen and phosphorus [1,2]. The A^2O process had been widely used in large-scale municipal sewage treatment plants (MSTPs) in China [3]. Conventional activated sludge processing is dependent on the metabolism of microorganisms, which can remove organic and inorganic pollutants or transform them into non-toxic compounds [4]. Efficiency and stability of the A^2O process are entirely dependent on microbial communities and their metabolic activity. However, there is currently a poor understanding of the microbial ecology of sewage treatment processing in both full- and pilot-scale systems [5]. Additionally, few studies have been conducted to investigate the bacterial community composition and shift due to A^2O processing at low temperatures.

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High-throughput sequencing has been successfully employed to profile microbial communities in various environmental samples of high taxonomic rank [6,7], including human gut microbial gene catalogues [8], bacterial virulence in drinking water [9], microbial communities in swine sewage anaerobic lagoons and traditional Chinese medicine wastewater [10,11], and bacterial diversity in activated sludge [4,12]. After that, it is essential to elucidate microbial composition and the function of nutrient removing in a specific process. In this study, activated sludge and sewage samples were collected from six tanks spanning the A^2O process for analysis by 16S rRNA gene using Illumina MiSeq sequencing. The objective of this study was to reveal microbial mechanisms involved in sewage treatment by analyzing the bacterial abundance, diversity, composition, and variability across the A^2O process, as well as to explore the correlation between the dominant bacterial community and removal of nitrogen and phosphorus. This work was a part of microbial database for activated sludge wastewater treatment plants and will help in understanding the role of microbial ecology in wastewater treatment systems and explore the environmental parameters involved in the selection of the different bacterial taxa at low temperatures.

2. Materials and methods

2.1. Plant overview and A^2O process performance

The Lanzhou Qilihe-Anning municipal sewage treatment plant (QA-MSTP; 36°05′15″N, 103°44′35″E), located in Lanzhou city in Northwest China, was selected as a sampling site. Local altitude is 1,540 m and mean annual temperature of approximately 10°C. The sewage treatment capacity exceeds 1.6×10^5 m^3/d. The influent was mainly from municipal sewage and a small amount of industrial sewage, and influent quality is shown in Table 1. The quality of effluent water reached Chinese first-B discharge standards. The A^2O process applied in QA-MSTP is composed of a primary settling tank (influent), three parallel series of anaerobic tanks, an anoxic tank and an oxic tank, a secondary sedimentation tank, and an effluent unit. The process is illustrated in Fig. 1.

Table 1
The values of indices of the influent and effluent sewage in winter 2014

Pollutant index		BOD_5 (mg/L)	COD_{Cr} (mg/L)	SS (mg/L)	TN (mg/L)	TP (mg/L)	DO (mg/L)	pH	*T* (°C)
Influent	Range	150–290	350–610	110–200	38–59	2.5–5.9	0.1–0.2	6.6–7.8	9–13
(IN sample)	Mean	210	430	160	53	3.6	0.1	7.3	10
Effluent	Range	8.2–16	34–51	15–18	4.6–12	0.53–1.1	0.3–0.7	6.3–7.1	10–14
(EF sample)	Mean	10.5	41	17	9.2	0.41	0.6	6.7	8
Removal efficiency (%)		95	90.5	89	79	89	–	–	–

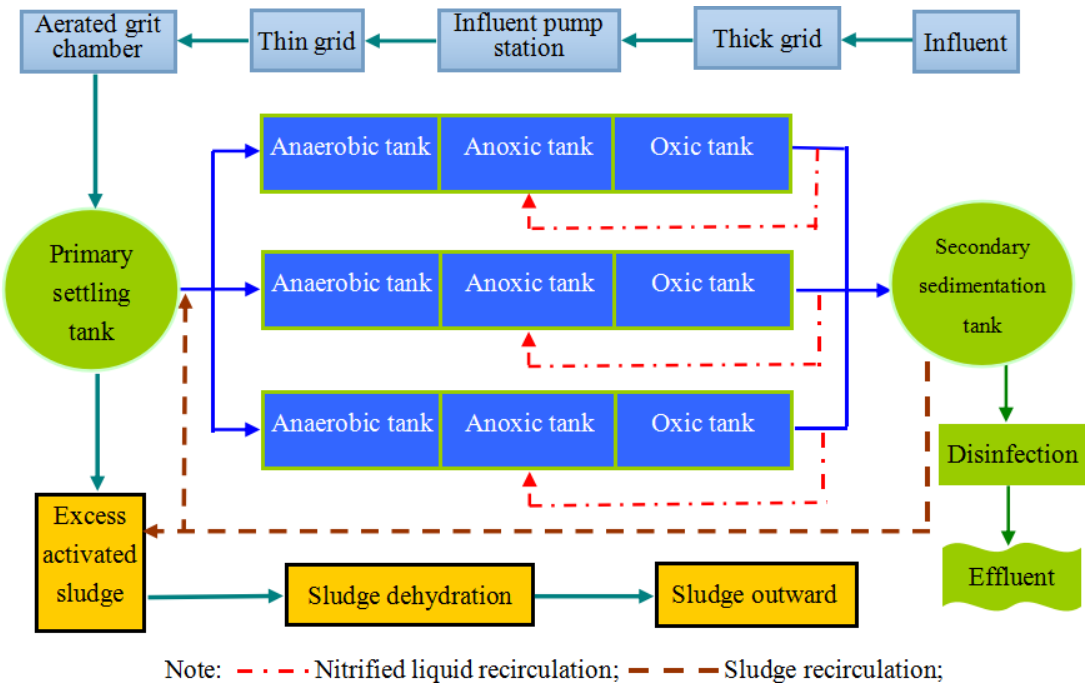

Fig. 1. The full-scale A^2O process working in the Lanzhou Qilihe-Anning municipal wastewater treatment plant.

