Science of the Total Environment 807 (2022) 150896

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effect of disinfectant exposure and starvation treatment on the detachment of simulated drinking water biofilms

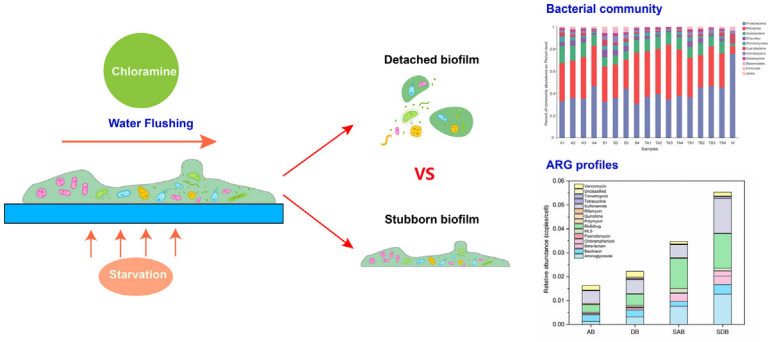
Jiping Chen [a], Weiying Li [a,b,*], Qiaowen Tan [a], Dongfang Sheng [a], Yue Li [a], Sheng Chen [a], Wei Zhou [a]

[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

HIGHLIGHTS

- The rate of biofilm detachment increased with the concentration of chloramine.
- Detached and stubborn biofilms have different bacteria community composition.
- Starvation treatment could enrich the ARGs in drinking water biofilm.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 June 2021
Received in revised form 3 October 2021
Accepted 5 October 2021
Available online 12 October 2021

Editor: Yolanda Picó

Keywords:
Biofilms
Microbial community
ARGs
Detachment
Starvation
Disinfectant

ABSTRACT

Biofilms were one of the main habitats of microbes in the drinking water distribution system. The variation of environmental conditions can lead to the detachment of biofilms and the deterioration of water quality. In this study, the effects of disinfectant exposure and starvation treatment on the detachment of biofilms were investigated. The results showed that detaching rate increased with the concentration of chloramine in the inlet water and 1.0 mg/L of chloramine led to the largest detached biomass. The starvation treatment resulted in less biofilm biomass but the detaching rates of treated biofilms were higher than those without starvation. The 16S rRNA sequencing results showed that detached and stubborn biofilms had a significant difference in microbial diversity and richness. The microbial community composition of the two types of biofilm showed the difference in the abundance of *Nitrospira*, *Bryobacter*, *Hyphomicrobium,* and *Pedomicrobium*. Chloramine exposure did not have a significant impact on the microbial community while the starvation treatment led to a higher abundance of chemolithotrophs bacteria. Metagenomic results indicated that detached biofilms had higher abundances of ARGs and starvation treatment could enrich the ARGs. The results of this research could provide the knowledge of biofilm sloughing and help understand the health risk of antibiotic resistance in drinking water.

1. Introduction

In drinking water distribution systems (DWDSs), biofilms are the predominant contributor of microbial growth, with more than 90% of the total biomass living in these matrix-enclosed microbial colonies (Flemming et al., 2002). Compared with those in the planktonic state, bacteria in biofilms can benefit from the presence of extracellular

* Corresponding author at: College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China.
E-mail address: 123lwyktz@tongji.edu.cn (W. Li).

https://doi.org/10.1016/j.scitotenv.2021.150896
0048-9697/

polymeric substance (EPS) matrix and are more resistant against external stressors, such as disinfectants (Gagnon et al., 2005), shearing force (Niederdorfer et al., 2016), and predators (Matz et al., 2005) in DWDS. Moreover, the high cell density and close distance between bacteria could facilitate horizontal gene transfer (HGT) between biofilm cells (Abe et al., 2020). The presence of biofilms in DWDS could impact the aesthetics of water (Fish et al., 2017), promote the decay of residual disinfectant (Xu et al., 2018), play a role in pipe corrosion (Teng et al., 2008) and harbor pathogenic microorganisms (Wang et al., 2018), posing threat to the safety of tap water in customers'house.

Planktonic bacteria firstly attach to the surface of inner pipes and gradually grow into a more complex multicellular mature biofilm with bacteria differentiation, EPS production, and intercellular communication (Flemming et al., 2016). As biofilms grow in size, the essential sources of energy and nutrients will become less while the waste products and toxins will accumulate. Thus biofilm cells have evolved mechanisms to escape the sessile mode of growth as a means of self-preservation and dissemination to new habitats (McDougald et al., 2011; Petrova and Sauer, 2016). The change of physical or chemical conditions can also trigger the detachment of biofilms. For example, high shear force promotes the sloughing of biofilms in DWDSs (Douterelo et al., 2020; Shen et al., 2015) and flushing is still one of the most common measures for water utilities to remove biofilms and loose deposits from DWDSs. Correlations have been found between daily hydraulic patterns and planktonic cell counts (Sekar et al., 2012). With the detachment of biofilms, the matrix material and bacterial cells will release into the bulk water, leading to aesthetic and pathogenic problems (Zhang et al., 2018). Moreover, biofilms have been also considered as favorable spots for the spread of antibiotic resistance genes (ARGs) and the detachment of biofilm clusters would increase the abundance of ARGs in tap water. Therefore, the impact of biofilm detachment on microbial safety needs equivalent attention with biofilm formation.

Previous studies have focused on the control of biofilm formation in DWDS, such as maintaining high disinfectant residue, reducing assimilable organic carbon (AOC) and changing the pipe material (Jing et al., 2018). Nevertheless, most of DWDSs have been operated for a long time and mature biofilms were observed on the surface of pipe walls. The effects of biofilms detached into the DWDS still need more research. Preceding research has revealed that *Legionella pneumophila* could harbor in drinking water biofilms and disinfectant exposure would impact the viability and infectivity of L. *pneumophila* detached with biofilm clusters (Shen et al., 2017). Nutrient starvation has also been considered as a potential trigger for biofilm detachment (Hunt et al., 2004). However, the impacts of disinfectant and nutrient starvation on the microbial community and ARGs profiles of detached biofilm in DWDSs have not been investigated.

