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Collaborative effect of secondary chlorination and organic matter content on biological safety in secondary water supply system

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ABSTRACT

Biofilm in secondary water supply system (SWSS) may reduce the biological safety of tap water. This study focused on the collaborative effect of secondary chlorination and organic matter content on the regrowth of biofilm bacteria and the diversity of microbial community in lab scale SWSS. Several biofilms cultivated in the same condition were used for secondary chlorination under different organic matter content and chlorination dose conditions. Bacteria regrowth yield under the highest organic matter content and the lowest free residual chlorine (FRC) condition was 2.84–3.11 times of that under the lowest organic matter content and the highest FRC condition. The collaborative effect ratio of organic matter content increase (per 1.00 mg/L) and secondary chlorination decrease (per 0.10 mg/L as Cl_2) was 0.99. Metagenomic sequencing was used to analyze the biofilm microbial community diversity in this experiment. *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Firmicutes* were the four major phyla of biofilm microbial communities. *Proteobacteria* presented significant increase after secondary chlorination in both high and low organic matter contents. The phylum *Bacteroidetes* was found to be dominant in beakers with high organic matter content while *Actinobacteria* was the most in beakers with low organic matter content. *Firmicutes* could almost be controlled by secondary chlorination with the dose more than 0.10 mg/L as Cl_2.

Keywords: Secondary chlorination; Organic matter content; Biofilm; Microbial community diversity; Biological safety

1. Introduction

Biological safety of tap water is extremely important to people's lives. Biofilm in secondary water supply system (SWSS) has many adverse factors on biological safety of tap water. Biofilm formation in pipes especially in the SWSS pipe may lead to health problems with opportunistic pathogens [1–3]. The increase of biofilm bacteria is influenced by several physical and chemical factors. Biofilm exists on the internal surface of water supply system pipes with diversity of bacteria despite of maintaining free residual chlorine (FRC) (no more than 0.05 mg/L as Cl_2) [4,5]. Thus, keeping enough FRC is always thought to be a necessary method to ensure the biological safety. Therefore, a complete understanding of impact of biofilm regrowth after secondary disinfection and microbial community diversity of the SWSS biofilm is of great importance in proposing effective control strategies, improving tap water quality and biological safety. Many research studies on biofilm growth and regrowth concentrated on the substrate and disinfectant types of biofilm [6,7]. For instance, Butterfield et al. [8] studied the biofilm development rates with amino acid, carbohydrates and humic substances under chlorination condition. Shen et al. [9] reported the cross-linked starch/polycaprolactone blends served as carbon source and biofilm supported for denitrification. Nevertheless, rare appears on the collaborative influence of secondary disinfectant dosage and organic matter content on SWSS biofilm regrowth.

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Several examples of outbreaks of microorganisms were reported to occur in water distribution systems because of the trace amount or absence of secondary disinfectant [10]. FRC decreases with the retention time and sometimes its values even reduce to zero. Thus chlorine was added to water once more as it enters the SWSS pipe in order to ensure water quality and biological stability [11]. However, chlorine cannot be overdosed as a high chlorine dose may cause the formation of higher levels of halogenated disinfection byproducts in tap water, which may cause adverse effects to human health [12]. So the dose of chlorine added to SWSS with different organic matter content needs to be further confirmed.

Additionally, the microbial community diversity has been reported to be related to biofilm bacteria growth by Codony et al. [13]. Microbial community diversity may affect the degradation rate of organic matters [14]. Moreover, microbial diversity may be influenced by the substrate load, hydraulic retention time and pH [15]. Permanganate oxygen consumption (COD_{Mn}, organic matter content was represented by permanganate oxygen consumption) is a regulation parameter as organic matter content of drinking water in Chinese drinking water standard with the limitation of 3.00 mg/L [16]. Although Sun et al. [1] revealed the positive correlation between COD_{Mn} and relative abundance of *Firmicutes* in different type of iron pipes in real drinking water distribution system, there is still a lack of knowledge on the influence of COD_{Mn} on biofilm bacteria regrowth and microbial diversity of SWSS after secondary chlorination.

