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Microbial community changes in a full-scale wastewater treatment system with a rotating biological contactor integrated into anaerobic-anoxic-oxic-oxic processes

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ABSTRACT

The objective of this study was to investigate the changes of microbial communities in different treatment units of a full-scale wastewater treatment plant (WWTP) equipped with a serially combined rotating biological contactor (RBC) and anaerobic-anoxic-oxic-oxic (A^2/O^2) processes. High-throughput sequencing results showed that functional communities including *Methylotenera*, *Nitrospiraceae*, *Terrimonas*, *Zoogloea*, *Lysobacter*, and *Rhodobacter* were the most dominant communities in the treatment system. The sequencing results demonstrated that the microbial communities along the different treatment units showed distinct community profiles. Additionally, dominant functional communities with *Methylotenera* and *Methanolobus* were significantly enriched on RBC films compared to those in the activated sludge of the RBC treatment unit, indicating that the RBC plays essential functions in the WWTP. In general, thoroughly screening the microbial communities in WWTP equipped with RBC and A^2/O^2 and monitoring the changes over seasons contributed to maintain and adjust the wastewater treatment system in case of decayed treatment efficiencies. Besides, our work could guide us to rapidly set up a new similar WWTP.

Keywords: Wastewater treatment system; Rotating biological contactor; Microbial community; Anaerobic-anoxic-oxic-oxic

1. Introduction

As a result of China's rapid agricultural development, industrialization, and urbanization, millions of tons of wastewater are discharged daily into receiving water bodies such as lakes and rivers, which has led to severe environmental problems. Excessive nitrogen in the discharged wastewaters can cause environmental problems and health risks, such as increases in phytoplankton biomass, eutrophication, and algal toxins [1–3]. Wastewater treatment plants

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(WWTPs) are the most commonly used facilities to reduce the pollutants in wastewater, which mainly include organic compounds, nitrogen, and phosphorus. However, most WWTPs in China are subjected to relatively low discharge standards, and considerable amounts of nitrogen are still discharged within the effluents. This makes WWTPs one of the most critical sources of nitrogen inputs into receiving waters [2].

Traditional wastewater treatment processes employ activated sludge technology to treat all forms of nitrogen in wastewaters. Presently, in various modern industrial and domestic wastewater treatment systems, anaerobic-aerobic-oxic (A^2/O) system is used because of its reliability, stability, and relatively high removal efficiency for nitrogen, as well as its low costs and energy consumption; this approach is universally used for biological wastewater treatment [4–7]. The biological wastewater treatment in an A^2/O system is usually achieved through activated sludge, which contains a complex and functionally complete microbial community being capable of removing organic matter, nitrogen, and phosphorus. Furthermore, to meet the increasing demand for environmental protection and stringent effluent standards in China, the universally used A^2/O systems are usually supplemented with additional treatment units to enhance the removal efficiency; one broadly used supplemental technology is the anaerobic-anoxic-oxic-oxic (A^2/O^2) treatment system.

Besides the process optimization, to increase the biomass and activity of the microbial community for efficient nitrogen removal in the activated sludge, numerous treatment configurations have been developed, such as the membrane bioreactor [8], moving bed biofilm reactor [9,10], and rotating biological contactor (RBC) [11,12]. Among these, the RBC system provides vast immobilized surfaces for complex microbial communities to attach and form biofilms, which are actively involved in various essential functions such as the removal of organic matter, nitrogen, and phosphorus from wastewater. Compared to conventional activated sludge systems, such technology has notable advantages including a highly complex and organized microbial community architecture with higher removal efficiency, smaller footprint and lower activated sludge production [13–15].

For the RBC system, requires an attached and complex microbial community for nutrient removal, and therefore, these systems require optimization and adaptive management, such as time for optimal microbial community establishment, the flexibility of treatment processes, system monitoring, and scaling up under different operating conditions, all of which are still challenging issues for its more universal use [16]. Whilst for the A^2/O^2 system, proper system optimization and characterization of the influent, circulated activated sludge, environmental conditions, and nutrient availability during the treatment processes collectively emerge as the primary issues [17–21]. Presently, some wastewater treatment plants in China have combined these systems to further improve the treatment effects. Factors during wastewater treatment, such as operational conditions required in each treatment unit, growth of the microbial populations, the status of the microbial community (attached biofilms in RBC systems and floating activated sludge in A^2/O^2 systems), and substrate concentrations may significantly affect the microbial dynamics and profiles and treatment effects. Therefore, the changes in the microbial community in each treatment unit are essential to the final treatment effects of WWTPs.

In this study, we investigated the architecture and changes of microbial communities in a WWTP containing a RBC combined with A^2/O^2 processes. Activated sludge and water samples were collected from the regulation pool (RP), RBC, anaerobic tank, anoxic tank, and the two continuous oxic tanks of this WWTP. High-throughput sequencing analyses on the 16S rRNA gene were used to investigate the changes of the microbial community, and a changing community profile was detected along the treatment path. Moreover, the differences with regard to the microbial profiles in the RBC activated sludge and in biofilms attached to RBC films were further compared, and significant community changes in terms of relative abundance were identified.