In this study, we monitored the detachment of simulated drinking water biofilms under water flushing. The effect of chloramine concentration and starvation treatment on the detachment of biofilms was explored using flow chambers. Furthermore, 16S rRNA sequencing and metagenomics methods were applied to analyze the microbial community and ARGs composition in both detached and stubborn biofilms. The results of this study explored the microbial characteristic of detached and stubborn biofilm under experimental conditions (disinfection exposure and starvation treatment), which could contribute to further research on the risk assessment and control of biofilm-associated pathogens in DWDS.

2. Methods and materials

2.1. Biofilm preparation and detachment operation

An annual reactor (AR, Model 1320LJ, BioSurface Technologies Corp., USA) was used to stimulate DWDS conditions as previously described (Li et al., 2018a). Twenty Polyvinyl chloride (PVC) slides (BioSurface Technologies Corp., USA) were placed in the slots provided on the wall of the inner cylinder. The rotational speed of the inner cylinder was 50 rpm, which offered a shear stress of 0.25 N/m^2, corresponding to a water velocity of around 0.3 m/s in a 100-mm-diameter pipe. The AR was inoculated with tap water of the laboratory in Shanghai, China, of which the water quality was summarized in Table 1. The AR was operated with a 2 h hydraulic residence time and maintained at 25 °C through a water jacket for 2 years.

After 2 years of growing biofilms with tap water, PVC slides were carefully removed from the AR according to the operation manual. A parallel flow chamber (seen in Fig. S1) was designed to detach biofilms from the slides, with the same tap water as influent water. The biofilm slides were installed upon the groove after several times rinse using sterile phosphate buffer saline (PBS, pH 7.4 at room temperature) to remove suspended bacteria. To avoid the influence of the bacteria in the influent water, filtration with the 0.22 μm polycarbonate membrane and autoclavation were used to remove the indigenous bacteria from tap water. Before the process of the detachment, the residue chlorine was neutralized using 0.1 M sodium thiosulfate. The inlet water with different concentrations of disinfectant was prepared by adding diluted chloramine solution to a final chloramine concentration of 0.2 mg/L, 0.4 mg/L, and 1.0 mg/L, which were common in local DWDS according to previous research (Li et al., 2018b). A chloramine stock solution was freshly prepared by mixing a sodium hypochlorite solution (NaClO) with an ammonium chloride (NH_4Cl) solution at a Cl:N molar ratio of 0.8 at pH 8.4. The prepared monochloramine solution was sat in the dark to react and reach equilibrium for 1 h before usage. Then the tap water containing different concentrations of chloramine was introduced into the flow chamber at a flow velocity of 0.4 m/s (shear stress of 0.54 N/m^2) to detach biofilms. The starvation treatment was conducted by changing the influent water of the AR to 0.9% NaCl solution, which provided an environment without nutrition for biofilms. The starvation process lasted for 48 h. Then slides were removed and inserted into the flow chamber to undergo the same washing process. For all the above conditions, the tap water with different concentrations of chloramine was introduced to the flow chamber for 10 min. The effluent water was collected at 10, 20, 30, 40, 50 and 60 s in the first minute and then at 2, 3, 5, 10 min for further analysis of detached bacteria, of which the volumes were 50, 50, 50, 50, 50, 50, 300, 300, 500, 1500 mL, respectively. The chloramine in the collected samples was immediately quenched using 0.1 M sodium thiosulfate.

2.2. Determination of total detached bacteria

Heterotrophic plate count (HPC) and Intact cells concentration (ICC) of collected samples were determined to explore the detachment of bacteria in biofilms. Water samples were diluted and plated on R2A agar. After being incubated at 22 °C for 7 days, the number of the total colonies was regarded as HPC. ICC analysis was conducted by flow cytometry as previously reported (Zhang et al., 2018). Briefly, 1 mL of a water sample was incubated with 10 μL SYBR green (100 × dilutions of a 10,000 × concentrate) (Life Technologies Ltd., USA) and 10 μL propidium iodide (50 g/mL) (Life Technologies Ltd., USA) and stained for 15 min at room temperature. Subsequently, the membrane-intact and membrane-disrupted cells were counted by FAC SCalibur flow

Table 1
The physiochemical parameters of feed water of the AR.

Water quality parameters	Tap water
Turbidity (NTU)	0.638 ± 0.247
Total chlorine (mg/L)	0.29 ± 0.07
Temperature (°C)	21.07 ± 1.2
pH	7.36 ± 0.34
Conductivity (μS/cm)	164.5 ± 17.2
DOC (mg/L)	1.848 ± 0.255
UV_{254} (cm^{-1})	0.0237 ± 0.0076

cytometer (BD Biosciences, USA). Water samples were collected in triplicate for the determination of HPC and ICC.

2.3. Biofilm sample collection and DNA extraction

After the flushing process, the residue biofilms on the slides were considered as stubborn biofilms. They were removed from the slides using the aseptic scraper and collected in sterile PBS. Approximately 50 mL of PBS containing turbid biofilms were obtained. The bacteria and clusters in effluent water were considered as detached biofilm as no bacteria existed in influent water. About 4 L water was collected in a sterile glass bottle from the tap of the laboratory. Then both biofilm and water samples were filtered through 0.22 μm pore-size cellulose ester filters (Millipore, Billerica, MA). DNA was extracted using the DNeasy® PowerWater® Kit (QIAGEN, USA) according to manufacturer protocol and was stored at −80 °C until further processing. The quantification and purification of DNA were determined by fluorometry using NANODROP (DS-11, DeNovix, USA).

2.4. 16S rRNA gene sequencing and analyses

Bacterial 16S rRNA genes were amplified with barcoded primer 338F/806R (338F: ACTCCTACGGGAGGCAGCAG, 806R: GGACTACHVGGGTWTCTAAT). PCR reactions were performed in triplicate as previously reported (Zhang et al., 2018). PCR products were pooled after PCR amplification and purified using the AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, CA, USA), and then quantified by QuantiFluor™-ST (Promega, USA). Finally, the purified amplicons were pooled in equimolar proportions, and then were paired-end sequenced (2 × 250) by Majorbio Co., Ltd. in Shanghai using the MiSeq PE300 platform(Illumina Inc., San Diego, CA, USA). Raw sequences were de-multiplexed and quality filtered by USEARCH 7.1 (http://drive5.com/uparse/) (Caporaso et al., 2012), in which the sequence containing >3 consecutive bases or obtaining a quality score < 20 were removed. And sequences with chimera were also ruled out. Then the optimized sequences were clustered into operational taxonomic units (OTUs) at a level of 97% sequence identity using UPARSE 7.1. Subsequently, effective sequences were aligned based on the SILVA database (http://www.arb-silva.de) using mothur and then were identified down to different levels using Ribosomal Database Project (RDP, http://rdp.cme.msu.edu/) Bayesian classifier at 70% threshold. The Illumina sequencing reads of all samples have been deposited at the NBCI Sequence Read Archive under accession number SRR 1267765–83.

