

Bacterial community change through drinking water treatment processes

X. Liao · C. Chen · Z. Wang · C.-H. Chang ·
X. Zhang · S. Xie

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Abstract The microbiological quality of drinking water has aroused increasing attention due to potential public health risks. Knowledge of the bacterial ecology in the effluents of drinking water treatment units will be of practical importance. However, the bacterial community in the effluents of drinking water filters remains poorly understood. The changes of the density of viable heterotrophic bacteria and bacterial populations through a pilot-scale drinking water treatment process were investigated using heterotrophic plate counts and clone library analysis, respectively. The pilot-scale treatment process was composed of preozonation, rapid mixing, flocculation, sedimentation, sand filtration postozonation, and biological activated carbon (BAC) filtration. The results indicated that heterotrophic plate counts decreased dramatically through the drinking water treatment processes. Clone library analysis indicated the significant change of bacterial community structure through the water treatment processes. *Betaproteobacteria* was dominant in raw water, the sand filter effluent and the BAC filter effluent. This work could provide some new insights on drinking water microbial ecology.

Keywords Biological activated carbon · Drinking water treatment system · Sand filter · Microbial community

Xiaobin Liao and Chao Chen have contributed equally to this study.

X. Liao · C. Chen · X. Zhang
School of Environment, Tsinghua University, Beijing 100084,
China

Z. Wang · C.-H. Chang · S. Xie (✉)
College of Environmental Sciences and Engineering, The Key
Laboratory of Water and Sediment Sciences (Ministry of
Education), Peking University, Beijing 100871, China
e-mail: xiesg@pku.edu.cn

Introduction

The microbiological quality of drinking water has aroused increasing attention due to potential public health risks. The conventional treatment process, composed of coagulation–flocculation, sedimentation, rapid sand filtration, and disinfection, is still widely used by drinking water producers to remove turbidity and pathogens. The conventional treatment process is not efficient in removal of biodegradable dissolved organic carbon (BDOC) that is mainly responsible for the microbial regrowth in drinking water distribution systems (DWDS). Biological activated carbon (BAC) filtration can perform well in reduction of organic pollutants after the attachment of the indigenous microbiota attached to the porous surface of granular activated carbon (GAC). BAC filters are important for their capacity to biodegrade undesirable compounds. In addition, the combination of ozonation with BAC filtration has proven to be an efficient method in reduction in BDOC (Yapsakli and Cecen 2010). However, biomass developed in biofilters might be a source of bacterial contamination in DWDS (Niemi et al. 2009; Liao et al. 2012). Therefore, concerns regarding the microorganisms in the effluents of filters have been raised (Norton and LeChevallier 2000; Poitelon et al. 2010). Pathogenic or opportunistic microorganisms from may enter DWDS as a result of improper management (Villarreal et al. 2010).

The heterotrophic plate count (HPC), as a culture-dependent method, has been widely used to estimate the density of viable heterotrophic bacteria in drinking water and is still considered very useful for assessing the general microbial water quality (Sartory et al. 2008). However, only a small fraction of the viable bacterial cells present in drinking water could be identified using the traditional culture-dependent methods (Eichler et al. 2006; Sartory et al.

2008). Molecular methods have been widely used to characterize the microbial community dynamics in drinking waters in DWDS (Berry et al. 2006; Revetta et al. 2010), and drinking water biofilters (Kasuga et al. 2007; Feng et al. 2012; Liao et al. 2012). Since the presence of certain bacteria may lead to water quality problems in the downstream DWDS, knowledge of the bacterial ecology in the effluents of drinking water treatment units will be of practical importance. Unfortunately, only very few works have investigated the microbial communities in the effluents of drinking water treatment units (Eichler et al. 2006; Poitelon et al. 2010). Therefore, the bacterial community in the effluents of drinking water filters remains poorly understood.

It is generally accepted that chlorination can result in a dramatic shift in the composition of the bacterial community (Norton and LeChevallier 2000; Eichler et al. 2006; Kormas et al. 2010; Poitelon et al. 2010), yet little information is available on the bacterial community change through other drinking water treatment units. Pinto et al. (2012) found that filtration by dual media rapid sand filters in a drinking water plant played a primary role in shaping the bacterial community in DWDS. The main aim of the current study was to assess bacterial community change through drinking water treatment processes. The bacterial populations in raw water, the sand filter effluent, and the BAC filter effluent were compared. For each water sample, genomic DNA was extracted followed by construction of 16S rRNA gene clone library. Water samples were collected from a combined pilot-scale drinking water treatment system treating lake water operated in Jiangsu Province (China) in June 2012. Molecular analysis was carried out between June and August in 2012 at College of Environmental Sciences and Engineering, Peking University, China.

