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JOURNAL OF ENVIRONMENTAL SCIENCES
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Impact of disinfection on drinking water biofilm bacterial community

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ARTICLE INFO

Article history:
Received 27 February 2015
Revised 22 April 2015
Accepted 23 April 2015
Available online 26 June 2015

Keywords:
Drinking water distribution systems
Disinfection
Microbial community
High-throughput sequencing
Firmicutes
Proteobacteria

ABSTRACT

Disinfectants are commonly applied to control the growth of microorganisms in drinking water distribution systems. However, the effect of disinfection on drinking water microbial community remains poorly understood. The present study investigated the impacts of different disinfectants (chlorine and chloramine) and dosages on biofilm bacterial community in bench-scale pipe section reactors. Illumina MiSeq sequencing illustrated that disinfection strategy could affect both bacterial diversity and community structure of drinking water biofilm. *Proteobacteria* tended to predominate in chloraminated drinking water biofilms, while *Firmicutes* in chlorinated and unchlorinated biofilms. The major proteobacterial groups were influenced by both disinfectant type and dosage. In addition, chloramination had a more profound impact on bacterial community than chlorination.

Introduction

Microbial growth in drinking water distribution systems (DWDS) can lead to a number of adverse problems, including proliferation of opportunistic pathogenic microorganisms (Berry et al., 2006; Emtiazi et al., 2004; Liu et al., 2013, 2014; Lu et al., 2014). Disinfectants are commonly applied to lower the numbers of microorganisms in DWDS, maintaining a disinfectant residual. In China, the recommended doses of chlorine and chloramine in the water industry were 0.3–4 and 0.5–3 mg/L, respectively (Ministry of Health, 2006). Even at a high dosage, disinfectant application cannot avoid microbial regrowth in DWDS, due to the presence of organic matter and nutrients (Lu et al., 2013; Mathieu et al., 2009; Zhu et al., 2014). Diverse bacterial species can be found both in bulk waters and on pipe surfaces (Berry et al., 2006; Lu et al., 2013; Martiny et al., 2005; Vaz-Moreira et al., 2013; Wu et al., 2014, 2015). Autochthonous microbes may promote the growth of potentially pathogenic bacteria (Berry et al., 2006; Eichler et al., 2006). Therefore, an in-depth knowledge of DWDS microbial community and its influential factors is crucial for the development of effective control strategies (Berry et al., 2006; Lu et al., 2013; Wu et al., 2015). So far, a variety of factors have been found to regulate the structure of DWDS microbial community, such as type of source water, water treatment processes, disinfection, pipe materials, temperature and water age (McCoy and VanBriesen, 2012; Sun et al., 2014;

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http://dx.doi.org/10.1016/j.jes.2015.04.008
1001-0742/

Wang et al., 2014; Wu et al., 2015). However, there is still a wide scope for elaborate investigations on the impact of changes in factors governing drinking water microbial communities (Wang et al., 2014). Moreover, the impact of different disinfectants and dosages on DWDS microbial community remains unclear.

Culture-dependent methods and low-profiling molecular biology approaches have greatly contributed to our understanding of drinking water microbes (Lu et al., 2013; Vaz-Moreira et al., 2013). In contrast, high-throughput sequencing, as a next generation sequencing technology, can provide a new opportunity to systematically compare the effects of physiochemical parameters on DWDS microbial communities (Wang et al., 2014; Wu et al., 2015). To date, pyrosequencing analysis has found many applications in characterizing DWDS microbial community (Liu et al., 2012, 2014; Sun et al., 2014; Wang et al., 2014; Wu et al., 2015), however, Illumina MiSeq sequencing, the more recently developed high-throughput sequencing technology, has gained increasing popularity due to its lower costs and greater throughput, compared to pyrosequencing (Caporaso et al., 2012). So far, information on Illumina MiSeq sequencing of drinking water microbial community is still very limited (Wu et al., 2015). Therefore, the main objective of the current study was to systematically investigate the impacts of different disinfectants and dosages on DWDS bacterial community using Illumina MiSeq sequencing.