2.5. Metagenomic sequencing and ARG analysis

Two groups of biofilm samples, namely the stubborn and detached biofilms with 0.2 mg/L chloramine exposure, and the ones after starvation treatment with the same condition were used for metagenomic sequencing (Fig. 1). DNA extracts were fragmented to an average size of about 400 bp using Covaris M220 (Gene Company Limited, China) for paired-end library construction. Paired-end sequencing was performed on Illumina NovaSeq (Illumina Inc., San Diego, CA, USA) by Majorbio (Shanghai, China). The size of the raw dataset for each sample was 11.63–13.61 Gb with an average length of 150 bp. The low-quality reads and adapters were filtered by fastp (https://github.com/OpenGene/fastp, version 0.20.0).

The generated clean reads were searched for ARGs by an ARG analysis pipeline (ARG-OAP, https://github.com/biofuture/Ublastx_stageone, version 2.0). The prescreened possible ARG sequences via UBLAST were aligned against Structured Antibiotic Resistance Genes (SARG) database using BLAST with the recommended parameters (e-value $\leq 10^{-5}$, sequencing identity ≥90%, and alignment length ≥ 25 amino acids) for ARG annotation and classification (Ma et al., 2017). ARG types and subtypes were identified by a package of customized scripts in ARGs-OAP, with "copies of ARGs per cell (capc)" as the unit of their relative abundance following previous studies (Jia et al., 2020; Yin et al., 2018). All the metagenomic sequencing data are available at NCBI Sequence Read Archive database with accession number SRR 12678982–85.

2.6. Statistical analyses

Non-metric multidimensional scaling (NMDS) was performed to evaluate the difference of microbial community among the samples based on Bray-Curtis distance. Wilcoxon rank-sum test was carried to assess the difference between water and biofilm samples at 95% confidence intervals. Bivariate correlation (2-tailed) analysis using Spearman methods was performed to explain the correlation between ARGs and microbial community composition. All statistical analyses were performed on SPSS 23.0. The p-value <0.05 was statistically significant, and $p < 0.01$ meant that the difference was more significant in statistics.

3. Results and discussion

3.1. Biofilm detachment kinetics under different disinfectant exposure

After being cultivated in AR for 2 years, PVC slides were moved to the flow chamber and the flushing process was conducted to detach mature biofilms. The concentration of released bacteria from the biofilm under different chloramine exposure was plotted as a function of release time (Fig. S2, Fig. 2). The proportion was the total detached bacteria in the experiment divided by the accumulative detached bacteria at the time (10 s, 20 s, 30 s, 40 s, 50 s, 1 min, 2 min, 3 min, 5 min, 10 min). The majority of bacteria was observed to be detached in the first minute, with a percentage of 61.1% ~ 94.6% for HPC results and 39.2% ~ 76.2% for ICC results in the total detached bacteria. This indicated that the sloughing biomass owing to flow shearing force was mainly attributed to the

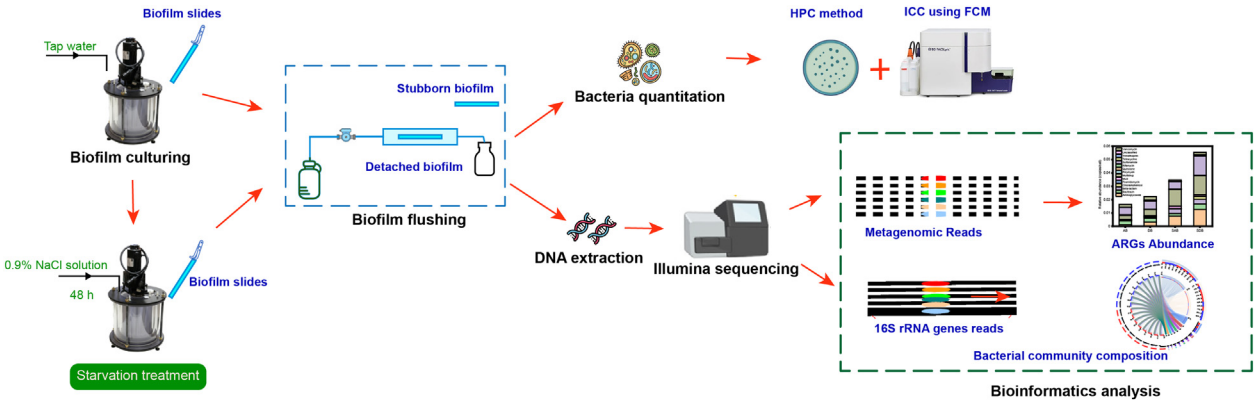


Fig. 1. The experimental procedure used for ARGs and microbial community analysis in this study.

first several minutes. A former study reported that the proportion of detached bacteria in the first minute was more than 80% using flow flushing (Shen et al., 2017). In operational drink water systems, the high-velocity flow was often utilized to remove the organic and inorganic material from pipe walls (mainly biofilms), and turbidity fluctuation was observed in short intervals after the flushing process (Douterelo et al., 2020).

The HPC results of detached bacteria showed that the detaching rates of bacteria were increased with the chloramine concentration in inlet water increasing (Fig. 2, Fig. S2). When the chloramine concentration was 1.0 mg/L, the proportion of detached bacteria in the first minute were more than 90% regardless of the starvation treatment. The chloramine could penetrate biofilms and react with the EPS and bacteria, leading to more biomass detachment. However, the ICC results did not show a significant difference in the proportion of detached bacteria in the first minute among the different chloramine concentrations. This was probably attributed to the discrepancy between the two methods. The HPC methods can only detect the culturable bacteria, while the chloramine would induce bacteria into the state of viable but unculturable (VBNC) (Chen et al., 2020b). Furthermore, the effect of chloramine on the detachment of biofilms was observed to decrease after the starvation treatment.