2. Materials and methods

2.1. WWTP description and sample collection

The samples were collected from a full-scale WWTP located in Xining City, Qinghai Province, China (101.7715°E, 36.6335°N), which has been reported in Han et al. [22]. Briefly, the main treatment units at this plant are continuously combined RBC and A^2/O^2 units (Fig. 1). The dissolved oxygen content of the RBC tank, anaerobic tank 1 (T1), anoxic tank 2 (T2), oxic tank 3 (T3), and oxic tank 4 (T4) were 0.88 ± 0.42 mg/L, 0.08 ± 0.04 mg/L, 0.34 ± 0.34 mg/L, 2.99 ± 1.11 mg/L, and 2.04 ± 1.28 mg/L, respectively. Water quality of the effluent was monitored online, and data met the discharging standard A [chemical oxygen demand (COD) < 50 mg/L, biochemical oxygen demand (BOD_5) < 10 mg/L, suspended solids (SS) < 10 mg/L, total nitrogen (TN) < 15 mg/L, NH_4–N < 5 mg/L, TP < 1 mg/L] according to the Discharge Standard of Pollutants for Municipal Wastewater Treatment Plant (GB18918-2002) (Fig. 2) [23].

Activated sludge samples were collected at the steady-state of the wastewater treatment system from the main treatment units, namely, the RP, RBC tank, T1, T2, T3, and T4 in July (the first sampling) and December (the second sampling) of 2016. Average atmospheric temperatures in Xining were 17.8°C in July and –6.0°C in December according to China Climate Bulletin. Collected samples were kept in an icebox and transferred back to the laboratory for further analyses including TN, ammonia nitrogen and nitrate–nitrogen concentrations. Fresh sludge samples were used for the DNA extraction and sequencing analyses.

2.2. DNA extraction and sequencing analyses

DNA was extracted by using the cetyltrimethyl ammonium bromide method [24,25] to facilitate comparisons with a previous study [26], which has been reported in Han et al. [22].

The 16S rDNA sequencing on the microbial communities in the sludge samples was performed by using the HiSeq2500 PE250 platform. In brief, the dissolved DNA products were diluted to 1 ng/μL for library construction by using sample-specific barcoded primers, which

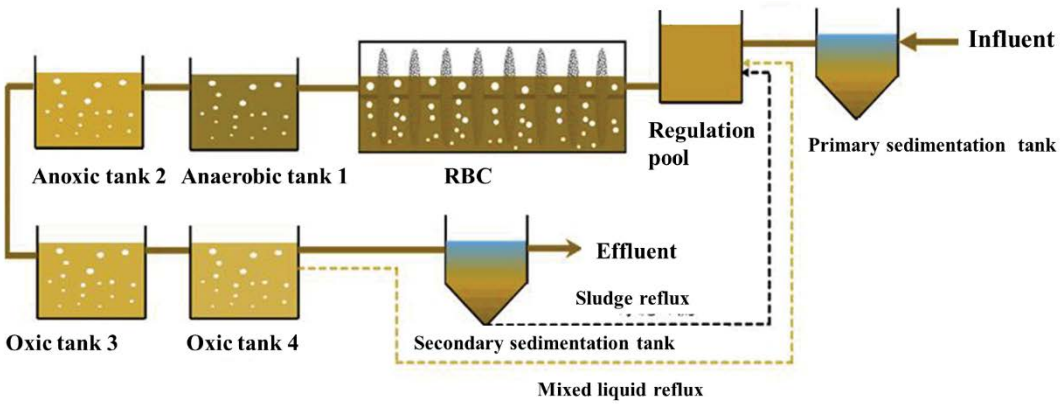

Fig. 1. Diagram of treatment units and processes used in the WWTP system.

were 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT), to amplify the V4 regions on 16S rRNA genes. After purifying the amplified products, the library was constructed based upon the manufacturer's instructions (TruSeq® DNA PCR-Free Sample Preparation Kit), and samples were quantified through Qubit and q-PCR. Subsequently, the sequencing analyses on samples from RP, RBC, T1, T2, T3, and T4 were performed on the HiSeq2500 PE250 platform.

2.3. Phylogenetic analyses on the microbial community

The generated sequences on the HiSeq2500 PE250 platform were processed according to established methods [27,28]; quality control on the tags was performed by using QIIME (V1.7.0) based upon the established instructions to yield effective tags [29]. The effective tags were subjected to clustering analysis through UPARSE (v7.0.1001), and sequences were grouped into operational taxonomic units (OTUs) with a similarity threshold of 97% [30]. The representative sequences of each OTU were subsequently annotated with MOTHUR against the SILVA database, and the phylogenetic relationships amongst representative sequences were established based upon the GreenGenes database [31,32]. Finally, the raw sequencing results were standardized, and a total number of 45,969 and 30,646 sequences representing 3,562 and 2,507 OTUs were generated for the first and second sequencing, respectively. The relative abundance of each OTU in each sample is presented through the percentage of each OTU abundance to the total number of sequences.

2.4. Statistical analyses

Alpha-diversity indices including OTU numbers (observed species) as well as Shannon, Simpson, and Chao 1 indices were calculated by using R ("vegan" package) based upon standardized sequencing results of each sample. A two-way analysis of variance analysis coupled with Sidak multiple comparisons tests was used to determine the statistical differences of alpha-diversity indices among these samples at the significant level of 0.05. Changes in the relative abundance of the corresponding microbial community between adjacent treatment units were statistically analyzed through a *t*-test at the significant level of 0.05. Microbial communities that significantly changed ($P < 0.05$) in relative abundance between adjacent treatment units were selected and evaluated through the ratios of relative abundance of corresponding OTUs in the next units to the last ones. The ratios were transformed to a logarithm of 10 (log10) scale for clearer presentation, and positive transformations indicated increases of communities, whilst negative transformations indicated decreases of communities in the next treatment units with respect to the last ones.