In this study, we investigated the collaborative impact of secondary chlorination and organic matter content on biofilm bacteria regrowth. The microbial community diversity was also analyzed with beakers in lab scale SWSS conditions simulating the time without using water. The purpose of this paper is to obtain a better understanding of SWSS biofilm regrowth and come up with some useful measures to control SWSS biofilm regrowth and ensure the biological safety of tap water.

2. Materials and methods

2.1. Experimental design

The study focused on the collaborative influence of secondary chlorination and COD_{Mn} concentration (C_C) on the regrowth of biofilm within a static SWSS in lab scale. Two factors were investigated with a two-level factorial design (Table 1). The experiment was carried out in nine beakers (B1–B9) operated in parallel, which were wrapped to simulate the dark environment of pipeline system. Each beaker was considered to represent a fraction of a SWSS in residential buildings in the idle time of using water. The whole experiment was conducted in triplicate. Total bacteria count, which is an important indicator in Chinese standard of drinking water and operating time of the first time were used to compare the collaborative effect of C_C and FRC on biofilm bacteria regrowth with mathematical formula (1):

$$\bar{v} = B / t \tag{1}$$

where $\bar{v}$ is the average regrowth rate of biofilm bacteria from the start of experiment till the total bacteria count reached maximum for the first time, unit is colony forming unit/(cm^2·h) (CFU/(cm^2·h)); B is the maximum total bacteria count achieved for the first time, unit is CFU/cm^2 and t is the experimental time when total bacteria count meets its maximum for the first time, unit is h.

C_C was determined according to Chinese drinking water standards, which regulated the maximum limit of COD_{Mn} to be 3.00 mg/L [16]. The first experiment C_C mean value was 2.70 mg/L. In case of some emergency organic matter pollution accident, the second experiment C_C of approximate mean 5.80 mg/L was chosen. The third mean value of C_C was chosen by averaging mean value of the first and the second C_C; it was 4.30 mg/L. Humic substances were the organic matter utilized as COD_{Mn} by adding concentrated humic substances stock solution to tap water. A concentrated humic substances stock solution was prepared by adding commercial humic acid powder (produced by Tianjin Zhiyuan Chemical Reagent Co., Ltd.) to sterile ultrapure water, adjusting the pH to 12 with 0.1 M sodium hydroxide solution, mixing for 24 h, and then centrifuging at 10,000.00 g at 4°C to remove particulate matter. Following the addition of humic substances stock solution to sterile ultrapure water, the pH was adjusted to 7.5 using 1 M hydrochloric acid.

FRC was selected based on Chinese drinking water standards [16], providing that when the supplying water leaving waterworks, its FRC must be higher than 0.30 mg/L as Cl_2 and the FRC of the endmost of drinking water pipe network should be higher than 0.05 mg/L as Cl_2. Thus, this experiment chose 0.10 mg/L as Cl_2, 0.150 mg/L as Cl_2 and 0.20 mg/L as Cl_2 as the secondary chlorination dosages. Chlorine solution was prepared by adding sodium hypochlorite (about 36.0%) to autoclaved ultrapure water. Then the chlorine solution was added to the residential building tap water on a discontinuous basis. The chlorine dose was slowly increased until FRC of 0.10 mg/L as Cl_2, 0.15 mg/L as Cl_2 and 0.20 mg/L as Cl_2 was obtained in the three sets of beakers, respectively. Each set had three beakers with different C_C. No chlorine was added again to each beaker during the whole experiment.

The biofilms were cultivated in two rotating annular reactors (BAR) on PVC slides. Each BAR was fed continuously with its C_C (mean 4.30 mg/L) until the biofilm reached a pseudo-steady state based on total bacteria count. FRC was very few (0.01–0.02 mg/L as Cl_2) overall the cultivation. It took about 72 d. The two BARs were operated with residential building tap water adding organic matter to provide influent C_C of approximate mean 4.30 mg/L. Biofilm grew in low C_C was much easier to slough than that in high C_C during the cultivation. Biofilm sloughing may lead to turbidity and bacteria number increase in SWSS.

