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RESEARCH ARTICLE

Characterization of suspended bacteria from processing units in an advanced drinking water treatment plant of China

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Abstract For the drinking water treatment plant (DWTP), the organic pollutant removal was the primary focus, while the suspended bacterial was always neglected. In this study, the suspended bacteria from each processing unit in a DWTP employing an ozone-biological activated carbon process was mainly characterized by using heterotrophic plate counts (HPCs), a flow cytometer, and 454-pyrosequencing methods. The results showed that an adverse changing tendency of HPC and total cell counts was observed in the sand filtration tank (SFT), where the cultivability of suspended bacteria increased to 34%. However, the cultivability level of other units stayed below 3% except for ozone contact tank (OCT, 13.5%) and activated carbon filtration tank (ACFT, 34.39%). It meant that filtration processes promoted the increase in cultivability of suspended bacteria remarkably, which indicated biodegrading capability. In the unit of OCT, microbial diversity indexes declined drastically, and the dominant bacteria were affiliated to *Proteobacteria* phylum (99.9%) and *Betaproteobacteria* class (86.3%), which were also the dominant bacteria in the effluent of other units. Besides, the primary genus was *Limnohabitans* in the effluents of SFT (17.4%) as well as ACFT (25.6%), which was inferred to be the crucial contributors for the biodegradable function in the filtration units. Overall, this paper provided an overview of community composition of each processing units in a DWTP as well as reference for better developing microbial function for drinking water treatment in the future.

Keywords Suspended bacteria · Flow cytometer · 454-Pyrosequencing · Biodegrading capability · Drinking water treatment plant

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Introduction

Drinking water safety has always been a social concern, which is directly related to human health. Recently, advanced drinking water treatment processes (AWTP) in the following of conventional water treatment processes (CWTP) were commonly employed in drinking water treatment plant (DWTP) to provide high-quality drinking water for consumers in China. As one of the popular AWTP, ozone-biological activated carbon (O_3-BAC) is widely adapted (Gerrity et al. 2011; Kirisits et al. 2001; Nishijima and Okada 1998; Song et al. 2015). Oxidation by ozone, adsorption of activated carbon, and biodegradable effect were well-known mechanisms involved in this technology (Li et al. 2006). Owing to these effects, O_3-BAC technology has shown excellent performance not only on eliminating disinfecting by-product precursors and tastes and odors but also on removing organic matters (Zhang et al. 2015), which were partly biodegraded by bacteria. However, the crucial bacteria involved in the biodegradation process were still vague.

Although the finished water quality of DWTPs was improved due to the application of O_3-BAC technology, previous studies revealed that it was still inevitably deteriorated during distribution (Chu et al. 2005; Manuel et al. 2007). Some opportunistic pathogens, such as *Legionella pneumophila*, *Mycobacterium avium*, and *Pseudomonas aeruginosa* (Holinger et al. 2014; King et al. 2016; Wang et al. 2012), were frequently detected in drinking water in recent years, and these opportunistic pathogens usually exhibited a chlorine-resistant property. Thus, the microbial safety of drinking water was facing new challenge. In practice, various methods had been proposed and tested to control opportunistic pathogens. Wang et al. (2013) had investigated the effect of granular activated carbon (GAC) pre-treatment and disinfectant on opportunistic pathogen occurrence. The results indicated that both chlorine and chloramine could strongly inhibit the colonization of *H. vermiformis* and *M. avium*, while GAC pre-treatment was not correlated with opportunistic pathogen abundance. Ceruero-Arago et al. (2014) studied the inactivating effects of UV irradiation (253.7 nm) on five strains of *Legionella* spp., and the result showed that a 3-log reduction was achieved with a fluence of 50 J/m^2. Silver cations (Hwang et al. 2007) and photocatalytic oxidation (Cheng et al. 2010) were also used to inactivate opportunistic pathogens. The results demonstrated that the tested opportunistic pathogens were completely inactivated within 8 h by silver cations (initial concentration of silver was 0.1 mg Ag/L), while a 4.5-log reduction in the viable cell count of *L. pneumophila* was obtained by photocatalytic oxidation tests (1000 mg/L of TiO_2 and 108 μW/cm^2 of UV_{365nm}) after 90 min treatment. However, the trace of opportunistic pathogen source was always neglected. Although large amounts of microbes were inactivated in the disinfection unit of DWTP, it is doubtless that raw water was the original source of bacteria, including pathogens in drinking water (Pinto et al. 2012). Getting insights into the variations of suspended bacteria counts and community along with processing units in drinking water treatment plants will be helpful for the control of opportunistic pathogens.