3. Results and discussion

3.1. Nitrogen removal performance

Concentrations of TN, ammonium, and nitrate in the influent, each treatment unit, and effluent were determined during the sampling campaign (Fig. 2). The results showed that in different seasons, ammonium as the main nitrogen form in the wastewater was effectively removed, whilst nitrate increased during the treatment process (Figs. 2A and B). In summer (Fig. 2A), ammonium reached 56.75 mg/L on average in the influent, and after the primary treatments (primary sedimentation and regulation pool), ammonium levels were reduced to 8.43 mg/L. After the A^2/O^2 process treatment from T1 to T4, ammonium levels were further reduced from 9.41 to 1.44 mg/L, and values reached 0.20 mg/L in the effluent, which met the GB18918-2002 standard A for discharging ammonium (<5 mg/L). Meanwhile, nitrate concentration increased along the treatment process, from 0.60 mg/L in the RBC to 4.61 mg/L in T4, and reached 11.09 mg/L in the effluent. Changes in the TN in the treated wastewaters were generally correlated with the dynamics of ammonium and nitrate, and the final discharged TN concentration was 11.32 mg/L, which did meet the GB18918-2002 standard A for discharging TN (<15 mg/L). In winter (Fig. 2B), a similar pattern was detected in that ammonium and TN were effectively removed from the wastewater, and nitrate was the main nitrogen form in the effluent. During winter, the influent ammonium level dropped to 40.44 mg/L, and after the primary treatment processes, ammonium decreased to 16.15 mg/L in the RBC; a dramatic decrease was observed when the wastewater was processed from T1 (14.42 mg/L)

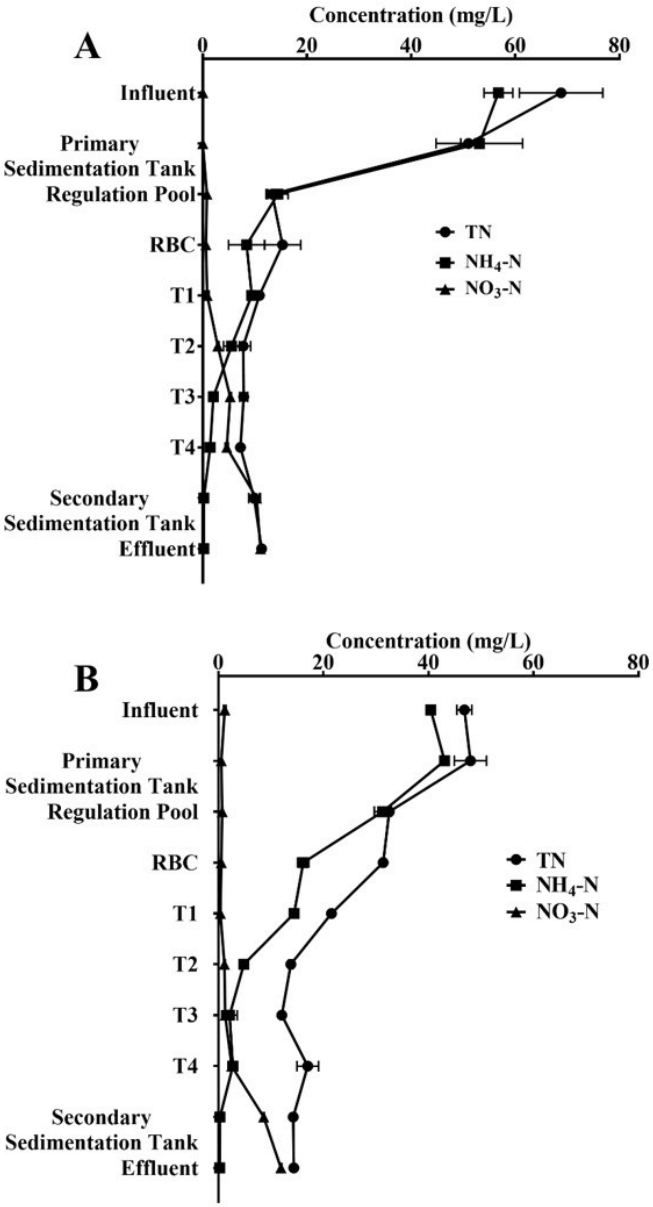

Fig. 2. Total nitrogen (TN), ammonium (NH_4–N), and nitrate (NO_3–N) in different treatment units; (A) July and (B) December of 2016. Scale bars represent the standard deviation of three replicates.

to T2 (4.84 mg/L). As for TN, the influent TN was 46.84 mg/L, and after the primary treatment processes, the TN concentration dropped to 31.38 mg/L in the RBC; a clear decrease in TN was detected from the RBC to T2 (13.79 mg/L). The final effluent ammonium and TN levels were 0.24 and 14.34 mg/L, respectively, and both values met the GB18918-2002 discharging standard A. Noticeably, the concentration of TN was greatly reduced when the wastewater was transferred from the primary sedimentation tank to the RBC units; however, this process was primarily related to a physiochemical sedimentation process but not microbial degradation. Nevertheless, microbial-mediated nitrogen removal also played an important role in the units with microbial activity as the core treatment processes [33]. Based on the results in Fig. 2, higher NO_3–N concentration in the effluent was detected in winter than in summer, indicating declined denitrification activities in winter. The declined denitrification level was attributed to the inhibition of low temperature on denitrifying bacterial activity. Although both ammonium and TN reached the discharge standard in summer and winter, season transition affected the nitrogen removal efficiency, particularly in terms of TN removal, as has been broadly reported in other studies [34–36]. This was due to the fact that lower temperatures decrease microbial activity during nitrogen removal in WWTPs [36,37]. Regardless, TN was markedly removed throughout the RBC to T2 treatment both in summer and winter, which demonstrates the importance of these treatment units in this system.