2.2. Activated sludge and sewage sampling and DNA extraction

From November 2014 to January 2015, activated sludge and sewage (approximately 3,000 mL) were sampled monthly at a depth of 30 cm below the surface of the primary settling tank (influent, IN), anaerobic tanks (A1), anoxic tank (A2), oxic tank (O), secondary sedimentation tank (SS), and the effluent unit (EF). All samples were kept in an ice box and sent to laboratory immediately. The samples were centrifuged at 14,000*g* for 10 min in refrigerated centrifuge (4°C), the supernatant was decanted, and at least 5 g of precipitate was weighed for DNA extraction. Genomic DNA was extracted using the method described in the Omega Mag-Bind® Soil DNA Kit Protocol (http://omegabiotek.com/store/product/soil-dna-kit/). The concentration and purity of raw DNA were assayed using a ND-2000 spectrophotometer (Nanodrop Inc., Wilmington, DE, USA). The integrity of DNA was tested by electrophoresis in a 0.8% (w/v) agarose gel applying 120 V for 30 min (Protein Simple Inc., AlphaImager HP, USA).

2.3. Sewage quality measurements

The sewage quality, including biochemical oxygen demand (BOD), bichromate chemical oxygen demand (COD_{Cr}), suspended solid (SS), ammonium (NH_4^+–N), total nitrogen (TN), and total phosphorus (TP), was measured according to the standard methods [13]. Dissolved oxygen (DO) and pH were measured using a multiparameter pH/ORP meter (WTW-340i, YSI Inc., Yellow Springs, OH).

2.4. PCR and Illumina MiSeq sequencing

The bacterial 16S rRNA gene was amplified with primers 520F (5′-AYTGGGYDTAAAGNG-3′) and 802R (5′-TACNVGGGTATCTAATCC-3′) targeting the hypervariable V4 region [12]. Amplicon product sequencing was performed on the Illumina MiSeq sequencing platform in Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). Illumina sequencing raw data was deposited in the NCBI Sequence Read Archive database (Accession Number: PRJNA357044).

2.5. Bioinformatic analysis

After sequencing, the raw paired-end FASTQ format sequences underwent quality filtering using Quantitative Insights into Microbial Ecology (QIIME, Version 1.7.0) to remove any low quality or ambiguous reads [14]. Then, the sequences were clustered into operational taxonomic units (OTUs) with a sequence similarity threshold of 97% using a Ribosomal Database Project (RDP) classifier [15]. Richness and diversity indices, including OTUs, Chao1 estimator, abundance-based coverage estimator (ACE), and Shannon and Simpson indices, were calculated using the Mothur software [16]. Redundancy analysis (RDA) and heatmap figures were completed by Vegan packages in R (http://www.R-project.org/).

3. Results and discussion

3.1. General performance of the full-scale A^2O process

The full-scale A^2O process has been operating for more than 6 months in a stable and good condition in the QA-MSTP (Table 2). The sludge return ratio, the mixed liquid recycle ratio, mixed liquid SSs, and sludge retention time (SRT) were set at 50%, 200%, 2.5–4 g/L, and 5 d, respectively. The nutrient removal rate of BOD_5, COD_{Cr}, TN, TP, and SS reached to 95%, 90.5%, 79%, 89%, and 89%, respectively (Table 1). This result conformed to the first grade B standards of municipal sewage discharge standard in China (GB8978-2002). Average data of these indices in different tanks between November 2014 and January 2015 are summarized in Table 3. The removal rate of TN and NH_4^+–N was lower than 80% at 10°C ± 2°C. All index values decreased gradually across the A^2O processing tanks except TP, which increased dramatically in the anaerobic tank and decreased in subsequent tanks (Table 3). This result was likely due to accumulation of phosphorous in the anaerobic tank and the subsequent release of the phosphorous in the following tanks. These results indicate that nitrogen and phosphorus were efficiently removed by the microbial communities present in the A^2O tanks at low temperature, and the microorganisms were well adapted to the operation mode, local sewage quality, and natural conditions of the A^2O process.

3.2. Bacterial richness and diversity across the full-scale A^2O process

Illumina MiSeq sequencing generated a total of 383,518 effective reads (Table S1). As shown in Table 4, a total of 11, 691 OTUs were acquired after homologous alignment and

Table 2
Monthly average operational parameters in the aeration tank in QA-MSTP from October 2014 to March 2015

Time	*T* (°C)	DO (mg/L)	Flow rate (1,000 m^3/d)	COD_{Cr} (mg/L)	NH_4^+–N (mg/L)	TN (mg/L)	TP (mg/L)	SRT (d)
Oct 2014	14	2.9	116	137	14	16	2.9	5.9
Nov 2014	12	2.3	107	149	14	23	3.3	6.0
Dec 2014	10	2.2	98	143	16	30	4.3	5.2
Jan 2015	9	2.4	96	169	18	24	3.2	5.1
Feb 2015	8	1.9	104	152	17	26	3.2	5.0
Mar 2015	10	2.1	97	154	15	25	3.5	5.9

Note: T, sewage temperature; DO, dissolved oxygen; COD_{Cr}, bichromate chemical oxygen demand; TP, total phosphorus; and SRT, sludge retention time.