In practice, both culture-based and culture-independent methods had been used to characterize bacteria community (Chiao et al. 2014; Gonzalez and Noble 2014; Liu et al. 2013; Pereira et al. 2010). For bacteria quantification, the traditional techniques usually consume long incubation times (2 to 3 days) and possess discrepancy between the number of cultivable and non-cultivable cells, since less than 1% of the bacteria present in aquatic environments were cultivable (De Roy et al. 2012). Flow cytometry (FCM) was an alternative tool for the analysis of bacteria in drinking water developed in recent years (Hammes et al. 2008). It was not only easy and rapid (results obtained in 15 min from sampling) but also quantitative, highly reproducible (less than 5% error), and sensitive (detection of change down to 3% from initial value) (Prest et al. 2014). For bacteria community, growing evidences have shown that high-throughput sequencing (Liao et al. 2015; Lin et al. 2015; Xu et al. 2012) is a powerful tool compared with conventional culture-dependent methods, which made it possible to demonstrate the variations of microbial communities in drinking water treatment processes comprehensively.

In this study, raw water and the effluent of each processing unit were collected from an advanced DWTP in China. Suspended bacteria count was detected with FCM as well as culture-dependent material and methods; suspended bacteria community was characterized by 454-pyrosequencing material and methods. This research mainly focused on (1) characterizing variations of suspended bacteria counts and community and (2) seeking crucial bacteria responsible for organics removal during the drinking water treatment processes. The results will not only be helpful for further understanding the bacterial ecology of DWTPs but also provide some reference for controlling opportunistic pathogens as well as developing functional bacteria for drinking water treatment.

Materials and methods

Description of the full-scale drinking water treatment plant

Source water from Lake T was treated through sequential conventional and advanced treatment units in the DWTP, whose capacity of drinking water production was about 0.3 million m^3/day. Then, the drinking water was pumped to the distribution system of city A. The whole drinking water supply system was illustrated by Fig. 1, which mainly presents the detailed drinking water processing units of the DWTP as well as the specific sampling points (marked with red bold font).

Sampling and water quality analysis

All water samples were sampled in the DWTP (Fig. 1). Bulk water (5 L) samples were collected with sterilized glass bottles at the outlet of each processing unit except for raw water, which was sampled at the inlet of a pre-ozonation contact tank. Seven water samples were acquired in all, and all samples were disposed within 12 h. Upon arrival at the lab, bulk water was used to analyze dissolved organic carbon (DOC) and heterotrophic plate counts (HPC). All these parameters were measured according to methods used in our previous study (Li et al. 2015). In addition, biodegradable dissolved organic carbon (BDOC) was detected by the methods described by Servais et al. (1995). All water quality parameters were detected in triplicate.

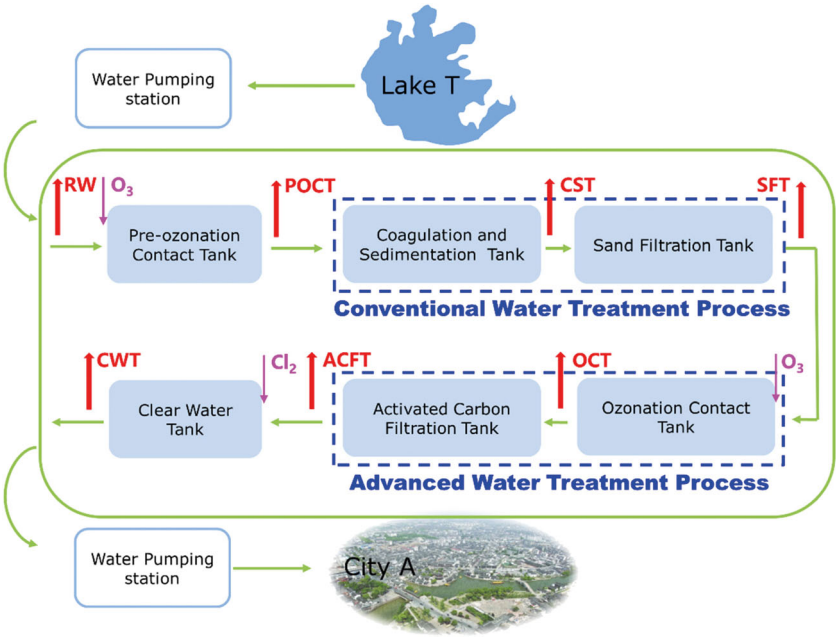

Fig. 1 The processing units and sampling points of the DWTP. Abbreviation: *RW* raw water, *POCT* pre-ozonation contact tank, *CST* coagulation and sedimentation tank, *SFT* sand filtration tank, *OCT* ozonation contact tank, *ACFT* activated carbon filtration tank, *CWT* clear water tank

