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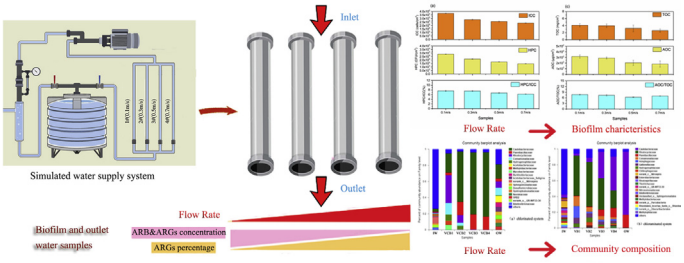
Effect of hydraulic conditions on the prevalence of antibiotic resistance in water supply systems

Junpeng Zhang [a], Weiying Li [a, b, *], Jiping Chen [a], Feng Wang [a], Wanqi Qi [a], Yue Li [a], Bing Xie [c]

HIGHLIGHTS

- Hydraulic condition effect on the prevalence of ARGs were developed.
- Biofilms in low flow rate pipeline had high ARB and ARGs concentration.
- High flow rate resulted in low contents of biomass and nutrient.
- Community composition of outlet water was more similar to biofilms than influent.
- The detachment of ARB and ARGs from biofilms to water leads to AR rising in water.

GRAPHICAL ABSTRACT



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ABSTRACT

The incidence of antibiotic resistance genes (ARGs) in tap water leads to potential risks to human health and draws more and more attention from the public. However, ARGs harbored in drinking water remain largely unexplored. In this study, a simulated water supply system was designed to study the effects of different pipe flow rates on the transmission of antibiotic resistance in water supply systems. We observed that the biofilm in low flow rate pipeline (0.1 m/s, 0.3 m/s) had higher concentration of both antibiotic resistant bacteria (ARB) and ARGs, while high flow rate (0.5 m/s and 0.7 m/s) resulted in low relative abundance of ARB and high relative abundance of ARGs in biofilms. The results showed that the high flow rate led to an abundance in non-culturable bacteria and a scarcity of nutrients in the biofilm, giving rise to its antibiotic resistance. High-throughput sequencing pointed out that the high content of *Caulobacteraceae* and *Paenibacillus* were determined in biofilms of high flow rate pipelines. Similarity analysis of microbial community composition of inlet water (IW), biofilms and outlet water (OW) showed that the composition of microbial community in OW was more similar to that in biofilms than in IW. Genera of bacteria in biofilms and OW (*Brevundimonas*, *Brevibacillus* and *Pseudomonas*) which had relationship with *sulI*, *sulII* in biofilms ($P < 0.05$) had higher relative abundance than that in IW. Different flow rate conditions had an impact on the biomass, microbial community, ARB and ARGs composition of biofilms. Thus, the detachment of biofilms can increased the antibiotic resistance of the water.

a College of Environmental Science and Engineering, Tongji University, Shanghai, 200092, China
b State Key Laboratory of Pollution Control and Resource Reuse, Tongji University, Shanghai, 200092, China
c School of Ecological and Environmental Science, East China Normal University, Shanghai, 200241, China
* Corresponding author. College of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Shanghai, PR China.
E-mail address: 123lwyktz@tongji.edu.cn (W. Li).

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1. Introduction

Antibiotic resistance (AR) is profoundly important to human health, but the environmental reservoirs of resistance determinants are poorly understood. Biofilms as surface-attached groups of microbial contain an extracellular matrix which makes it less susceptible to antimicrobial agents than the planktonic cells in drinking water supply systems (DWSSs). The biofilm formation can lead to the accumulation of pathogens, antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in DWSSs (Guo et al., 2018; Zhang et al., 2018). Bacteria of biofilms can protect themselves from bactericide and increase scrutiny concerns over the existence and proliferation of opportunistic pathogen, which could be potentially exposed to consumers (Flemming et al., 2016; Liu et al., 2016). Biofilms are responsible for biofouling, contamination of process water and hygienic deterioration of drinking water quality (Simpson, 2008). The biofilm detachment caused by its own growth or the hydraulic conditions in DWSSs will bring bacteria from biofilms to bulk water. These bacteria can in turn form biofilms again in other areas of the pipeline and continue to detach to bulk water, which had influence on the water quality of tap water. Therefore it is particularly important to dig into mechanism of antibiotic resistance transmission through biofilms in DWSSs.

The antibiotic resistance has been frequently detected in raw water, DWSSs and tap water in different countries (Su et al., 2018; Zheng et al., 2018). Many factors might affect the ARGs and ARB concentration in tap water, including disinfectant concentration, heavy metal ion and so on (Jia et al., 2015; Xu et al., 2017b). Hydraulic conditions have been proved to have an effect on biofilm formation. When the shear force of the water flow rose from 0.2 to 10 Pa, 56% of the biofilm cells were removed. The detachment of the biofilm depends on the process of erosion and aggregation of the biofilm clusters, in which the clusters of 50–300 μm^3were reduced, while the content of small clusters (less than 50 μm^3) and larger clusters (greater than 600 μm^3) increased (Mathieu et al., 2014). Furthermore, it was confirmed that the extracellular polymer of the biofilm becomes tight as the hydrodynamic pressure increased. The biofilms were a significant sink for ARGs in the aquatic environment (Balcazar et al., 2015). The hydraulic condition changed the bacterial composition in biofilms and might affect the concentration of ARB and ARGs in biofilms. Douterelo studied three different hydraulic states (stagnation, low water flow changes, high water flow changes), indicating that the hydraulic condition affected the biofilm community composition, and the high-flow-fluctuating pipeline has higher organisms and tighter biofilm structure (Douterelo et al., 2014, 2016). Fish developed the characteristics of the biofilm under these three different hydraulic conditions, pointing out that the change of hydraulic condition was more likely to affect the EPS content of the biofilm (Fish et al., 2016, 2017). Xu examined the effect of hydraulic condition on the biofilm of sewage pipes and rendered that the porosity and dissolved oxygen of biofilms decreased with increasing flow rate (Xu et al., 2017a). To summarize, it has been commonly acknowledged that the different hydraulic conditions of the DWSS can affect microbial community composition, the tightness of the biofilm structure and lead to the interaction between biofilms and the bulk water. Understanding the mechanism of the influence of hydraulic conditions on antibiotic resistance prevalence in DWSS will provide a basis for improving biosafety of drinking water.

In this study, we investigated the impact of hydraulic conditions on the antibiotic resistance of biofilms and tap water, by establishing a simulated water supply system (SWSS) with different flow rate. Both ARB and ARGs were determined in biofilms and bulk water. High throughout sequencing was adopted to study the community shift in the operation period at different hydraulic condition. We aimed to address the following questions: (i) What is the influence of the hydraulic condition on the existence of ARB and ARGs in biofilms and water? (ii) What is the relationship between biofilm characteristics and antibiotic resistance in SWSS under different flow rate? (iii) Provide a fundamental baseline on what is the relationship between community shift and antibiotic resistance variation in biofilms and water?

2. Materials and methods

2.1. Simulated water supply system operation and monitoring

A simulated water supply system (Fig. 1) consisting of four branch pipes was designed. The SWSS used the circulating water supply mode. The raw water with fixed disinfectant concentration was placed in the circulating water tank, and then water was supplied by peristaltic pump (BT 300L, Baoding Reef Fluid Co., Ltd.). By controlling the feed water flow rate of each pipe, the flow rates of each branch pipes were 0.1 m/s, 0.3 m/s, 0.5 m/s and 0.7 m/s, respectively. The flow rate was checked in the morning and evening every day. Eight cast iron coupons ($H \times W \times L = 15\ cm \times 1.0\ cm \times 1.0\ cm$) were installed in the middle of each pipe. The surface area of each cast iron coupon was 60 cm^2. In order to simulate the actual pipe network water supply system, the experiment was carried out under disinfection conditions, containing a chlorine disinfection system and a chloramine disinfection system. The initial concentration of chlorine in circulating water tank was prepared by sodium hypochlorite solution to prepare disinfectant. The free chlorine concentration of water tank and each pipeline was maintained at 1.0 mg/L. The chloramine disinfectant was prepared by mixing ammonium sulfate solution and sodium hypochlorite solution according to Cl:N = 4:1 (mass ratio). Finally, the initial concentration of chloramine in the circulating water tank was 1.0 mg/L. The water in the circulating water tank was replaced every 11 h, thus the residence time of each pipeline was 11 h. The water temperature was maintained at room temperature (18–23 °C). The effluent water quality of each pipeline was measured every day and was shown in Tables S1 & S2. The SWSS was continuously operated under this condition for eight weeks.