Table 3
The average values of nutrient indices in the full-scale A²O process tanks during November 2014 to January 2015

Pollutant index	Samples in different tanks						Removal efficiency (%)
	IN	A1	A2	O	SS	EF	
BOD_5 (mg/L)	210	158	51	17	12	10.5 (20)	95
COD_{Cr} (mg/L)	430	327	203	154	43	41 (60)	90.5
TN (mg/L)	53	42.0	29.3	25.7	14.4	11.2 (15)	79
TP (mg/L)	3.6	7.9	6.1	3.6	1.1	0.41 (1.0)	89
NH_4^+–N (mg/L)	45.5	38.2	31.6	16.3	10.5	9.6 (15)	79
DO (mg/L)	0.10	0.10	0.40	2.3	1.0	0.6	–
T (°C)	10	11	11	10	9	8	–

Note: Influent tank (IN), anaerobic tank (A1), anoxic tank (A2), oxic tank (O), secondary sedimentation tank (SS), and effluent tank (EF).

Table 4
The bacterial richness and diversity indices across the full-scale A²O process

Sample	Reads	OTUs	Good's coverage (%)	Chao1	ACE	Shannon	Simpson
IN	54,047	1,973	89	11,175	21,017	5.8273	0.0104
A1	103,538	2,736	90	15,058	24,003	5.8303	0.0165
A2	64,852	1,678	88	10,453	17,616	5.7930	0.0102
O	113,467	2,799	90	15,651	24,927	6.1607	0.0082
SS	24,378	1,402	89	7,203	11,722	5.9671	0.0093
EF	23,236	1,103	90	6,986	10,802	5.8912	0.0078

Note: The operational taxonomic units (OTUs) were defined based on the sequence similarity of 97%. α = 3% Chao1 estimator, abundance-based coverage estimator (ACE).

clustering based on a sequence similarity of 97%, Chao1 and ACE richness indices, and Shannon and Simpson diversity indices with cut off levels of 3%. In this experiment, coverage values varied from 88% to 90%, indicating that all the DNA libraries sufficiently reflected the structure of microbial communities [17]. Based on OTU numbers, the bacteria community was the richest in the O tank (2,799 OTUs), followed by the A1 (2,736 OTUs), IN (1,973 OTUs), A2 (1,678 OTUs), SS (1,402 OTUs), and least rich in the EF tank (1,103 OTUs). Similarly, enrichment of the Chao1 index was highest in the O (15,651) and A1 tanks (15,058), intermediate in the IN (11,175) and A2 tanks (10,453), and low in the SS (7,203) and EF tanks (6,986). ACE index exhibited a similar variation trend as well (Table 4). These results demonstrate that bacterial community richness varied greatly across the A²O process. This dynamic change could be ascribed to different operating conditions in different tanks, for example, DO, BOD, and SRT (Table 3). The bacterial community enrichment at low temperature was relatively low when compared with the activated sludge (AS) samples from tropical areas (average sewage temperature 22°C), such as Hong Kong, Guangzhou in Southern China [12]. Additionally, the abundance of bacterial community in the full-scale A²O process was significantly higher than the pilot A²O process in which there were 506, 495, and 508 OTUs in the anaerobic, anoxic, and oxic chambers, respectively [18]. The Shannon and Simpson diversity indices are typically used to evaluate the diversity of the bacterial community within a specific treatment system [14]. In this study, no significant differences in Shannon and Simpson indices were observed between different tanks, indicating that the diversity of the bacterial community had no significant changes throughout the A²O process.

3.3. Bacterial community composition across the full-scale A²O process

Bacterial community composition at the phylum level is shown in Fig. 2. The predominant OTUs were classified to seven phyla, including *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, *Chloroflexi*, *Verrucomicrobia*, and *TM7*. The total percentage of these dominant phyla ranged from 89% to 95% in each tank (Table S2). *Proteobacteria* was the most dominant bacterial community across the full-scale A²O process, followed by *Bacteroidetes*, *Actinobacteria*, and *Firmicutes*. These results demonstrate a similar bacterial community composition to that seen in activated sludge and sewage from moderate temperature climates [4,12,19,20]. The phylum composition of the dominant community was similar between different tanks, although the percentage of the dominant phylum had changed a great deal across the A²O process. To date, many results agree that a variety of factors, such as influent characteristics and operational conditions (pH, DO, and *T* (°C)), might cause these variations [21–24].

At the genus level, 4,188 genera were classified by the RDP classifier across all samples. The top 20 dominant genera were selected and listed in Table S3. The major genera (>1%) in each tank are summarized in Fig. 3. The composition of predominant bacterial genera varied widely across the