Compared with the normal group (no starvation), the biofilms with starvation were observed to detach a higher proportion of bacteria in the first minute. Starvation has been considered as one of the main factors that would trigger the dispersal of biofilms (McDougald et al., 2011). Previous research has reported that *Pseudomonas fluorescens* biofilms detached with nutrients decrease (Delaquis et al., 1989), and biofilms of *Pseudomonas putida* rapidly dispersed when suffering from carbon starvation (Gjermansen et al., 2010). The starvation could change the structure and composition of biofilms and make them more susceptible to water flow flushing. The total detached biomass after the starvation was significantly lower than that without starvation since the original biomass of biofilms was reduced owing to the limited nutrients. However, the proportion of detached bacteria in the first minute after the starvation was higher than that without starvation, which indicated that biofilms with starvation tended to release more bacteria when undergone water flushing. Former researchers have described the carbon starvation of *P. putida* led to a decrease in levels of the adhesin LapA, which was a major component of the EPS of biofilms (Gjermansen et al., 2010). The change of drinking water source and the promotion of treatment process might result in the lower organic matter in finished water and therefore lead to the detachment of pipe wall biofilms. The further correlation between the nutrients in bulk water and the detachment of biofilms needs more research.

3.2. The microbial community of stubborn and detached biofilms

To compare the microbial diversity of detached and stubborn biofilms, Shannon index, Simpson index, and Chao index were calculated in Fig. S3. According to the results, detached biofilms had a higher Shannon index (Student's *t*-test, $p < 0.01$) and Chao index (Student's t-test, $p < 0.05$) than stubborn biofilms, as well as a lower Simpson index (Student's t-test, $p < 0.01$), which indicated detached biofilms had higher bacteria community diversity than stubborn ones. Bacteria in DWDS live in different niches including bulk water, biofilms on the pipe wall, loose deposits as well as suspended particles (Liu et al., 2014; Liu et al., 2013). Former studies have reported that the microbial community diversity and richness in the bulk water and loose deposits were higher than those in the biofilm (Ling et al., 2016; Vavourakis et al., 2020). The detached biofilms mostly came from the superficial part of biofilms which exchanged more frequently with bulk water and were comprised of more complicated bacterial species. In comparison, stubborn ones were more adaptive to the sessile life and different from the planktonic or flexible bacteria which can change the life mode according to the environmental condition (Yan et al., 2017). The stubborn biofilms only had 28 unique OTUs while the number in detached ones was 179 (Fig. S4). However, the shared OTUs made up the majority of the total

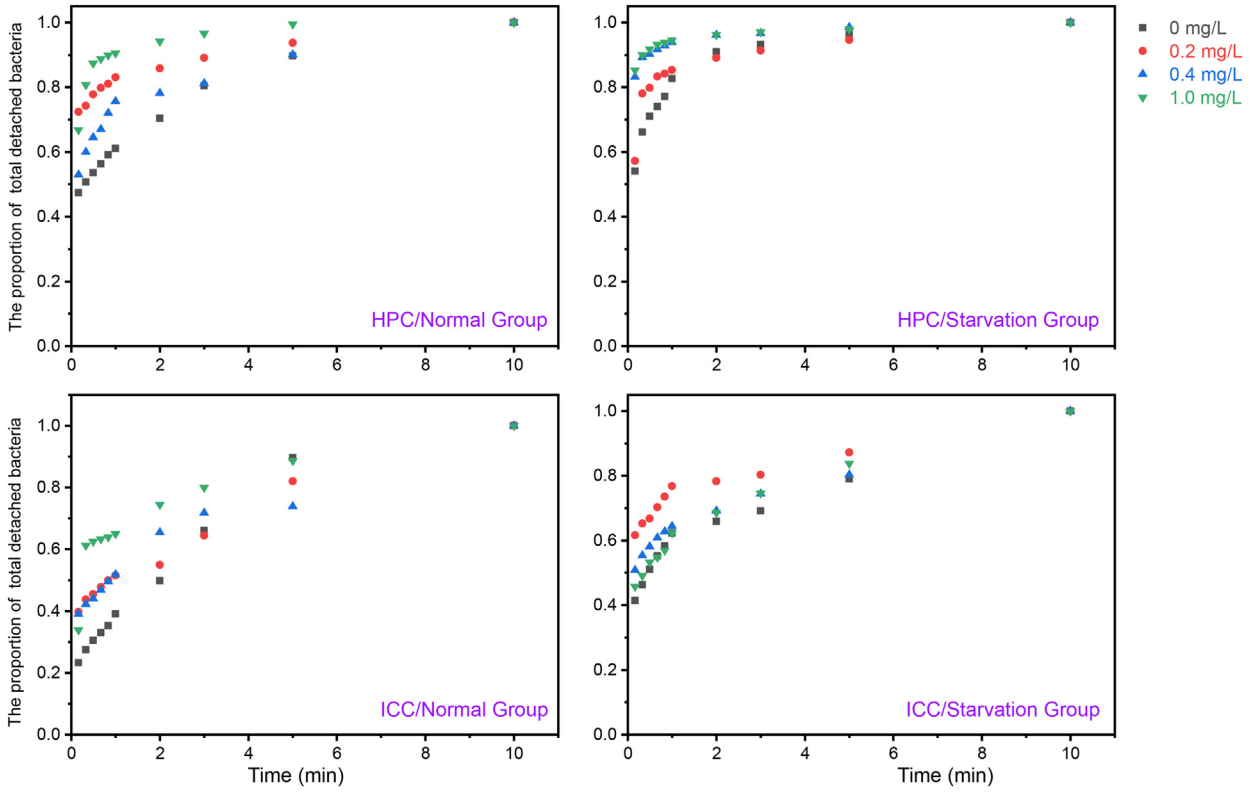


Fig. 2. The variation pattern of the proportion of detached bacteria from biofilms with time. HPC and ICC methods were used to determine the bacteria numbers. (a) the HPC results in the normal group; (b) the HPC results in the starvation group; (c) the ICC results in the normal group; (d) the ICC results in the starvation group.