3.2. Microbial community in activated sludge of the RBC-A^2/O^2 wastewater treatment system

High-throughput sequencing analyses on the 16S rRNA gene targeting the overall microbial community were performed on the first (in July) and the second (in December) batch of the collected activated sludge samples. After sequencing was performed on each sample, the sequencing results were standardized to yield 45,969 and 30,646 sequences representing 3,562 and 2,507 OTUs for the first and second sequencing, respectively. Alpha-diversity indices showed that within each sampling (July and December collections) there were no significant differences ($P > 0.05$) among the treatment units (Table 1).

The finding that significant differences existed among these treatment units and changes of seasons can affect the microbial community had been reported in other studies [34–36]. Considering that a better nitrogen removal efficiency was achieved in summer (Fig. 2A) since warm condition contributed to nitrifying and denitrifying bacterial activities in sludge, and therefore the higher microbial diversity was as expected. Additionally, the difference in microbial diversity between these two samples may also attribute to the operational conditions and maintenance of the treatment system in this WWTP [15,38].

Next, the dominant communities in each treatment units were sorted out based upon their relative abundances, and the top 450 OTUs (the first sequencing) and 400 OTUs (the second sequencing) making up over 95% of the abundance of the total community in each sample were selected for further analyses. The results showed that *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, and *Chloroflexi* were the most dominant phyla throughout the whole process (Fig. 3), which is in accordance with other studies on microbial profiles in activated sludge treatment systems [39,40]. The main reason was that common nitrifying and denitrifying bacteria, such as *Nitrosomonas*, *Nitrosococcus*, *Paracoccus denitrificans*, *Pseudomonas stutzeri* and so on belonged to *Proteobacteria*. The relative abundances of *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Chloroflexi* ranged from 47.11%–50.00%, 13.78%–14.22%, 8.44%–12.44%, 7.56%–9.11%, and 4.67%–5.11%, respectively, for the first sequencing (Fig. 3A), and 50.75%–55.75%, 11.50%–13.5%, 3.50%–7.25%, 10.75%–12.25%, and 4.25%–6.25%,

respectively, for the second sequencing (Fig. 3B). Therefore, the dominant microbial in phylum level was *Proteobacteria* no matter the seasons, and heterotrophic bacteria dominated in microbial communities. Additionally, within each sequencing analysis, there were no significant differences detected in the community structures amongst these treatment units, which also was a indicator of the relatively stable treatment system used by this WWTP. The convergences of microbial communities among the whole treatment units were attributed to the continuous flow in WWTP. Besides, the stable microbial communities in WWTP might contribute to the high removal efficiencies of ammonia nitrogen.

3.3. Microbial community changes along with the RBC-A^2/O^2 wastewater treatment system

Changes in the microbial community along the treatment system were determined by comparing the changes in the

Table 1
Alpha-diversity indices of microbial communities in each treatment unit

Treatment unit	Sampling in summer (n = 6)				Sampling in winter (n = 3)			
	Observed species	Shannon	Simpson	Chao 1	Observed species	Shannon	Simpson	Chao 1
RP	1,270 ± 92.45	6.87 ± 0.19	0.96 ± 0.007	1,534 ± 158.5	810.3 ± 54.5	5.73 ± 0.36	0.91 ± 0.03	917.4 ± 81.6
RBC water	1,198 ± 114.7	6.78 ± 0.38	0.95 ± 0.015	1,359 ± 130.5	885.3 ± 11.5	5.99 ± 0.12	0.92 ± 0.01	1,013 ± 25.2
RBC film	N.D.	N.D.	N.D.	N.D.	872.7 ± 18.8	6.94 ± 0.12	0.97 ± 0.005	968.7 ± 23.0
T1	1,174 ± 94.8	6.66 ± 0.29	0.95 ± 0.014	1,405 ± 160.4	856.7 ± 71.9	6.09 ± 0.25	0.93 ± 0.01	966.1 ± 103
T2	1,217 ± 88.9	6.72 ± 0.28	0.95 ± 0.013	1,391 ± 123.4	883.7 ± 44.4	6.04 ± 0.24	0.93 ± 0.015	1,022 ± 6.5
T3	1,153 ± 93.8	6.72 ± 0.26	0.95 ± 0.01	1,336 ± 128.9	831.3 ± 20.8	5.89 ± 0.04	0.92 ± 0.002	938.3 ± 16.0
T4	1,271 ± 58.7	6.86 ± 0.23	0.96 ± 0.008	1,539 ± 97.41	910.3 ± 41.2	6.14 ± 0.10	0.93 ± 0.004	1,046 ± 33.1

N.D.: not determined;
For the first sequencing, biofilms attached to RBC films were not sampled.