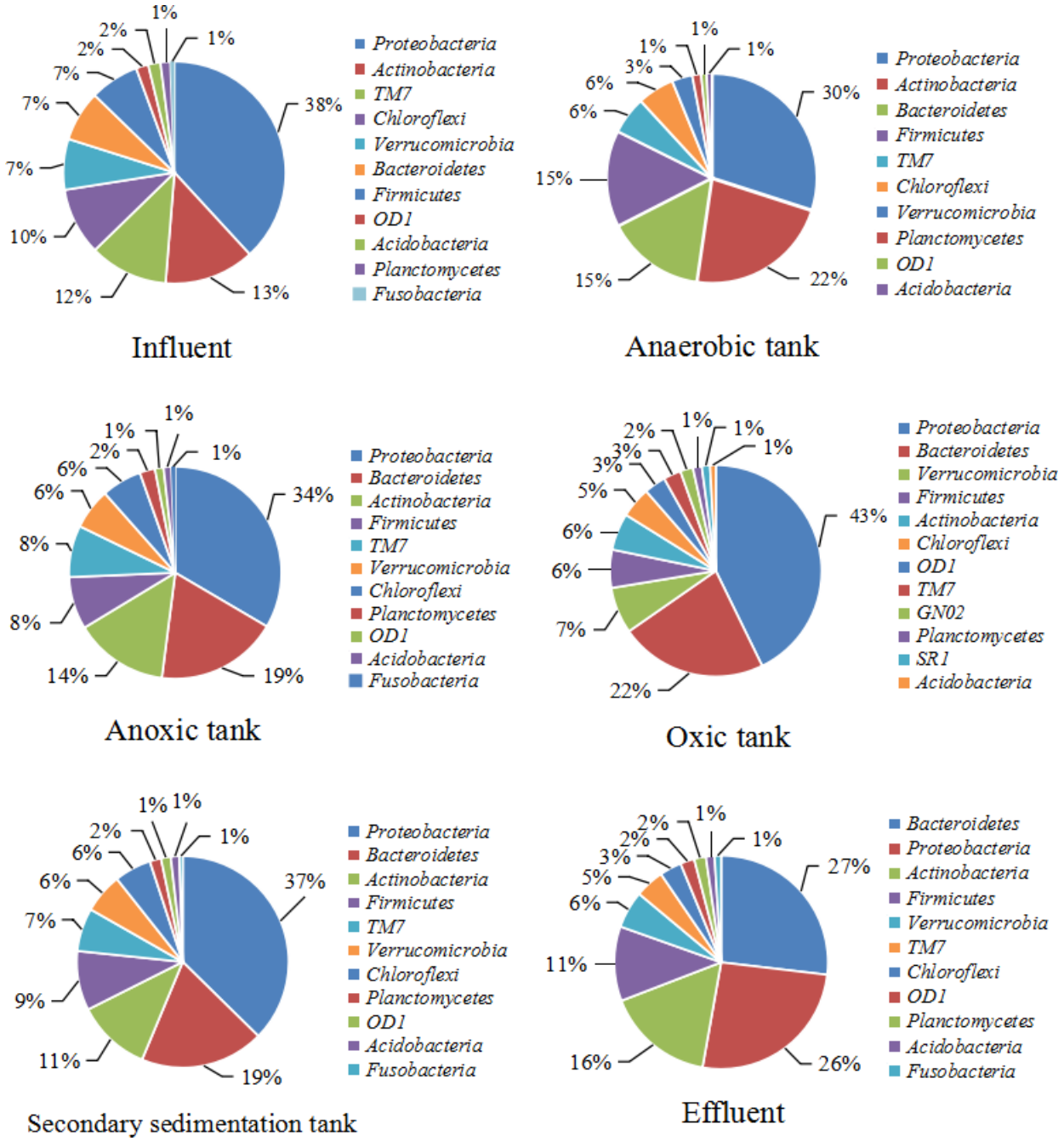

Fig. 2. Pie chart showing the dominant bacterial community composition in the full-scale A²O tanks at the phylum level.

A²O tanks. In the IN tank, *TM7* (23%) was the most abundant, followed by *Luteolibacter* (16%). It should be noted that *TM7* was less than 1% in the other five tanks. This could be due to an inability of *TM7* to survive in sewage after separation from a human host at lower temperature because of its unique epibiotic and parasitic lifestyle [25,26]. *Candidatus* Microthrix was the most abundant genus (38%) in the A1 tank, but it rapidly dropped to 9%, 5%, 11%, and 15% in the A2, O, SS, and EF tanks, respectively. *Candidatus* Microthrix is a key polyphosphate-accumulating organism responsible for phosphorus removal and are commonly enriched in the anaerobic tank [27,28]. Moreover, *Candidatus* Microthrix decreased in abundance in O tank, indicating its proliferation was suppressed by higher DO concentration.

Interestingly, *Saprospira* dominated in the A2 (21%), O (22%), SS (30%), and EF tank (33%), but not in the A1 and IN tank. *Saprospira* is frequently observed in aquatic environments and AS [9], having a demonstrated ability to remove complex organic compounds through aerobic heterotrophic metabolism [29]. In addition, members of *Saprospiraceae* are also known to prey on and lyse bacterial cells as a nutrient source [30]. Thus, *Saprospira* populations may play an important role in regulation of populations of *Cyanobacteria* and algal populations in aquatic environments.