Flow cytometry

Total bacteria counts were determined by flow cytometry (BD FACSVerse, USA) equipped with a 488 nm solid state laser. According to the standardized protocol proposed in the Swiss guideline for drinking water analysis, water samples were stained with SYBR® Green I before detecting. Firstly, samples (500 μL) were pre-heated to 35 °C (5 min), then stained with 5 μL SYBR® Green I (1:100 dilution in DMSO; Molecular Probes), and incubated in the dark for 15 min at 35 °C in the end (Prest et al. 2014). Green fluorescence was collected in the FL1 channel at 533 nm (FL1), while red fluorescence was collected in the FL3 channel at 670 nm (FL3) with the trigger set on the green fluorescence. All samples were detected in triplicate. FCM data analysis was performed using FlowJo 7.6.1 software. In detail, signal of total bacteria was selected using electronic gating on density plots of green fluorescence (FL1; 533 nm) to separate positive signals from noise. Based on dot counts in the gate and record volume acquired during measurement, total cell counts (TCC) was calculated. All samples were detected in triplicate.

DNA extraction and preparation for 454-pyrosequencing

In addition to detecting water parameters, water samples were also filtered with 0.22-μm polycarbonate membranes (Millipore, USA) by vacuum pump (SHZ-DIII, Ling Ke, China) before DNA extraction, aiming at concentrating suspended bacteria on the membrane. According to the manufacturer's protocol, total DNA was extracted immediately by using PowerWater® DNA Isolation Kit (MOBIO, USA) and 50 μL DNA sample was obtained finally. It should be noted that DNA extraction of the CWT sample was failed due to its low biomass in bulk water. All DNA samples were preserved in a refrigerator keeping at −80 °C. The filtering volume and DNA concentration of each sample was summarized in Table S1.

The PCR was conducted using forward primer 27f: 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 519r: 5′-GGTTACCTTGTTACGACTT-3′ to amplify the 16S ribosomal DNA (rDNA) gene of the DNA sample, and each 50-μL reaction mixture included Premix Ex *Taq*™ Hot Start (25 μL), forward primer (1.2 μL), reverse primer (1.2 μL), DNA template (2 μL), and ddH_2O (20.6 μL). The PCR program for bacterial amplification was performed by initial denaturation at 94 °C for 3 min, followed by 32 cycles of amplification (30 s denaturation at 94 °C, 30 s annealing at 55 °C, and 1 min elongation at 72 °C), and 7 min at 72 °C to finish elongation, and finally preserved at 15 °C.

The 454-pyrosequencing method was used to characterize bacterial communities and to examine their relative abundance and diversity in water samples. Firstly, the PCR products amplified with tag-encoded primers (27F and 519R) were purified with QIAquick Gel Extraction Kit (Qiagen, Germany), and then the extracted DNA was sent to the company for bacterial 16S rDNA gene pyrosequencing. Prior to sequencing, the quantity of purified PCR amplicons was determined using the Quant-iT™ PicoGreen® dsDNA Reagent Kit (Life Technologies™ Corporation, Grand Island, NY). The same amount (in moles) of PCR amplicons of different samples was pooled together and subjected to emulsion PCR to

generate amplicon libraries, as recommended by 454 Life Sciences (Margulies et al. 2006).