Materials and methods

Water samples

A pilot-scale drinking treatment system was constructed to treat surface water retrieved from a small lake, in Jiangsu Province. The whole treatment process was composed of

preozonation, rapid mixing, flocculation, sedimentation, sand filtration postozonation, and BAC filtration (Fig. 1). In this study, the BAC filter was operated in downflow mode. Before this study, the pilot-scale drinking treatment system had been operated for approximately ten months allowing for biofilm maturation. The operating parameters of the pilot-scale system were shown in Table 1.

Water samples in three replicates were collected from raw water, the sand filter effluent, and the BAC filter effluent, referred to as Samples A, B, and C, respectively. The three samples were collected in the order of Sample C, B and A. The replicate water samples at each location were collected at an interval of a week. HPCs were measured using R2A medium according to the standard methods (APHA, Awwa, WEF 1995). Temperature was measured using a JPBJ-608 DO meter (Shanghai, China), while pH was measured using a PHSJ3F pH meter (Shanghai, China). Turbidity was measured according to the standard methods described by China Environmental Protection Agency (2002). Samples for dissolved organic carbon (DOC) concentration were filtered through 0.45- μm -pore-size Whatman GF/F filters and analyzed with a SHIMADZU Total Organic Carbon Analyzer (Shimadzu, Japan).

Clone library analysis

The triplicate water samples (1 L) from each sampling location were pooled. The pooled water samples were filtered through 0.22- μm -pore-size membranes (diameter 50 mm; Millipore). The membrane filter was cut into quarters with a sterile scalpel, and each quarter was stored at $-20\text{ }^{\circ}\text{C}$ for further molecular analysis (Huang et al. 2011). DNA for water sample was extracted using the E.Z.N.A.[®] Water DNA kit (Omega, USA) according to the manufacturer's protocol. 16S rRNA genes were amplified using bacterial primers 27F (5'-GAGTTTGATCMTGGC TCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') according to the literatures (Zhang et al. 2012; Wang et al. 2012). The PCR products were cloned into pMD19-T vector (Takara Corp, Japan) with ampicillin selection and blue/white screening. The clones containing correct size

Fig. 1 Schematic of the pilot-scale drinking treatment system. Sample A, B, and C represent raw water, the sand filter effluent, and the biological activated carbon (BAC) filter effluent, respectively

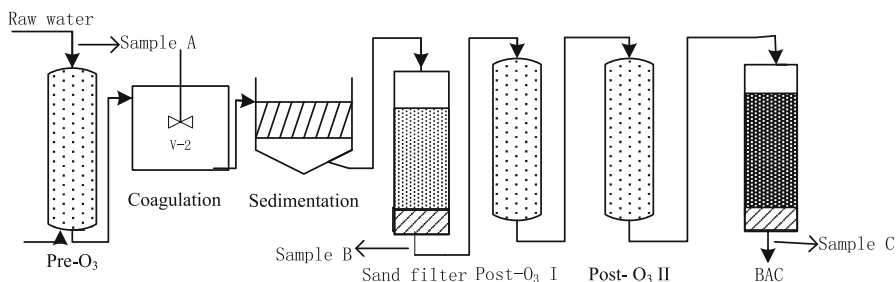


Table 1 Operating parameters of the pilot-scale drinking treatment system

Process	Parameters	Designed or nominal values
Pre-O ₃	Contact time	7.5 min
	Ozone dose	0.5 mg L ⁻¹
Flocculation	Paddle type	Three stages
	Retention time	18.66 min
	Coagulant dose	50 mg L ⁻¹ (as 10 % liquid aluminum)
Sedimentation	Inclined tube type	7.97 min
	Retention time	
Sand filter	Bed depth	1,150 mm
	Grain size	0.8 mm
	Filtration velocity	8 m h ⁻¹
Post-O ₃ I	Contact time	7.5 min
	Ozone dose	0.5 mg L ⁻¹
Post-O ₃ II	Contact time	7.5 min
	Ozone dose	0.5 mg L ⁻¹
BAC filtration columns	Bed depth	1,000 mm
	Filtration velocity	8 m h ⁻¹
	Size	∅ 400 mm × 2,210 mm