OTUs in both stubborn and detached biofilms, with a proportion of 96.3% and 80.4%, respectively. According to the theory of fitness cost, when the living environment is stable, bacteria specializing in biofilm formation and planktonic growth have the advantage over flexible ones that can transition between the biofilm and planktonic mode (Madsen et al., 2015; Nadell and Bassler, 2011). In drinking water biofilms, these biofilm specialists seem to occupy a limited proportion of the total bacteria community and most of the bacteria live in the biofilm were able to leave from biofilms to become suspended bacteria.

The microbial community composition of the detached and stubborn biofilms at phylum and genus level are presented in Fig. 3 and Fig. S5, respectively. Proteobacteria was the most abundant bacterial phylum in all biofilm samples, with abundances of 30.8% ~ 46.5%, which was also the main bacteria phylum in tap water (75.5%). Nitrospirae and Acidobacteria were abundant in biofilm samples, with a proportion of 26.1% ~ 49.1% and 7.8% ~ 15.1%, respectively. Chloroflexi, Planctomycetes, Cyanobacteria, Actinobacteria were also found in biofilms samples, with abundances of more than 1%. In comparison, the abundance of Nitrospirae in tap water was 6.75%. *Nitrospira* from the phylum of Nitrospirae is Nitrite-oxidizing bacteria and could utilize nitrite to synthesize organic matter. *Nitrospira* are non-planktonic organisms that usually reside as aggregates in biofilms (Fujitani et al., 2014) and many researchers have reported the existence of *Nitrospira* in drinking water biofilms as well as loose deposits (Vavourakis et al., 2020; Waak et al., 2019). Stubborn biofilms had a higher abundance of *Nitrospira* than the detached biofilms (Wilcoxon rank-sum test, $p < 0.05$, Fig. S6), which indicated the genus of *Nitrospira* was resistant to the shear force and preferred the settled lifestyles. *Bryobacter*, *Hyphomicrobium,* and *Pedomicrobium*, which were considered as chemolithotrophs, were also found to have a higher abundance in stubborn biofilms than in detached ones. Former studies have demonstrated that chemolithotrophs could benefit from the gradational interfaces between electron acceptors and reduced inorganic compounds and form microbial mats at the sediment-water interface in water bodies (Fossing et al., 1995; Schulz et al., 1999). These chemolithotrophs were more resistant to the flushing process and could produce organic nutrients for other biofilm-related bacteria, which deserves more attention to control biofilm growth in DWDS.

3.3. The impact of disinfectant and starvation on the microbial community

To compare the microbial community composition of detached biofilms under different concentrations of chloramine exposure, the Kruskal-Wallis rank sum test was conducted (Fig. S7). Although chloramine exposure accelerated the detachment of bacteria in biofilms (Fig. 2, Fig. S2), the bacterial relative abundance of the accumulative detached biofilms did not have a significant difference ($p > 0.05$). Since the main driving force to detach biofilms was the water shear force and the contact time of the disinfectant and biofilms was too short (10 min), chloramine did not significantly change the biofilm structure. Former studies have reported the limited efficiency of disinfectants on the removal of single and dual-species biofilms (Gomes et al., 2018; Zhang et al., 2018). Bacteria living in biofilms are more resistant to disinfectant exposure and short-time chloramine exposure did not play an important role in the detachment of biofilms clusters. Therefore, water flow with different chloramine concentrations did not change the microbial community composition of detached biofilms.

The microbial community compositions before and after the starvation are also shown in Fig. 3 and Fig. S5. Stubborn biofilms between these with and without starvation had a significant difference in the abundance of Nitrospira, Deltaproteobacteria, Blastocatellia_Subgroup_4 and Bacteroidia (Wilcoxon rank-sum test, $p < 0.05$). However, the detached biofilms only had a significant difference in the abundance of Deltaproteobacteria and Blastocxatellia_Subgruop_4. The nutrients condition has been correlated with the formation and development of biofilms and starvation was reported to induce dispersion. A previous study has reported that *Nitrospira* did not form biofilm alone but promoted the development of biofilms in oligotrophic conditions through nitrification (Keshvardoust et al., 2019). The starvation conditions of biofilms lead to a higher abundance of Nitrospira and these bacteria were more resistant to water flushing.

3.4. The composition of ARGs in detached and stubborn biofilms

Metagenomic analysis revealed that a total of 96 ARG subtypes belonging to 15 ARG types were identified in at least one of the 4 biofilm samples. Multidrug, Sulfonamide, Aminoglycoside, Bacitracin, and Beta-lactam were the dominant types of ARGs in biofilms, with an

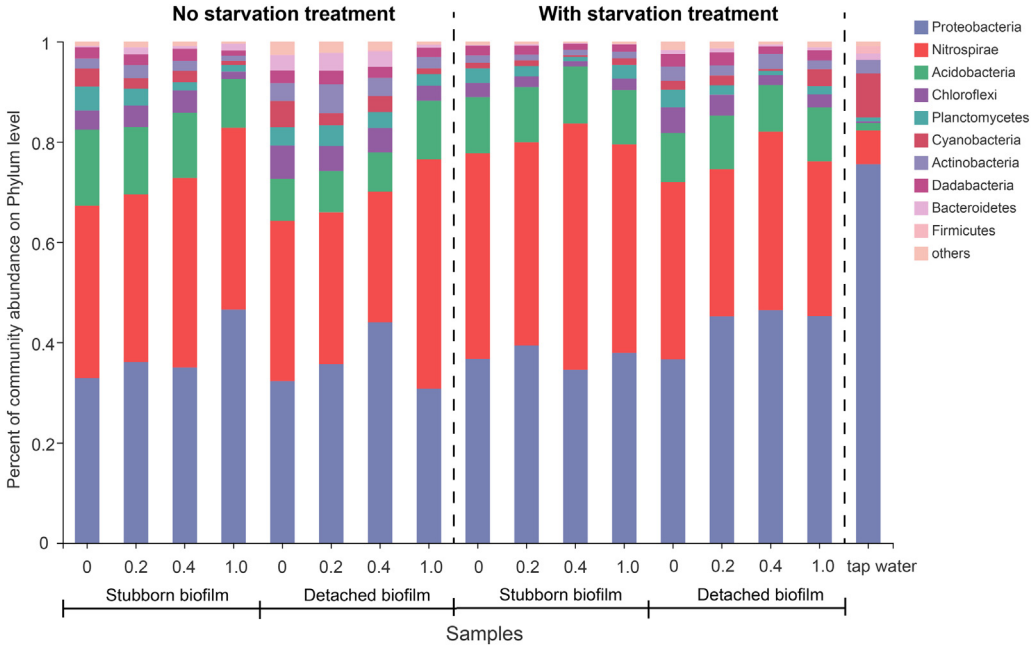


Fig. 3. The microbial community composition at phyla level of different biofilm samples as well as bulk water sample. The numbers on the x axis represent different chloramine concentrations used for flushing.

