JOURNAL OF ENVIRONMENTAL SCIENCES 51 (2017) 21–30

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

Characterization of bacterial community dynamics in a full-scale drinking water treatment plant

Cuiping Li[1], *Fangqiong Ling*[2], *Minglu Zhang*[3], *Wen-Tso Liu*[2], *Yuxian Li*[4], *Wenjun Liu*[1,*]

1. *School of Environment, Tsinghua University, Beijing 100084, China. E-mail: lcp870000@163.com*
2. *Department of Civil and Environment Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*
3. *School of Food and Chemical Engineering, Beijing Technology and Business University, Beijing 100048, China*
4. *Water Quality Monitoring Center, Beijing Waterworks Group, Beijing 100085, China*

ARTICLE INFO

Article history:
Received 3 February 2016
Revised 5 May 2016
Accepted 20 May 2016
Available online 2 September 2016

Keywords:
Heterotrophic plate count
Flow cytometry
454 pyrosequencing
Bacterial community
Dynamics

ABSTRACT

Understanding the spatial and temporal dynamics of microbial communities in drinking water systems is vital to securing the microbial safety of drinking water. The objective of this study was to comprehensively characterize the dynamics of microbial biomass and bacterial communities at each step of a full-scale drinking water treatment plant in Beijing, China. Both bulk water and biofilm samples on granular activated carbon (GAC) were collected over 9 months. The proportion of cultivable cells decreased during the treatment processes, and this proportion was higher in warm season than cool season, suggesting that treatment processes and water temperature probably had considerable impact on the R2A cultivability of total bacteria. 16s rRNA gene based 454 pyrosequencing analysis of the bacterial community revealed that *Proteobacteria* predominated in all samples. The GAC biofilm harbored a distinct population with a much higher relative abundance of *Acidobacteria* than water samples. Principle coordinate analysis and one-way analysis of similarity indicated that the dynamics of the microbial communities in bulk water and biofilm samples were better explained by the treatment processes rather than by sampling time, and distinctive changes of the microbial communities in water occurred after GAC filtration. Furthermore, 20 distinct OTUs contributing most to the dissimilarity among samples of different sampling locations and 6 persistent OTUs present in the entire treatment process flow were identified. Overall, our findings demonstrate the significant effects that treatment processes have on the microbial biomass and community fluctuation and provide implications for further targeted investigation on particular bacteria populations.

Introduction

Drinking water treatment plants (DWTPs) produce potable water that meets public health regulations from natural water sources by a series of treatment processes, typically including coagulation, sedimentation, filtration and disinfection. These treatment processes result in profound changes in the physicochemical and biological profiles of the raw water (Au, 2004; Chen et al., 2007). The stable performance of the treatment processes is crucial to the safety of the treated water and the microbial communities in drinking water are particularly important for public health because it is directly

* *Corresponding author*. E-mail: wjliu@tsinghua.edu.cn (Wenjun Liu).

http://dx.doi.org/10.1016/j.jes.2016.05.042

linked to the occurrence of pathogens. As microbial quantity and composition varied spatially and temporally in drinking water systems (DWSs) and sometimes the temporal patterns were striking in both treated and untreated water (Sharp et al., 2006; Henne et al., 2013; Isaac-Renton et al., 1996), end-product monitoring alone is inadequate to keep the high level of confidence in drinking water safety. Holistic characterization of microbial features in DWTPs is necessary.

Treatment processes such as coagulation, sedimentation and filtration can physically remove some of the microorganisms. Disinfection inactivates most of the microorganisms, but those that can endure the disinfection stress are then transported to distribution networks and proliferated there even at a low organic nutrient concentration (Boe-Hansen et al., 2002; Liu et al., 2002; Lu et al., 2014). Various parameters have been developed to control the biological quality, among which the total cell count is one of the most widely used parameters (Carter et al., 2000; Chen et al., 2007; LeChevallier et al., 1991). Cultivation-based heterotrophic plate count (HPC) method has a long history to be recommended in most guidelines, but it detects only a small fraction of the total microbes (Allen et al., 2004; Bartram et al., 2003). Flow cytometry (FCM) coupled with nucleic acid targeting stains has been recently developed and proven to be an sensitive tool for measuring the total cell concentration in water. The two methods can be integrated to elucidate the dynamic of cultivable and uncultivable microbial biomass in DWSs (Hammes et al., 2008).

In terms of community composition, 16S rRNA gene based sequencing revealed that DWSs harbored diverse microbes, including some opportunistic pathogens and disinfectant resistant bacteria in the distribution networks (Pinto et al., 2012; Berry et al., 2006; Holinger et al., 2014; Hwang et al., 2012; Lautenschlager et al., 2014; Lin et al., 2014; Mi et al., 2015). The microorganisms that survived after water treatment processes are considered to be an important source for the potential pathogens at tap faucets. Moreover, the seeded bacteria in some bio-enhanced filters that used to remove specialized pollutants posed a potential risk of leaking from filter biofilms (Zhang et al., 2013).

Some studies concurred that filtration and disinfection had more significant effects on the microbial community compared with other processes, such as coagulation and sedimentation, and that the biofiltration process may determine the characteristics of the downstream microbiome (Holinger et al., 2014; Kwon et al., 2011; Pinto et al., 2012; Wang et al., 2013). Meanwhile, another study showed no major changes occurred after sand filtration (Eichler et al., 2006). The discrepancies found among studies may result from different factors such as study areas, treatment process chains, time scale of sampling and sequencing methods. The temporal fluctuation is a critical consideration for rigorous statistical tests and high validity of information on the processes. However, longitudinal surveys on the dynamics of microbial communities through treatment processes were still limited.

In the present study, the temporal and spatial changes in microbial communities of a full-scale drinking water treatment plant (DWTP) in Beijing, China, were investigated over 9 months. HPC, FCM and 454 pyrosequencing were used to measure the microbial biomass and bacterial compositions of both bulk water and granular activated carbon (GAC) biofilms. An in-depth characterization of the microbial dynamic patterns in a DWTP was conducted, the results of this study may help to extend our knowledge about the microbial quality of water in DWTPs. The primary objective of this work was to determine (1) how the treatment processes and the temporal variation contribute to the microbial biomass and the bacterial community structure, (2) the influence of GAC biofilm on the water microbiology and (3) the distinct and persistent bacteria that present throughout the treatment processes.