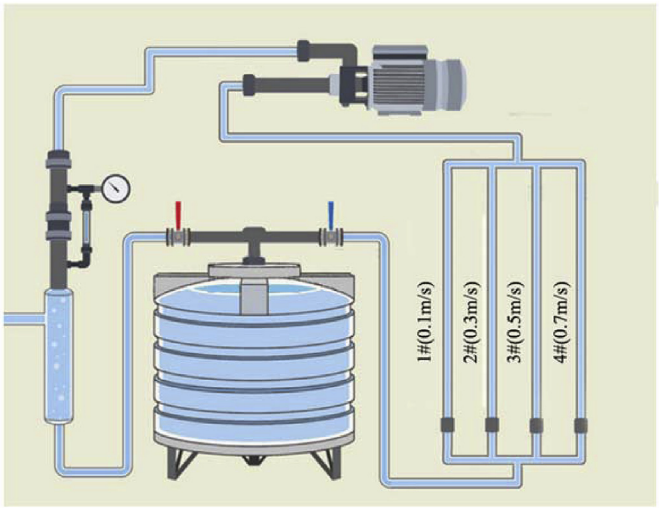

Fig. 1. Simulated water supply system with different flow rates.

2.2. Water and biofilm sampling and DNA extraction

The inlet water (IW) and mixed outlet water (OW) (5 L each) was sampled at the 2nd, 4th, 6th and 8thweek by using sterile glass bottle. 4L water of each samples was filtered through a polycarbonate membrane (0.22 μm, Millipore, USA) to extract DNA. Biofilm samples were collected at the eighth week by swabbing the coupon surface with sterile cotton, which was then put into a glass bottle filled with 100 ml sterile phosphate buffer. The bottle was placed into an ultrasonic vibration chamber (SB-800D, Ningbo Scientz Biotechnology Co., LTD, China) for 5 min of ultrasound treatment in ice water to detach the biomass from the cotton to the phosphate buffer (Proctor et al., 2016). Subsequently, the phosphate buffer was filtered through a 0.22 μm membrane for further DNA extraction. FastDNA SPIN Kit (MP Biomedicals, CA) was used for DNA extraction. The DNA concentration and purity were determined by Nano DropND-2000 (NanoDrop Technologies, Wilmington, DE).

2.3. Biological and chemical parameters determination

After sampling, the disinfectant residual was immediately neutralized. Turbidity, DOC, UV_{254}, pH, ammonia, total particle count, assimilable organic carbon (AOC) concentration, intact cell concentration (ICC), and total cell concentration (TCC) were determined. ICC and TCC was determined by using flow cytometry (Prest et al., 2013). SYBR green was added to samples (Life Technologies Ltd., USA) for TCC determination. Both SYBR green and propidium iodide were added (Life Technologies Ltd., USA) for ICC determination. The ICC and TCC were counted by FACSCalibur flow cytometer (BD). AOC analysis was conduct by using an assay determined with flow cytometry previously (Li et al., 2018). The other chemical parameter investigated in this experiment were shown in Table S3.

2.4. Antibiotic resistance bacteria determination method

Four kinds of antibiotics frequently determined in surface water of south China (Jiang et al., 2013; Zhang et al., 2018) were chosen to investigate antibiotic resistant bacteria, as shown in Table S4. Heterotrophic plate count (HPC) and ARB were measured as previous described (Zhang et al., 2018), by usingR2A agar and R2A agar with antibiotics. After incubated at 22 °C for 7days, the number of total colonies was regarded as HPC concentration and ARB concentration which was shown in Figs. S1–S5. The ARB percentage were calculated as ARB concentration divided HPC concentration. The proportion of uncultivable cells was calculated as follows:

$$\text{Proportion of uncultivable cells} = (1 - HPC/ICC)*100\%$$

2.5. Real-time qPCR

Two macrolide ARGs (*ermA*, *ermB*), four tetracycline ARGs (*tetA*, *tetB*, *tetC*, *tetM*), two sulfonamide ARG (*sulI*, *sulII*), four β-Lactam ARGs (bla_{SHV}, bla_{TEM}, bla_{CTX}, *ampC*), one MGE (*intl1*), and the 16S rRNA gene were quantified using SYBR-Green real-time qPCR. The primer sequences and PCR conditions were shown in Table S3. Positive controls contained cloned and sequenced PCR amplicons that were obtained from the raw water of water treatment in south China. Concentrations of the standard plasmids (ng/μL) were determined with the Nano DropND-2000, and their copy concentrations (copies/μL) were then calculated. The 25 μL reactions of qPCR typically contained 1 μL SYBR premix Ex Taq II (Tli RNaseH Plus) (TaKaRa), 0.3 μL ROX Reference Dye (TaKaRa), 10 mM of each primer, and 2 μL of DNA templates. Real-time PCR was run by using an ABI 7500 system (ABI, USA) with the following program: 95 °C for 30 s, 40 cycles consisting of: (i) 95 °C for 5 s; (ii) annealing temperature for 30 s; (iii) 72 °C for 30 s to collect the fluorescent signals. The melting process was automatically generated using the ABI 7500 software. Ten-fold dilution of plasmids carrying the target gene were used as calibration standards, ranging from 10^8 copies to 10^2 copies. Standard curves were constructed in each PCR run and the copy numbers of genes in each sample were interpolated using these standard curves. All of the standards, samples, and negative control (sterile water) were quantified in triplicate. Reliable correlation coefficients ($R^2 > 0.99$) for standard curves over five orders of magnitude were obtained. Relative abundances of the ARGs and MGEs were normalized to bacterial 16S rRNA genes for comparison. Absolute abundance was calculated based on number of gene copies per water sample volume.

2.6. Illumina MiSeq-sequencing

The 16S rRNA genes were amplified from all DNA extracts using barcoded primers 515F/907R (515F 5′-barcode-GTGCCAGCMGCCGCGG)-3′ and 907R 5′- CCGTCAATTCMTTTRAGTTT -3′), the reaction system was as follows: 95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30s and a final extension at 72 °C for 10 min. PCR products were separated on 2% agarose gels. The extraction and purification was performed using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.). After preliminary assessment by electrophoresis, the PCR products were accurately quantified with the QuantiFluor -ST blue fluorescent quantitative system (Promega company), and then the samples were prepared at the corresponding proportions according to the sequencing requirements of each sample. One end of the DNA fragment is complementary to the primer base and is fixed on the chip. Finally, purified amplicons were sequenced on an Illumina MiSeq platform.

2.7. Data analysis

After sequencing, QIIME (version 1.9.1) was used to process the sequencing data. The 300 bp reads were truncated where the average quality score was less than 20, and the truncated reads were shorter than 50 bp. Only sequences with overlap longer than 10 bp were retained and assembled according to their overlapping sequence. After removing the chimeric sequences, the sequences with a similarity greater than 97% using UPARSE (version 7.1 http://drive5.com/uparse/) were classified as belonging to the same OTU, and taxonomic analysis of the representative out sequences was performed. Based on the results of OTU cluster analysis, OTU can be analyzed for multiple diversity indices and determination of sequencing depth. Based on taxonomic information, the determination data was analyzed using R software and Origin 9.1. P value below 0.05 was regarded as significant for all tests.