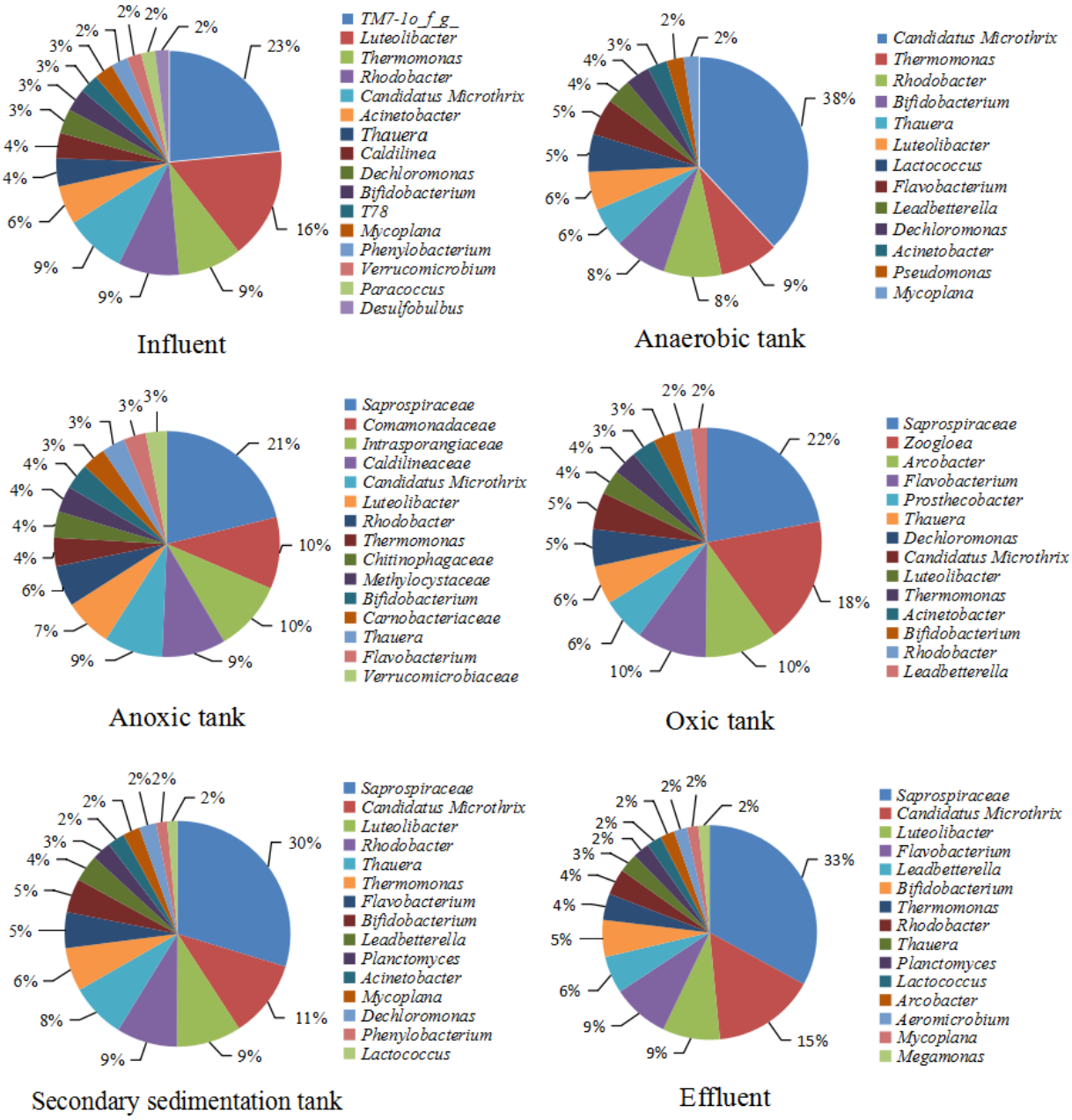

Fig. 3. Pie chart showing the dominant bacterial community composition in the full-scale A²O tanks at the genus level.

Other major genera, including *Thermomonas* (9% in both IN and A1), *Thauera* (6% in both A1 and O), *Rhodobacter* (9% in IN, 8% in A1, 6% in A2, and 9% in SS), and *Flavobacterium* (5% in A1, 10% in O, and 9% in EF), were detected in the tanks as specified. Among them, *Thauera* is known to cause denitrification and biodegradation of organic compounds and is frequently detected in sewage treatment bioreactors [31,32]. *Rhodobacter* was remarkably metabolically diverse, as it can grow heterotrophically via fermentation, aerobic, and anaerobic respiration.

The composition of core genera varied widely in different tanks and was consistent with biodegradation actions of the different tanks, such as release of phosphorus in the A1 tank, nitrogen removal in the A2 tank, and nitration and absorption of phosphorus in the O tank. Additionally, the bacterial community composition at low temperatures was different from the AS and sewage sample collected at higher temperatures [12]. Specific microbial community compositions developed due to specific environmental factors, including temperature, DO, and TN, which are considered to be more crucial factors in shaping microbial community structure than other potential factors [5,33,34].

3.4. Bacterial community shift across the full-scale A²O process

A hierarchical cluster analysis was performed to understand the bacterial community shift throughout the A²O process and the results are shown in Fig. 4. The results