1. Materials and methods

1.1. Experiment setup and chemical analysis

In this study, the effect of disinfection on DWDS bacterial community was evaluated using bench-scale pipe section reactors (Fig. 1). Cast iron pipes (25–30 years old; length of 10 cm; diameter of 100 cm) used for the construction of bench-scale pipe section reactors were originally collected in a real DWDS transporting treated surface water. A stirring polyethylene paddle was driven by a motor at the rotating rate of 300 r/min to provide the hydraulic shear. The tap water (ground water previously receiving no disinfection treatment) in the campus of Tsinghua University was used as raw water. The physicochemical parameters of tap water are as follows: pH 7.82 ± 0.02, sulfate 70.5 ± 5.0 mg/L, chloride 19.5 ± 2.5 mg/L, alkalinity 145 ± 10 mg/L as $CaCO_3$, hardness 196 ± 10 mg/L as $CaCO_3$, conductivity 561 ± 20 μS/cm, turbidity 0.22 ± 0.12 NTU, DO 7.64 ± 0.40 mg/L, DOC 0.65 ± 0.15 mg/L, NH^{4+}-N < 0.02 mg/L, NO_2^--N < 0.003 mg/L, and NO_3^--N 0.46 ± 0.12 mg/L. Water pH and conductivity were measured by an electrode probe (HQ11d, HACH, Loveland, Colorado, USA). Turbidity was determined using a Turbidimeter (2011P, HACH, Loveland, Colorado, USA), while dissolved oxygen (DO) using a LDO probe (HQ30d, HACH, Loveland, Colorado, USA). Dissolved organic carbon (DOC) was measured using a TOC analyzer (5000A, Shimadzu, Kyoto, Japan). The concentrations of ammonium, nitrite and nitrate in waters were conducted according to the standard methods described by China Environmental Protection Agency (2002).

The tap water was amended with different levels of NaClO or NH_2Cl for disinfection tests. Pipe section reactors A0–A5 were fed with waters containing NaClO at the levels of 0, 0.04, 0.17, 0.56, 1.02 and 1.76 mg Cl_2/L, respectively, while reactors

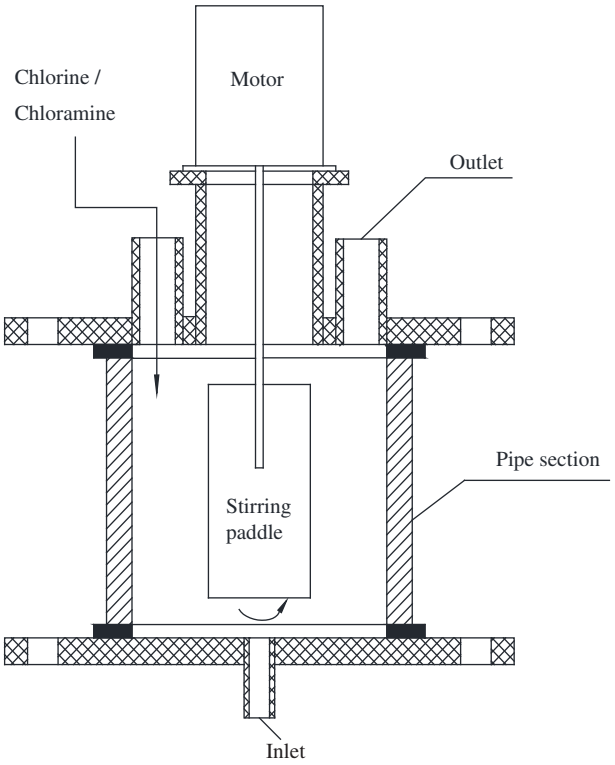

Fig. 1 – Schematic diagram of the bench-scale pipe section reactor.

B0–B6 with waters containing NH_2Cl at the levels of 0, 0.06, 0.20, 0.42, 0.78, 1.16 and 1.41 mg Cl_2/L, respectively. Chlorine and chloramine residual were measured using a HACH Pocket Colorimeter II Chlorine. These reactors were operated in a batch mode for about 2 months at 25°C prior to biofilm sampling. The water in each reactor was totally renewed every two days.