Table 1
Two-level factorial experimental design of the beaker investigation

FRC (mg/L)	C_C (mg/L)		
	2.70	4.30	5.80
0.10	B1	B2	B3
0.15	B4	B5	B6
0.20	B7	B8	B9

The experiment was started immediately after the slides with biofilm were immersed in the prepared experiment water. Water and biofilm samples were taken every 2 h during the first 24 h after the startup of experiment and once every 24 h in the following 48 h. All experiments were performed under dark sterile condition at room temperature (20°C ± 2°C).

2.2. Chemical analyses

Water samples from the beakers were collected and analyzed for COD_{Mn} and FRC in the same hour. To ensure accuracy and precision of the methods, each sample was measured three times in parallel and averaging.

COD_{Mn} analyses were performed according to standard analysis method using titration in acid environment [17].

FRC was measured by portable residual chlorine testing equipment (S-CL501, Sinche Co., Ltd., Guangdong, China) based on the *N*,*N*-diethyl-*p*-phenylenediamine spectrophotometry [17].

2.3. Microbial analyses

Biofilm was sampled by removing a slide from the beaker, aseptically scraping biofilm from the slide with cotton swabs, they were then put into test tube with sterile ultrapure water followed by ultrasonic cleaning at 40 kHz for 20 min using an ultrasonic cleaner (KQ-500B, Kunshan Ultrasonic Instrument Co., Ltd., Jiangsu, China) to slough biofilm off the cotton swabs, homogenizing at 20,000 rpm for 1 min using a tissue homogenizer (Model M37610-33, Barnstead International, IOWA, USA). Each sample was analyzed for total bacteria count using plate culture method: a 1.00 mL aliquot of the sample or its decimal dilutions were mixed with melted nutrient agar medium and incubated for 24 h at 37°C.

Metagenomics method was used in this study to access the genomic potential of an environmental habitat either directly or after enrichment for specific communities [18].

2.3.1. DNA extraction

Biofilm genomic DNA was extracted using 50.00 mg of biofilm and sampled from the beaker which was used for total community DNA extraction using E.Z.N.A. Soil DNA Kit (OMEGA).

2.3.2. Polymerase chain reaction amplification

Qubit2.0 DNA Kit (Life) was used to quantify genomic DNA precisely. The V3–V4 region of 16S rDNA was amplified by polymerase chain reaction (PCR) with primer 341F (CCCTACACGACGCTCTTCCGATCTG) and primer 805R (GACTGGAGTTCCTTGGCACCCGAGAATTCCA) to conserve regions of the 16S rRNA genes. The PCR conditions were settled as follows: 94°C preheating for 3 min, five cycles of denaturation at 94°C for 30 s, annealing at 45°C for 20 s and extension at 65°C for 30 s, and a following 20 cycles as this processes at 94°C for 20 s, 55°C for 20 s, 72°C for 30 s and a final extension of 5 min at 72°C, respectively. PCR amplification was repeated three times according to the above method.

2.3.3. Clone and sequencing of metagenomics

Clone library construction and metagenomic sequencing were carried out by Sangon Biotech (Shanghai) Co. Ltd. (Shanghai, China). Sequencing was implemented on MiSeq Sequencing instrument (Illumina) with 2 × 300 standard mode. The sequencing data were analyzed by Uclust (version 1.1.579).

3. Results and discussion

3.1. Biofilm bacteria regrowth

Before the slides were put into beakers, the total bacteria count of biofilm was 1.25×10^4–2.10×10^4 CFU/cm^2.

The experimental FRC (0.10, 0.15 and 0.20 mg/L as Cl_2) was a few times higher than the FRC during the biofilm cultivation, which impacts the biofilm bacteria number. A sharp decline in bacteria number was exhibited soon after the biofilm was put into beakers (0 h: B1 = 15, B2 = 35, B3 = 50, B4 = 9, B5 = 12, B6 = 20, B7 = 5, B8 = 8, B9 = 10 CFU/cm^2). This phenomenon was similar with the study of Fass et al. [19] who reported that chlorination had a strong influence on bacteria growth with decreasing the cell production by 1-log. The total bacteria counts of biofilm during the experiment were shown in Fig. 1.