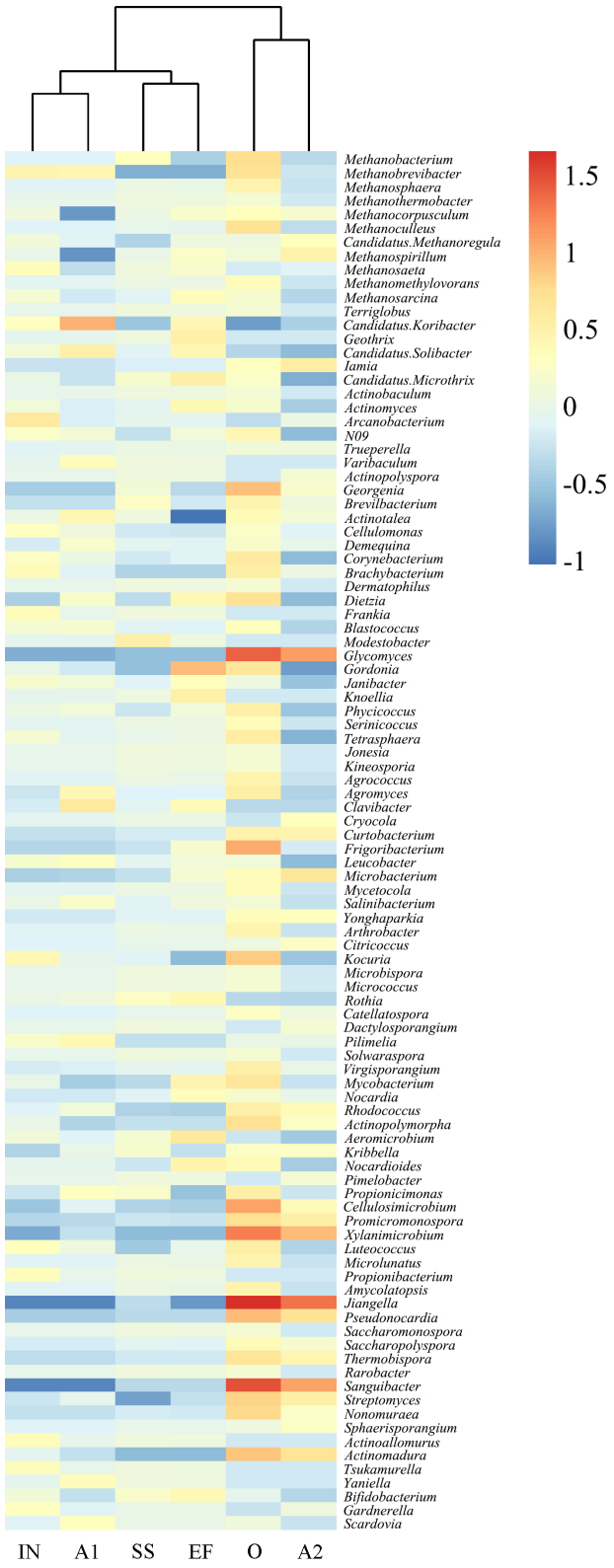

Fig. 4. Hierarchical cluster analysis of bacterial communities in the full-scale A²O process. OTUs were ordered by genus (top 100). The bacterial phylogenetic tree was calculated using the R (heatmap) method (IN: influent tank, A1: anaerobic tank, A2: anoxic tank, O: oxic tank, SS: secondary sedimentation tank, and EF: effluent tank).

reveal that the samples in IN and A1, O and A2, SS and EF were clustered together, indicating that each pair had a similar abundance of the bacterial community. However, the abundance and composition of top 100 genera shifted in the different A²O tanks. As shown in Fig. 4, the core genera in the O tank were the most abundant, perhaps due to the bacterial community expansion from recirculated AS when there were enough nutrients (recirculated nitrified liquid) and oxygen present (DO = 3.3 in the O tank) [35]. In the SS and EF tanks, the bacterial community decreased dramatically due to the sedimentation, decay, and lysis of bacterial cells, resulting in disinfection. Comparison of dynamic changes among the top 100 genera across A²O tanks indicated that the shift of the bacterial community was possibly associated with DO, nitrate recirculation ratios and the return sludge ratio at low temperatures. These results agree with the report that shifts of microbial communities in different tanks are affected by process-based operational conditions [36,37].

A total of 22,109 OTUs were clustered in the A1, A2, O, SS, and EF tanks; among them, 941 OTUs were shared in all tanks, accounting for 4.26% of the total OTUs (Fig. 5). There were 3,019, 1,606, 3,102, 790, and 739 unique OTUs in the A1, A2, O, SS, and EF tanks, respectively, accounting for 41.86% of the total OTUs. There were 3,322 (A1), 2,497 (A2), 3,256 (O), 1,879 (SS), and 1,899 (EF) shared OTUs, accounting for 58.14% of the total OTUs. The majority of the shared OTUs were assigned to phyla *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*. The data analysis demonstrated that most of the bacterial community structure was stable across the A²O process, although there were significant changes in number and abundance of the bacterial community compositions in each tank.

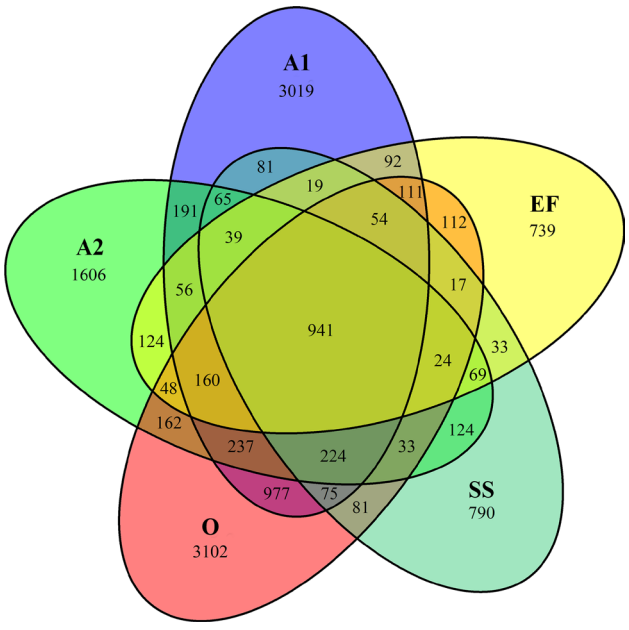

Fig. 5. Venn diagram of the bacterial genera in the A²O process (A1: anaerobic tank, A2: anoxic tank, O: oxic tank, SS: secondary sedimentation tank, and EF: effluent tank).