1.2. Molecular analyses

Biofilms were removed from pipes as previously described (Sun et al., 2014). Total genomic DNA was recovered from biofilms using the Powersoil DNA extraction kit (Mobio Laboratories, Carlsbad, CA, USA), and then amplified using the primer sets 515F (5′-GTGC CAGCMGCCGCGG-3′)/R907 (5′-CCGTCAATTCMTTTRAGTTT-3′) targeting V4–V5 hypervariable regions of bacterial 16S rRNA genes (Wang et al., 2015). The amplicons were subjected to Illumina MiSeq sequencing. The reads from the original DNA fragments were merged using FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/) and the quality filtering was performed according to the literature (Caporaso et al., 2010). The sequences obtained from Illumina MiSeq sequencing analysis in the present study were deposited in the NCBI short-read archive under accession number SRP049933. UPARSE pipeline was used to cluster bacterial sequences into operational taxonomic units (OTUs) with a maximum distance of 3% and further generated the Shannon diversity index for each biofilm sample (Edgar, 2013). The OTU-based beta diversity analysis was carried out using UniFrac, and Bray–Curtis similarity matrices with QIIME (http://qiime.org/index.html) were used for Unweighted Pair

Group Method with Arithmetic mean (UPGMA) clustering. The taxonomic identities of the bacterial sequences were determined using the RDP classifier (Wang et al., 2007).

2. Results

2.1. Bacterial community diversity

The obtained valid Illumina reads for each biofilm sample ranged between 11,322 and 28,659 and were normalized to 11,322 to compare the difference of OTUs and Shannon index among samples. In this study, Sample AB0 represents the composite biofilm sample from the reactors without any disinfection treatment. Samples A1–A5 are referred to the chlorinated biofilm samples from pipe reactors receiving waters containing 0.04, 0.17, 0.56, 1.02 and 1.76 mg Cl_2/L NaClO, respectively, while Samples B0–B6 represent the chloraminated biofilm samples from reactors receiving waters containing 0.06, 0.20, 0.42, 0.78, 1.16 and 1.41 mg Cl_2/L NH_2Cl, respectively. The number of OTUs in these biofilm bacterial communities varied from 95 to 275 (Table 1). The OTUs of Samples A4, A5, B4, B5 and B6 were much lower than those of Sample AB0, suggesting that a high dosage of chlorine and chloramine could remarkably lower the number of bacterial OTUs in model DWDS. The Shannon indices of biofilm samples ranged between 1.87 and 3.34. Sample AB0 had higher bacterial diversity than Samples A4, A5 and B6, but lower than the other biofilm samples. This indicated that the impact of both two disinfectants on DWDS bacterial community depended on dosages. DWDS bacterial diversity could be promoted by low disinfectant dosage, but lowered by high dosage.

2.2. Bacterial community composition

In this study, a total of 9 bacterial phyla were frequently identified in DWDS biofilm samples, including *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Chlorobi*, *Planctomycetes*, *Cyanobacteria*, *Nitrospirae*, *SHA-109*, and *Actinobacteria* (Fig. 2). Sample AB0 (DWDS biofilm with no previous exposure to disinfectant) was mainly composed of *Firmicutes* (61.3%) and *Proteobacteria* (31.6%). For the model DWDS receiving chlorinated waters, *Firmicutes* (42%–66.8%) and *Proteobacteria* (28.3%–49.9%) also predominated in biofilm samples (Samples A1–A5). The rise of chlorine dosage increased the relative abundance of *Proteobacteria*, but decreased the proportion of *Firmicutes*. A positive correlation was observed between chlorine dosage and the relative abundance of *Proteobacteria* ($R^2 = 0.78$, $P < 0.05$), but the *Firmicutes* proportion showed negative correlation with chlorine dosage ($R^2 = 0.93$, $P < 0.05$) (Fig. 3). In addition, *Bacteroidetes* (3.8%–13.4%) was always the third largest bacterial group in chlorinated and unchlorinated DWDS biofilm samples.

For the chloraminated DWDS biofilm samples, *Proteobacteria* (accounting for 67.3%–96%) predominated in Samples B2–B6, but was much less abundant in Sample B1 (17.9%). *Firmicutes* also were dominant in Samples B2–B4 (9.2%–12.7%), but existed with a much lower proportion in other samples (2%–2.7%). However, these chloraminated DWDS biofilm samples had a much lower proportion of *Firmicutes* than the unchloraminated one. *Bacteroidetes* illustrated a large variation among chloraminated DWDS biofilm samples (0.3%–35.8%). *Chlorobi*, *Planctomycetes* and *Nitrospirae* were dominant only in Sample B1. Moreover, a positive correlation was observed between chloramine dosage and the relative abundance of *Firmicutes* ($R^2 = 0.5$, $P < 0.05$), but the *Firmicutes* proportion showed no significant correlation with chloramine dosage ($P > 0.05$) (Fig. 4). In addition, a relatively high proportion of *Cyanobacteria* (6.3% or 6.7%) was found in Samples B3 and B4. Therefore, these results showed that the impact of disinfectant on DWDS biofilm bacterial community could depend on disinfectant type and dosage.