3.1.1. Impact of FRC on biofilm bacteria regrowth

The total bacteria count of each beaker was rarely detected by the end of the first 2 h. It was consistent with the conclusion of other study that bacteria were significantly correlated with the concentration of free chlorine [20]. In middle C_C condition and high C_C condition (C_C = 4.30 and C_C = 5.80 mg/L), biofilm bacteria recovery regrowth was obvious in the 4th h, total bacteria count increased 38.67 times compared with that of 0 h. The FRC was 0.08 mg/L as Cl_2 in B8 and B9, 0.02 mg/L as Cl_2 in B5, 0.04 mg/L as Cl_2 in B6 and 0.03 mg/L as Cl_2 in B2 and B3 at that time (Fig. 2). Total bacteria counts of B1–B4 and B6 reached maximum values for the first time at the time when FRC came down to as low as 0.01–0.03 mg/L as Cl_2 in the 10th h while B5, B7–B9 reached their maximum total bacteria counts in the 12th h with FRC of 0.02–0.04 mg/L as Cl_2. From then on, biofilm bacteria

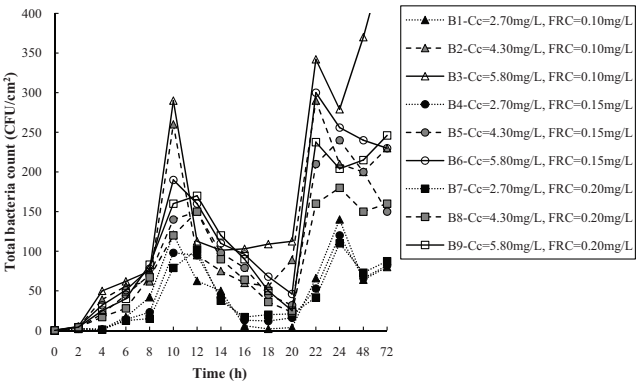

Fig. 1. Variations of biofilm bacteria number in two-level factorial design beakers during the experiment.

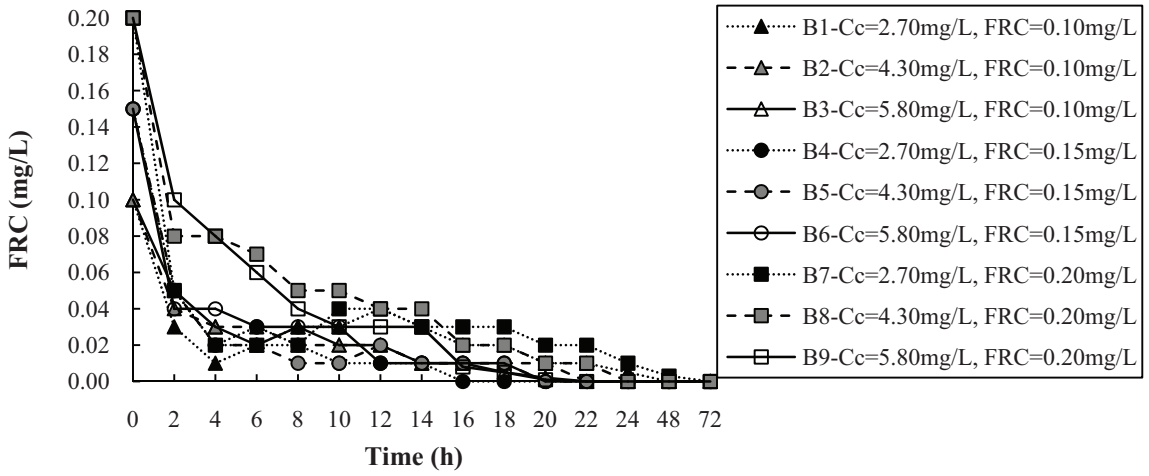

Fig. 2. FRC decay in nine beakers with different secondary chlorination dosage and C_C.

counts had a slow drop. The second stationary phase of B2, B3, B6 and B9 came in the 22th h with the total bacteria counts were 684–2,380 times of those of 0 h. The FRC were zero for all beakers by that time. This level of total bacteria count (10^5 CFU/cm^2) was similar with those that reported tap water previously [13].