Table 2 Water quality parameters at the time of sampling

Parameter	Sample A	Sample B	Sample C
Temperature (°C)	12.2 ± 0.3	12.2 ± 0.3	12.2 ± 0.3
pH	7.78 ± 0.01	7.35 ± 0.02	7.31 ± 0.01
Turbidity (NTU)	1.05 ± 0.02	0.57 ± 0.03	0.21 ± 0.02
DOC (mg L ⁻¹)	4.25 ± 0.1	3.35 ± 0.07	2.35 ± 0.05
HPCs (CFU mL ⁻¹)	380 ± 20	43 ± 7	10 ± 2

Sample A, B, and C represent raw water, the sand filter effluent, and the BAC filter effluent, respectively

were sequenced. Chimera-free sequences with ≤3 % difference were clustered into operational taxonomic units (OTUs) using DOTUR program as well as the OTU-based richness and diversity indices were calculated (Schloss and Handelsman 2005). The Ribosomal Database Project analysis tool ‘classifier’ (<http://rdp.cme.msu.edu/classifier/classifier.jsp>) was utilized to classify the taxonomic identities of the obtained bacterial sequences (Wang et al. 2007). Phylogenetic trees were constructed using MEGA software version 4.0 (Tamura et al. 2007). The sequences obtained in this study were submitted to GenBank under accession numbers JX464674–JX464708, and JX472014–JX472030 for Sample A, JX464709–JX464763 for sample B, and JX464764–JX464818 for Sample C, respectively.

Table 3 Operational taxonomic units (OTUs)-based richness and Shannon index (calculated at 0.03 difference level) of three clone libraries

Library	Number of clones	Number of OTUs	Chao1	Shannon index
Sample A	52	33	79	3.26
Sample B	55	25	46	2.86
Sample C	55	18	36	2.13

Sample A, B, and C represent raw water, the sand filter effluent, and the BAC filter effluent respectively. These data represent the composite results from three samples

Results and discussion

Water quality

Table 2 showed the bacteriological and physicochemical analyses of raw water, the sand filter effluent, and the BAC filter effluent. The results indicated that turbidity, DOC, and HPCs decreased dramatically through the treatment processes. The HPCs in the sand filter effluent and the BAC filter effluent were relatively low, compared with the worldwide recommend drinking water HPC limits (100–500 cfu mL⁻¹) (Pavlov et al. 2004).

Bacterial community structures

In this study, the triplicate water samples from each sampling location were pooled.

16S rRNA gene clone library was constructed for each pooled sample, and 162 total chimera-free sequences were obtained in this study. Sequences with ≥97 % similarity were grouped into OTUs, and the richness and diversity indices were calculated (Table 3). The results indicated 33, 25, and 18 distinct OTUs in Sample A, B, and C, respectively. Richness (Chao1) and diversity indices (Shannon index) decreased dramatically through the treatment processes. Some types of bacterial species could be removed during the treatment process, so bacterial diversity can be expected to decrease. However, rarefaction curve for each bacterial library failed to level off completely, suggesting that further sequencing would have resulted in more OTUs (Fig. 2).

Alphaproteobacteria, *Betaproteobacteria*, *Actinobacteria*, and *Bacteroidetes* were shared among all the three samples (Fig. 3). *Alphaproteobacteria* was abundant in Sample A (15.4 %) and Sample C (16.4 %), but significantly decreased in Sample B (3.6 %). *Betaproteobacteria* was also abundant in Sample A (17.3 %) and obtained a significant increase in Sample B (32.7 %) and Sample C

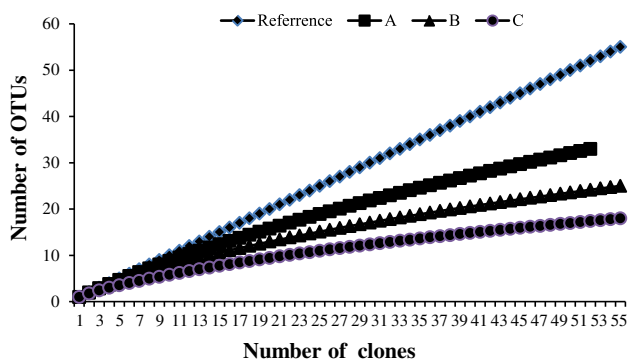


Fig. 2 Rarefaction curves indicating bacterial 16S rRNA gene richness within the three clone libraries. Sample A, B, and C represent raw water, the sand filter effluent, and the BAC filter effluent, respectively. The reference line represents 1:1, indicating infinite diversity