3.5. Correlation between bacterial community and nutrient removal

The RDA was performed to demonstrate the correlation between nutrient removal and the dominant bacterial community using R software (Version 3.3.0). As shown in Fig. 6, the bacterial communities were clustered in the A1, O, and A2 tanks at the phylum (Fig. 6(a)) and genus levels (Fig. 6(b)). The dominant bacterial phyla, such as *Firmicutes* and *Actinobacteria*, *Proteobacteria* and *Bacteroidetes*, were positively correlated, but a negative correlation was observed between *Candidatus* Microthrix and other genera. Shifts in the dominant bacterial community were closely related to NH_4^+, COD, and TP concentration at low temperature. Additionally, the similarity of bacterial community between different tanks was indicated according to sample distance (Fig. 6), where the bacterial communities in the A1 and O tanks were dissimilar when compared with other samples, but high similarity was exhibited between the IN, A2, SS, and EF tanks. On the other hand, the removal of NH_4^+ and COD, TN and BOD, and TP mainly occurred in the A1, A2, and O tanks, respectively, however, the bacterial community in the IN, SS, and EF tank had little to do with nutrient removal. These results indicate that the shifts in the bacterial community across the A²O process were affected by TP and NH_4^+, demonstrating that the efficiency of nutrient removal was correlated to bacterial community composition, abundance and environmental variables.

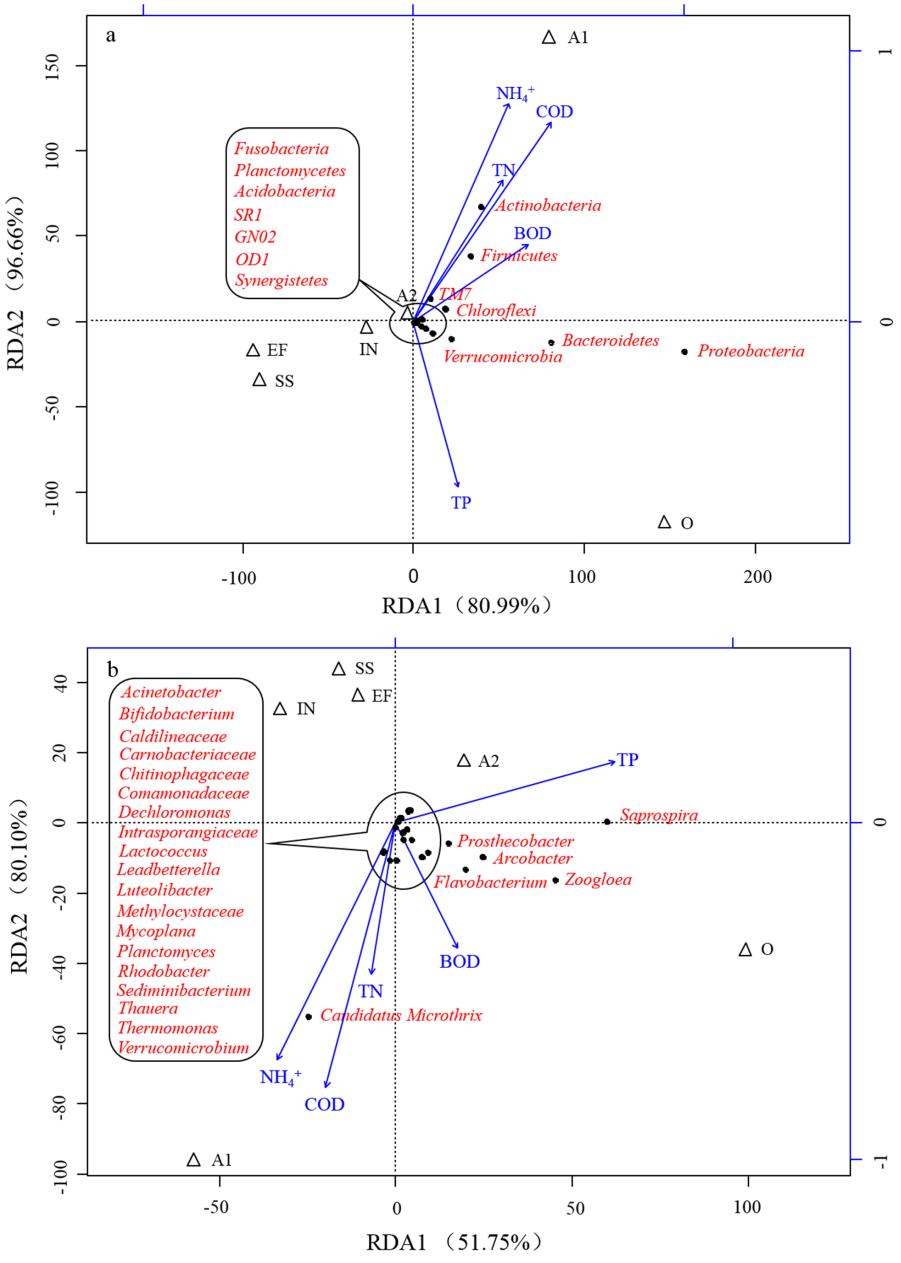

Fig. 6. RDA ordination diagram showing the correlation between nutrient removal and bacterial communities at the phylum level (a) and the genus level (b).

Furthermore, the presence of *Actinobacteria*, *Firmicutes*, and *TM7* was closely related to nitrogen removal, and *Candidatus* Microthrix was related to TN and NH_4^+ removal. Therefore, it may be concluded that *Candidatus* Microthrix is not only responsible for phosphorus removal but also helpful in the removal of TN and NH_4^+ [38]. *Saprospira*, *Zoogloea*, and *Arcobacter* were positively correlated with TP removal (Fig. 6). Despite the relevance of these dominant bacterial communities to the removal of nutrients, there was no doubt that a variety of bacterial communities were involved in the biodegradation of nitrogen and phosphorus in the specific A^2O system.