1. Materials and methods

1.1. Drinking water treatment processes and sampling schedule

The DWTP monitored in this study produces 60% of the drinking water of Beijing, China. During the sampling period, its water source consists of two reservoirs (Miyun reservoir in Beijing and Huangbizhuang reservoir in Hebei) as well as groundwater from Huairou aquifer. The average volume mix ratio of these water sources is 4:1:2. The treatment processes include pre-chlorination, coagulation, clarification and coal-sand dual media filtration as the conventional treatment and GAC filtration as the advanced treatment (Fig. 1). The GAC tanks are backwashed every 6 days. Free chlorine is added to the GAC effluent at a concentration of 1.2–1.8 mg/L for 5 hr. 0.2 mg/L ammonia is added post clear well to produce a chloramine residual of 0.7–0.8 mg/L in the distribution system.

Samples were collected in 6 months over a period of 9 months in 2012 (May, August, October, November and December) and 2013 (January). The pre-chlorinated raw water (RW), the coal-sand filter effluent (SE), the GAC tank effluent (CE) and the finished water (FW) were water samples and the GAC particles (CB) were biofilm samples. The GAC particles were taken from the top of the filter tank. Samples were collected in sterile bottles, which were taken to the laboratory within 4 hr. Water quality parameters were listed in Appendix A Table S1.

1.2. Heterotrophic plate count

1-mL aliquots of ten-fold serial dilutions of each water sample were mixed with 20 mL R2A agar (Difco, BD, USA) and incubated at 20°C for 7 days. All HPC determinations were performed in triplicate.

1.3. Total cell concentrations measured by flow cytometry

Total cell concentrations were determined according to the method introduced by Hammes et al. (2008) with a Cell Lab Quanta SC flow cytometer (Beckman Coulter, Inc., Brea, CA, USA). The total cell concentration of all the samples should be maintained between 3×10^3 and 2×10^6 cells/mL.

1.4. DNA extraction

10–40 L of bulk water were filtered through a 0.22-μm pore size membrane (47 mm diameter, Millipore, USA) with a 90-mm Filter Holder (Millipore, USA). For GAC biofilm samples, about

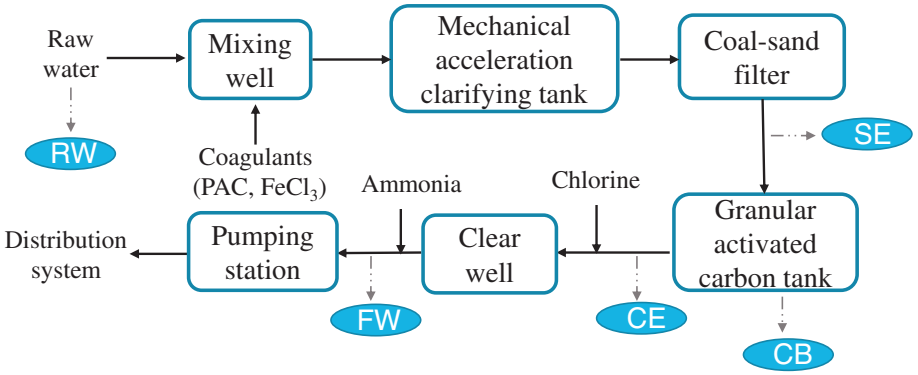

Fig. 1 – Schematic diagram of the drinking water treatment process. RW: raw water; SE: coal–sand filter effluent; CB: GAC particles; CE: GAC tank effluent; FW: finished water; GAC: granular activated carbon.

15 g wet carbon particles were rinsed twice with sterile PBS buffer to remove planktonic cells and then immersed in 30 mL sterile PBS buffer. Ultrasonication (500 W, 40 kHz, 20 min) was applied to detach bacteria from carbon particles combining vortexing or hand-shaking for 30 sec every 10 min. Ice bags were used to prevent the thermal effect caused by ultrasonication. After ultrasonication, the suspension was transferred and centrifuged at 10,000 ×*g* for 10 min at 4°C to collect the pellet of microbes and the supernatant was discarded.

The total DNA was extracted from the filter membranes and the pellets of GAC biofilm with an EZNA® Soil DNA kit (Omega Bio-Tek, USA) according to the manufacturer's protocol. The concentration and purity of the total DNA were measured by a NanoDrop 2000 spectrophotometry (Thermo Fisher Scientific, Wilmington, DE, USA).

1.5. 454 *pyrosequencing and data analysis*

The hypervariable V1-V3 region of the 16S rRNA gene was amplified using the universal bacterial primers 27F (5′-AGA GTTTGATCCTGGCTCAG-3′) and 533R (5′-TTACCGCGGCTGC TGGCAC-3′). Pyrosequencing was performed on the Roche 454 FLX pyrosequencing platform at SinoGenoMax (Beijing, China). Pyrosequencing results were processed using the Quantitative Insights Into Microbial Ecology (QIIME) (v 1.5.0) pipeline (Caporaso et al., 2010). Briefly, raw sequences were first filtered with a minimum average quality score ≥ 25, a sliding window value of 50 and size between 200 and 700 bp; then denoising was applied to reduce erroneous sequences with imprecise signals. After denoising, the Uclust algorithm was used for operational taxonomic unit (OTU) picking (97% similarity). Representative sequences of each OTU were selected for alignment to the Greengenes aligned reference set by PyNAST with default settings. Chimera Slayer was used to identify chimeras, which were then removed from the representative sequences. Taxonomy assignment was performed with the RDP classifier using the Greengenes reference at a 0.8 minimum confidence level. Alpha and beta diversities were calculated at a sequencing depth of 950 after removing the singleton sequences. Principal coordinate analysis (PCoA) was conducted with QIIME to visualize pairwise Bray–Curtis distance among samples.

Analysis of Similarity (ANOSIM) and Similarity Percentage (SIMPER) was performed based on the Bray–Curtis dissimilarity distance matrices at OTU level with the software PAST. ANOSIM is a non-parametric test of significant difference between two or more groups based on any distance measure. Distances are converted into ranks prior to calculating the statistic *R*. *R* was calculated by Eq. (1):

$$R = \frac{r_b - r_w}{\frac{1}{4}n(n-1)} \quad (1)$$

where, r_b is the mean rank of all distances between groups, r_w is the mean rank of all distances within the same group, and n is the total number of samples (Clarke, 1993). *R* ranges from –1 to 1 and larger *R* indicates higher degree of separation between groups. The significance is computed by random permutation of group membership. SIMPER is used to weight the contribution of each OTU to the dissimilarity between groups confirmed to be significant by ANOSIM.

Venn diagram was plotted to identify the numbers of shared OTUs among different sampling locations with the R package. OTUs that were present in at least 50% of samples at each sampling location were picked out respectively and they were the source data for the Venn diagram.