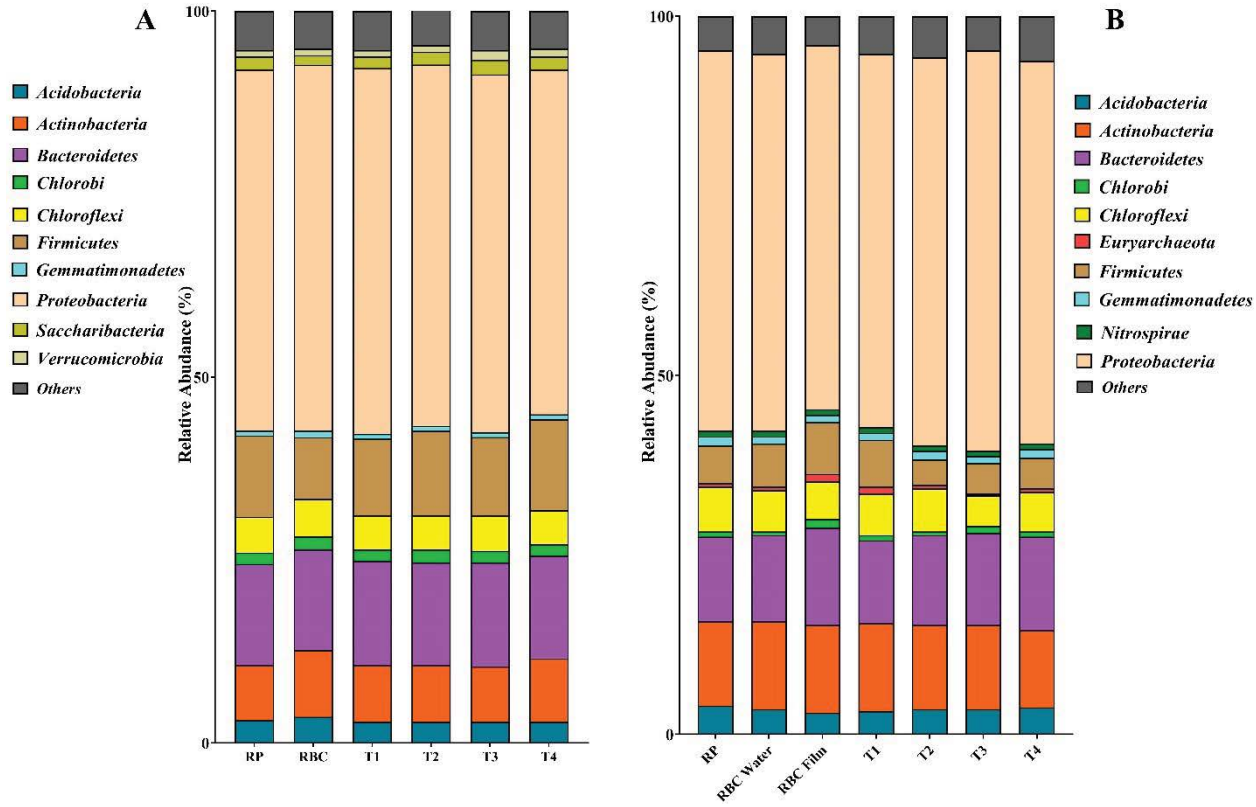

Fig. 3. Dominant microbial community in each unit of the wastewater treatment process at the phylum level for (A) the first and (B) the second sequencing analysis in the regulation pool (RP), rotating biological reactor (RBC), anaerobic tank 1 (T1), anoxic tank 2 (T2), oxic tank 3 (T3), and oxic tank 4 (T4).

relative abundance of the microbial community in the adjacent previous treatment unit with the next unit. In this study, the changes of the microbial community from the genus level were investigated in the major treatment units including the RP, RBC, and A^2/O^2.

For the first sequencing (in July 2016), most of the genera remained relatively stable with no significant changes in the relative abundances (Table 1) ($P > 0.05$), whilst some showed significant changes ($P < 0.05$) during the continuous wastewater treatment process (Fig. 4). After wastewater entered the RBC from the RP, relative abundances of *Leucobacter* (OTU2299), *Chlorobiales* (OTU110), *Sphingomonadaceae* (OTU2143), *Methylophilaceae* (OTU168), and *Lysobacter* (OTU65 and OTU729) significantly increased, whilst those of *Rhodocyclaceae* (OTU3208 and OTU10) and *Nitrospiraceae* (OTU6) significantly decreased (Fig. 4A). However, the microbial community exhibited clear changes in the anaerobic tank (T1) compared with that in the RBC. The dominant genera in the RBC, which included *Leucobacter* (OTU2299), *Chlorobiales* (OTU110), *Methylophilaceae* (OTU168), and *Lysobacter* (OTU65 and OTU729) significantly decreased in T1, and the relative abundances of *Actinobacteria* (OTU18), *Comamonadaceae* (OTU1939), *Nitrosomonas* (OTU12), and *Rhodocyclaceae* (OTU10) significantly increased ($P < 0.05$) (Fig. 4B). The microbial community profiles in the anaerobic tank (T1) and anoxic tank (T2) did not change greatly, but *Romboutsia* (OTU5) and *Nitrospiraceae* (OTU6) increased and *Saprospiraceae* (OTU4) decreased ($P < 0.05$) (Fig. 4C). Changes from anoxic (T2) to oxic conditions (T3) also affected the microbial community, and *Sphingobacteriales* (OTU8), *Comamonadaceae* (OTU28), and *Saprospiraceae* (OTU4) significantly increased, whilst *Nitrospiraceae* (OTU6), *Chlorobiales* (OTU2), and *Methylotenera* (OTU1) decreased ($P < 0.05$) (Fig. 4D). Changes in the community also occurred between the two oxic tanks (Fig. 4E), in which *Bifidobacterium* (OTU9), *Tetrasphaera* (OTU25), *Romboutsia* (OTU5), and *Trichococcus* (OTU3) significantly increased, and those enriched in T3, such as *Sphingobacteriales* (OTU8) and *Comamonadaceae* (OTU28), significantly decreased (Fig. 4E). The differences in microbial communities between treatment units might be attributed to two main reasons, that is, various operating conditions (aeration condition, mechanical stirring condition, retention time and so on) and altered wastewater flow composition through former units (declined ammonia nitrogen and TN).