Sequence data were analyzed using a combination of software UPARSE (USEARCH version v7.0.1090) (Edgar 2013), QIIME (version 1.8) (Kuczynski et al. 2012), and R (version 3.0.2). The raw 16S ribosomal RNA (rRNA) gene sequences were analyzed with script split_libraries.py in QIIME to remove the primers, demultiplex reads, and then filter reads according to Phred quality scores with default parameters (a minimum sequence length of 200 bp, a maximum sequence length of 1000 bp, and a minimum average quality score of 25, a maximum homopolymer length of 6 bp, and no ambiguous bases or mismatches in the primer sequence and no barcode errors). The demultiplexed reads were clustered at 97% sequence identity into operational taxonomic units (OTUs) using the UPARSE pipeline (http://drive5.com/usearch/manual/uparse_cmds.html). The OTU representative sequences were aligned against the Greengenes reference template set (http://greengenes.lbl.gov/Download/Sequence_Data/Fasta_data_files/core_set_aligned.fasta.imputed) based on PyNAST (version 1.2.1) (Caporaso et al. 2010). The Ribosomal Database Project (RDP) Classifier (version 2.7) (Wang et al. 2007) was employed for taxonomy assignment against RDP 16S rRNA training set 9 with confidence score ≥0.8.

Data analysis

The cultivability was equal to the ratio of HPC/TCC, which represented the proportion of heterotrophic bacteria in TCC. For the alpha-diversity metrics, Shannon, Chao, and ACE were calculated. For the beta-diversity metrics, the weighted UniFrac distance matrix (Lozupone and Knight 2005) was calculated and visualized with principal component analyses (PCAs) in QIIME. Besides, linear fitting analysis between the relative abundance of specific genera and the BDOC removal ratio in each water treatment processing unit was conducted with Origin 9.1.

Results and discussion

Suspended bacteria counts

According to Fig. 2, HPC kept an increasing tendency before ozone contact tank (OCT), where it reduced firstly, then it increased again in ACFT and decreased to the lowest value in the subsequent CWT. TCC was maintained almost at the same level until CST, while a decline was observed at sand filtration tank (SFT) as well as OCT, then it increased slightly in ACFT and decreased drastically in CWT. Obviously, an adverse tendency occurred at SFT with respect to HPC and TCC, and this change could be well illustrated by changes of

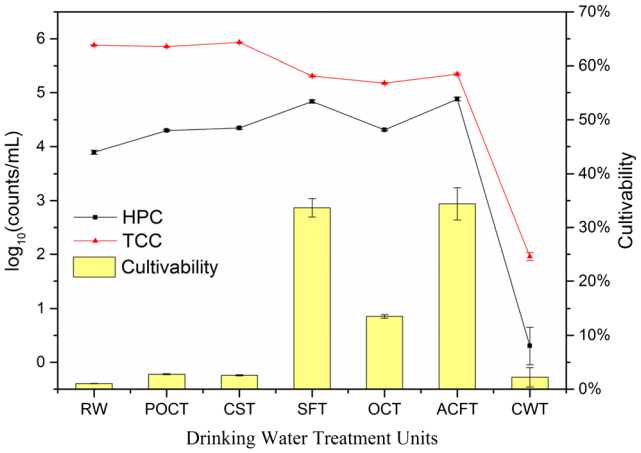

Fig. 2 Bacteria counts and cultivability variations along with drinking water treatment units

cultivability. The cultivability kept at about 2% in RW, POCT, CST, and CWT, but stayed higher than 13% in OCT and 33% in SFT and ACFT (Table S2). The effects of water treatment processes on cultivability were evident.

Based on the above discussion, it can be concluded that obvious variations of the HPC, TCC, and cultivability occurred at SFT. It is well known that the intercepting function was involved in the sand filtering process, and bacteria could be intercepted during filtration. Therefore, the TCC concentration declined in the effluent of SFT. The intercepted bacteria proliferated in the sand layer by consuming organic matter and biofilms gradually formed at the same time. It was obvious that all these bacteria belonged to heterotrophic bacteria. Due to the hydraulic effects, the mature biofilm could detach from the sand surface, which made contribution to the increase of HPC in the effluent of SFT. The decline of TCC and the rise of HPC caused a drastic increase of cultivability in SFT. The reasons for high levels of HPC, TCC, and cultivability in ACFT were similar to that in SFT. High value of cultivability commonly means high portion of heterotrophic bacteria in TCC, which implies prominent microbial degrading capability. This could be well explained by the removal ratio of organics (Figs. S1 and S2). The DOC and BDOC removal ratio of SFT reached 13.4 and 33%, while that of ACFT reached 19.2 and 56.2%, respectively. However, it should be noted that the DOC and the BDOC removal ratio were also reached as high as 16.2 and 31.7% in CST, but the value of cultivability was about 2.5%, which was far less than that of SFT (33.6%) and ACFT (34.4%). The difference of cultivability demonstrated that organics removal was mainly determined by the complex precipitation (Wang et al. 2015) in CST, rather than microbial degradation.