2. Results and discussion

2.1. *Planktonic biomass changes through treatment processes*

Microbial biomass in raw water based on HPC and total cell concentration (FCM) ranged from 1.4×10^3 to 5.9×10^4 CFU/mL and 2.7×10^5 to 3.9×10^5 cells/mL, respectively (Fig. 2). The biomass was significantly higher in May, August and October 2012 than in other months, and positive correlation was observed between the biomass and the water temperature (Appendix A Table S2) (Pearson's *R* = 0.841 for HPC and 0.877 for total cell concentration). Conventional treatment processes had an average removal rate of 61.6% for HPC and 37.7% for total cell concentration, whereas the average removal rates of GAC filtration were 32.1% and 8.3% for HPC and total cell concentration, respectively. No cultivable bacteria (HPCs) were detected in all the finished water samples, which met the

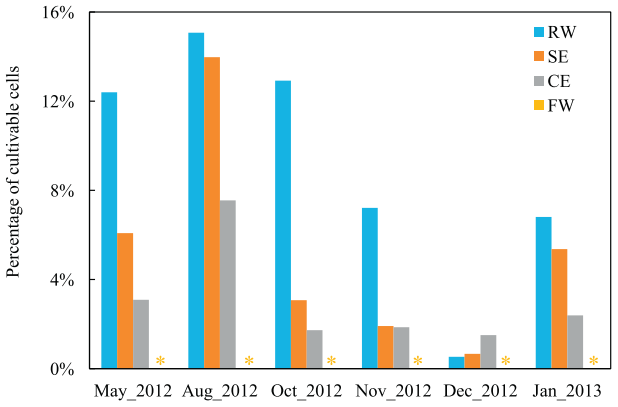

Fig. 2 – The percentage of R2A cultivable cells (heterotrophic plate count/total cell concentration) along treatment processes (* indicates no cultivable bacteria (HPCs) was detected in the sample). HPCs: heterotrophic plate count.

standard for drinking water quality in China. However, the total cell concentration in finished water was in the range of 7×10^3–61×10^3 cells/mL, suggesting a large number of uncultivable bacteria survived after disinfection and would seed in the distribution system.

The percentage of cultivable cells (HPC/total cell concentration) ranged from 0% to 15% (Fig. 2). Interestingly, the averaged percentage of cultivable cells decreased as the treatment process progressed. It's 9.2% in RW, 5.2% in SE and 3.0% in CE. In addition, the proportions of cultivable cells decreased in November and December 2012 and January 2013, when the temperature of the water decreased significantly. The reasons for variation of R2A cultivable cell proportion were complicated. Converse variation of different bacteria groups and the phenotype switch of the same species might both lead to the phenomenon. According to literature, total cell concentration detected by FCM was commonly divided into two distinct clusters: high nucleic acid bacteria (HNA) and low nucleic acid bacteria (LNA). LNA cells are considerably much smaller than HNA cells and harder to cultivate (Lautenschlager et al., 2010; Wang et al., 2009). Lautenschlager observed a clear shift from more HNA bacteria to more LNA bacteria in the serial filtration processes (Lautenschlager et al., 2014). In our study, the coal–sand filter and GAC tank might preferably remove the cultivable HNA with lager cell size as well and resulted in the decrease of R2A cultivable bacteria proportion. Low temperature in winter months might also preferably restrain the growth of cultivable bacteria and even induce the viable-but-nonculturable (VBNC) stages of some species. Laboratory studies with pure cultures have shown that low nutrient and low temperature have been shown to be the main causes of the induction of VBNC stages in some bacteria strains, including indicator organisms such as *Escherichia coli* and water-borne pathogens such as *Vibriocholerae* (Mizunoe et al., 2000; Xu et al., 1982). Disinfection was another environment pressure that causes the adoption of a VBNC state. Hoefel ever found that *Stenotrophomonas maltophila* was initially cultivable by HPC in raw water and settled water, but undetected by HPC after disinfection whilst remaining active (Hoefel et al., 2005). Decreased R2A cultivability of total bacteria might result in a general underestimation of biomass with HPC method. Further detailed investigation targeted on discrimination and sorting of different bacteria groups by FCM combining other molecular methods would allow a better understanding of microbial biomass fluctuation.

2.2. Diversity and composition of the microbial community

The bacterial 16S rRNA gene V1–V3 region of all bulk water and biofilm DNA samples was analyzed by 454-pyrosequencing. After quality filtration of the raw data, 62,399 effective sequences (8405–974 per sample) were obtained in total and 1615 OTUs were assigned. To fairly compare the 30 samples at the same sequencing depth, 950 sequences were randomly picked from each sample for further analysis. The averaged Good's coverage of each sampling group ranged from 90% to 92% indicating the majority of bacterial communities had been well covered. The averaged alpha diversity indices of Shannon, Chao 1 were highest in the CB samples and lowest either in CE or FW samples (Appendix A Table S3), indicating higher richness and bacterial diversity within GAC biofilms than in bulk water samples.

A total of 30 phyla were detected across all of the biofilm and water samples (Fig. 3a). *Proteobacteria* (53.3%), *Bacteroidetes* (10.7%), Firmicutes (9.9%) and *Actinobacteria* (8.4%), *Planctomycetes* (7.3%), and *Cyanobacteria* (5.0%) dominated in the water samples, constituting about 82.3% of the total sequences in all water samples. *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* are the three most common dominating phyla in drinking water systems (Lautenschlager et al., 2014; Lin et al., 2014; Pinto et al., 2012).

In comparison to water samples, the three dominant phyla in carbon biofilm were *Proteobacteria* (48.3%), *Acidobacteria* (22.9%) and *Planctomycetes* (16.3%). *Acidobacteria* was also abundant but not that dominant in the biofilm samples of other studies (Pinto et al., 2012; Liao et al., 2013). It is a newly devised phylum with 26 recognized subdivisions. Bacteria in this phylum are widely distributed and abundant in soils and freshwater sediments, but numbers of cultured representatives are very limited (Newton et al., 2011). Genomic analyses of some isolated strains from the phylum indicated that they are best adapted to low-nutrient conditions, capable of utilizing diverse polysaccharides, and even involved in biofilm formation (Rawat et al., 2012; Ward et al., 2009). In our study, more than 80% of *Acidobacteria* in the GAC biofilm samples belonged to the class of *Chloracidobacteria*. However, still little is known about their metabolic and genetic functions.