Similar trend was also observed for the second sequencing (in December 2016) (Fig. 5). From the RP to RBC, only a few genera significantly increased, for example, *Ferruginibacter* (OTU16) and *Sphingomonadaceae* (OTU38 and OTU39), whilst relative abundances of *Methylophilaceae* (*Methylotenera*, OTU1987, OTU1174, OTU1, and OTU1928) and *Zoogloea* (OTU4) significantly decreased (Fig. 5A). Comparing the results for T1 with those for the RBC, *Nitrospiraceae* (OTU3), *Acinetobacter* (OTU18), and *Zoogloea* (OTU4) showed significant increases, whilst *Rhizobiales* (OTU11), *Pseudomonas* (OTU23), and *Methylophilaceae* (OTU1 and OTU1928) showed significant decreases (Fig. 5B). When changing from T1 to T2, significant decreases in the microbial community were detected for *Sphingomonadaceae* (OTU39), *Psychrobacter* (OTU27), *Romboutsia* (OTU7), *Trichococcus* (OTU1793 and OTU2), and *Acinetobacter* (OTU18), and only Beta *Proteobacteria* (OTU2004), *Dechloromonas* (OTU6), and *Nitrospiraceae* (OTU3) showed significant increases (Fig. 5C). Changing from anoxic (T2) to oxic conditions (T3), more genera were affected compared with the first sequencing (Fig. 5D), and *Sphingomonadaceae* (OTU39 and OTU38), *Rhizobiales* (OTU11), *Saprospiraceae* (OTU5), and *Pseudomonas* (OTU23) significantly increased, whilst *Comamonadaceae* (OTU62), *Trichococcus* (OTU2), *Acidimicrobiaceae* (OTU14), and *Nitrospiraceae* (OTU1430 and OTU3) significantly decreased. On the contrary, microorganisms that significantly increased in T3 included *Sphingomonadaceae* (OTU39 and OTU38), *Pseudomonas* (OTU23), and *Rhizobiales* (OTU11), whilst those that significantly decreased in T3 included *Nitrospiraceae* (OTU3, OTU67 and OTU1430) and *Comamonadaceae* (OTU62), but these significantly increased in T4 (Fig. 5E). Comparing with the alterations in summer, more significant differences in common bacterial numbers were observed in C, D and E, that is, in T1, T2, T3 and T4

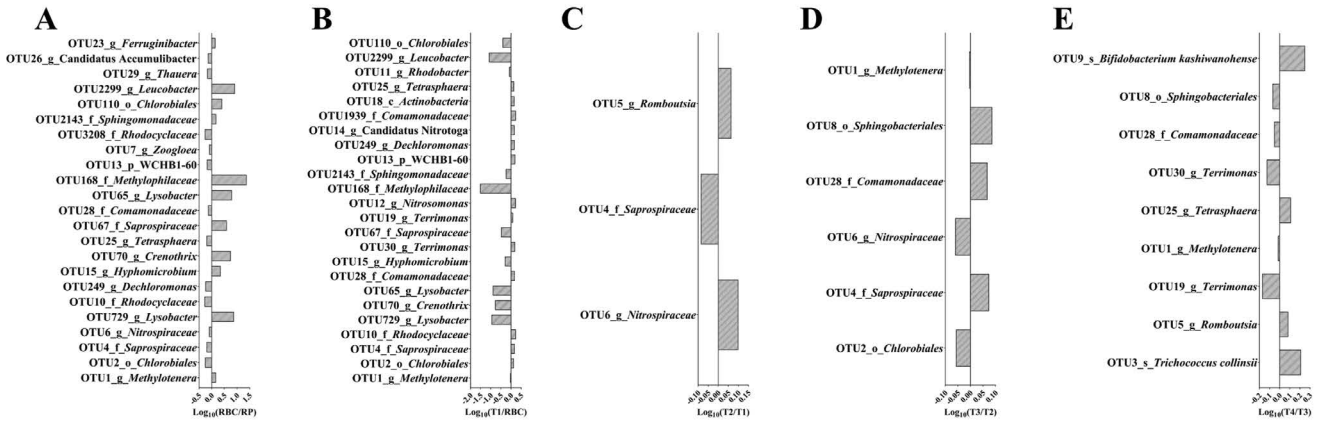

Fig. 4. Ratios of common bacterial abundances in one unit to the former unit in summer. (A) RBC/RP, (B) T1/RBC, (C) T2/T1, (D) T3/T2, and (E) T4/T3. Communities with significant changes ($P < 0.05$) in relative abundances, taken as mean values, are sorted out. Differences in the community between adjacent treatment units are presented as the ratios of relative abundance in the next unit compared to the last one, and values were subsequently transformed into log10 data for clearer presentation.

rather than in other units. Therefore, low temperature might contribute to homogenize the microbial communities among these four units. Meanwhile, less survival bacteria in influent due to extremely cold whether resulted to the decreases in common bacterial numbers in RP and RBC.

For the first sequencing, more relative abundances with significant changes were detected between the RP and RBC, as well as between the RBC and T1. This finding may have been correlated with the dynamic changes in the operational and environmental conditions (Fig. 4), which can actively affect the functional microorganisms within these treatment units. Since the operational conditions between the anaerobic tank (T1) and anoxic tank (T2) were similar, fewer microorganisms were affected. When changing from anoxic (T2) to oxic conditions (T3), aeration emerged as one of the main factors in shaping the microbial community [41]. Interestingly, between the two oxic tanks, more OTUs were also significantly affected between T4 and T3 than those affected between T2 and T3. The result indicates that continued aeration could further change the microbial community which has become recalcitrant to the changing environment in T3.