3. Results and discussion

3.1. Effect of flow rate on ARB concentration and percentage in the SWSS

As shown in Fig. S6(a), the IW and OW of the SWSS were sampled every two weeks, and the four kinds of ARB were determined. The average concentration of four ARB (tetracycline-resistant bacteria, sulfamethoxazole-resistant bacteria, clindamycin-resistant bacteria and norfloxacin-resistant bacteria) in IW of chlorine disinfected SWSS were 183.5 ± 5.2 CFU/ml,

162.5 ± 12.6 CFU/ml, 206.9 ± 4.2 CFU/ml and 84.3 ± 3.6 CFU/ml, while in OW were 253.7 ± 6.8 CFU/ml, 160.3 ± 4.8 CFU/ml, 167.2 ± 3.5 CFU/ml, and 83.5 ± 3.6 CFU/ml, respectively. The concentration of tetracycline-resistant bacteria in OW increased significantly, while concentrations of the other three ARB were close to or slightly lower than those of IW. As shown in Fig. S6(b), the average ARB percentage were 6.11 ± 0.01%, 5.42 ± 0.42%, 6.87 ± 0.04%, 2.81 ± 0.12% in IW and were 16.92 ± 0.32%, 10.69 ± 0.12%, 11.15 ± 0.10%, and 5.57 ± 0.11% in OW. The proportion of ARB in OW significantly increased, indicating that the chlorine disinfected SWSS will lead to an increase in the proportion of ARB. As shown in Figs. S6(c) and (d), the concentration of ARB in OW was higher than that in IW, indicating that ARB concentration increased in SWSS. Compared with the chlorine disinfected SWSS, the chloraminated system had higher ARB concentration and lower ARB percentage, indicating that the chlorine disinfection performed better to suppress ARB growth in water, while the ARB percentage was better controlled by the chloramine disinfection.

As shown in Fig. 2, ARB concentrations and ARB percentage of biofilms at different flow rates were investigated at the eighth week. In chlorine disinfected SWSS, the order of concentration of ARB in four different flow rate biofilms was 0.1 m/s > 0.3 m/s > 0.5 m/s > 0.7 m/s. ARB had the highest concentration in the lowest flow rate (0.1 m/s) pipeline and the highest ARB percentage in the pipeline with the highest flow rate (0.7 m/s). In the chloramine disinfection pipeline, the order of concentration of tetracycline-resistant bacteria and sulfamethoxazole-resistant bacteria in four different flow rate biofilms was 0.3 m/s > 0.1 m/s > 0.5 m/s > 0.7 m/s; while the order of concentration of clindamycin-resistant bacteria and norfloxacin-resistant bacteria in four different flow rate biofilms was 0.1 m/s > 0.3 m/s > 0.5 m/s > 0.7 m/s. The results indicated that ARB had a high concentration at low flow rate (0.1 m/s and 0.3 m/s) pipeline.

The order of ARB percentage in biofilms samples in chlorine disinfected SWSS is 0.1 m/s > 0.3 m/s > 0.5 m/s > 0.7 m/s. The lowest ARB percentage was found in the lowest flow rate (0.1 m/s) pipeline while the highest proportion was found in the fastest flow rate pipeline. The order of percentage of tetracycline-resistant bacteria and sulfamethoxazole-resistant bacteria in four different flow rate biofilms was 0.5 m/s > 0.1 m/s > 0.3 m/s > 0.7 m/s under chloramine disinfected conditions, while the order of percentage of clindamycin-resistant bacteria and norfloxacin-resistant bacteria in four different biofilm samples is 0.1 m/s > 0.3 m/s > 0.5 m/s > 0.7 m/s. The results rendered that clindamycin-resistant and norfloxacin-resistant bacteria accounted for a higher percentage in the slower flow rate (0.1 m/s), while tetracycline and sulfamethoxazole resistance were higher at faster flow rates. In general, both under chlorine and chloramine disinfection conditions, the concentration and proportion of ARB were high when the flow rate was low.

3.2. Effects of flow rates on the concentration and relative abundance of ARGs in SWSS

As shown in the Fig. S7(a) & (b), the concentrations of the nine resistance genes (*tetA*, *tetB*, *tetM*, *sulI*, *sulII*, *ermA*, *ampC*, *Integron* I, bla_{TEM}) were investigated in IW and OW of the SWSS under different flow rates. The concentration of*tetA*, *tetB*, *tetM*, *sulII*, *ermA* and *Integron* I in IW was higher than that of OW. Among nine different ARGs of OW, tetracycline resistance gene had the highest concentration, while *ampC* had the lowest one. Comparing the relative abundances of nine resistance genes in IW and OW, it can be found that the relative abundance of *tetA*, *tetB*, *tetM*, *sulII*, *ermA* and *IntegronI* increased significantly after water supply. Combined with the results that ICC concentration of IW was lower than that in OW, and the residual disinfectant had been retained in OW, which limited the regrowth of bacteria in water, it can be concluded that only biofilm detachment and chlorine disinfectant can affect the bacteria concentration in OW. It can be inferred that the formation and detachment of biofilm might lead to the increase of ARGs concentration, while chlorine disinfection has a selective effect on bacteria carrying ARGs in water, leading to the increase of relative abundance of ARGs. As shown in the Fig. S7(c) & (d), comparing the concentrations of ARGs in IW and OW of the chloramine disinfection system, the relative contents of *tetA*, *tetB*, *tetM*, *sulI*, *sulII*, *ermA* and bla_{TEM} increased significantly in OW (ANOVA, $P < 0.05$). Compared with chlorine disinfected effluent, ARGs concentration was higher in chloramine disinfection condition while the relative abundance of ARGs was lower. The relative abundance of ARGs was higher in OW than in IW samples under both chlorine and chloramine disinfection.

As shown in Fig. 3(a), the concentrations of nine ARGs (*tetA*, *tetB*, *tetM*, *sulI*, *sulII*, *ermA*, *ampC*, *Integron I*, bla_{TEM}) in the biofilm of the coupon with different flow rates in chlorine disinfection were respectively 1.00×10^3 to 1.18×10^4 copies/cm^2, 1.35×10^4 to 1.49×10^5 copies/cm^2, 9.01×10^3 to 1.21×10^5 copies/cm^2, 1.40×10^3 to 7.79×10^3 copies/cm^2, 2.50×10^2 to 6.62×10^2 copies/cm^2, 4.89×10^2 to 2.36×10^3 copies/cm^2, 4.55 to 8.05 copies/cm^2, 2.31×10^3 to 1.72×10^4 copies/cm^2 and 1.29×10^3 to 1.29×10^4 copies/cm^2. Comparing the biofilms of pipelines with different flow rates, it can be found that the pipeline with the lowest flow rate (0.1 m/s) had the highest ARGs concentration and 16S concentration, indicating that the low flow rate resulted in high biomass and ARGs. With the increase of flow rate, the content of *tetA*, *tetB*, *tetM*, *sulI*, *sulII* and *ermA* decreased in biofilm, while the concentration of *Integron I* and bla_{TEM} increased with the flow rate. As shown in Fig. 3(b), the relative abundance of nine ARGs in the biofilm with different flow rates of chlorine disinfection was 0.17%–0.20%, 2.09%–2.59%, 1.60%–2.11%, 0.06%–0.43%, 0.01%–0.11%, 0.04%–0.24%, 0.01%–0.02%, 0.33%–0.80% and 0.19%–0.24%, respectively. As shown in Fig. 3(c) and (d), under chloramine disinfection, biofilms of pipelines with flow rates of 0.1 m/s and 0.3 m/s had higher ARGs concentration than that of pipelines with flow rate of 0.5 m/s and 0.7 m/s (ANOVA, $P < 0.05$). Comparing with ARGs concentration of biofilms under chloramine disinfection, ARGs concentration in biofilms was lower under chlorine disinfection conditions. Both in chlorinated and chloraminated system, biofilms had high abundance of *tetA*, *tetB*, *tetM*, *sulI*, *sulII* and *ermA* in low flow rate biofilms, the mechanism of antibiotic resistance was efflux pumping and dihydropteroate synthase, which was probably caused by the enrichment of pollutant such as heavy metal in the biofilms. However under the high flow rate most antibiotic resistance mechanisms were mainly β-lactam and integron. The relative abundance of ARGs in the biofilm reached the highest level, when the flow rate of pipelines was at 0.5 m/s. Thus, the flow rate played an important role on antibiotic resistance of biofilms in water supply system.