The *Cyanobacteria* population was also abundant with averaged relative abundance of about 5.0% in water samples, but this decreased to 0.2% in the GAC biofilm samples, which contradicted a recent study that showed *Cyanobacteria* was more abundant in biofilm sample than in water samples (Lin et al., 2014). Whereas other observations were consistent that *Cyanobacteria* was more abundant in bulk water (Lautenschlager et al., 2014; Pinto et al., 2012).

The dominant subclasses of *Proteobacteria* were *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria* (Fig. 3b). Along the treatment process flow, the relative abundances of *Alphaproteobacteria* and *Betaproteobacteria* decreased from 41.8% and 12.4% in raw

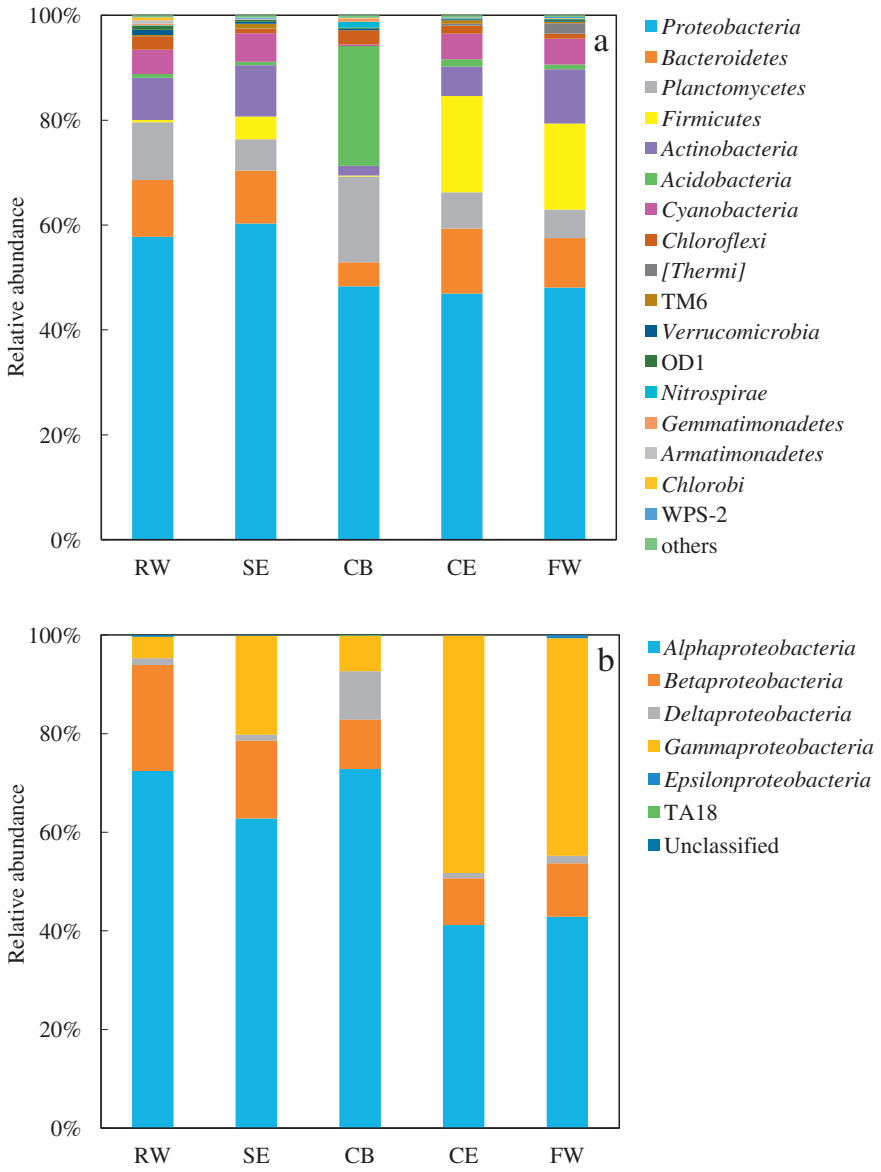

Fig. 3 – Relative abundances of bacterial communities at the phylum level (a) averaged over 9 months (phyla with relative abundances below 0.1% in all samples were combined with unclassified bacteria as others) and relative abundances of subclasses of *Proteobacteria* (b).

water to 20.6% and 5.2% in finished water, respectively. In contrast, the relative abundances of *Gammaproteobacteria* and *Bacilli* (belonging to the phylum *Firmicutes*) increased from about 2.5% and 0.2% to 21.2% and 16%, respectively. *Deltaproteobacteria* was more abundant in CB samples than in water samples.

2.3. *Temporal and spatial dynamics of the microbial community*

To visually demonstrate the temporal and spatial dynamics of the microbial community, PCoA based on the Bray–Curtis distance metric was performed at OTU level (Fig. 4). The plot showed that (1) the GAC biofilm samples and the water samples occupied distinct positions, (2) changes of water samples according to sequence of the treatment process flow took place along principal coordinate 2, and (3) no clear clustering pattern was found among samples from the same sampling date, indicating that the dynamics of the microbial communities of the water samples were mainly affected by the treatment processes, not by the sampling time. It was also noteworthy that all the biofilm samples clustered very closely except for the sample from May 2012, indicating a mature biofilm with a stable community structure had established in the carbon particles. The stability was confirmed by Bray-Curtis similarities within different sampling groups, the higher of which indicated more stable community structures (Keith et al., 2010). The Bray–Curtis similarity within CB samples was 0.54, while values within RW, SE, CE and FW samples were all below 0.23 (Appendix A Table S3).

The clustering patterns of samples in PCoA plot were confirmed by one-way ANOSIM test based on spatial and temporal grouping categories respectively. The statistical

significance level was set at 0.05. To counteract the problem of multiple comparisons in ANOSIM, Bonferroni-corrected *p*-values were used in pairwise test. Bonferroni correction is multiplying the uncorrected *p*-value with the number of pairwise comparisons between groups. It is more conservative and avoids the presence of false positive. In spatial variation test, samples from the same process were assigned as a group, so there were 5 groups and 10 pairwise comparisons. The global ANOSIM results revealed significant differences with a global *R* of 0.52 and p-value of 0.0001. From the results of pair-wise ANOSIM tests (Table 1), CB was distinct from all the other groups, and especially from RW, with a high *R* of 0.969; while RW vs SE, SE vs CE, SE vs CE and CE vs FW showed no significant differences (Bonferroni-corrected $p > 0.05$). RW was significantly different from CE and FW, indicating that the microbial communities of the water samples were greatly changed after GAC filtration. This was consistent with another finding that filtering shapes the bacterial community of post-filtration samples based on the OTU grouping strategy (Pinto et al.,2012). In temporal test, as biofilm samples were distinct, they were excluded when water samples were grouped by sampling time; there were 6 groups and 15 pairwise comparisons. The global ANOSIM ($R = 0.34$, $p = 0.001$) still indicated significant but a little weaker differences among groups. Whereas the pair-wise comparison (Table 2) showed that Bonferroni-corrected *p*-values between different groups were all above 0.05, indicating there was no distinct difference among water samples in different months. The non-significant pairwise result for temporal test difference was also in accordance with the random scattering of water samples in the same sampling date in the PCoA plot.