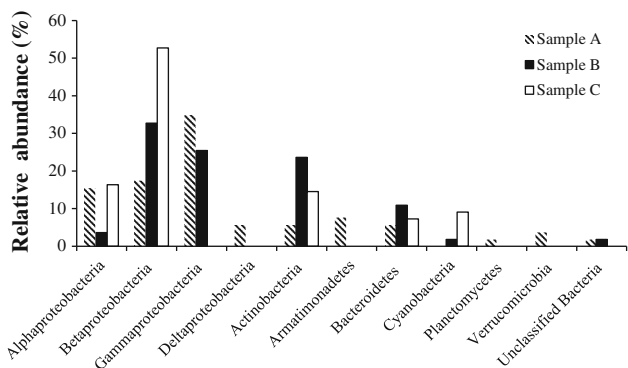


Fig. 3 Changes of the bacterial groups through the treatment processes. Sample A, B, and C represent raw water, the sand filter effluent, and the BAC filter effluent, respectively

(52.7 %). *Gammaproteobacteria* was the largest bacterial group in Sample A (34.6 %), but significantly decreased in Sample B (25.5 %) and was not detected in Sample C. *Actinobacteria* was a minor component in Sample A (5.8 %), but became one of the major groups in the other samples (23.6 or 14.5 %). Therefore, the major bacterial groups and their relative abundance changed greatly through the treatment processes.

Bacterial community change through the drinking water treatment units has still been poorly characterized. Eichler et al. (2006) found that no major changes of bacterial community structures occurred after flocculation and sand filtration. In contrast, Pinto et al. (2012) found that filtration by dual media rapid sand filters could shape the bacterial community in waters. Based on the significant difference between bacterial communities in raw water and on BAC, Soonglerdsongpha et al. (2011) suggested that bacterial community structure changed along the BAC filtration process. Previous studies showed the shift of bacterial community structure inside BAC systems (Liao et al. 2012, 2013) and granular activated carbon–sand dual media filter (Feng et al. 2013a). However, an earlier work using culture-dependent method indicated that BAC filtration did not change the nature of the bacterial populations associated with drinking water (Burlingame et al. 1986). Preozonation can usually effectively remove bacteria in water, while Burlingame et al. (1986) showed that preozonation of the water before BAC filtration had no noticeable effect on the bacterial genera found as compared with BAC filtration unit having no preozonation. Further efforts will be necessary in order to elucidate the bacterial