Fig. 5 illustrates the composition of the proteobacterial community in each DWDS biofilm sample. Gammaproteobacterial organisms predominated in proteobacterial communities in Sample AB0 (undisinfected DWDS biofilm) and Samples A1–A3 (DWDS biofilms with exposure to low or medium chlorine dosage). The high chlorine dosage decreased the proportion of *Gammaproteobacteria* but increased the dominance of *Betaproteobacteria*. In addition, the proteobacterial communities in all the chloraminated DWDS biofilm samples were mainly composed of *Alphaproteobacteria* and *Betaproteobacteria*. Therefore, both disinfectant type and dosage could influence the major components of proteobacterial community in DWDS biofilm.

Table 1 – Community richness and diversity indices for biofilm samples in drinking water distribution systems.

Sample	OTUs [a]	Shannon index [a]
AB0	247	2.49
A1	263	3.29
A2	275	3.23
A3	226	2.62
A4	146	2.12
A5	117	2
B1	226	2.82
B2	244	3.34
B3	199	3
B4	115	2.86
B5	117	2.58
B6	95	1.87

[a] Tags are normalized to 11,322.

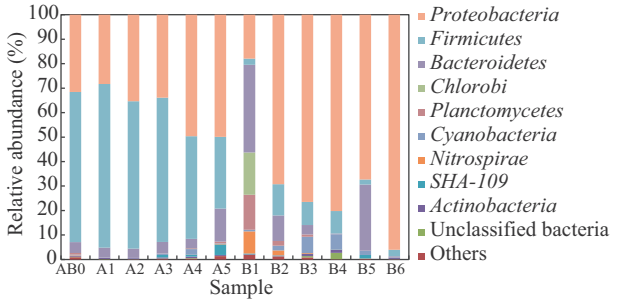

Fig. 2 – Comparison of the quantitative contribution of the sequences affiliated with different phyla to the total number of sequences from biofilm samples in drinking water distribution systems. Sequences not classified to any known phylum are included as unclassified bacteria. The rare species with relative abundance less than 0.1% are included as others.

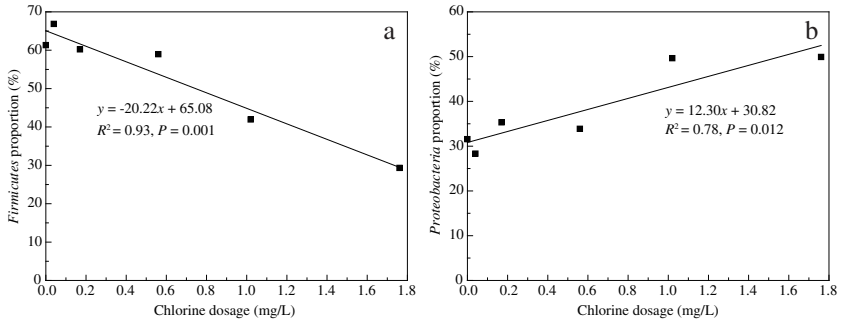

Fig. 3 – Relationship between chlorine dosage and *Firmicutes* (a) and *Proteobacteria* (b) proportion.

Fig. 6 shows the result of UPGMA clustering of DWDS biofilm samples. Sample AB0 (undisinfected DWDS biofilm) was distantly separated from all the chloraminated DWDS biofilm samples, but could be grouped with the chlorinated DWDS biofilm samples. This suggested that chloramination had a more profound impact on DWDS biofilm bacterial community than chlorination. Samples B1–B3 and Samples B4–B6 fell into two distinct groups, indicating the strong impact of chloramine dosage on DWDS biofilm bacterial community. Moreover, Samples A1 and A2, Samples A3 and A4, and Sample A5 formed three distinct groups. This also illustrated the strong impact of chlorine dosage on DWDS biofilm bacterial community. However, it is to be noted that Sample AB0 was more closely grouped with Samples A3 and A4 than Samples A1 and A2.