The two methods PCoA and ANOSIM corroborated each other and revealed that the dynamics of microbial communities among all samples were best explained by treatment processes not by sampling time. In a study at Ann Arbor, PCoA plots also illustrated that the spatial dynamics showed the similar pattern irrespective of the three seasons (Pinto et al., 2012). Roeselers et al. (2015) found that different treatment plants, rather than the sampling time, differentiated drinking water microbial communities. El-Chakhtoura's short-term study (hourly, daily and weekly scales) also demonstrated that no significant variation in bacterial community profiles occurred in the treated and distributed water samples over time (El-Chakhtoura et al., 2015). Conversely, long-term studies of drinking water distribution systems found seasonality to be the main contributor to microbial dynamics of suspended and biofilm communities apart from the locations, pipe materials and pipe ages (Ling et al., 2015; Pinto et al., 2014). To conclude, treatment strategy is the most important factor that differentiates microbial communities in the entire drinking water system from source to tap. Profound variations usually took place after filtration processes. It is necessary to conduct more detailed surveys to distinguish the influence of treatment strategies especially filtration patterns on microbial community in water.

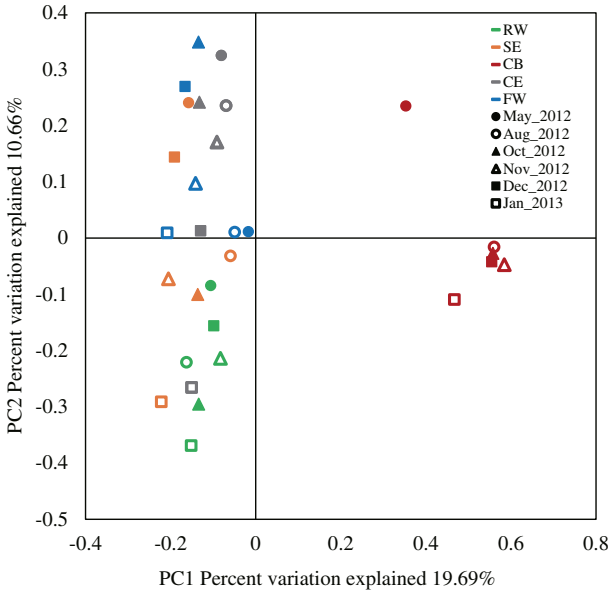

Fig. 4 – Principal coordinate analysis (PCoA) plot based on the Bray–Curtis distance metric illustrating the variability of samples over time and along the treatment processes. Data points are colored according to sampling positions, and the sampling dates are distinguished by different shapes.

2.4. Distinct and persistent bacterial taxa

To further investigate distinct OTUs associated with the spatial dynamics of community composition when exposed to different treatment processes, SIMPER analysis was performed. The results showed that the top 20 OTUs contributing most to the dissimilarity among different sampling groups were responsible for 37% of the difference and they accounted for 40% of total sequences (Table 3). 18 of them belonged to the 20 most abundant OTUs in all samples as well. This might be explained by the sensitivity of SIMPER to relative abundance (Clarke, 1993). The relative abundance of each OTU varied uniquely and different sampling locations were dominated by different OTUs.

Three OTUs in Table 3 were classified to the family *Sphingomonadaceae*. Their relative abundances were all higher in water samples and declined slightly with the treatment processes. Members of this family are ubiquitous in drinking water systems because of their remarkable ability to survive oligotrophic and even chlorinated environments (Srinivasan et al., 2008). They are strictly aerobic and many species can be isolated on culture media with yellow pigmentation. Vaz-Moreira et al. (2011) previously reported that the counts of cultivable *Sphingomonadaceae* were higher in treated water and further identification demonstrated that high levels of antibiotic resistance existed in five genera of this family isolated from different sites in the same drinking water system. *Sphingomonadaceae* were also highly represented in free living amoebae and biofilm (Delafont et al., 2013; Zhang et al., 2012) which might pose a threat to water quality. Four OTUs, which were classified to *Bacillaceae*, *Legionellaceae*, *Pseudomonadaceae* and *Moraxellaceae* respectively, were more abundant in FW samples indicating their likely resistance to disinfectant. Previous studies also found genera of these families in disinfected water (Cervero-Aragó et al., 2015; Mir et al., 1997; Wang et al., 2012). While another two

Table 1 – ANOSIM statistics for pairwise comparisons of samples grouped by treatment process (the global R = 0.52, p = 0.0001). *

(R, p_B)	CB	RW	SE	CE
CB				
RW	(0.969,0.019)			
SE	(0.935,0.025)	(0.081,1)		
CE	(0.874,0.026)	(0.652,0.024)	(0.017,1)	
FW	(0.738,0.022)	(0.490,0.024)	(0.057,1)	(0.119,0.953)

* p_B represents Bonferroni-corrected p-value. Values in bold indicate a Bonferroni-corrected p-value < 0.05 (uncorrected p-values < 0.005).

OTUs also affiliated to *Moraxellaceae* and abundant in CE samples did not occur in FW samples. They were classified to the same genus *Acinetobacter*, but different species. The OTU classified to family Ellin6075 was the most abundant OTU in CB samples (accounted for 15.9% of total sequences in CB samples and 78.5% of *Acidobacteria* in all samples). Though the phylum *Acidobacteria* has been poorly studied and few cultivated representatives are known, some species of the family Ellin6075 were isolated under selective laboratory conditions (Crowe et al., 2014; Joseph et al., 2003) indicating the possibility of further analysis. In water samples its relative abundance increased slightly in the CE samples by 0.4% compared to SE samples indicating the predominant bacteria in biofilm might seed the effluent through sloughing of biofilms.