The variance of functional communities among treatment units was also well correlated to nitrogen removal along with the treatment system. For instance, during the summer period, ammonium was greatly reduced from the RBC to T2, which was correlated to the continuously increased abundance in the microbial community including *Nitrosomonas* and *Nitrospiraceae* (Figs. 4B and C) [42–44]. Additionally, microorganism potentially worded for denitrification, such as those including *Methylophilaceae* [45], *Zoogloea* [46], *Rhodobacter* [47], and *Pseudomonas* [48], were significantly enriched and became dominant along the treatment process, which should be responsible for the efficient TN removal [49]. Regarding the OTUs and relative abundances, *Methylophilaceae*, *Nitrospiraceae*, *Dechloromonas*, *Zoogloea*, *Trichococcus*, *Phyllobacteriaceae*, *Terrimonas*, *Rhodobacter*, and *Pseudomonas* were the most dominant community members in this WWTP, correlating to further analyses of the performance of the WWTP [50,51].

3.4. Variability in the microbial community between RBC films and RBC activated sludge

The RBC is one of the essential treatment units in this WWTP; therefore, the variance of the microbial community on RBC films and in the floating activated sludge was further investigated. There were a considerable amount of microorganisms on RBC films that differed from the counterparts in the activated sludge (Fig. 6). In comparisons, *Methanolobus* (OTU205), *Methanomethylovorans* (OTU55), *Enterococcus* (OTU503 and OTU17), *Saprospiraceae* (OTU143), *Lactococcus* (OTU186), *Crenothrix* (OTU158), *Microbacteriaceae* (OTU985), *Xanthomonadaceae* (OTU69), *Leucobacter* (OTU42), *Microbacterium* (OTU131), and *Novosphingobium* (OTU1877) were significantly higher on the RBC films than those in the activated sludge ($P < 0.05$), whilst *Chloroflexi* (OTU44), *Sphingomonadaceae* (OTU38 and OTU39), *Nitrospiraceae* (OTU3 and OTU1430), and *Methylotenera* (OTU1, OTU1987 and OTU1174) were significantly lower on RBC films than those in the activated sludge ($P < 0.05$).

The variances in microbial profiles may indicate that there is a core functional community determining the functions and removal effects of the RBC in this WWTP. The RBC provides a unique environment for the attachment of microorganisms where the microbial community can form and mature into functional biofilms for wastewater treatment [12,14,43,52,53]. In this study, distinct microbial biofilms formed on the RBC films, in that some functional communities were significantly enriched, whilst others were significantly lower on the RBC films (Fig. 6). Those significantly enhanced communities may determine the major functions of the RBC treatment unit. For instance, enhanced communities (*Rhizobiales*, *Thermomonas*, and *Rhodobacter*) with denitrification and aerobic denitrification ability enabled the RBC to enhance the nitrogen removal efficiency [54–57]. Additionally, the degradation of carbon-based compounds may also be enhanced in the RBC as a result of the corresponding communities. Previous studies have shown that *Mycobacterium* can degrade many carbon-based compounds such as polycyclic aromatic

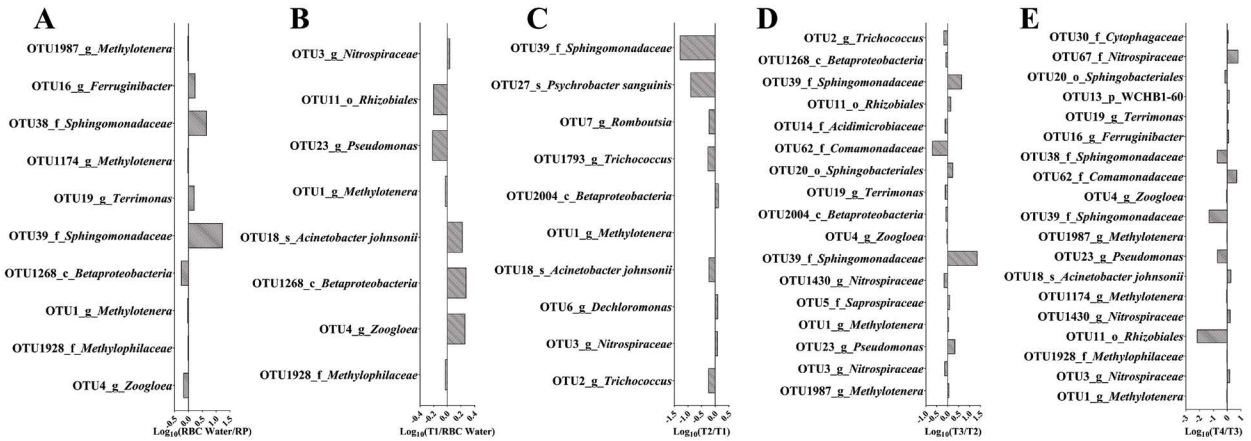

Fig. 5. Ratios of common bacterial abundances in one unit to the former unit in winter. (A) RBC/RP, (B) T1/RBC, (C) T2/T1, (D) T3/T2, and (E) T4/T3. Communities with significant changes ($P < 0.05$) in relative abundances, taken as mean values, are sorted out. Differences in the community between adjacent treatment units are presented as the ratios of relative abundance in the next unit relative to the last one, and values were subsequently transformed into log10 data for clearer presentation.