3. Discussion

High-throughput sequencing has found increasing applications in characterizing microbial community in model or real DWDS. Using pyrosequencing analysis, Gomez-Alvarez et al. (2014) identified a total of 491 OTUs in biofilm samples from bench-scale annular reactors simulating DWDS, while Wang et al. (2014) reported a total of 62–132 OTUs in biofilm bacterial community in model DWDS. In addition, pyrosequencing of biofilm bacterial communities on cast iron pipes in a real DWDS revealed 642–1532 OTUs and Shannon index of 3.36–5.29 (Sun et al., 2014). Our recent study using Illumina MiSeq sequencing analysis indicated that bacterial communities attached on DWDS pipes had 363–582 OTUs and Shannon index of 5.11–7.12 (Wu et al., 2015). In this study, Illumina MiSeq sequencing of pipe biofilm bacterial communities revealed 95–275 OTUs and Shannon index of 1.87–3.34, much lower than those previously reported in real DWDS (Sun et al., 2014; Wu et al., 2015). So far, the impact of disinfection on DWDS biofilm bacterial diversity remains elusive. However, the present work provided the direct evidence for the impact of two disinfectants (chlorine and chloramine) on DWDS biofilm bacterial diversity. DWDS bacterial diversity was found to be increased by low disinfectant dosage, but lowered by high dosage.

The predominance of proteobacterial microorganisms in DWDS has been well-documented both in bulk waters (Lu et al., 2013; Poitelon et al., 2009; Tokajian et al., 2005; Vaz-Moreira et al., 2013; Williams et al., 2004; Wu et al., 2014), and in biofilms (Douterelo et al., 2013; Gomez-Alvarez et al., 2014; Krishna et al., 2013; Lee et al., 2005; Liu et al., 2013; Sun et al., 2014; Wu et al., 2015), yet the impact of disinfectant type and dosage on drinking water biofilm proteobacterial community remains still unclear. In this study, *Proteobacteria* predominated in the majority of chloraminated DWDS biofilm samples, but showed a much lower proportion in DWDS samples that were exposed to no or the lowest chloramine dosage, which suggested that disinfection using chloramine stimulated the dominance of *Proteobacteria*. Although the increase of chlorine dosage also promoted the increase in the proportion of *Proteobacteria*, it appeared to be the largest bacterial group only in the two DWDS samples that were exposed to the highest chlorine dosages. These results indicated that disinfection using chloramines favored the predominance of proteobacterial microorganisms in DWDS biofilm. To date, the relative importance of different

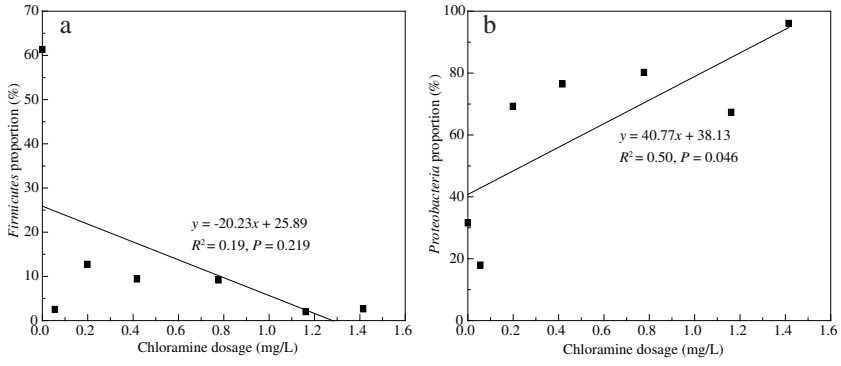

Fig. 4 – Relationship between chloramine dosage and *Firmicutes* (a) and *Proteobacteria* (b) proportion.

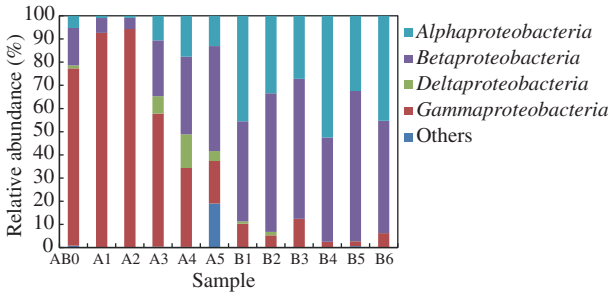

Fig. 5 – Comparison of the quantitative contribution of the sequences affiliated with different proteobacterial classes to the total number of proteobacterial sequences from biofilm samples in drinking water distribution systems. Sequences not classified to any known proteobacterial class are included as others.