Compared with RW and SFT, TCC in POCT and OCT varied little, while a sharp decline was observed in CWT. According to the design information provided by DWTP, the hydraulic retention times of POCT, OCT, and CWT were

3 min, 12 min, and 3–8 h, respectively. In addition, previous studies showed that oxidants could react with nucleotides and lipids, but three to seven orders of magnitude slower than with amino acid side chains (Gray et al. 2013). Therefore, it was inferred that DNA was intact in POCT and OCT, but was not in CWT due to the long hydraulic retention time. Moreover, it is well known that SYBR Green I could penetrate membranes and bind with DNA unless it is destroyed, so TCC declined drastically in the CWT. However, HPC decreased not only in the OCT but also in the CWT, which was because the cultivation-dependent method was only valid for live bacteria. For cultivability, it kept at about 2% in POCT and CWT, but stayed at 13.5% in OCT. The difference could be attributed to the high proportion of heterotrophic bacteria in the influent (cultivability 33.7%) as well as short contacting time (12 min) of the OCT. All these reasons led to the exceptional value of cultivability in OCT.

Suspended bacteria diversity

Approximately 32,433 sequence reads of 16S rRNA genes with an average length of approximately 480 bp were obtained after trimming and chimera removal, and the effective reads of each sample were all higher than 75%. Eighty-seven OTUs for bacteria were acquired eventually. The results of 454-sequencing are shown in Table 1.

The alpha-diversity was illustrated by diversity indexes (Shannon) and species richness estimators (Chao and ACE) in this research. According to Table 1, it can be found that varying patterns of alpha-diversity indexes were similar. In detail, the first peak value of microbial diversity appeared at the POCT, then it continually declined to the valley value in the unit of OCT but increased to the highest value in the unit of ACFT finally. It should be noted that CWTP played little impact on microbial diversity compared with the ozone biological carbon process. The drastic decline of microbial diversity in OCT was attributed to the strong oxidizing ability of ozone, while the increase of microbial diversity in ACFT was closely related to the development of biofilms in the activated carbon layer.

The variations of microbial diversity were closely related to the working mechanisms of each water treatment unit. The CT value applied in the POCT was 3 min/mg/L (ozone concentration 1.0 mg/L, contacting time 3 min) aiming at killing algae, aiding coagulation, and oxidizing high molecular weight organic matters into smaller ones, which was advantageous for the utilization of bacteria. Therefore, the microbial diversity indexes in the effluent of POCT reached the first peak value. In the following CWTP, microbial diversity indexes fluctuated within a small range. Lower microbial diversity level was observed in the effluent of CST due to the precipitation of particle-associated bacteria. The coagulants, including particle-associated bacteria, were removed by SFT, but the biofilm formed in the sand layer simultaneously. Paul et al. (2012) investigated the effect of shear stress on biofilms developed under different shear stresses, and the result showed that biofilm detachment would prevail for the superficial layers and compression for the deep layers under low shear stress (<2 Pa). In this study, the filtration rates of SFT and ACFT were about 2.2×10^{-3} m/s (8 m/h) and 4.2×10^{-3} m/s (15 m/h), which generated very low shear stress. Therefore, biofilm detachment surely happened during the filtering process. The level of microbial diversity in the effluent of SFT was determined by the balance of the bacteria interception and the biofilm detachment.

For O_3-BAC, the microbial diversity varied greatly. The CT value applied in the OCT increased to 24 min/mg/L (ozone concentration 2.0 mg/L, contacting time 12 min), which was 8 times of that applied in the POCT, resulting in relative sufficient reactions between ozone and bacteria. Large amounts of bacteria were killed, and microbial diversity decreased consequently. Meanwhile, much of the organic matter in the OCT was also oxygenated to be low molecular weight ones, which were easily utilized by bacteria. Besides, some residual ozone also existed in the effluent of OCT, which also was beneficial for bacteria proliferation. Thus, the microbial diversity increased to the highest value in the ACFT.