Comparing the relative abundance of ARGs in biofilms and OW at different flow rates, the results showed that a significant higher relative abundance of ARGs (such as *tetA*, *tetB* and *tetM* under chlorination disinfection conditions, *sulI* and *sulII* under chloramine disinfection conditions) was obtained in biofilms and OW than in IW. It implied that the biofilm has an effect on the water quality of OW. Comparing the relative abundance of ARGs with ARB percentage, ARB percentage was positively related to flow rate, while the relative abundance of ARGs is negatively correlated to flow rate. The reason for the difference was caused by the different determination method of ARB and ARGs. ARB was measured by culture based method and ARGs were determined by culture independent method. The hydraulics are thought to influence accumulation and adhesive strength. When the flow rate was high, the biofilm

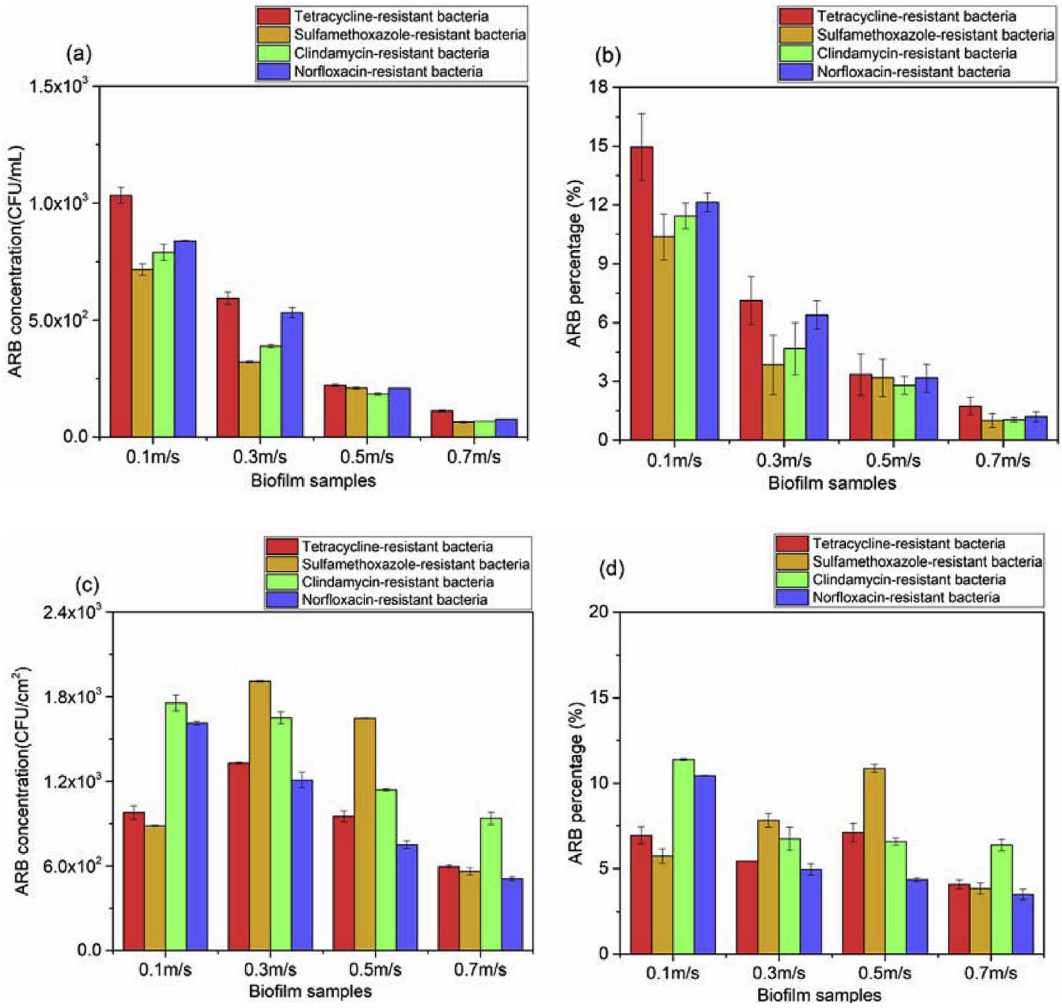

Fig. 2. Variation of ARB concentration and ARB percentage in biofilm samples under different flow rates (a and b, chlorinated system, c and d, chloraminated system).

structure was dense and compact. The bacteria composition were mostly anaerobic microorganisms due to the low dissolved oxygen concentration (Beyenal and Lewandowski, 2002; Rochex et al., 2008). Thus, lots of bacteria in biofilms formed at high flow rate cannot be cultivated, while ARGs were investigated by culture independent methods, and the relative abundance of ARGs were relatively high.

3.3. Relationship between biofilm characteristics and antibiotic resistance in SWSS

As shown in Fig. 4(a) and (b), the HPC, ICC, and HPC/ICC levels in biofilms at different flow rates were compared. The results showed that the ICC, HPC, and HPC/ICC were lower in the biofilm at higher flow rates. The HPC/ICC ratio decreased with increasing flow rate, indicating a higher proportion of non-cultivable bacteria in the biofilm at high flow rates, possibly due to differences in biofilm structure at different flow rates. Sharpe have also shown that lower flow rates or shear stresses (different steady states were tested) resulted in thicker biofilms (Sharpe, 2012). At low flow rates, the biofilm structure was loose and easy to contact with the water phase, the HPC/ICC ratio is high when the flow rate is low. High shear stress and turbulent flow conditions favor the production of more dense and compact biofilms by the production of extracellular polymers (Douterelo et al., 2013). When the flow rate was high, the biofilm surface structure was relatively thin and tight. Because the high flow rate can affect the detachment rate of the biofilm. Most of microorganisms in biofilms cannot be cultivated by HPC method, accounting for a relatively low proportion of HPC/ICC. Lin induced the entry of *E. coli* into the VBNC state by using a low concentration disinfectant, and the results showed that the VBNC status of the bacterial anti-stress gene and resistance gene content was up-regulated (Lin et al., 2017). High flow rates in this study resulted in biofilms with a higher proportion of non-cultivable bacteria, and the presence of higher levels of antibiotic resistance in these bacteria resulted in increased biofilm resistance.

As shown in Fig. 4(c) and (d), comparing the organic composition in the biofilms of different flow rates, the highest TOC, AOC and AOC/TOC were obtained when the flow rate was 0.1 m/s under chlorine disinfection condition, while the maximum values reached at a flow rate of 0.3 m/s under chloramine disinfection condition. It was implied that the biofilms with low flow rate had high TOC, AOC concentration and AOC/TOC ratio. Mathieu pointed out that biofilms can cause medium-sized biofilm clusters (50–300 μm^3) to fall off at high flow rates (Mathieu et al., 2014). When the AOC and TOC concentration in the biofilm were high, the biofilm had high ICC, HPC, ARB and ARGs concentration, while the relative abundance of ARB and ARGs were low. The reason was that when the flow rate was low, biofilm was thick, containing a loose structure surface, which resulted in a high content of organic matter, biomass, ARB