Although bacterial community changed significantly at each step of treatment processes, some OTUs were commonly found throughout treatment processes and over different sampling time. OTUs that were present in at least 50% of samples at each sampling location were depicted in a Venn diagram (Fig. 5). Six of these OTUs, which constituted 16.1% of the total sequences, were shared by all sampling groups. According to the conceptual framework of the core community proposed by Shade and Handelsman (2012), they were defined persistent OTUs in the entire treatment process flow. Three of the six OTUs were also among the 20 most abundant OTUs in all samples. Five of the six OTUs were classified to the class *Alphaproteobacteria* and the other one was classified to the genus *Chryseobacterium*, belonging to the class *Flavobacteriia*. *Alphaproteobacteria* is dominant in most drinking water systems (El-Chakhtoura et al., 2015; Williams et al., 2004) and has been reported to be competitive in low nutrient freshwater with availability of degrading complex organic compounds (Newton et al., 2011). The genus *Chryseobacterium* was reported to be an amoebae-resisting bacterium in a DWTP and the species *Chryseobacterium meningosepticum* which could colonize water taps was pathogenic in humans (Hoque et al., 2001; Thomas et al., 2008). In addition, the number of shared OTUs among CB and CE samples was 27, 7 more than that among SE and CB samples. This supported the finding that CE water was slightly influenced by CB samples. Persistent OTUs in systems were usually difficult to remove completely and remained in finished water as a potential threat. Therefore comprehensive and systematic studies on distinct and core OTUs will make important contributions to the understanding of microbial characters of drinking water systems.

3. Conclusions

This study provides a comprehensive view of the dynamics of the microbial biomass and bacterial community during treatment processes over time. The important findings achieved were as follows: (1) The treatment processes and water temperature probably had remarkable influences on the R2A cultivability of total bacteria. (2) The variation of bacterial communities with treatment processes rather than the temporal fluctuation was the primary dynamic pattern. (3) The GAC filter harbored a mature biofilm with a stable and distinct microbial community. Profound changes in the microbial community in water occurred after GAC filtration. (4) Distinct bacterial taxa at each process and persistent taxa throughout the treatment process flow were identified.

Acknowledgments

This work was supported by the China Major Science and Technology Program for Water Pollution Control and Treatment (No. 2012ZX07404-002) and the Special Fund of State Key Joint Laboratory of Environment Simulation and Pollution Control (No. 14K09ESPCT).

Table 2 – Analysis of similarity (ANOSIM) statistics for pairwise comparisons of water samples grouped by sampling months (the global R = 0.34, p = 0.001). *

(R, p_B)	May	Aug	Oct	Nov	Dec
May					
Aug	(0.130, 1)				
Oct	(0.313,0.863)	(0.120,1)			
Nov	(0.417,0.431)	(0.146,1)	(0.396,1)		
Dec	(0.260,0.431)	(0.208,1)	(0.427,1)	(0.208,1)	
Jan	(0.604,0.408)	(0.500,0.453)	(0.635,0.515)	(0.781,0.408)	(0.370,0.903)

* p_B represents Bonferroni-corrected p-value. All Bonferroni-corrected p-values > 0.05 (uncorrected p-values > 0.003).

Table 3 – SIMPER analysis results displaying top 20 OTUs responsible for dissimilarity among different sampling groups.

No.	Contrib. %	Cumulative %	Mean abund. CB	Mean abund. RW	Mean abund. SE	Mean abund. CE	Mean abund. FW	Family
OTU1909	5.294	5.294	1.34%	17.05%	8.84%	4.92%	4.11%	*Sphingomonadaceae*
OTU1597	3.693	8.987	15.89%	0.00%	0.02%	0.37%	0.04%	Ellin6075
OTU1992	3.541	12.53	3.18%	0.37%	3.07%	9.86%	5.42%	*Weeksellaceae*
OTU1441	3.147	15.67	0.00%	0.00%	1.42%	12.21%	0.46%	*unclassified_Bacillales*
OTU2387	2.507	18.18	0.00%	0.04%	0.04%	0.02%	10.84%	*Bacillaceae*
OTU1673	1.973	20.16	0.00%	1.28%	1.84%	1.63%	4.92%	*Legionellaceae*
OTU1599	1.872	22.03	4.13%	4.58%	2.32%	2.32%	0.54%	*unclassified_Sphingomonadales*
OTU1611	1.572	23.6	0.00%	0.00%	6.53%	0.28%	0.09%	*Sphingomonadaceae*
OTU689	1.522	25.12	0.04%	1.98%	1.97%	3.16%	1.45%	*Gemmataceae*
OTU903	1.499	26.62	0.00%	1.08%	3.11%	3.26%	0.04%	*Synechococcaceae*
OTU1592	1.238	27.86	1.34%	0.04%	1.28%	2.40%	2.45%	unclassified_HOC36
OTU1949	1.202	29.06	0.05%	0.02%	1.26%	0.26%	4.11%	*Pseudomonadaceae*
OTU1825	1.174	30.23	0.02%	0.00%	0.12%	4.98%	0.00%	*Moraxellaceae*
OTU1164	1.15	31.38	0.00%	3.24%	1.89%	0.47%	0.54%	*Pelagibacteraceae*
OTU2319	1.106	32.49	0.02%	0.00%	0.47%	4.44%	0.09%	*Moraxellaceae*
OTU1459	1.065	33.56	0.00%	1.65%	2.56%	0.70%	0.70%	C111
OTU223	0.9754	34.53	0.11%	2.40%	2.37%	0.42%	0.12%	*Sphingomonadaceae*
OTU1274	0.8756	35.41	0.07%	0.00%	0.65%	0.26%	3.18%	*Moraxellaceae*
OTU2280	0.8225	36.23	0.04%	2.47%	1.02%	0.19%	0.05%	*Comamonadaceae*
OTU89	0.8007	37.03	0.02%	0.02%	0.00%	2.93%	0.61%	*Bacillaceae*

RW: raw water; SE: coal–sand filter effluent; CB: GAC particles; CE: GAC tank effluent; OTU: operational taxonomic units; SIMPER: similarity percentage.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2016.05.042.