4. Conclusions

In conclusion, the bacterial community structure, diversity, and variation during sewage treatment in an A^2O process were investigated. The microbial community was composed of predominant phyla, including *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, *Chloroflexi*, *Verrucomicrobia*, and *TM7*. The bacterial diversity showed non-significant changes across the A^2O process tanks; however, the bacterial abundance and composition varied greatly across the A^2O tanks. Environmental factors and operational parameters, such as DO, NH_4^+, COD, and TP in the sewage, were the key factors that influenced the variation of the dominant bacteria and community composition. *Actinobacteria*, *Firmicutes*, and *TM7* contributed to the nitrogen removal, whereas *Candidatus* Microthrix was responsible for the TN and NH_4^+ removal, in the full-scale A^2O process at low temperatures.

Acknowledgments

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Supplementary information

Table S1
Sample reads in different tanks throughout the full-scale A²O process

Sample	Reads	Quality reads	Percentage (%)
IN	60,926	54,047	89
A1	118,906	103,538	87
A2	72,255	64,852	90
O	128,991	113,467	88
SS	25,808	23,236	90
EF	26,991	24,378	90
Total	433,877	383,518	88

Table S2
The percentage of dominant bacteria phylum in different tanks throughout the full-scale A²O process

Phylum	IN	A1	A2	O	SS	EF
Proteobacteria	38%	30%	34%	43%	37%	26%
Bacteroidetes	7%	15%	19%	22%	19%	27%
Actinobacteria	13%	22%	14%	6%	11%	16%
Firmicutes	7%	15%	8%	6%	9%	11%
Chloroflexi	10%	6%	6%	5%	6%	5%
Verrucomicrobia	7%	5%	6%	7%	6%	6%
TM7	12%	6%	8%	5%	7%	6%

Table S3
The top 20 dominant bacteria communities across the full-scale A^2O process

Taxonomy					The percentage of dominant flora						
Phylum	Class	Order	Family	Genus	Total	IN	A1	A2	O	SS	EF
Bacteroidetes	*Saprospirae*	*Saprospirales*	*Saprospiraceae*	–	8.1%	4.5%	6.2%	9.9%	11.5%	7.5%	9.0%
Actinobacteria	*Acidimicrobiia*	*Acidimicrobiales*	*Microthrixacea*	*Candidatus Microthrix*	4.3%	2.2%	9.3%	3.9%	5.4%	1.8%	3.4%
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	–	4.2%	5.3%	4.2%	4.8%	2.7%	3.5%	4.8%
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Intrasporangiaceae*	–	3.7%	5.6%	4.2%	4.7%	3.6%	0.8%	3.2%
Chloroflexi	*Anaerolineae*	*Caldilineales*	*Caldilineaceae*	–	3.6%	5.6%	3.5%	4.3%	2.2%	2.3%	3.6%
TM7	*TM7-1*	–	–	–	3.4%	6.1%	3.3%	4.6%	2.3%	1.5%	2.7%
Verrucomicrobia	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Luteolibacter*	2.6%	4.1%	1.4%	3.2%	3.0%	1.2%	2.8%
Firmicutes	*Bacilli*	*Lactobacillales*	*Carnobacteriaceae*	–	2.0%	1.0%	5.4%	1.6%	1.7%	0.6%	1.8%
Proteobacteria	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	*Rhodobacter*	2.0%	2.3%	2.1%	2.7%	1.4%	0.8%	2.6%
Bacteroidetes	*Flavobacteriia*	*Flavobacteriales*	*Flavobacteriaceae*	*Flavobacterium*	1.9%	0.4%	1.3%	1.5%	3.0%	3.3%	1.6%
Bacteroidetes	*Saprospirae*	*Saprospirales*	*Chitinophagaceae*	–	1.8%	0.5%	1.4%	1.8%	2.9%	2.1%	2.2%
Proteobacteria	*Gammaproteobacteri*	*Xanthomonadales*	*Xanthomonadaceae*	*Thermomonas*	1.8%	2.4%	2.1%	1.9%	1.4%	1.2%	1.9%
Actinobacteria	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	1.5%	0.8%	1.9%	1.7%	1.9%	1.0%	1.5%
Proteobacteria	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	*Thauera*	1.5%	1.0%	1.4%	1.6%	1.0%	1.8%	2.4%
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Methylocystacea*	–	1.2%	1.3%	0.9%	1.7%	1.3%	0.6%	1.5%
Proteobacteria	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	*Zoogloea*	1.2%	0.3%	0.1%	0.1%	0.5%	6.1%	0.1%
OD1	–	–	–	–	1.1%	1.6%	0.7%	1.1%	1.2%	0.8%	1.2%
Verrucomicrobia	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	–	1.1%	1.5%	0.6%	1.4%	1.1%	1.1%	1.2%
Bacteroidetes	*Cytophagia*	*Cytophagales*	*Cytophagaceae*	*Leadbetterella*	1.0%	0.3%	0.9%	1.1%	2.0%	0.8%	1.2%
Proteobacteria	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	–	1.0%	1.3%	1.0%	0.8%	0.4%	1.3%	1.0%
No blast hit					0.2%	0.1%	0.1%	0.1%	0.2%	0.8%	0.1%

Note: –, Not identified.