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Secondary disinfection ensures biosafety of domestic hot water and its impact on biofilm bacterial community

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ABSTRACT

To ensure the biosafety of the domestic hot water system (DHWS) in the case of residual chlorine exhaustion and biofilm exfoliation, the secondary disinfection effects of chlorine alone or in combination with ultraviolet (UV) on microorganisms and its impact on biofilm bacterial community were investigated. A biofilm annular reactor was used to simulate DHWS conditions on a lab-scale and reproducibly. The results showed that even in the case of biofilm exfoliated into domestic hot water seriously, the chlorine combined with UV (chlorine-UV) disinfection could achieve high-level inactivation of suspended bacteria in a short time. Chlorine-UV disinfection effectively reduced the diversity of the bacterial community and affected bacterial community structure. It decreased the relative abundance of pathogenic bacteria, including *Legionella*, *Staphylococcus*. Chlorine-UV disinfection is suitable for the secondary disinfection of DHWS, which can ensure biosafety and effectively reduce bacterial contamination.

Keywords: Domestic hot water; Secondary disinfection; Biosafety; Bacterial community; Pathogenic bacteria

1. Introduction

Microbial growth is recognized as a major problem in drinking water distribution systems (DWDS) [1]. Water temperature is an essential factor influencing bacterial growth kinetics and competition processes [2]. Elevated water temperatures and a relatively long hydraulic retention time (HRT) in domestic hot water systems (DHWS) can lead to the acceleration of disinfectant consumption, which favors the multiplication of highly diverse mix of microorganisms and may cause more serious biosafety problems [3]. The inner wall of the pipeline in DHWS provides opportunities for biofilm formation. Biofilm protects the resident microbes against environmental stresses or disinfectants. Furthermore, it can function as a reservoir of potential pathogens and may release planktonic bacteria back into the water, causing water pollution [4]. The exfoliation of biofilm can also have a considerable adverse impact on domestic hot water quality and may pose a threat to human health. Therefore, it is necessary to consider adding disinfectant to domestic hot water for secondary disinfection.

Chlorine is one of the most widely used disinfectants in drinking water. Its application cannot fully avoid microbial regrowth even at a high dosage, due to the presence of organic matter and nutrients [5]. Ultraviolet (UV) provides an increasingly common alternative. It is safe to use, does

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not form mutagenic by-products and requires only a short contact time compared to chemical disinfectants. However, UV provides no residual protection, so that ideally it should be paired with a disinfectant capable of maintaining long term activity. As a result, chlorine combined with UV (chlorine-UV) disinfection has received widespread attention, which can get better disinfection efficiency than that single treatment.

Chlorine-UV as a water treatment method was a good disinfection option for chlorine-resistant (coliphages. MS2) and UV-resistant (human adenoviruses) microorganisms, and it can destroy the viral genome through the combined action of chlorine radicals and hydroxyl radicals [6–8]. Chlorine-UV disinfection was more effective than single chlorine to control the regrowth of opportunistic pathogens with the same residual chlorine concentration, and enhancing the microbial safety in drinking water [9]. Although the effects of chlorine-UV disinfection on pathogenic bacteria and viruses in drinking water have been widely recognized, limited efforts have been made to investigate its application on domestic hot water. Due to the difference between water quality and operation mode between DWDS and DHWS, it is still worth further study.

The composition of biofilm bacterial communities in the pipe wall of DWDS has been found to impact the stability of water quality, and it was affected by many factors including source water type, water age, pipe material, water purification strategies and so on [10–12]. Different bacterial community structure was captured between chlorine and chlorine-UV disinfection [9], and chlorine-UV had obvious advantages in reducing the number of microbial species and community complexity [13]. At present, the research on bacterial species in domestic hot water is mainly focused on *Legionella* [14,15]. The impact of chlorine-UV disinfection on the biofilm bacterial community in DHWS remains unclear, which is essential for the development of effective control strategies.

This study aimed to fill the knowledge gap discussed above by investigating the effect of chlorine-UV disinfection on domestic hot water pollution and compared it with chlorine disinfection alone. The impact of secondary disinfection on biofilm bacterial community structure and diversity were also studied using Illumina MiSeq sequencing.