Despite of the different C_C, the total bacteria count reached its maximum value for the first time was earlier in low FRC (0.10 mg/L as Cl_2) beakers than that in high FRC (0.20 mg/L as Cl_2) beakers. Moreover, the maximum values of two regrowth periods were much higher in low FRC condition, accordingly. This was a consequence of the presence of chlorine. Biofilm bacteria correlated negatively with the concentration of FRC [8]. This experiment showed similar correlation with that study.

3.1.2. Impact of organic matter content on biofilm bacteria regrowth

Total bacteria counts of low C_C (2.70 mg/L) were obviously lower than those of high C_C (5.80 mg/L). Compared with B7 and B9, it was easy to find that the total bacteria count of biofilm regrowth of B9 was almost more than double that of B7 at each sampling time and those of B8 were between B7 and B9. The difference of regrowth in biofilm bacteria between B1 and B3 was bigger than that of B7 and B9; it was more than two times in practically. The maximum total bacteria count of the second regrowth cycle was higher than the first one for each condition (1.15 times for B1, 1.12 times for B2, 1.18 times for B3 and 1.22 times for B4, 1.60 times for B5, 1.58 times for B6, 1.08 times for B7, 1.20 times for B8 and 1.40 times for B9). This fact brings out similar results to an earlier report [21]. The growth and death of bacteria may result in biofilm compaction due to permanent bacteria production. Finally, the process and the sufficient concentration of organic matter in inflow would yield high biofilm bacteria number.

All the beakers presented significant increase of total biofilm bacteria count after 24 h. FRC in each beaker was close to zero in that period so the only limitation of biofilm bacteria regrowth might be the organic matter content in beakers, for example, C_C. The organic matter that the bacteria utilized in that period was the residual of COD_{Mn} which was added at the beginning of the experiment and a fraction of dead bacteria cells. However, the regrowth of biofilm bacteria in B1, B4 and B7 was not as obvious as that of B2, B3, B5, B6, B8 and B9 in 16th–20th h. It may be because of the lack of organic matter. Therefore, it probably led to a conclusion that the regrowth of biofilm bacteria is influenced by the concentration of organic matter content in residential building SWSS condition.

Comparing the regrowth regularity of biofilm bacteria of B3, B6 and B9, it is showed that the FRC had obviously impacted on the biofilm bacteria regrowth, especially on the maximum total bacteria number. The similar impaction was observed in B1, B4 and B7 (with lower C_C), but was not as clear as observed in B3, B6 and B9 (with higher C_C). Organic matter content had a little influence on the regrowth of biofilm bacteria under the condition when FRC was more than 0.05 mg/L as Cl_2. However, it became a limitation of biofilm bacteria growth as FRC went down to less than 0.05 mg/L as Cl_2.

3.1.3. Collaborative effect of FRC and organic matter content on biofilm bacteria regrowth

The restored regrowth of biofilm bacteria was due to the organic matter in beakers which provided sufficient organic matter for bacteria growth and the amounts of residual chlorine was not enough to prevent the regrowth of biofilm bacteria [6].

The value of average regrowth rate of biofilm bacteria by the time of total bacteria count reached maximum for the first time ($\bar{\upsilon}$) was obtained from Fig. 1. For comparing the collaborative effect of FRC and C_C on biofilm bacteria regrowth, using K_f represents the average effect of three C_C levels (2.70, 4.30 and 5.80 mg/L) on biofilm bacteria regrowth and K_f equals the average value of $\bar{\upsilon}$ of three C_C levels under the fixed FRC. K_c represents the average effect of three FRC levels (0.10, 0.15 and 0.20 mg/L as Cl_2) on biofilm bacteria regrowth, and it equals the average value of $\bar{\upsilon}$ of three FRC levels under the fixed C_C. So the impact of FRC on biofilm bacteria regrowth can be seen through variation of K_f which leaves out the effect of C_C (Fig. 3(a)). Likewise, the impact of C_C on biofilm bacteria regrowth can be seen through variation of K_c which leaves out the effect of FRC (Fig. 3(b)).