Suspended bacteria community

Pyrosequencing results revealed that the effluents of different water treatment units harbored a diverse bacterial community. As shown in Fig. 3, Proteobacteria (54.7~99.9%) was the dominant phylum among all the water samples, whereas Bacteroidetes (0~27.2%), Armatimonadetes (0~14.6%), Chloroplast (0.1~10.9%), and Actinobacteria (0~2.1%) were

Table 1 454-Sequencing results and alpha-diversity parameters of different samples

Sample	Raw reads	Effective reads ratio (%)	OTU	Shannon	ACE	Chao
RW	2065	76.76	32	2.5475	38	37
POCT	5815	79.07	63	3.4286	74	72
CST	5830	79.35	50	3.1598	54	52
SFT	3208	77.74	51	3.1146	64	62
OCT	2902	80.63	15	1.3688	19	18
ACFT	12,613	79.01	79	3.4996	81	80

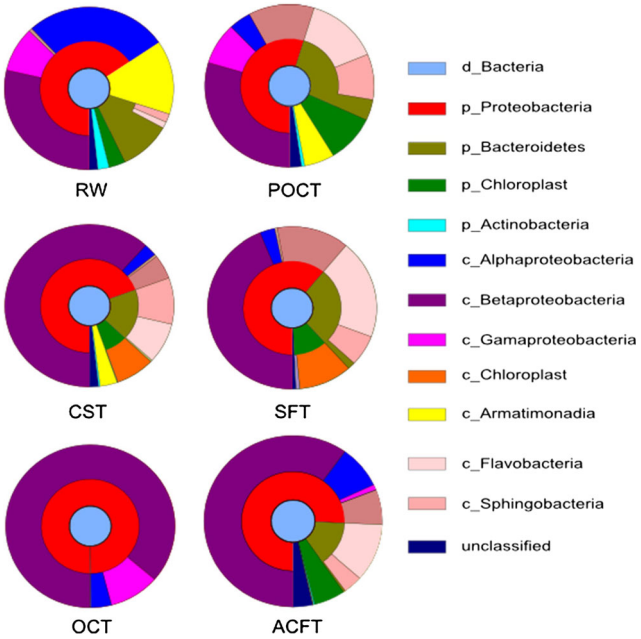

Fig. 3 Microbial community composition of different water processing units in the DWTP. The *inner circle* represents kingdom level (*d*), the *middle circle* represents phylum level (*p*), and the *external circle* represents class level (*c*)

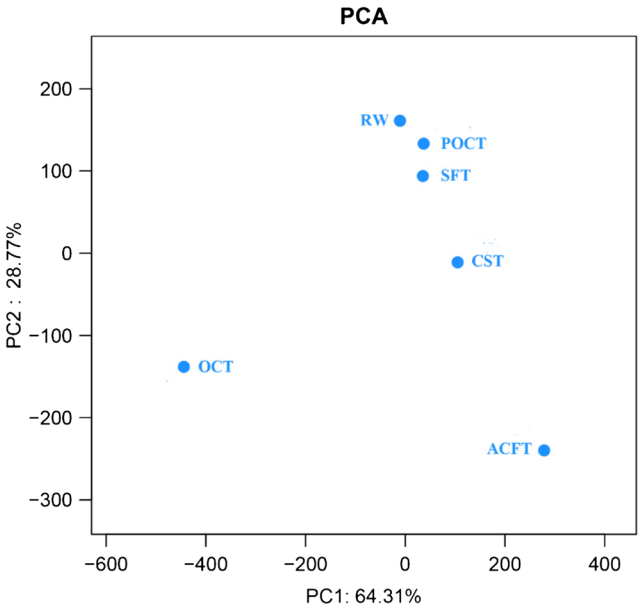

Fig. 4 PCA analysis of suspended bacteria community in the effluent of different water treatment units

also found to be the minor groups. At the class level, Betaproteobacteria was dominant in all samples regarding the relative abundance (28.6~86.3%).

Compared with RW, the relative abundance of Proteobacteria, Actinobacteria, and Armatimonadetes in the POCR decreased in different degrees, while Bacteroidetes and Chloroplast increased. In the following CST, the relative abundance of Proteobacteria increased to 69.4%, while that of Bacteroidetes decreased nearly to 10% and other phyla varied little. However, the phylum category kept no change until SFT, where it decreased to three phyla. Moreover, one more phylum disappeared in the following OCT, where nearly all the bacteria belonged to Proteobacteria (99.9%). In the effluent of ACFT, the relative abundance of many phyla increased with the exception of Proteobacteria, which reduced from 99.9 to 75.6%. Generally, AWTP (OCT and ACFT) played a more important role on bacterial community composition compared with CWTP (CST and SFT).