2. Materials and methods

2.1. Cultivation of biofilm

The biofilm culture device used in the study is schematically shown in Fig. 1. In this study, laboratory tap water was heated to 45°C ± 1°C (temperature lower limit of domestic hot water in DHWS) by means of aluminum tube heat exchange to simulate domestic hot water, and used as the influent water of biofilm annular reactor (BAR), with water quality parameters as follows: dissolved organic carbon (DOC) 1.052–1.420 mg/L; turbidity 0.342–0.674 NTU; UV_{254} 0.007–0.018 cm^{-1}; and residual chlorine 0.02–0.08 mg/L. The silicone tube which connected the aluminum tube and the inlet port of the reactor was insulated to keep the influent water warm. BAR was used to simulate conditions relevant to DHWS. The BAR consisted of an outer cylinder containing an inner rotating drum operated at a rotational speed of 60 rpm, which approximately corresponded to a shear stress of 0.08 N/m^2. The HRT was controlled by the volumetric flow rate of the influents entering the BAR. The total working volume was approximately 800 mL, the HRT for the reactor was 4 h, corresponding to a total flow rate of 3.33 mL/min. The BAR accommodated 18 removable polyvinyl chloride coupons (17.2 cm^2 per coupon), and they were flush-mounted on the side of the inner rotating cylinder to support biofilm growth. The reactor was equipped with an inlet, outlet, and biofilm sampling port so that the coupons could be removed at any time during the operation of BAR. Prior to experimental use, the BAR was disinfected with sodium hypochlorite. The biofilm was cultured in BAR with continuous flow for a while to establish steady-state biofilms.

2.2. Experimental design

After the biofilm reached steady-state, the coupons were removed from the BAR. To simulate the domestic hot water pollution caused by the exfoliation of biofilm, an appropriate amount of biofilm scraped from the coupon surface was added to the beaker with 1 L BAR effluent water, and it was placed in a water bath to maintain water temperature required for the experiment. When investigating the effect of disinfection on the biofilm bacterial community, the coupons were placed along the beaker wall. The chlorine concentration used in the experiment was 0.3 mg/L, which is the lower limit of residual chlorine in the effluent of water treatment plant according to Standards for Drinking Water Quality (GB 5749-2006, Standards for Drinking Water Quality, China, 2006). UV irradiation was produced by a low-pressure mercury lamp (Hanovia, USA, λ = 253.7 nm) with a power of 5 W (for bacteria in water) or 10 W (for biofilm), and the exposure time was 1 min. The UV lamp was placed vertically in a 1 L beaker (inner diameter = 105 mm and length = 145 mm) wrapped in tin foil to prevent UV light. Samples were taken for microbiological detection at regular time intervals. During the disinfection phase, residual chlorine was quenched by adding sterile 10% sodium thiosulfate to each tube. The operation throughout the experiment was carried out in sterile rooms and the inactivation efficiency was assessed by Eq. (1).

$$\text{Inactivation efficiency} = \lg\left(\frac{N_0}{N_t}\right) \tag{1}$$

where N_0 is an initial concentration of suspended bacteria (CFU/mL) or biofilm (CFU/cm^2) without disinfection, N_t is the concentration of suspended bacteria (CFU/mL) or biofilm (CFU/cm^2) after disinfection.

2.3. Analytical methods

2.3.1. Chemical analysis

DOC content was measured by a total organic carbon analyzer (Vario TOC, Elementar, Germany), and turbidity values were measured by a turbidity meter (2100N, HACH, USA). The concentration of residual chlorine was measured using a portable residual chlorine rapid analyzer (SCL-501,