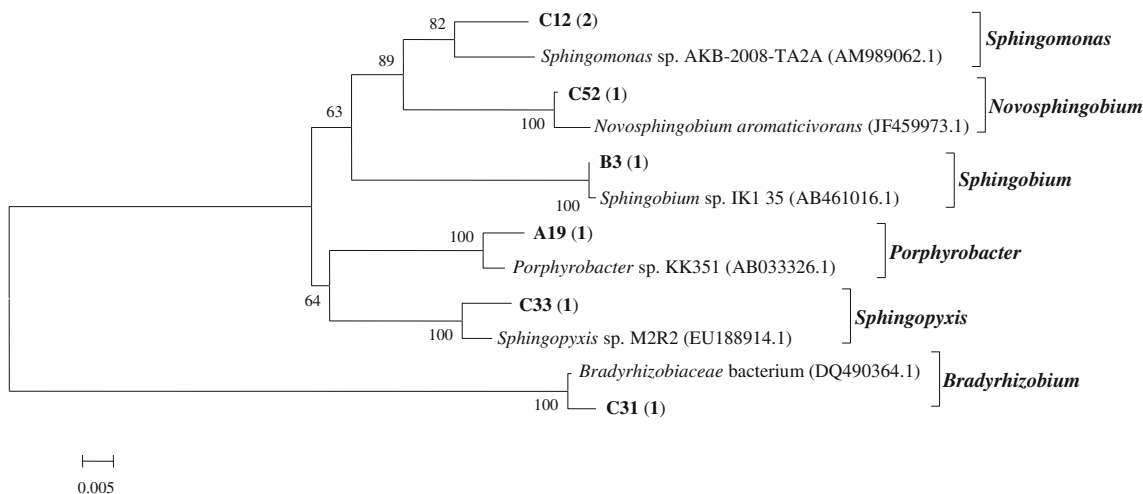


Fig. 4 Phylogenetic tree of representative 16S rRNA gene sequences assigned with putative genus within *Alphaproteobacteria* and reference sequences from GenBank. The obtained sequences beginning with ‘A,’ ‘B,’ and ‘C’ were referred to sequences recovered from Sample A, Sample B, and Sample C, respectively. Sample A, B, and C represent raw water, the sand filter effluent, and the BAC filter

effluent, respectively. The bold number in parentheses represents the numbers of the sequences in the same genus. Numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analysis of 1,000 resampled datasets. Bootstrap values <50 are not shown. The bar represents 0.5 % sequence divergence

community change through the drinking water treatment units. In this study, the preozonation followed by flocculation and sand filtration greatly altered the bacterial community in water. BAC filtration could further play an important role in shaping the aquatic bacterial community.

The phylogenetic composition of freshwater bacterial community has been well documented. Freshwater bacteria commonly belong to *Cyanobacteria*, *Bacteroidetes*, *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*, *Actinobacteria* and *Verrucomicrobia* (Zwart et al. 2002; Huang et al. 2011). The structure of the

bacterioplankton communities is dependent on a wide variety of factors. In this study, most of the bacterial sequences in each sample were affiliated with these groups. Moreover, substantial knowledge on microbial ecology and bacteria in real and model DWDs has been obtained (Berry et al. 2006; Lu et al. 2013). *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* have usually been found to be the major groups in chlorinated drinking water (Domingo et al. 2003; Williams et al. 2004; Eichler et al. 2006). However, *Cyanobacteria* were found to be one of major groups in chlorinated drinking water in a real