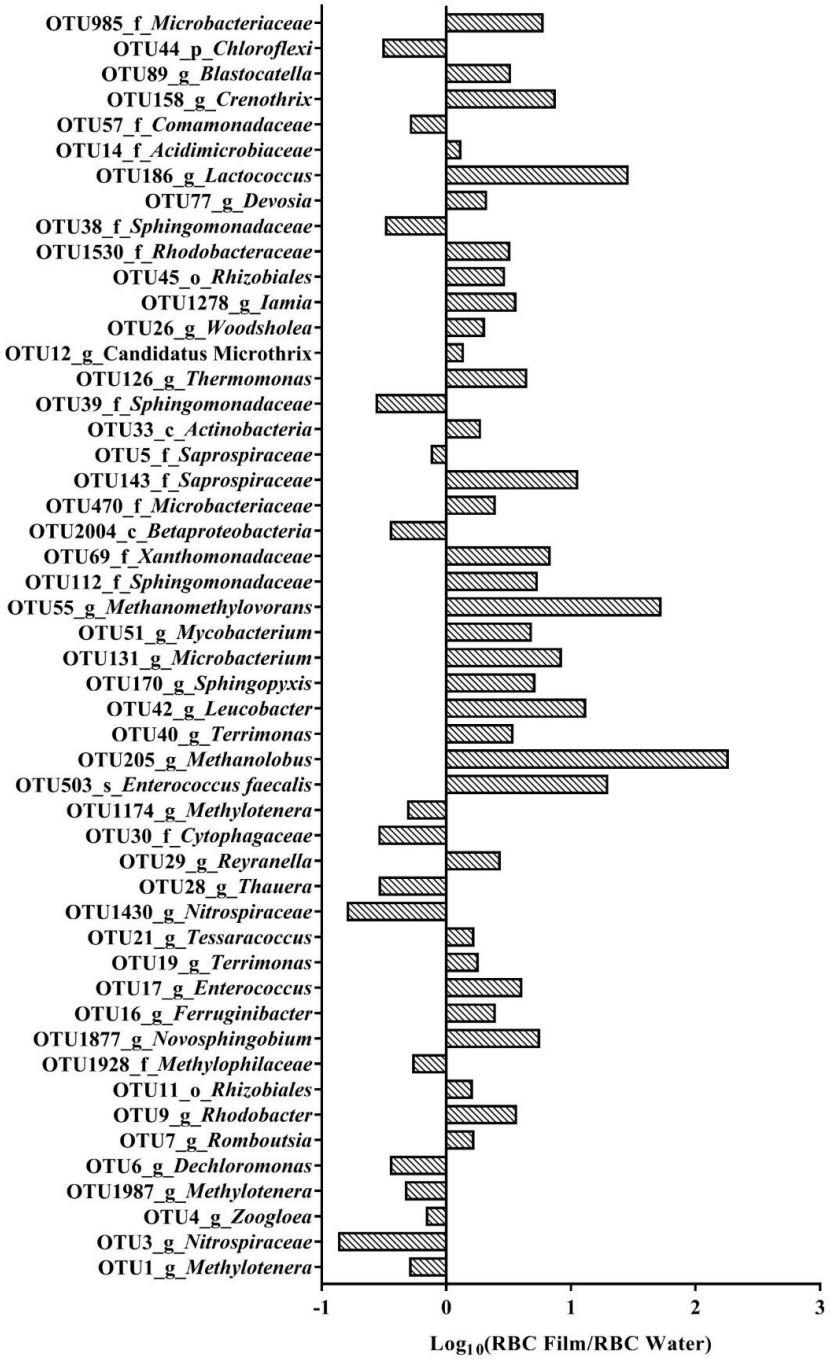

Fig. 6. Variance of the microbial community on RBC films and in RBC activated sludge. Communities with significant changes ($P < 0.05$) in relative abundance, taken as mean values, are sorted out. Differences in the community are presented as the ratios of relative abundance on RBC films to RBC activated sludge, and values were subsequently transformed into log10 data for clearer presentation.

hydrocarbons, phenanthrene, and pyrene [43,58,59], whilst *Leucobacter* is related to the biodegradation of other xenobiotic substrates during wastewater treatment processes [60]. The diversity and distinctiveness of microbial community on RBC films indicate that wastewater characteristics and operational conditions determine the microbial community composition so that different wastewater contents and operational parameters will result in varied microbial community compositions [15,38,43,60,61].

4. Conclusion

In general, the changes in the microbial community in a WWTP with a RBC serially combined with A^2/O^2 units

were investigated along with the core treatment system. The effluent TN and ammonium concentrations of this WWTP were found to meet the Chinese discharge standards. High-throughput sequencing analyses showed that there were general similarities in the microbial community compositions of the core treatment units, including in the regulation pool, RBC, anaerobic tank, aerobic tank, and continuous oxic tanks. However, some functional microbial communities related to nitrogen removal were significantly enriched/reduced in certain treatment units. The RBC provides a unique environment for the development of microbial communities on the films and in the tanks, and microorganisms (*Rhizobiales*, *Thermomonas*, and *Rhodobacter*) contributed to aerobic denitrification were found to be enriched on the films, whilst those contributed to denitrification (*Nitrospiraceae*) were found to be enriched in the tanks. The complete profiles of microbial communities in each treatment unit in WWTP equipped with RBC and A^2O^2 were paired with the efficiencies of biological nitrogen removal, which contributed to cope with dramatic changes of pollution load and adjust the operating conditions in case of declined treatment efficiency. In addition, such information could assist a rapid set-up of a new similar WWTP.

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Author contributions

S.X and H.S. designed and directed the experiments. Y.B., Z.H., and S.F. performed the experiments, analyzed the data and wrote the main text of the manuscript. X.Z., Y.Z., Z.X., Z.B., and X.W. contributed to the interpretation of the results. Z.B. and S.F. supervised the project all along. All authors reviewed and improved the manuscript.

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