average proportion of 78.7% ~ 89.2% in the total ARG abundance (Fig. 4). A previous study analyzed the biofilms of the reclaimed water distribution system and reported similar results of ARG abundance (Zhang et al., 2020). Among the 96 detected subtypes, the top 40 most abundant ARG subtypes are shown in Fig. 4. *Sul*1 was the most abundant subtype in all samples, ranging from 14.9% to 23.8% of total ARG abundance. Former studies using marker-gene-based methods have also reported a high abundance of *sul*1 in drinking water as well as biofilms (Chen et al., 2020a; Zhang et al., 2019). Bacitracin resistance gene *bac*A also had high relative abundance in biofilms, followed by aminoglycoside resistance gene aph(3″)-I and aph(6)-I. Multidrug resistance genes including 37 subtypes were detected in biofilm samples, of which *mex*T, *mex*F, and the gene encoding multidrug transporters had high relative abundance.

The total abundance of ARGs in stubborn biofilms (0.016 and 0.0347 capc) was lower than that in detached biofilms (0.022 and 0.055 capc). The ARG subtypes including *sul*1, *bac*A, aph(3″)-I, aph(6)-I, and *mex*F also showed similar tendencies with the total abundance of ARGs. As regards the effect of starvation, for both stubborn and detached biofilms, the total abundance of ARGs increased after the starvation (Fig. 5). Most ARG subtypes also showed higher abundance in starved biofilms, except for the vancomycin-resistant gene *van*R, which was observed to be more abundant in stubborn biofilms before starvation. The enrichment of ARGs in detached biofilms and starved ones indicated that the detachment of biofilms in DWDS had higher risks and need further research.

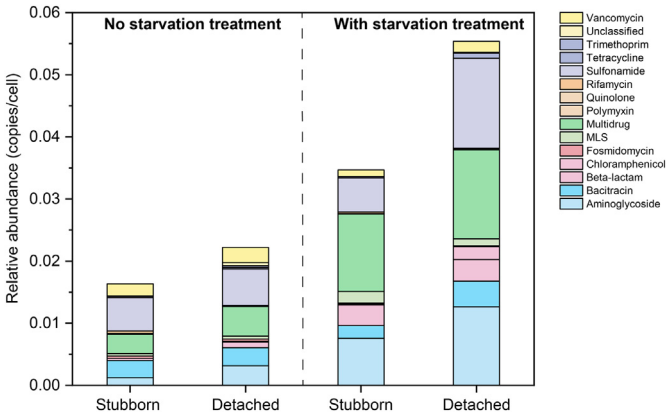


Fig. 5. Relative abundance of different ARG types in biofilms.

4. Conclusions

- The chloramine concentration impacted the detaching rate of biofilms, while the starvation treatment reduced the impact of chloramine, with a smaller proportion of detached bacteria in the first minute. Starvation treatment led to a higher detaching rate of bacteria in biofilms.