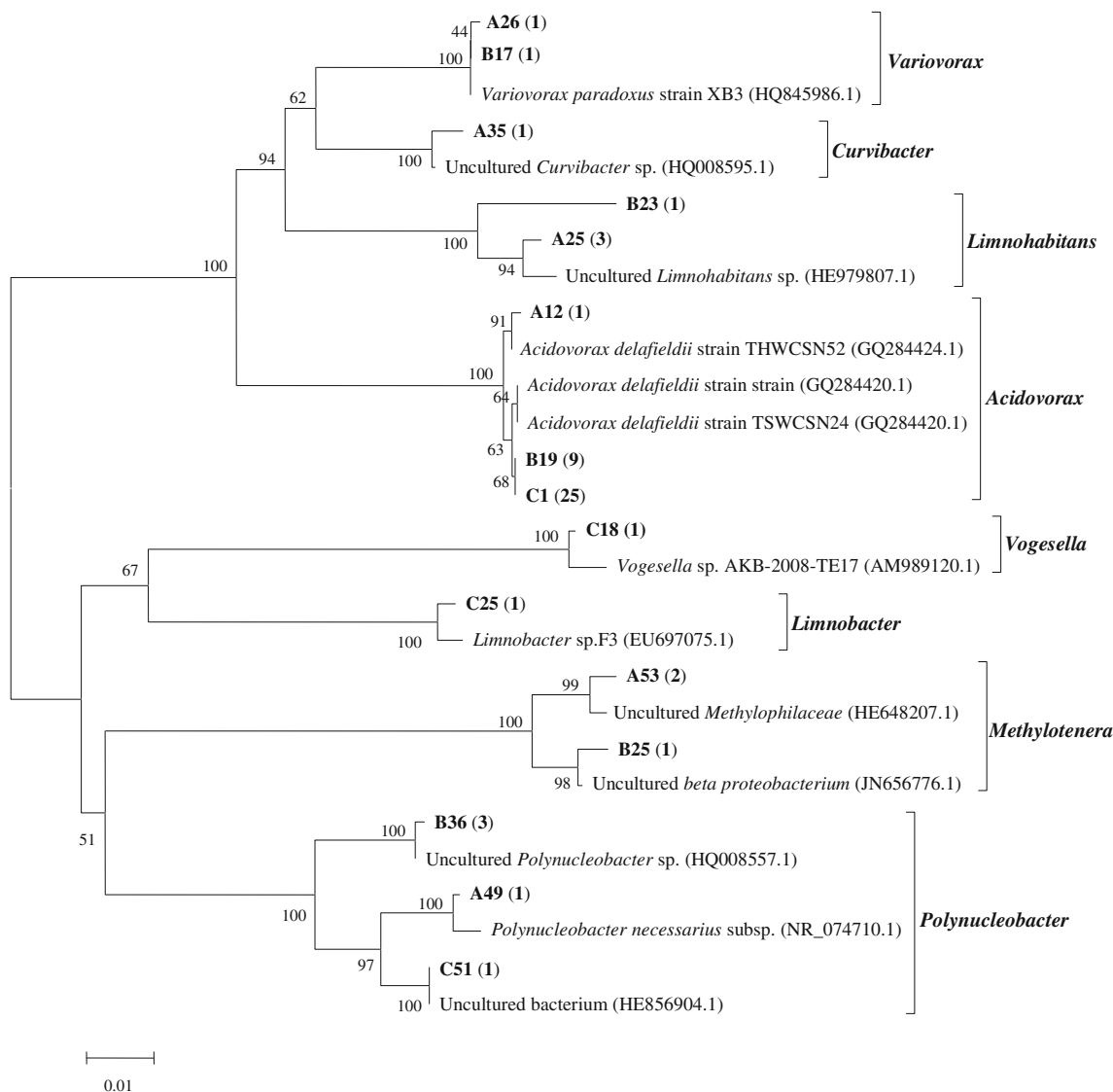


Fig. 5 Phylogenetic tree of representative 16S rRNA gene sequences assigned with putative genus within *Betaproteobacteria* and reference sequences from GenBank. The obtained sequences beginning with ‘A,’ ‘B,’ and ‘C’ were referred to sequences recovered from Sample A, Sample B, and Sample C, respectively. Sample A, B, and C represent raw water, the sand filter effluent, and the BAC filter

effluent, respectively. The bold number in parentheses represents the numbers of the sequences in the same genus. Numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analysis of 1,000 resampled datasets. Bootstrap values <50 are not shown. The bar represents 1 % sequence divergence

DWDs (Lu et al. 2013). Moreover, the composition of the bacterial community in chlorinated (or chloraminated) water might be much different from that in non-chlorinated (or non-chloraminated) water (Norton and LeChevallier 2000; Berry et al. 2006; Eichler et al. 2006; Kormas et al. 2010; Poitelon et al. 2010). Unfortunately, very few research works have provided the phylogenetic description of bacterial community in the sand filter effluent and the BAC filter effluent. Using culture-dependent technique, Norton and LeChevallier (2000) found that *Pseudomonas* (affiliated with *Gammaproteobacteria*) and *Sphingomonas* (affiliated with *Alphaproteobacteria*) were the predominant isolates in the BAC filter effluent. A recent study using clone library analysis indicated that *Alphaproteobacteria* and *Betaproteobacteria* were predominant in the BAC filter effluent (Poitelon et al. 2010). In this study, *Alphaproteobacteria*, *Betaproteobacteria*, and *Actinobacteria* were found to be major bacterial groups in the BAC filter effluent.

Feng et al. (2013b) found that *Nitrospirae* and *Alphaproteobacteria* were the dominant bacterial groups in a full-scale drinking water biosand filter. *Nitrospirae*, *Alphaproteobacteria*, *Acidobacteria*, and *Betaproteobacteria* were the dominant groups in a granular activated carbon–sand dual media filter for drinking water treatment (Feng et al. 2013a). Li et al. (2010) found that *Betaproteobacteria*

was the most abundant bacterial group both in bench-scale and pilot-scale BAC reactors. *Alphaproteobacteria*, *Gammaproteobacteria*, and *Acidobacteria* were the major bacterial groups in a pilot-scale up-flow BAC filtration system (Liao et al. 2012).

A recent study indicated *Alphaproteobacteria* and *Acidobacteria* were the largest two bacterial groups inside a downflow BAC filtration system (Liao et al. 2013). Moreover, in the last GAC sampling point before leaving the BAC system, *Betaproteobacteria* and *Actinobacteria* were not detected or detected in very low abundance (Liao et al. 2013). The BAC filtration system was the same as that used in this study. However, in the BAC filter effluent, *Acidobacteria* was not detected, while *Betaproteobacteria* and *Actinobacteria* were the dominant groups. Therefore, no direct link was found between the composition of microbial community in the BAC filter and that in its effluent.