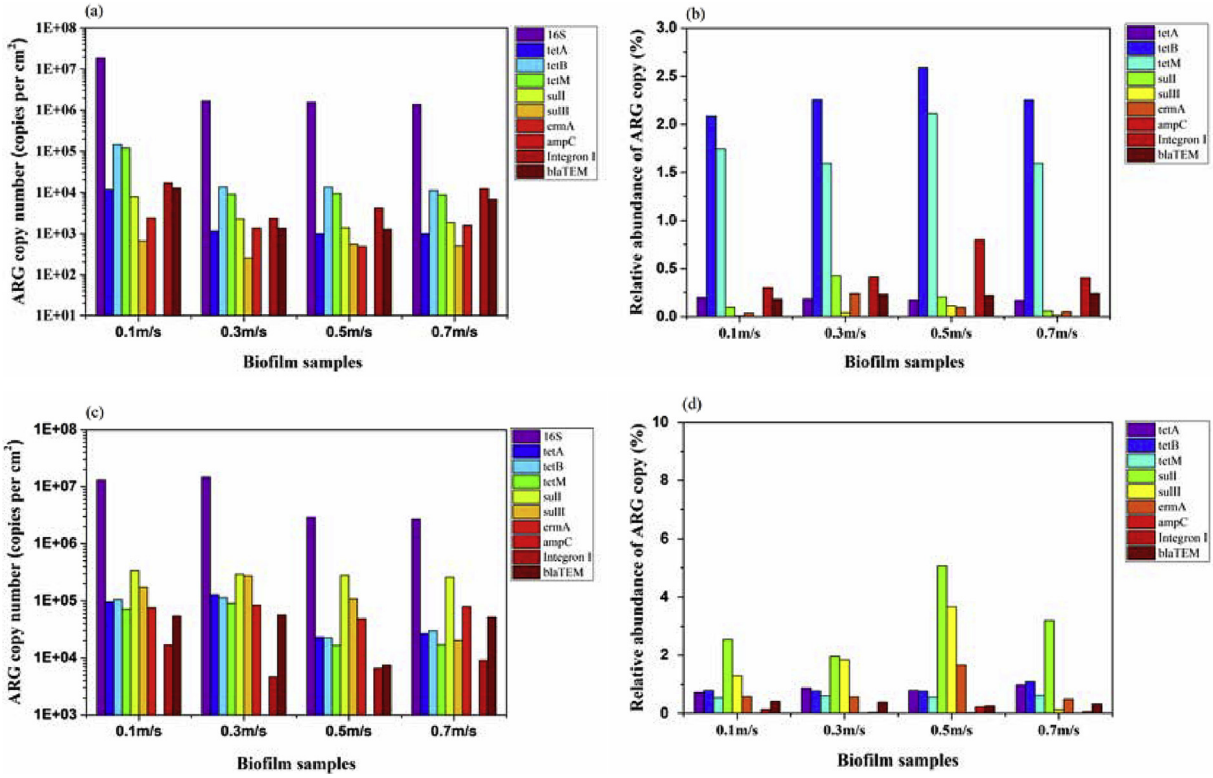

Fig. 3. Variation of ARGs concentration and ARGs percentage in biofilm samples under different flow rates (a and b, chlorinated system, c and d, chloraminated system).

and ARGs. When the flow rate was high, the biofilm structure was tight. Since the environment at the bottom of the biofilm tended to have less accumulation of oxygen, nutrients and metabolites, it is more suitable for anaerobic bacteria and facultative bacteria survive and easy to induce bacteria to enter the VBNC state (Stewart and Franklin, 2008). Beloin showed that bacteria in oxygen-rich biofilms are more susceptible to antibiotics than bacteria in biofilms with limited oxygen (Beloin et al., 2004). Other studies have shown that when biofilms are transferred to anaerobic conditions, the inactivation rate of antibiotics decreased (Hill et al., 2005). Nutrients and oxygen imitation leads to a slow bacterial growth rate and metabolic rate in biofilms. Xu showed that an increase in flow rate leads to a decrease in the porosity and dissolved oxygen of biofilms (Xu et al., 2017b). Therefore, different flow rates may produce different shear forces on the biofilm of the pipeline, affecting the biofilm structure, which resulted in different nutrient and oxygen gradients and different bacterial antibiotic resistance.

3.4. The effect of microbial community shifts on antibiotic resistance in tap water and biofilm samples under different rate

The flow rate not only affects the biomass in the biofilm but also affects the microbial community composition in the biofilm (Douterelo et al., 2013, 2014, 2018), which may lead to differences in antibiotic resistance in the biofilm at different flow rates. In this study, high-throughput sequencing was used to explore the community composition of the IW, biofilm and OW in SWSS under different flow conditions. The sequencing results showed that compared with the IW, the total number of OTU in biofilm and OW samples was low both in chlorinated system and chloraminated system.

As illustrated in Fig. S8, the phylum of biofilm and water samples in SWSS under chlorine disinfection conditions were mainly *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Acidobacteria*, *Nitrospirae*, *Bacteroidetes*, *Chloroflexi*, *Cyanobacteria*, *Planctomycetes*, *Spirochaetae*, *Parcubacteria*. *Proteobacteria* was dominant in both water and biofilm samples, accounting for 61.61%–87.84%. *Firmicutes* had the second abundance in biofilm samples, which accounted for 4.37%–20.15%.While *Firmicutes* occupied 2.71% in IW and 14.32% in OW, showing a significant increase in OW, indicating that the interaction between biofilms and water affected microbial community in water. At the class level, the relative abundances of *Alphaproteobacteria*, *Betaproteobacteria*, and *Bacilli* in biofilm and OW samples were higher than that in IW. At the family level, the four families with the highest proportion of biofilms are: *Caulobacteraceae*, *Paenibacillus*, *Rhodocyclaceae*, and *Comamonadaceae*, with the highest proportions of 79.85%, 16.49%, 34.51%, and 14.31%, respectively. The relative content of these four families in biofilms and OW was higher than that in IW. Comparing the microbial community composition in different flow rate pipelines, the higher concentration of *Caulobacteraceae* and *Paenibacillus* in the high-flow pipeline biofilms indicated that these two families may have ability to secrete extracellular polymer to resist high flow rate.

Under chloramine disinfection conditions, the microorganisms in the IW, biofilm and OW samples, which were shown in Fig. S8, were mainly *Proteobacteria*, *Firmicutes*, *Acidobacteria*, *Bacteroidetes*, *Nitrospirae*, *Chloroflexi*, *Actinobacteria*, *Cyanobacteria*, *Planctomycetes*, *Parcubacteria*. *Proteobacteria* also dominated in water and biofilm samples, accounting for 61.69%–85.12%. *Firmicutes* accounted for 2.71% in IW, and 17.71% in OW samples. At the class level, the relative abundances of *Alphaproteobacteria* and *Bacilli* in biofilm and OW samples were higher than IW; the relative abundance of *Deltapotoobacteria*, *Gammaproteobacteria* and *Nitrospira* in water was higher than that of biofilm samples. At the family level, shown in Fig. 5, the highest proportion of biofilms were *Caulobacteraceae* and *Paenibacillus*, with the highest proportions of