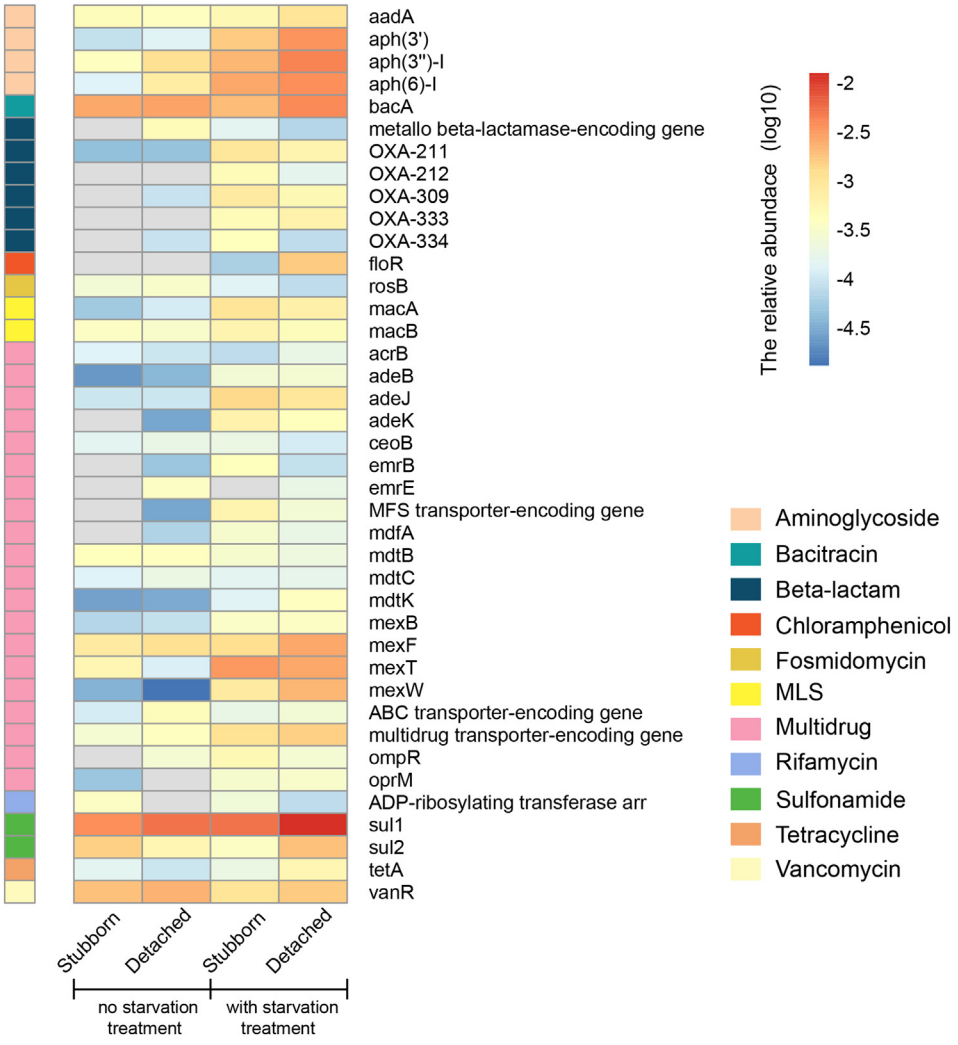


Fig. 4. The relative abundance of ARGs in stubborn and detached biofilms.

- The detached biofilms had higher bacteria diversity and richness compared with stubborn biofilms. The abundances of chemolithotrophs bacteria including *Nitrospira*, *Bryobacter*, *Hyphomicrobium,* and *Pedomicrobium* in stubborn biofilms were higher than those in the detached biofilms.
- Chloramine concentration did not change the microbial community compositions of the detached biofilms. Stubborn biofilms between these with starvation and without starvation had a significant difference in the abundance of Nitrospira, Deltaproteobacteria, Blastocatellia_Subgroup_4, and Bacteroidia.
- The total abundance of ARGs in stubborn biofilms was lower than that in detached biofilms and the total abundances of ARGs were observed to increase after the starvation.

CRediT authorship contribution statement

Jiping Chen: Conceptualization, Investigation, Writing – original draft, Visualization. **Weiying Li:** Writing – review & editing, Supervision, Funding acquisition. **Qiaowen Tan:** Software. **Dongfang Sheng:** Investigation. **Yue Li:** Investigation. **Sheng Chen:** Methodology, Writing – review & editing. **Wei Zhou:** Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare no competing financial interests related to the publication of this study.

Acknowledgments

This project was supported by the National Natural Science Foundation of China (51979194) and the China National Critical Project for Science and Technology on Water Pollution Prevention and Control (Project NO. 2018ZX07110-008).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.150896.

References

Abe, K., Nomura, N., Suzuki, S., 2020. Biofilms: hot spots of horizontal gene transfer (HGT) in aquatic environments, with a focus on a new HGT mechanism. FEMS Microbiol. Ecol. 96.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., et al., 2012. Ultra-high-throughput microbial community analysis on the illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624.

Chen, J., Li, W., Zhang, J., Qi, W., Li, Y., Chen, S., et al., 2020a. Prevalence of antibiotic resistance genes in drinking water and biofilms: the correlation with the microbial community and opportunistic pathogens. Chemosphere 259, 127483.

Chen, S., Zeng, J., Wang, Y., Ye, C., Zhu, S., Feng, L., et al., 2020b. Modelling the effect of chlorination/chloramination on induction of viable but non-culturable (VBNC) Escherichia coli. Environ. Technol. 41, 3443–3455.

Delaquis, P.J., Caldwell, D.E., Lawrence, J.R., McCurdy, A.R., 1989. Detachment of Pseudomonas fluorescens from biofilms on glass surfaces in response to nutrient stress. Microb. Ecol. 18, 199–210.

Douterelo, I., Dutilh, B.E., Arkhipova, K., Calero, C., Husband, S., 2020. Microbial diversity, ecological networks and functional traits associated to materials used in drinking water distribution systems. Water Res. 173, 115586.

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. 593–594, 571–580.

Flemming, H.C., Percival, S.L., Walker, J.T., 2002. Contamination potential of biofilms in water distribution systems. Water Sci. Technol. Water Supply 2, 271–280.

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.

Fossing, H., Gallardo, V.A., Jørgensen, B.B., Hüttel, M., Nielsen, L.P., Schulz, H., et al., 1995. Concentration and transport of nitrate by the mat-forming Sulphur bacterium thioploca. Nature 374, 713–715.

Fujitani, H., Ushiki, N., Tsuneda, S., Aoi, Y., 2014. Isolation of sublineage I nitrospira by a novel cultivation strategy. Environ. Microbiol. 16, 3030–3040.

Gagnon, G.A., Rand, J.L., O'Leary, K.C., Rygel, A.C., Chauret, C., Andrews, R.C., 2005. Disinfectant efficacy of chlorite and chlorine dioxide in drinking water biofilms. Water Res. 39, 1809–1817.

Gjermansen, M., Nilsson, M., Yang, L., Tolker-Nielsen, T., 2010. Characterization of starvation-induced dispersion in pseudomonas putida biofilms: genetic elements and molecular mechanisms. Mol. Microbiol. 75, 815–826.

Gomes, I.B., Lemos, M., Mathieu, L., Simoes, M., Simoes, L.C., 2018. The action of chemical and mechanical stresses on single and dual species biofilm removal of drinking water bacteria. Sci. Total Environ. 631–632, 987–993.

Hunt, S.M., Werner, E.M., Huang, B., Hamilton, M.A., Stewart, P.S., 2004. Hypothesis for the role of nutrient starvation in biofilm detachment. Appl. Environ. Microbiol. 70, 7418–7425.

Jia, S., Bian, K., Shi, P., Ye, L., Liu, C.H., 2020. Metagenomic profiling of antibiotic resistance genes and their associations with bacterial community during multiple disinfection regimes in a full-scale drinking water treatment plant. Water Res. 176, 115721.

Jing, H., Sahle-Demessie, E., Sorial, G.A., 2018. Inhibition of biofilm growth on polymer-MWCNTs composites and metal surfaces. Sci. Total Environ. 633, 167–178.

Keshvardoust, P., Huron, V.A.A., Clemson, M., Constancias, F., Barraud, N., Rice, S.A., 2019. Biofilm formation inhibition and dispersal of multi-species communities containing ammonia-oxidising bacteria. NPJ Biofilms Microbiomes 5, 22.