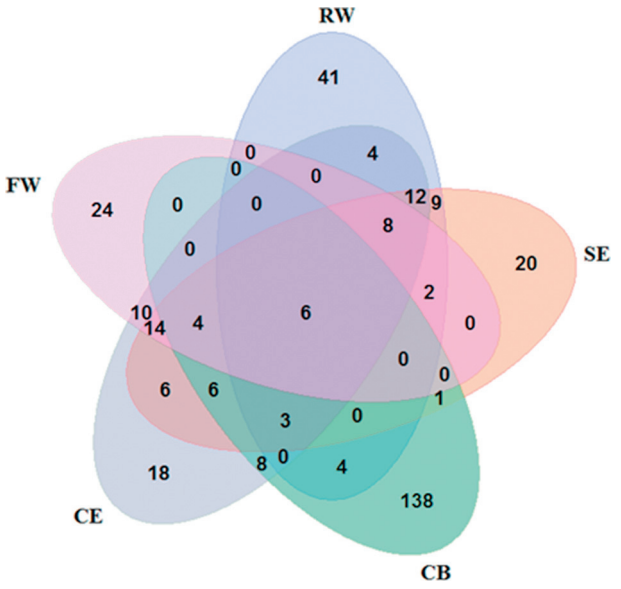

Fig. 5 – Venn diagram showing the numbers of shared operational taxonomic units (OTUs) among samples from different sampling locations (the numbers in overlaps of the diagram indicated shared OTUs that were detected in at least 50% of samples at each of the two or more corresponding sampling locations).

REFERENCES

Allen, M.J., Edberg, S.C., Reasoner, D.J., 2004. Heterotrophic plate count bacteria-what is their significance in drinking water? Int. J. Food Microbiol. 92 (3), 265–274.

Au, K., 2004. Water Treatment and Pathogen Control: Process Efficiency in Achieving Safe Drinking-Water. IWA Publishing.

Bartram, J., Cotruvo, J., Exner, M., Fricker, C., Glasmacher, A., 2003. Heterotrophic Plate Counts and Drinking-Water Safety: The Significance of HPCs for Water Quality and Human Health. IWA Publishing.

Berry, D., Xi, C., Raskin, L., 2006. Microbial ecology of drinking water distribution systems. Curr. Opin. Biotechnol. 17 (3), 297–302.

Boe-Hansen, R., Albrechtsen, H., Arvin, E., Jørgensen, C., 2002. Bulk water phase and biofilm growth in drinking water at low nutrient conditions. Water Res. 36 (18), 4477–4486.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7 (5), 335–336.

Carter, J.T., Rice, E.W., Buchberger, S.G., Lee, Y., 2000. Relationships between levels of heterotrophic bacteria and water quality parameters in a drinking water distribution system. Water Res. 34 (5), 1495–1502.

Cervero-Aragó, S., Rodríguez-Martínez, S., Puertas-Bennasar, A., Araujo, R.M., 2015. Effect of common drinking water disinfectants, chlorine and heat, on free *Legionella* and amoebae-associated *Legionella*. PLoS One 10 (8), e134726.

Chen, C., Zhang, X., He, W., Lu, W., Han, H., 2007. Comparison of seven kinds of drinking water treatment processes to enhance organic material removal: a pilot test. Sci. Total Environ. 382 (1), 93–102.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117.

Crowe, M.A., Power, J.F., Morgan, X.C., Dunfield, P.F., Lagutin, K., Rijpstra, W., Vyssotski, M., Damste, J.S., Houghton, K.M., Ryan,

J., 2014. *Pyrinomonas methylaliphatogenes* gen. nov., sp. nov., a novel group 4 thermophilic member of the phylum *Acidobacteria* from geothermal soils. Int. J. Syst. Evol. Microbiol. 64 (1), 220–227.

Delafont, V., Brouke, A., Bouchon, D., Moulin, L., Héchard, Y., 2013. Microbiome of free-living amoebae isolated from drinking water. Water Res. 47 (19), 6958–6965.

Eichler, S., Christen, R., Höltje, C., Westphal, P., Bötel, J., Brettar, I., Mehling, A., Höfle, M.G., 2006. Composition and dynamics of bacterial communities of a drinking water supply system as assessed by RNA-and DNA-based 16S rRNA gene fingerprinting. Appl. Environ. Microbiol. 72 (3), 1858–1872.

El-Chakhtoura, J., Prest, E., Saikaly, P., van Loosdrecht, M., Hammes, F., Vrouwenvelder, H., 2015. Dynamics of bacterial communities before and after distribution in a full-scale drinking water network. Water Res. 180–190.

Hammes, F., Berney, M., Wang, Y., Vital, M., Köster, O., Egli, T., 2008. Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. Water Res. 42 (1), 269–277.

Henne, K., Kahlisch, L., Höfle, M.G., Brettar, I., 2013. Seasonal dynamics of bacterial community structure and composition in cold and hot drinking water derived from surface water reservoirs. Water Res. 47 (15), 5614–5630.

Hoefel, D., Monis, P.T., Grooby, W.L., Andrews, S., Saint, C.P., 2005. Profiling bacterial survival through a water treatment process and subsequent distribution system. J. Appl. Microbiol. 99 (1), 175–186.

Holinger, E.P., Ross, K.A., Robertson, C.E., Stevens, M.J., Harris, J.K., Pace, N.R., 2014. Molecular analysis of point-of-use municipal drinking water microbiology. Water Res. 49, 225–235.

Hoque, S.N., Graham, J., Kaufmann, M.E., Tabaqchali, S., 2001. Chryseobacterium (Flavobacterium) meningosepticum outbreak associated with colonization of water taps in a neonatal intensive care unit. J. Hosp. Infect. 47 (3), 188–192.

Hwang, C., Ling, F., Andersen, G.L., LeChevallier, M.W., Liu, W., 2012. Microbial community dynamics of an urban drinking water distribution system subjected to phases of chloramination and chlorination treatments. Appl. Environ. Microbiol. 78 (22), 7856–7865.

Isaac-Renton, J., Moorehead, W., Ross, A., 1996. Longitudinal studies of Giardia contamination in two community drinking water supplies: cyst levels, parasite viability, and health impact. Appl. Environ. Microbiol. 62 (1), 47–54.

Joseph, S.J., Hugenholtz, P., Sangwan, P., Osborne, C.A., Janssen, P.H., 2003. Laboratory cultivation of widespread and previously uncultured soil bacteria. Appl. Environ. Microbiol. 69 (12), 7210–7215.

Keith, A.R., Bailey, J.K., Whitham, T.G., 2010. A genetic basis to community repeatability and stability. Ecology 91 (11), 3398–3406.

Kwon, S., Moon, E., Kim, T., Hong, S., Park, H., 2011. Pyrosequencing demonstrated complex microbial communities in a membrane filtration system for a drinking water treatment plant. Microbes Environ. 26 (2), 149–155.

Lautenschlager, K., Boon, N., Wang, Y., Egli, T., Hammes, F., 2010. Overnight stagnation of drinking water in household taps induces microbial growth and changes in community composition. Water Res. 44 (17), 4868–4877.