As shown in Fig. 4, it could be found that O_3-BAC (OCT and ACFT) was far away from other water treatment units, which implied the divergence amplification of the community structure among them. Almost all phyla were killed in OCT except for Proteobacteria, which demonstrated that this phylum exhibited oxidation-resistant ability to some extent. The results were consistent with previous studies (Chao et al. 2013; Shi et al. 2013). As for ACFT, the divergence was enlarged due to the increase in microbial diversity (Table 1).

At the level of genus, the community structure of water samples is shown in Table S3. It could be found that the occurrence of the dominant genus shift along with the water treatment process was not apparent before O_3-BAC. *Flavobacterium*, *Limnohabitans*, *Polynucleobacter*, etc., were the primary genera present in the effluents before O_3-BAC. However, it changed to *Limnobacter* in the OCT and recovered to *Limnohabitans* in the ACFT eventually. In general, limited variations of the dominant genera along with the drinking water processing units were observed.

The crucial bacteria responsible for the biodegradation of organics

According to the discussion above, SFT and ACFT promoted the proliferation of heterotrophic bacteria, which indicated enhanced microbial function. It is well-known that the biodegradation of organics is associated with biofilm developed on filter media, rather than the suspended bacteria present in the bulk water. However, Pinto et al. (2012) found that the bacterial community of the effluent of the filter tank was governed by a biofilm community formed on the filter media and statistical analysis showed a significantly similar community structure between them ($P = 0.032$). Besides, Paul et al. (2012) reported that biofilm detachment frequently occurred at the outside layer of the biofilm under low shear stress condition (<2 Pa). Therefore, bacterial community composition in the effluent of the filtration tank could reflect the community structure of bio-