Li, H., Li, S., Tang, W., Yang, Y., Zhao, J., Xia, S., et al., 2018b. Influence of secondary water supply systems on microbial community structure and opportunistic pathogen gene markers. Water Res. 136, 160–168.

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

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

Liu, G., Verberk, J.Q., Van Dijk, J.C., 2013. Bacteriology of drinking water distribution systems: an integral and multidimensional review. Appl. Microbiol. Biotechnol. 97, 9265–9276.

Liu, G., Bakker, G.L., Li, S., Vreeburg, J.H., Verberk, J.Q., Medema, G.J., et al., 2014. Pyrosequencing reveals bacterial communities in unchlorinated drinking water distribution system: an integral study of bulk water, suspended solids, loose deposits, and pipe wall biofilm. Environ. Sci. Technol. 48, 5467–5476.

Ma, L., Li, B., Jiang, X.T., Wang, Y.L., Xia, Y., Li, A.D., et al., 2017. Catalogue of antibiotic resistome and host-tracking in drinking water deciphered by a large scale survey. Microbiome 5, 154.

Madsen, J.S., Lin, Y.C., Squyres, G.R., Price-Whelan, A., de Santiago, Torio A., Song, A., et al., 2015. Facultative control of matrix production optimizes competitive fitness in Pseudomonas aeruginosa PA14 biofilm models. Appl. Environ. Microbiol. 81, 8414–8426.

Matz, C., McDougald, D., Moreno, A.M., Yung, P.Y., Yildiz, F.H., Kjelleberg, S., 2005. Biofilm formation and phenotypic variation enhance predation-driven persistence of vibrio cholerae. Proc. Natl. Acad. Sci. U. S. A. 102, 16819–16824.

McDougald, D., Rice, S.A., Barraud, N., Steinberg, P.D., Kjelleberg, S., 2011. Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. Nat. Rev. Microbiol. 10, 39–50.

Nadell, C.D., Bassler, B.L., 2011. A fitness trade-off between local competition and dispersal in vibrio cholerae biofilms. Proc. Natl. Acad. Sci. U. S. A. 108, 14181–14185.

Niederdorfer, R., Peter, H., Battin, T.J., 2016. Attached biofilms and suspended aggregates are distinct microbial lifestyles emanating from differing hydraulics. Nat. Microbiol. 1, 16178.

Petrova, O.E., Sauer, K., 2016. Escaping the biofilm in more than one way: desorption, detachment or dispersion. Curr. Opin. Microbiol. 30, 67–78.

Schulz, H.N., Brinkhoff, T., Ferdelman, T.G., Marine, M.H., Teske, A., Jorgensen, B.B., 1999. Dense populations of a giant sulfur bacterium in namibian shelf sediments. Science 284, 493–495.

Sekar, R., Deines, P., Machell, J., Osborn, A.M., Biggs, C.A., Boxall, J.B., 2012. Bacterial water quality and network hydraulic characteristics: a field study of a small, looped water distribution system using culture-independent molecular methods. J. Appl. Microbiol. 112, 1220–1234.

Shen, Y., Monroy, G.L., Derlon, N., Janjaroen, D., Huang, C., Morgenroth, E., et al., 2015. Role of biofilm roughness and hydrodynamic conditions in legionella pneumophila adhesion to and detachment from simulated drinking water biofilms. Environ. Sci. Technol. 49, 4274–4282.

Shen, Y., Huang, C., Lin, J., Wu, W., Ashbolt, N.J., Liu, W.T., et al., 2017. Effect of disinfectant exposure on legionella pneumophila associated with simulated drinking water biofilms: release, inactivation, and infectivity. Environ. Sci. Technol. 51, 2087–2095.

Teng, F., Guan, Y.T., Zhu, W.P., 2008. Effect of biofilm on cast iron pipe corrosion in drinking water distribution system: corrosion scales characterization and microbial community structure investigation. Corros. Sci. 50, 2816–2823.

Vavourakis, C.D., Heijnen, L., Peters, M., Marang, L., Ketelaars, H.A.M., Hijnen, W.A.M., 2020. Spatial and temporal dynamics in attached and suspended bacterial communities in three drinking water distribution systems with variable biological stability. Environ. Sci. Technol. 54, 14535–14546.

Waak, M.B., Hozalski, R.M., Halle, C., LaPara, T.M., 2019. Comparison of the microbiomes of two drinking water distribution systems-with and without residual chloramine disinfection. Microbiome 7, 87.

Wang, H., Shen, Y., Hu, C., Xing, X., Zhao, D., 2018. Sulfadiazine/ciprofloxacin promote opportunistic pathogens occurrence in bulk water of drinking water distribution systems. Environ. Pollut. 234, 71–78.

Xu, J., Huang, C., Shi, X., Dong, S., Yuan, B., Nguyen, T.H., 2018. Role of drinking water biofilms on residual chlorine decay and trihalomethane formation: an experimental and modeling study. Sci. Total Environ. 642, 516–525.
Yan, J., Nadell, C.D., Bassler, B.L., 2017. Environmental fluctuation governs selection for plasticity in biofilm production. ISME J. 11, 1569–1577.
Yin, X., Jiang, X.T., Chai, B., Li, L., Yang, Y., Cole, J.R., et al., 2018. ARGs-OAP v2.0 with an expanded SARG database and hidden markov models for enhancement characterization and quantification of antibiotic resistance genes in environmental metagenomes. Bioinformatics 34, 2263–2270.
Zhang, J., Li, W., Chen, J., Qi, W., Wang, F., Zhou, Y., 2018. Impact of biofilm formation and detachment on the transmission of bacterial antibiotic resistance in drinking water distribution systems. Chemosphere 203, 368–380.
Zhang, J., Li, W., Chen, J., Wang, F., Qi, W., Li, Y., 2019. Impact of disinfectant on bacterial antibiotic resistance transfer between biofilm and tap water in a simulated distribution network. Environ. Pollut. 246, 131–140.
Zhang, G., Guan, Y., Zhao, R., Feng, J., Huang, J., Ma, L., et al., 2020. Metagenomic and network analyses decipher profiles and co-occurrence patterns of antibiotic resistome and bacterial taxa in the reclaimed wastewater distribution system. J. Hazard. Mater. 400, 123170.