According to the results of linear fit in Fig. 3(a) and (b), the collaborative effect of FRC and organic matter content on biofilm bacteria regrowth could be 0.99 (expressed as [C_C increases 1.00 mg/L]/[FRC decreases 0.10 mg/L as Cl_2]). In this experiment, the intervals of C_C and FRC were 1.60 and 0.50 mg/L as Cl_2, respectively. Then, (1.60 mg/L)/(0.50 mg/L as Cl_2) = 3.2 which indicated that C_C, videlicet, organic matter content was the main factor compared with FRC in the condition of FRC > 0.5 mg/L as Cl_2 in this experiment.

3.2. Structure of microbial community in biofilm

This experiment chose four typical samples (B1, B3, B7 and B9) to analyze their microbial community using metagenomic sequencing.

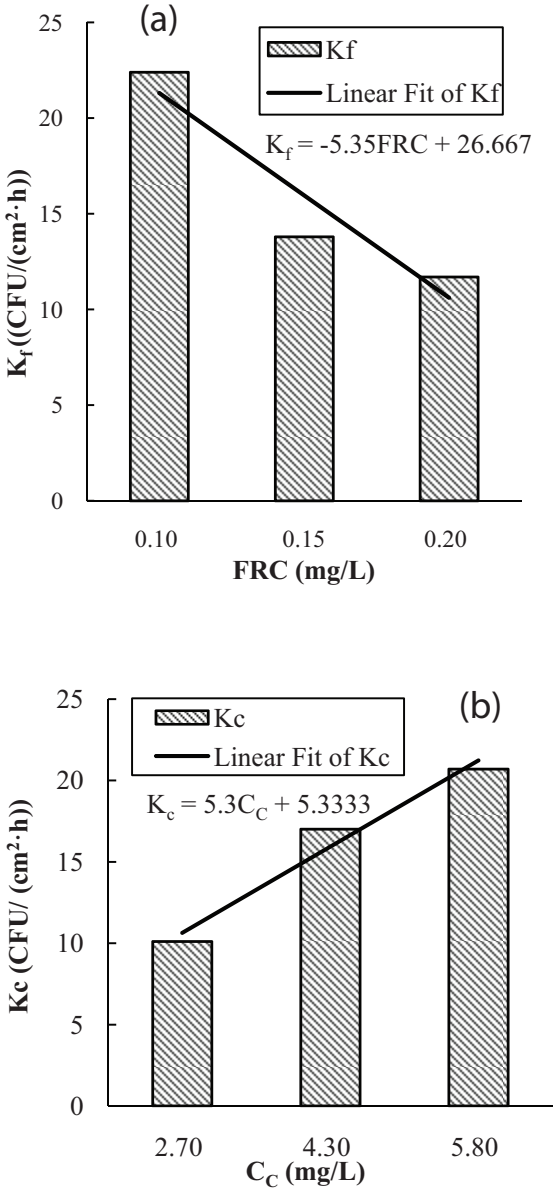

Fig. 3. Variation diagrams of K_f and K_c. (a) Linear fitting chart of K_f and (b) linear fitting chart of K_c.