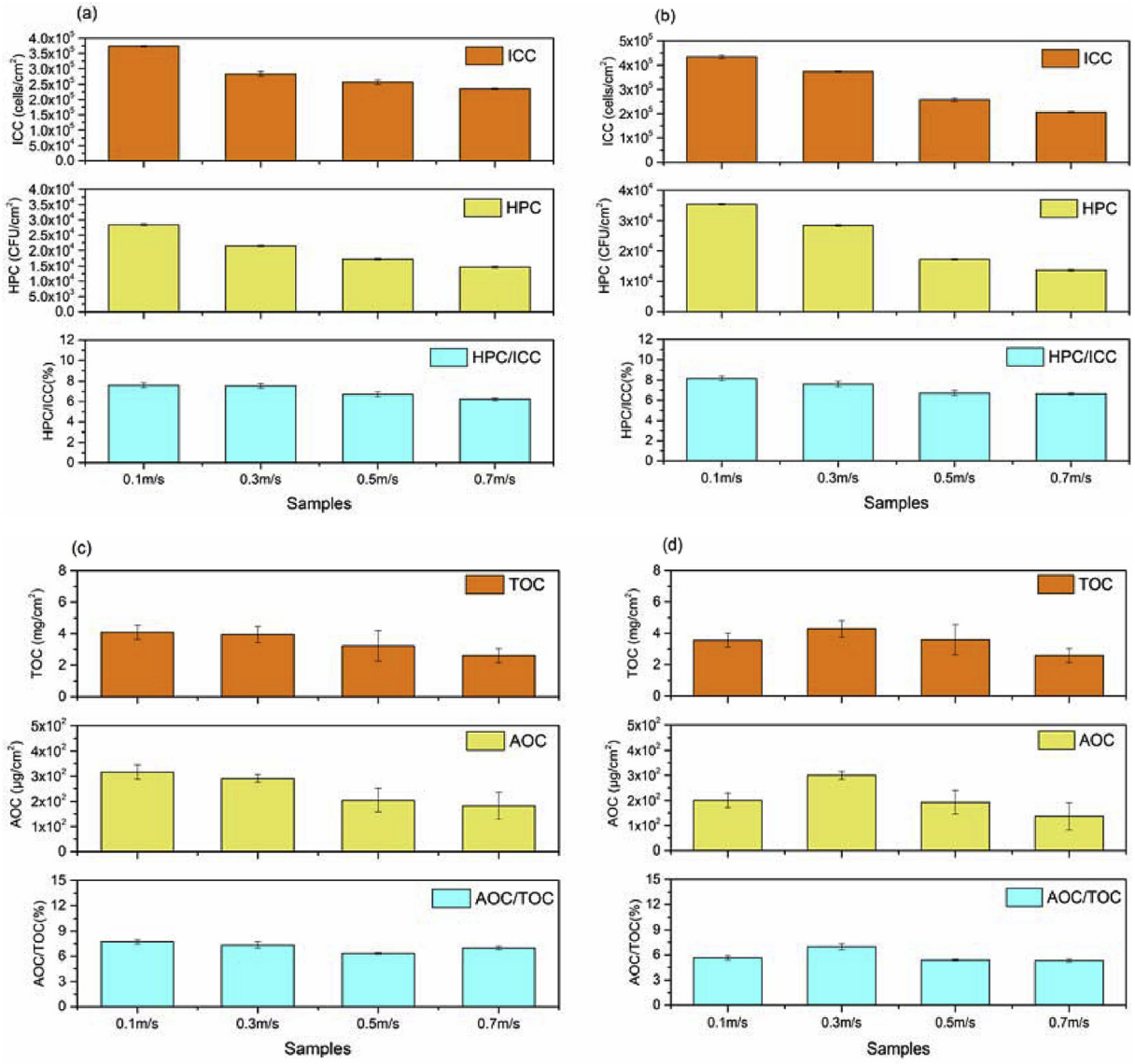

Fig. 4. HPC, ICC, HPC/ICC, TOC, AOC and AOC/TOC in biofilm samples under different flow rate (a and c, chlorinated system, b and d, chloraminated system).

79.81% and 16.11%, respectively. The relative content of these two microorganisms in biofilms and OW was higher than that of IW. A significant increase of these microorganisms in OW indicated that the interaction of biofilms and water had an effect on the microbial community in OW. Comparing the microbial community composition in different flow rate pipelines, the higher content of *Caulobacteraceae* and *Paenibacillus* were found in high flow rate pipelines, it implied that the high flow rate can select the community composition in biofilms and finally affect the water. The comprehensive results indicated that the biofilm interacted with water and affected the water quality of OW.

As shown in Fig. 6, cluster analysis was carried out on the microbial community composition of IW, biofilm and OW samples. The results showed that under chlorine disinfection, the community composition of OW was similar to biofilm samples of the pipeline with a flow rate of 0.1 m/s, while community composition of biofilms in pipeline with flow rate of 0.3 m/s and 0.5 m/s was similar, which suggested differences in biofilms at different flow rates. In the chloramine disinfection system, the microbial community composition was similar in biofilms of pipeline with a flow rate of 0.1 m/s and 0.3 m/s, or 0.5 m/s and 0.7 m/s. The community composition of OW was relatively close to the biofilm samples. The results showed that the biofilm detachment into the water will affect the microbial composition of the OW. In the chlorine disinfection system, the relative contents of *Brevundimonas* and *Brevibacillus*in biofilm samples accounted for 14.07%–79.80% and 3.35%–16.49%, respectively, which were correlated to the relative abundance of *tetB* and *integron* I of different flow rates pipeline. It is speculated that the increase of these genus in biofilms enhances the antibiotic resistance of biofilms. In the chloramine disinfection system, the relative contents of *Brevundimonas*, *Brevibacillus* and *Pseudomonas* in the biofilm accounted for 2.67%–42.69%, 0.69%–11.68% and 0.43%–1.77%, respectively. A positive correlation was found between the relative abundance of these three bacteria and *sulI*, *sulII* in biofilms, indicating that these bacteria were selected in the biofilm. These genera in the OW significantly increased than IW due to the interaction between biofilms and water. Therefore, biofilms in the water supply system can select a variety of ARB depending on their protection against oligotrophic environment, and the detachment of these ARB or ARGs from the biofilm

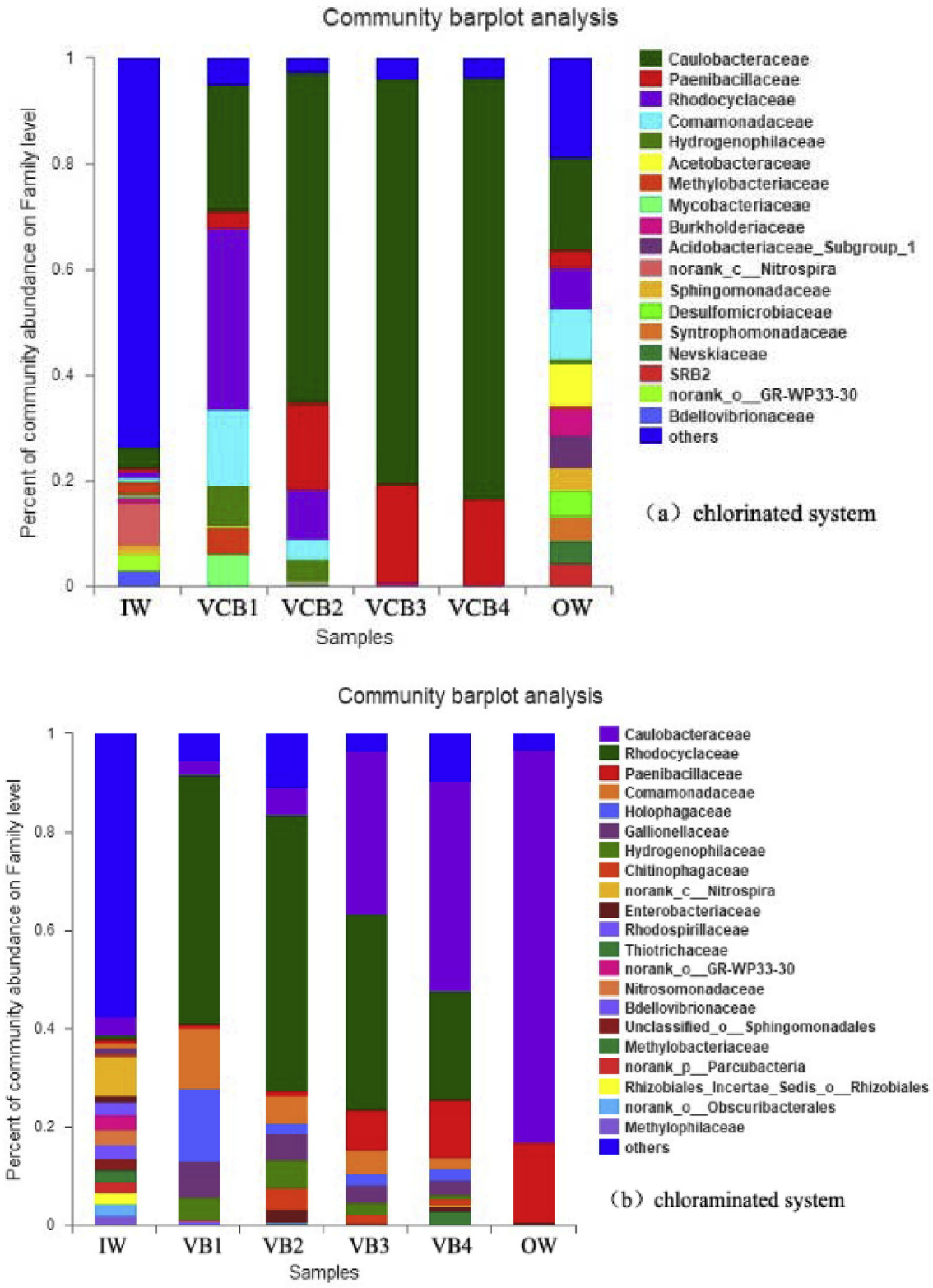

Fig. 5. The community barplot analysis on family level of inlet water, biofilm and mixed outlet water samples under different flow rates in the (a) chlorinated system and (b) chloraminated system, VCB was short for biofilm samples for different flow rate in chlorinated system while VB was short for biofilms in chloraminated system.

increased the resistance of the water.