Lautenschlager, K., Hwang, C., Ling, F., Liu, W., Boon, N., Köster, O., Egli, T., Hammes, F., 2014. Abundance and composition of indigenous bacterial communities in a multi-step biofiltration-based drinking water treatment plant. Water Res. 62, 40–52.

LeChevallier, M.W., Schulz, W., Lee, R.G., 1991. Bacterial nutrients in drinking water. Appl. Environ. Microbiol. 57 (3), 857–862.

Liao, X., Chen, C., Wang, Z., Wan, R., Chang, C., Zhang, X., Xie, S., 2013. Changes of biomass and bacterial communities in biological activated carbon filters for drinking water treatment. Process Biochem. 48 (2), 312–316.

Lin, W., Yu, Z., Zhang, H., Thompson, I.P., 2014. Diversity and dynamics of microbial communities at each step of treatment plant for potable water generation. Water Res. 52, 218–230.

Ling, F., Hwang, C., LeChevallier, M.W., Andersen, G.L., Liu, W., 2016. Core-satellite populations and seasonality of water meter biofilms in a metropolitan drinking water distribution system. ISME J. 10 (3), 582–595.

Liu, W., Wu, H., Wang, Z., Ong, S.L., Hu, J.Y., Ng, W.J., 2002. Investigation of assimilable organic carbon (AOC) and bacterial regrowth in drinking water distribution system. Water Res. 36 (4), 891–898.

Lu, P., Zhang, X., Zhang, C., Niu, Z., Xie, S., Chen, C., 2014. Biostability in distribution systems in one city in southern China: characteristics, modeling and control strategy. J. Environ. Sci. 26 (2), 323–331.

Mi, Z., Dai, Y., Xie, S., Chen, C., Zhang, X., 2015. Impact of disinfection on drinking water biofilm bacterial community. 37 (11), 200–205.

Mir, J., Morato, J., Ribas, F., 1997. Resistance to chlorine of freshwater bacterial strains. J. Appl. Microbiol. 82 (1), 7–18.

Mizunoe, Y., Wai, S.N., Ishikawa, T., Takade, A., Yoshida, S., 2000. Resuscitation of viable but nonculturable cells of *Vibrio parahaemolyticus* induced at low temperature under starvation. FEMS Microbiol. Lett. 186 (1), 115–120.

Newton, R.J., Jones, S.E., Eiler, A., McMahon, K.D., Bertilsson, S., 2011. A guide to the natural history of freshwater lake bacteria. Microbiol. Mol. Biol. Rev. 1 (75), 14–49.

Pinto, A.J., Xi, C., Raskin, L., 2012. Bacterial community structure in the drinking water microbiome is governed by filtration processes. Environ. Sci. Technol. 46 (16), 8851–8859.

Pinto, A.J., Schroeder, J., Lunn, M., Sloan, W., Raskin, L., 2014. Spatial–temporal survey and occupancy-abundance modeling to predict bacterial community dynamics in the drinking water microbiome. Mbio 5 (3), 1–13.

Rawat, S.R., Männistö, M.K., Bromberg, Y., Häggblom, M.M., 2012. Comparative genomic and physiological analysis provides insights into the role of *Acidobacteria* in organic carbon utilization in Arctic tundra soils. FEMS Microbiol. Ecol. 82 (2), 341–355.

Roeselers, G., Coolen, J., Wielen, P.W., Jaspers, M.C., Atsma, A., Graaf, B., Schuren, F., 2015. Microbial biogeography of drinking water: patterns in phylogenetic diversity across space and time. Environ. Microbiol. 17 (7), 2505–2514.

Shade, A., Handelsman, J., 2012. Beyond the Venn diagram: the hunt for a core microbiome. Environ. Microbiol. 14 (1), 4–12.

Sharp, E.L., Parsons, S.A., Jefferson, B., 2006. Seasonal variations in natural organic matter and its impact on coagulation in water treatment. Sci. Total Environ. 363 (1), 183–194.

Srinivasan, S., Harrington, G.W., Xagoraraki, I., Goel, R., 2008. Factors affecting bulk to total bacteria ratio in drinking water distribution systems. Water Res. 42 (13), 3393–3404.

Thomas, V., Loret, J.F., Jousset, M., Greub, G., 2008. Biodiversity of amoebae and amoebae-resisting bacteria in a drinking water treatment plant. Environ. Microbiol. 10 (10), 2728–2745.

Vaz-Moreira, I., Nunes, O.C., Manaia, C.M., 2011. Diversity and antibiotic resistance patterns of *Sphingomonadaceae* isolates from drinking water. Appl. Environ. Microbiol. 77 (16), 5697–5706.

Wang, Y., Hammes, F., Boon, N., Chami, M., Egli, T., 2009. Isolation and characterization of low nucleic acid (LNA)-content bacteria. ISME J. 3 (8), 889–902.

Wang, H., Edwards, M., Falkinham, J.O., Pruden, A., 2012. Molecular survey of the occurrence of *Legionella* spp., *Mycobacterium* spp., *Pseudomonas aeruginosa*, and amoeba hosts in two chloraminated drinking water distribution systems. Appl. Environ. Microbiol. 78 (17), 6285–6294.

Wang, H., Pryor, M.A., Edwards, M.A., Falkinham, J.O., Pruden, A., 2013. Effect of GAC pre-treatment and disinfectant on

microbial community structure and opportunistic pathogen occurrence. Water Res. 47 (15), 5760–5772.

Ward, N.L., Challacombe, J.F., Janssen, P.H., Henrissat, B., Coutinho, P.M., Wu, M., Xie, G., Haft, D.H., Sait, M., Badger, J., 2009. Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. Appl. Environ. Microbiol. 75 (7), 2046–2056.

Williams, M.M., Domingo, J., Meckes, M.C., Kelty, C.A., Rochon, H.S., 2004. Phylogenetic diversity of drinking water bacteria in a distribution system simulator. J. Appl. Microbiol. 96 (5), 954–964.

Xu, H., Roberts, N., Singleton, F.L., Attwell, R.W., Grimes, D.J., Colwell, R.R., 1982. Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. Microb. Ecol. 8 (4), 313–323.

Zhang, M., Liu, W., Nie, X., Li, C., Gu, J., Zhang, C., 2012. Molecular analysis of bacterial communities in biofilms of a drinking water clearwell. Microbes Environ. 27 (4), 443.

Zhang, D., Li, W., Gong, H., Zhang, L., Gong, X., Liu, B., 2013. Evaluation of long term stability of seeded bacteria in a bio-enhanced activated carbon filter used for treating drinking water. Int. Biodeterior. Biodegrad. 85, 701–708.