Shannon's index as diversity parameter of chlorinated biofilm was 1.86, 1.93, 1.04 and 1.36 for B1, B3, B7 and B9, respectively, indicating that bacteria diversity of B3 was richer than that of B9 and B1 was richer than B7. It may be because of the effect of FRC on biofilm bacteria regrowth. The microbial community diversity of B3 and B9 was richer than those of B1 and B7, respectively. That may state that the organic matter content has some influence on the microbial community diversity. Phyla of biofilm bacteria changed a little before and after biofilm were chlorinated. However, the proportion of main phyla changed a lot. The major phylum for each beaker after chlorination was *Proteobacteria* (it mainly consisted of two classes, for example, *α-proteobacteria* and *β-proteobacteria*). It accounted for 97.6%, 96.8%, 97.0% and 98.3% of the sequences which were related to the known bacteria, respectively (Fig. 4). There were significant increases of *Proteobacteria* both in high and low C_C condition. However, a large shift in the proportions of *α-proteobacteria* and *β-proteobacteria* was shown in the four beakers. Class *α-proteobacteria* represented the most abundance class in B1 (96.7%), B3 (96.4%) and B9 (96.8%) but was the second largest bacteria group in B7 (40.5%). Class *β-proteobacteria* showed the largest abundance in B7 (55.9%), while it was a minor component of bacteria community in other biofilm samples (<0.5%). In addition, *Firmicutes* was with high proportion before chlorination but almost could not be found especially in high FRC chlorinated biofilm. *Bacteroidetes* was a phylum that is common to all biofilm samples of small number. *α-Proteobacteria* was found to be the largest component of biofilm bacteria community in real drinking water distribution system and tap water [22,23]. Sun et al. [1] found that biofilm with surface water had higher *Firmicutes* than that with groundwater and they also showed the correlation between COD_{Mn} and *Firmicutes* [1]. A phylum of *Actinobacteria* was detected with proportions of 1.2% and 2.4% in B1 and B7 but it was occurred in B3 and B9 with much lower proportions of 0.4% and 0.0%. Either low or high abundance of *Actinobacteria* in drinking water distribution system biofilm has been reported by other previous studies [1,24], which was in agreement with the result obtained in this experiment.

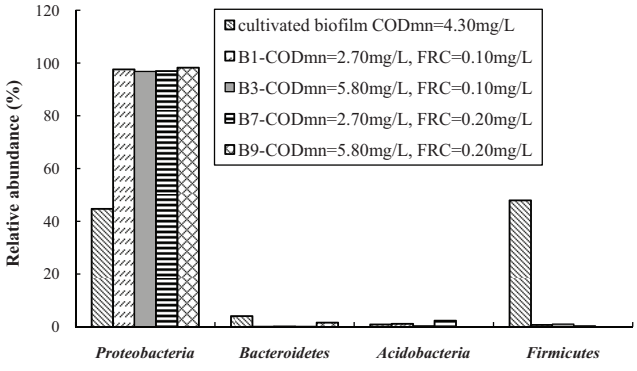

Fig. 4. Relative abundance of the main phyla identified in B1, B3, B7 and B9 biofilm samples (Only phyla with a relative abundance greater than 1% were shown. These four dominated phyla together accounted for more than 99.68% of sequences identified. Total numbers of sequences of B1, B3, B7 and B9 were 27,671, 43,375, 25,702 and 32,532).

Actinobacteria was linked to biodegradation of various environmental contaminants and it was also reported as the starch hydrolyzing organisms by starch staining combined with fluorescence in situ hybridization analysis of samples taken from certain wastewater treatment plants [25–27]. Humic substances are macromolecular substance as starch which should be the first biodegradation to monomers that can be further utilized by heterotrophic bacteria as a carbon source for microbial growth. These were consistent with the bacterial regrowth trend described in this paper. It probably explained the phenomenon that biofilm bacteria in B3 grew faster than that in B9, and biofilm bacteria in B1 grew faster than that in B7.

A total of 132, 148, 48 and 44 genera were identified from B1, B3, B7 and B9, respectively. There were only tiny proportions of sequences which were not related to the known groups at genera level (0.2%, 0.4%, 0.2% and 0.03% for each beaker, respectively). A number of genera from diverse bacterial phyla were detected in each biofilm samples implying high bacteria diversity in lab scale SWSS biofilm. This experiment mainly detected 11 genera in B1, B3, B7 and B9 biofilm samples which together accounted for more than 95.6% of sequences identified (Table 2). There was a distinctive difference of dominant phylotypes among the beakers at genus level, which was probably due to the different organic matter content and FRC in all beakers. For the beakers with high C_C, the predominant genus was *Novosphingobium* which constituted 84.9% of the identified genera in B9 biofilm samples while *Blastomonas* occupied the most percentage in B3 biofilm samples. On the other hand, for lower C_C beakers, *Methylobacterium* and *Paucibacter* were detected to be the dominant genera in B1 and B7, respectively, as the different values of FRC of both beakers. In addition, the genus *Sphingobium* was only detected in the beakers with high C_C (B3 and B9), and *Paucibacter* was only detected in the beakers with low C_C (B1 and B7). The genus *Pseudomonas* seemed to prefer high FRC environment and it was listed as pathogenic bacteria by the World Health Organization.