4. Conclusions

The existence of ARGs in tap water leads to potential risks to human health. A SWSS was designed to study the effects of

different pipe flow rates on the prevalence of antibiotic resistance in water supply systems. The results showed that the biofilms in low flow rate pipelines had high ARB and ARGs concentration, while high flow rate biofilms resulted in low relative abundance of ARB and high relative abundance of ARGs. The high flow rate would lead to a high proportion of non-culturable bacteria in the biofilm, which could lead to an increase of antibiotic resistance in biofilms. High-throughput sequencing pointed out that the high content of *Caulobacteraceae* and *Paenibacillus* were determined in high flow rate pipelines. The results that these two families both had high concentration in biofilms of high flow rate pipeline and OW implies that the high flow rate can select the community composition in biofilms and finally affect the water. Similarity analysis of microbial community compositions of IW, biofilm and OW showed that the composition of microbial community in OW was more similar to that in biofilms than in IW. Genera (*Brevundimonas*, *Brevibacillus* and *Pseudomonas*), which had muti-antibiotic resistance and easy to survive in oligotrophic environment, in biofilms and OW had higher relative abundance than that in IW. Thus, under different flow rate, biofilms in the water supply system can select a variety of ARB depending on their protection against oligotrophic environment, and the detachment of these ARB or ARGs from the biofilm can increased the antibiotic resistance of the water.

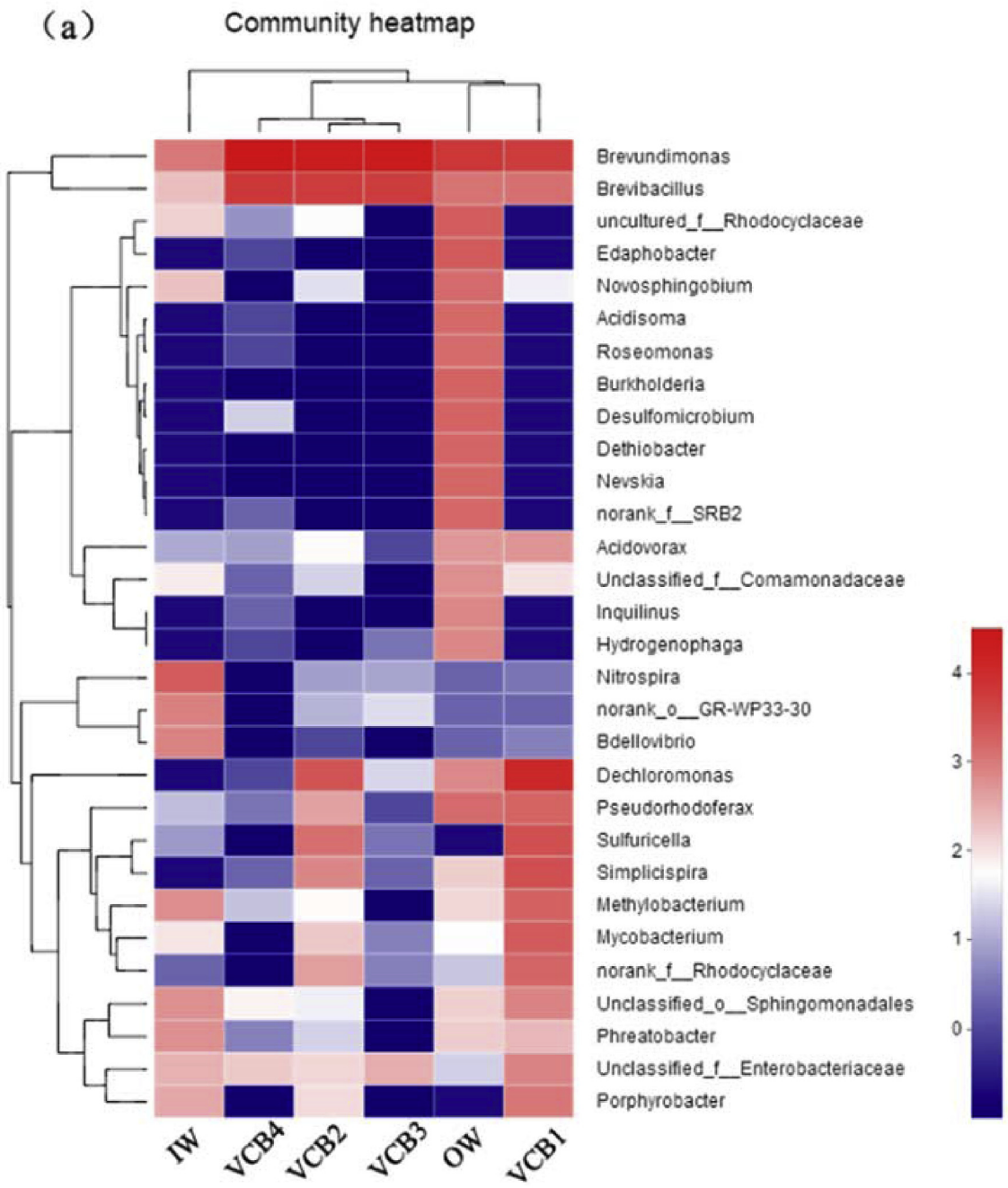

Fig. 6. Heat map showing the most abundant genera in water and biofilm samples and the community cluster analysis of inlet water, biofilm and mixed outlet water samples under different flow rates in the (a) chlorinated system and (b) chloraminated system, VCB was short for biofilm samples of different flow rate pipeline in chlorinated system while VB was short for biofilms in chloraminated system.