Less than half of sequences recovered from Sample A could be classified to the genus level, but most of sequences recovered from Sample B and C could be affiliated with the putative bacterial genera. The putative genera were affiliated with *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Cyanobacteria*, and *Planctomycetes* (Figs. 4, 5, 6, 7). The distribution of identified bacterial genera is much different from one previous study (Norton

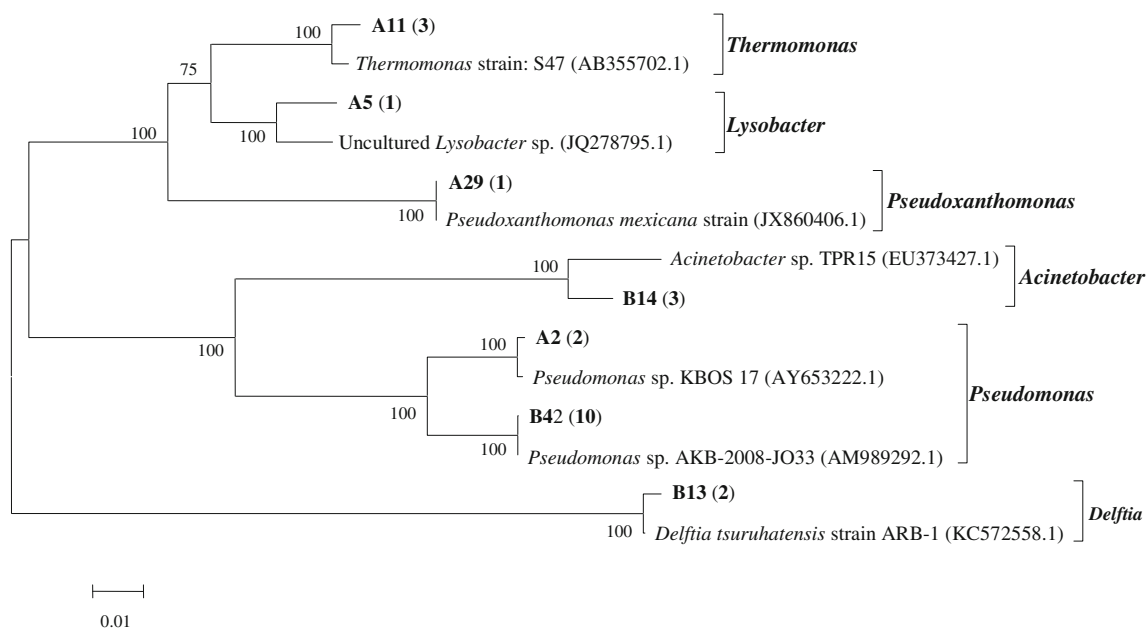


Fig. 6 Phylogenetic tree of representative 16S rRNA gene sequences assigned with putative genus within *Gammaproteobacteria* and reference sequences from GenBank. The obtained sequences beginning with ‘A,’ ‘B,’ and ‘C’ were referred to sequences recovered from Sample A, Sample B, and Sample C, respectively. Sample A, B, and C represent raw water, the sand filter effluent, and the BAC filter

effluent, respectively. The bold number in parentheses represents the numbers of the sequences in the same genus. Numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analysis of 1,000 resampled datasets. Bootstrap values <50 are not shown. The bar represents 1 % sequence divergence