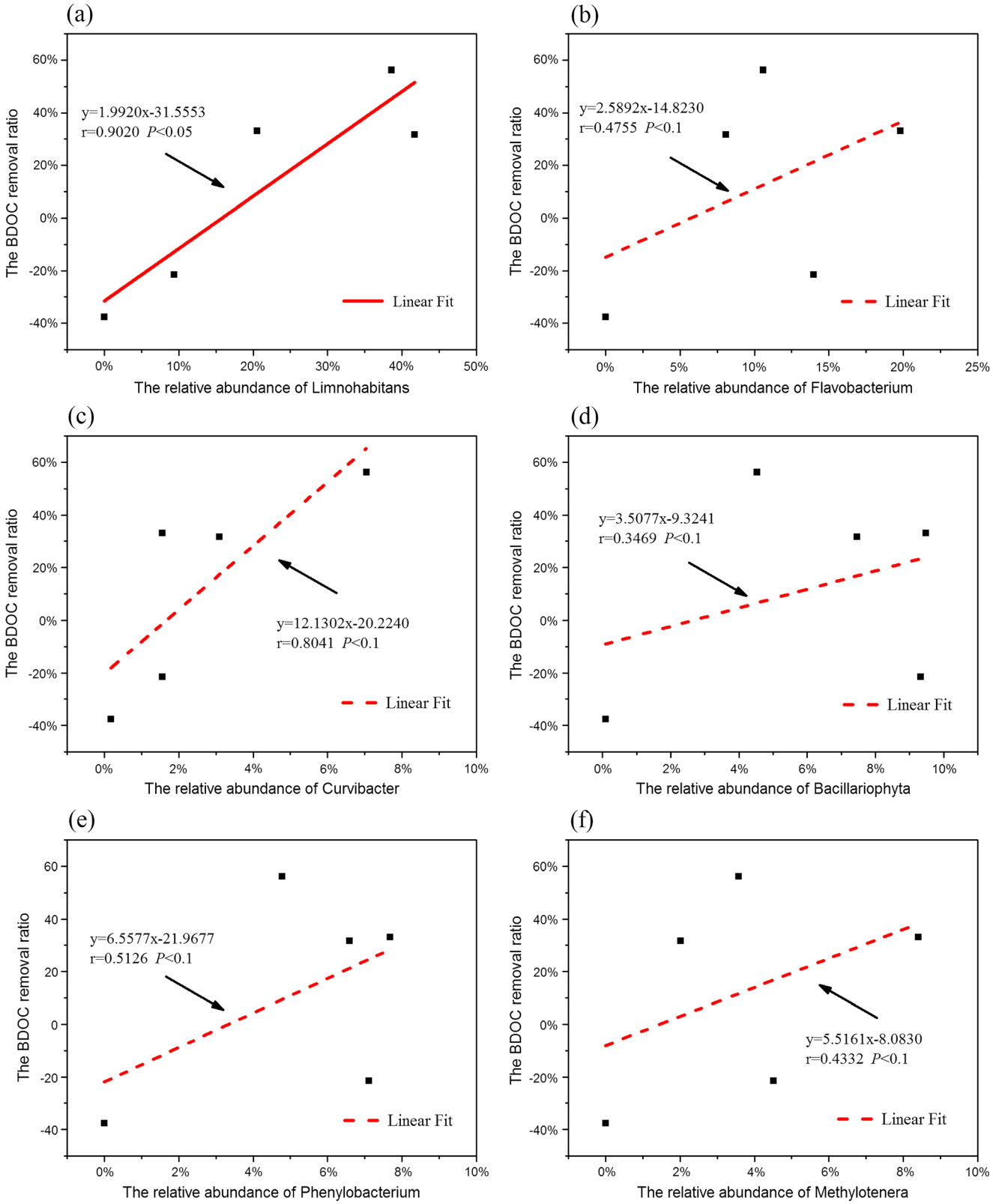

Fig. 5 Linear fitting analysis between the removal ratio of organics and *Limnohabitans* (**a**), *Flavobacterium* (**b**), *Curvibacter* (**c**), *Bacillariophyta* (**d**), *Phenylobacterium* (**e**), and *Methylotenera* (**f**). *Solid lines* represent significant correlation ($P < 0.05$); *dashed lines* represent correlation ($P < 0.1$)

film on the filter media to some degree. By analyzing community composition of the effluent of SFT and ACFT, the crucial bacteria might be screened out. According to Table S3, *Limnohabitans*, *Flavobacterium*, *Curvibacter*, *Bacillariophyta*, *Phenylobacterium*, and *Methylotenera* were six dominant genera in SFT and ACFT. However, the BDOC removal ratio of ACFT was about 1.7 times higher than that of SFT (Fig. S2), while only the relative abundance of *Limnohabitans* and *Curvibacter* increased in ACFT (Table S3). So, it was deduced that *Limnohabitans* and *Curvibacter* might be associated with BDOC removal.

Linear fitting was conducted between the relative abundance of all primary genera and the BDOC removal ratio of each processing unit (Fig. 5); the results showed that they were positively correlated, while only the relative abundance of *Limnohabitans* significantly correlated with the BDOC removal ratio ($P < 0.05$). It suggested that all these selected bacteria were conducive to organics removal, but *Limnohabitans* might play a more important role. Previous studies demonstrated that *Limnohabitans* was one kind of genus often detected in freshwater systems (Freese and Schink 2011; Grujcic et al. 2015; Hahn et al. 2010; Jezbera et al. 2013; Simek et al. 2010; Simek et al. 2011) and it was well-known for its high rates of substrate uptake (Kasalicky et al. 2013), which indicated that the growth of *Limnohabitans* was beneficial to remove organic pollutants in water. Based on the above analysis, it could be inferred that *Limnohabitans* was crucial for the organics biodegradation during drinking water treatment. This was an exciting finding. However, this is only a preliminary conclusion and there are still much more works to be further carried out. As the survival condition of *Limnohabitans* was clear, the biodegrading capacity of it could be developed by adjusting parameters during drinking water treatment, which was helpful for promoting drinking water quality.

Conclusions

This paper provided a detailed overview on variations of suspended bacteria counts and community along with water treatment processing units in an advanced DWTP of China. The conclusions are summarized as follows:

1. For the suspended bacteria counts, an adverse changing tendency of HPC and TCC was observed in the unit of SFT, where the cultivability increased to about 33%. The filtering units (SFT and ACFT) showed high levels of cultivability, which indicated an enhancement of biodegradable function.
2. Suspended bacteria diversity varied little along with drinking water producing units except for a drastic decline in OCT, which was attributed to the high oxidation capacity of ozone.
3. The dominant bacteria almost kept fixed during the whole process of drinking water production. Proteobacteria (54.7~99.9%) phylum and Betaproteobacteria class (28.6~86.3%) were predominant in all samples. The primary genus was *Limnohabitans* (9.4~41.7%) in samples except for OCT, which was replaced by *Limnobacter* (48.4%).
4. *Limnohabitans* genus was inferred to be the crucial contributor for the biodegradation of organics.

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