proteobacterial classes in DWDS biofilm bacterial community remained unresolved (Wu et al., 2015). Previous studies showed the dominance of either *Alphaproteobacteria* (Douterelo et al., 2013; Gomez-Alvarez et al., 2014; Krishna et al., 2013), or *Betaproteobacteria* (Lee et al., 2005; Liu et al., 2014; Sun et al., 2014), while both of them could be dominant (Wang et al., 2014; Wu et al., 2015). Moreover, *Gammaproteobacteria* could also be the largest bacterial group in DWDS biofilms (Douterelo et al., 2013; Wu et al., 2015). Due to the difference in either the geographic region or the methods used, different bacterial groups may predominate (Vaz-Moreira et al., 2013), and the dominant proteobacterial classes can be found to vary among different DWDS (Wu et al., 2015). In this study, both *Alphaproteobacteria* and *Betaproteobacteria* were the dominant proteobacterial members in all the chloraminated DWDS biofilms. In contrast, *Gammaproteobacteria* predominated in the proteobacterial communities in both undisinfected DWDS biofilm and biofilms exposed to low or medium chlorine dosage, while *Betaproteobacteria* became the largest proteobacterial class in DWDS biofilms exposed to the highest chlorine dosage. These results illustrated that the composition of the dominant proteobacterial classes could be influenced by both disinfectant type and dosage.

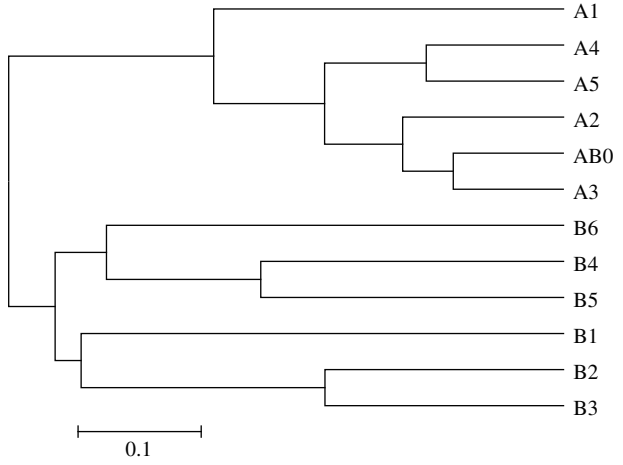

Fig. 6 – UPGMA clustering of biofilm samples in drinking water distribution systems based on relative abundance of bacterial phyla.

Firmicutes has usually been found to be a minor component of DWDS bacterial communities in both in bulk waters (Kormas et al., 2010; Lu et al., 2013; Pinto et al., 2012; Vaz-Moreira et al., 2013), and biofilms (Lin et al., 2013; Liu et al., 2014; Revetta et al., 2013). Our previous study showed that *Firmicutes* was usually a rare species in pipe biofilms from a real urban DWDS, while in few cases it could be a major component of biofilm bacterial community (Wu et al., 2015). Sun et al. (2014) reported that *Firmicutes* was a minor bacterial group in DWDS pipes transporting treated ground waters, while it dominated in pipes transporting treated surface waters. However, the factors regulating the abundance of *Firmicutes* in drinking water have rarely been addressed (Sun et al., 2014; Wu et al., 2015). Information on the impact of disinfectant on *Firmicutes* in DWDS is still lacking. In this study, *Firmicutes* was found to predominate in unchlorinated and chlorinated biofilm samples, but the rise of chlorine dosage lowered its advantage. In contrast, *Firmicutes* became much less important in chloraminated DWDS biofilms. Therefore, disinfection strategy could have a profound impact on the proportion of *Firmicutes* in DWDS bacterial community.

4. Conclusions

Bacterial diversity in DWDS biofilm was increased by low disinfectant dosage, but lowered by high dosage. DWDS bacterial community structure could be affected by disinfectant type and dosage. *Proteobacteria* and *Firmicutes* were dominant under chloramination and chlorination, respectively.

Acknowledgments

This work was financially supported by the State Environmental Protection Key Laboratory of Microorganism Application and Risk Control (No. MARC2012D010), the National Water Special Program (No. 2012ZX07404-002), and the International Science & Technology Cooperation Program of China (No. 2010DFA91830).

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