Table 2
Comparison of percentage of the sequences affiliated with the frequently identified genera to the total number of sequences from cultivated biofilm samples

Genus	Class	B1[a]	B3[a]	B7[a]	B9[a]
Blastomonas	*α-Proteobacteria*	0.02	95.1	0.04	6.7
Caulobacter	*α-Proteobacteria*	–	0.01	35.3	–
Methylobacterium	*α-Proteobacteria*	90.7	0.02	0.01	–
Novosphingobium	*α-Proteobacteria*	5.8	0.3	0.9	84.9
Rhizobium	*α-Proteobacteria*	0.01	0.03	3.7	–
Sphingobium	*α-Proteobacteria*	–	0.03	–	2.8
Sphingopyxis	*α-Proteobacteria*	–	–	–	2.1
Paucibacter	*β-Proteobacteria*	0.01	–	55.0	–
Pseudomonas	*γ-Proteobacteria*	0.03	0.03	0.3	1.4
Rhodococcus	*Actinobacteria*	1.1	0.1	0.01	–
Lapillicoccus	*Actinobacteria*	–	–	1.1	–

[a]Percentage of the sequences (%).
"–" not detected.

The genus *Blastomonas* was also referred to as a common inhabitant of disinfected water [28]. Moreover, *Blastomonas* and *Methylobacterium* genera were known to coaggregate [29]. Thomas et al. [30] demonstrated that the coaggregation had contributed to accelerate the growth rate of *Blastomonas*. It might be the reason for the high regrowth rate and biofilm bacteria number of B3 biofilm samples (Fig. 1). *Novosphingobium* was reported to be able to metabolize volatile compounds, such as phenylethyl acetate [31]. *Caulobacter* has the ability to use chlorogenic acid, *p*-hydroxybenzoate, phenol and benzoate degradation as the sole carbon and energy source [32].

Paucibacter belongs to the order of *Burkholderiales*. Some researchers have found organisms similar to *Paucibacter* strains only in the granulated activated carbon reactor sample from California [33].

4. Conclusions

The study of impact of secondary chlorination on biofilm regrowth and microbial community diversity in SWSS with different organic matter contents rises to the following conclusions:

(1) Organic matter content (COD_{Mn} < 5.8 mg/L) had a little effect on biofilm regrowth when FRC was more than 0.05 mg/L as Cl_2 in lab scale SWSS. FRC less than 0.05 mg/L as Cl_2 could not limit the regrowth of biofilm when organic matter content was above the Chinese standard (COD_{Mn} > 3.00 mg/L). FRC should be kept more than 0.05 mg/L as Cl_2 when C_C is more than 3.00 mg/L in order to ensure the biological safety.
(2) The collaborative effect ratio of secondary chlorination and organic matter content on biofilm bacteria regrowth was 0.99 (expressed as [C_C increases 1.00 mg/L]/[FRC decreases 0.10 mg/L as Cl_2]). In this experiment, the collaborative effect ratio was 3.2, which indicated that the influence of organic matter content was prior to FRC on the regrowth of biofilm in SWSS.
(3) The dosage of secondary chlorination should be more than 0.10 mg/L as Cl_2 (in FRC) in eligible supply water according to Chinese standard (COD_{Mn} < 3.00 mg/L).
(4) Organic matter content does not have obvious influence on microbial community diversity. Only the phylum *Proteobacteria* was significantly affected by organic matter content and it could restore regrowth quickly after secondary chlorination in this lab scale SWSS. FRC could effectively restrain the regrowth of *Firmicutes* but it might restore pathogenic bacteria from the genus *Pseudomonas* to regrowth in this experimental condition.

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