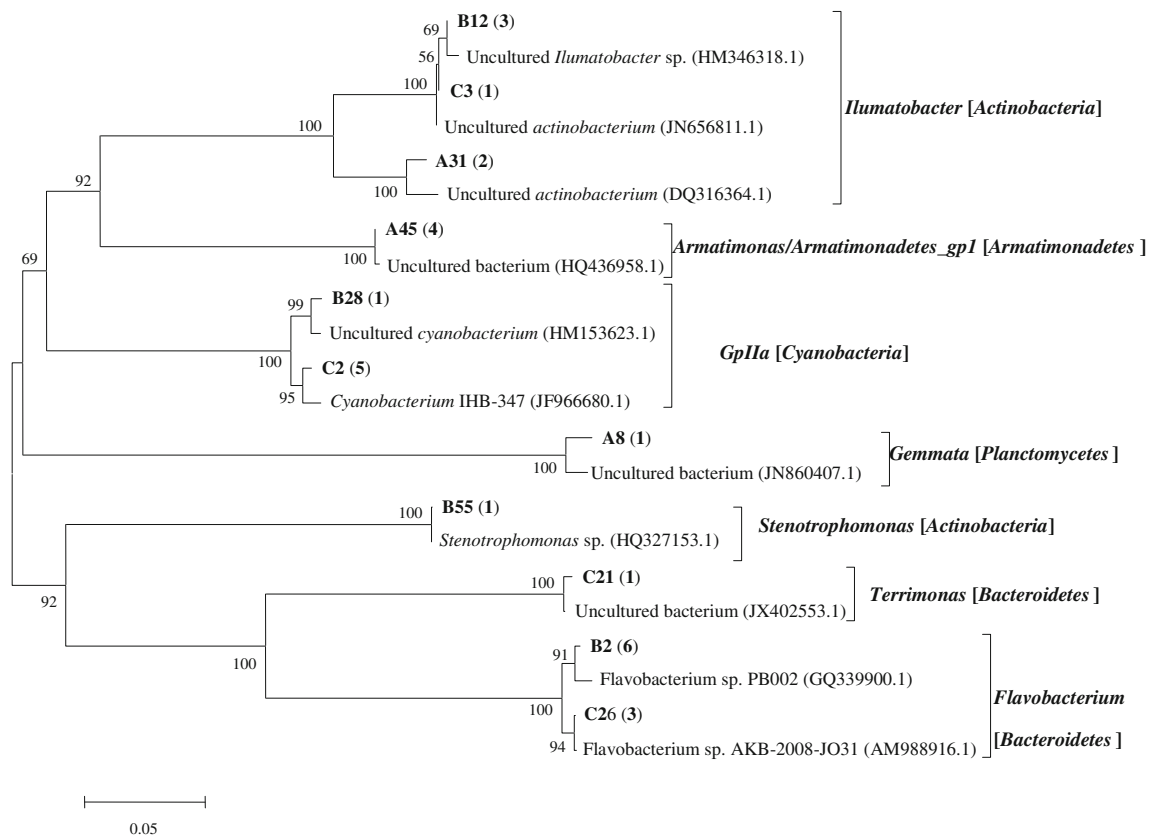


Fig. 7 Phylogenetic tree of representative 16S rRNA gene sequences assigned with putative genus within *Actinobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Cyanobacteria*, and *Planctomycetes*, and reference sequences from GenBank. The obtained sequences beginning with ‘A,’ ‘B,’ and ‘C’ were referred to sequences recovered from Sample A, Sample B, and Sample C, respectively. Sample A, B, and C

represent raw water, the sand filter effluent, and the BAC filter effluent, respectively. The bold number in parentheses represents the numbers of the sequences in the same genus. Numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analysis of 1,000 resampled datasets. Bootstrap values <50 are not shown. The bar represents 5 % sequence divergence

and LeChevallier 2000). The genus compositions of the three samples were much different, and only *Acidovorax*, *Polynucleobacter*, and *Ilumatobacter* were shared among the three samples. *Armatimonas/Armatimonadetes_gp1*, *Limnohabitans*, and *Thermomonas* were the three largest genus groups in Sample A. *Pseudomonas*, *Acidovorax*, and *Flavobacterium* were dominant in sample B. However, *Acidovorax* was predominant in Sample C.

In this study, HPCs decreased dramatically through the treatment processes, and the HPCs in the sand filter effluent and the BAC filter effluent complied with the international quality standards (Pavlov et al. 2004). However, this might not necessarily ensure good microbiological quality of drinking water. In the sand filter effluent and the BAC filter effluent, microorganisms from several bacterial genera (*Pseudomonas*, *Acinetobacter*, *Sphingomonas*, and *Novosphingobium*) associated with opportunistic infections were found (Figs. 4, 6). Therefore, intense monitoring of the bacterial community changes through drinking water treatment processes will be necessary.

Conclusion

Treatment processes resulted in the change of HPCs. Molecular analysis indicated bacterial population diversity dramatically changed through the treatment processes, while *Betaproteobacteria* was dominant in raw water, the sand filter effluent, and the BAC filter effluent.

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