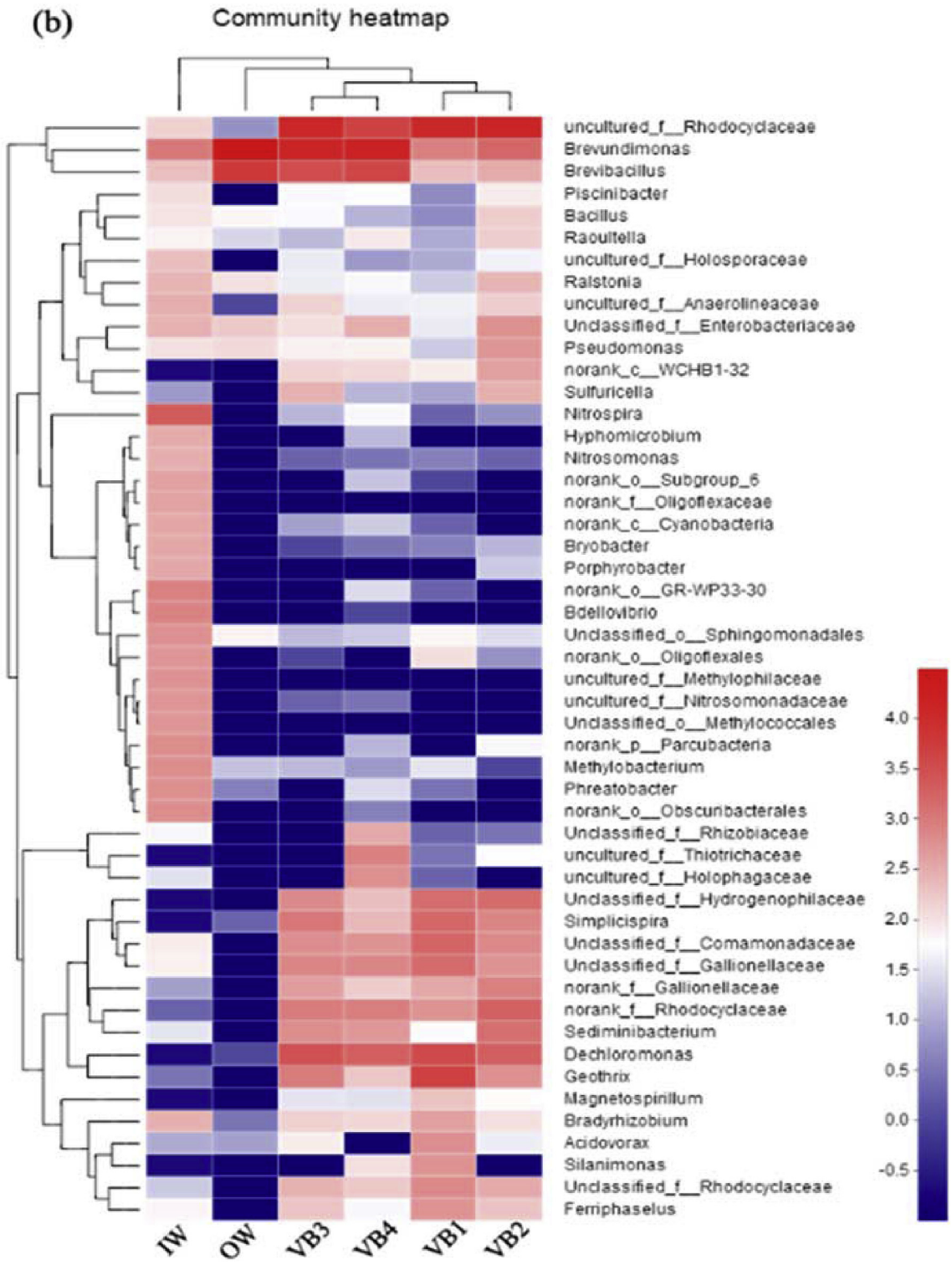

Fig. 6. (*continued*).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.06.157.

References

Balcazar, J.L., Subirats, J., Borrego, C.M., 2015. The role of biofilms as environmental reservoirs of antibiotic resistance. Front. Microbiol. 6, 1216–1227.

Beloin, C., Valle, J., Latour-Lambert, P., Faure, P., Kzreminski, M., Balestrino, D., Haagensen, J.A.J., Molin, S., Prensier, G., Arbeille, B., Ghigo, J.M., 2004. Global impact of mature biofilm lifestyle on Escherichia coli K-12 gene expression. Mol. Microbiol. 51, 659–674.

Beyenal, H., Lewandowski, Z., 2002. Internal and external mass transfer in biofilms grown at various flow velocities. Biotechnol. Prog. 18, 55–61.

Douterelo, I., Fish, K.E., Boxall, J.B., 2018. Succession of bacterial and fungal communities within biofilms of a chlorinated drinking water distribution system. Water Res. 141, 74–85.

Douterelo, I., Husband, S., Boxall, J.B., 2014. The bacteriological composition of biomass recovered by flushing an operational drinking water distribution system. Water Res. 54, 100–114.

Douterelo, I., Husband, S., Loza, V., 2016. Dynamics of biofilm regrowth in drinking water distribution systems. Appl. Environ. Microbiol. 82, 4155–4168.

Douterelo, I., Sharpe, R.L., Boxall, J.B., 2013. Influence of hydraulic regimes on bacterial community structure and composition in an experimental drinking water distribution system. Water Res. 47, 503–516.

Fish, K., Osborn, A.M., Boxall, J.B., 2017. Biofilm structures (EPS and bacterial communities) in drinking water distribution systems are conditioned by hydraulics and influence discolouration. Sci. Total Environ. 593, 571–580.

Fish, K.E., Osborn, A.M., Boxall, J., 2016. Characterising and understanding the impact of microbial biofilms and the extracellular polymeric substance (EPS) matrix in drinking water distribution systems. Environ. Sci. Water Res. Technol. 2, 614–630.

Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., Kjelleberg, S., 2016. Biofilms: An emergent form of bacterial life. Nat. Rev. Microbiol. 14, 563–575.

Guo, X.P., Yang, Y., Lu, D.P., Niu, Z.S., Feng, J.N., Chen, Y.R., Tou, F.Y., Garner, E., Xu, J., Liu, M., Hochella, M.F., 2018. Biofilms as a sink for antibiotic resistance genes (ARGs) in the Yangtze Estuary. Water Res. 129, 277–286.

Hill, D., Rose, B., Pajkos, A., Robinson, M., Bye, P., Bell, S., Elkins, M., Thompson, B., MacLeod, C., Aaron, S.D., Harbour, C., 2005. Antibiotic susceptibilities of Pseudomonas aeruginosa isolates derived from patients with cystic fibrosis under aerobic, anaerobic, and biofilm conditions. J. Clin. Microbiol. 43, 5085–5090.

Jia, S., Shi, P., Hu, Q., Li, B., Zhang, T., Zhang, X.-X., 2015. Bacterial community shift drives antibiotic resistance promotion during drinking water chlorination. Environ. Sci. Technol. 49, 12271–12279.

Jiang, L., Hu, X., Xu, T., Zhang, H., Sheng, D., Yin, D., 2013. Prevalence of antibiotic resistance genes and their relationship with antibiotics in the Huangpu River and the drinking water sources, Shanghai, China. Sci. Total Environ. 458, 267–272.

Li, W., Zhang, J., Wang, F., Qian, L., Zhou, Y., Qi, W., Chen, J., 2018. Effect of disinfectant residual on the interaction between bacterial growth and assimilable organic carbon in a drinking water distribution system. Chemosphere 202, 586–597.

Lin, H., Ye, C., Chen, S., Zhang, S., Yu, X., 2017. Viable but non-culturable E. coli induced by low level chlorination have higher persistence to antibiotics than their culturable counterparts. Environ. Pollut. 230, 242–249.

Liu, S., Gunawan, C., Barraud, N., Rice, S.A., Harry, E.J., Amal, R., 2016. Understanding, monitoring, and controlling biofilm growth in drinking water distribution systems. Environ. Sci. Technol. 50, 8954–8976.

Mathieu, L., Bertrand, I., Abe, Y., Angel, E., Block, J., Skali-Lami, S., Francius, G., 2014. Drinking water biofilm cohesiveness changes under chlorination or hydrodynamic stress. Water Res. 55, 175–184.

Prest, E.I., Hammes, F., Kotzsch, S., van Loosdrecht, M.C.M., Vrouwenvelder, J.S., 2013. Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method. Water Res. 47, 7131–7142.

Proctor, C.R., Gachter, M., Kotzsch, S., Rolli, F., Sigrist, R., Walser, J.-C., Hammes, F., 2016. Biofilms in shower hoses - choice of pipe material influences bacterial growth and communities. Environ. Sci. Water Res. Technol. 2, 670–682.

Rochex, A., Godon, J.-J., Bernet, N., Escudie, R., 2008. Role of shear stress on composition, diversity and dynamics of biofilm bacterial communities. Water Res. 42, 4915–4922.

Sharpe, R., 2012. Laboratory Investigations into Processes Causing Discoloured Potable Water. University of Sheffield.

Simpson, D.R., 2008. Biofilm processes in biologically active carbon water purification. Water Res. 42, 2839–2848.

Stewart, P.S., Franklin, M.J., 2008. Physiological heterogeneity in biofilms. Nat. Rev. Microbiol. 6, 199–210.

Su, H.-C., Liu, Y.-S., Pan, C.-G., Chen, J., He, L.-Y., Ying, G.-G., 2018. Persistence of antibiotic resistance genes and bacterial community changes in drinking water treatment system: From drinking water source to tap water. Sci. Total Environ. 616, 453–461.

Xu, J.W., Li, M.Z., He, Q., Sun, X.F., Zhou, X.R., Su, Z.P., Ai, H.N., 2017a. Effect of flow rate on growth and oxygen consumption of biofilm in gravity sewer. Environ. Sci. Pollut. Control Ser. 24, 427–435.

Xu, Y., Xu, J., Mao, D.Q., Luo, Y., 2017b. Effect of the selective pressure of sub-lethal level of heavy metals on the fate and distribution of ARGs in the catchment scale. Environ. Pollut. 220, 900–908.

Zhang, J.P., Li, W.Y., Chen, J.P., Qi, W.Q., Wang, F., Zhou, Y.Y., 2018. Impact of biofilm formation and detachment on the transmission of bacterial antibiotic resistance in drinking water distribution systems. Chemosphere 203, 368–380.

Zheng, J., Chen, T., Chen, H., 2018. Antibiotic resistome promotion in drinking water during biological activated carbon treatment: Is it influenced by quorum sensing? Sci. Total Environ. 612, 1–8.