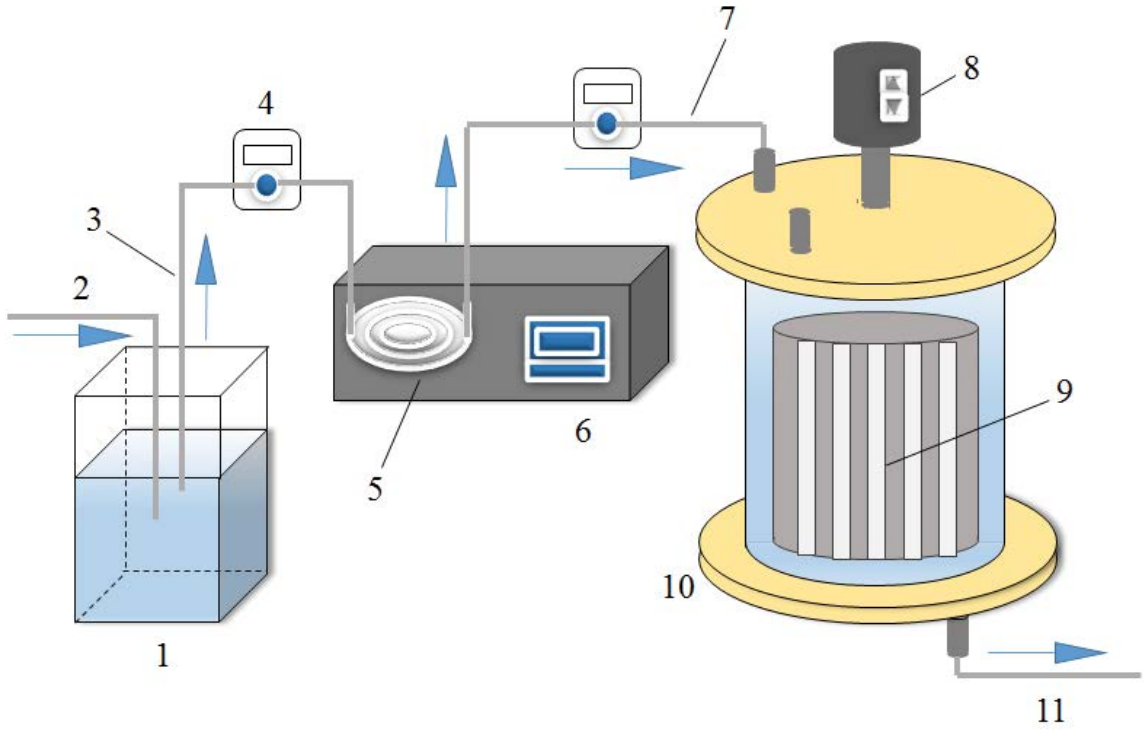

Fig. 1. Schematic diagram of the biofilm culture device.

Qingshijie, China) with a measuring range of 0.01–5.00 mg/L and absorbance at 254 nm (UV_{254}) were determined using a UV-spectrophotometer (UV-2600, SOPTOP, China).

2.3.2. Microbial analysis

The biofilm was sampled by removing the coupon from the reactor and the bacteria attached to the surface of the coupon was rinsed with sterile water. After aseptically collecting the biofilm by scraping the surface repeatedly with 2 to 3 cotton swabs, these were put into a test tube containing 10 mL sterilized ultra-pure water and then placed in the ultrasonic cleaning device for 20 min with a frequency of 40 kHz. The biofilm on the cotton swabs was fully dissolved in sterilized ultra-pure water and homogenized at 20,000 rpm for 1 min using a tissue homogenizer (Model M37610-33, Barnstead International, IOWA, USA). A standard method was used to determine the total number of bacteria. 1 mL of serially diluted sample was transferred to a plate containing a nutrient agar medium. The incubation time was 24 h at 37°C and then counted. Membrane filter technique was used to determine the number of *Escherichia coli* (*E. coli*) according to standard examination methods for drinking water (GB/T5750.12-2006, Standard examination methods for drinking water-Microbiological parameters, China, 2006). Samples were filtered aseptically through sterile 0.45 μm filter membranes using a vacuum aspirator. The filter membranes were then transferred using sterile forceps to plates containing pre-dried fuchsin basic sodium sulfite agar. These plates were incubated upside down at 37 °C± 1°C for 22–24 h after which all positive coliforms were enumerated. Dilutions were plated on R2A agar using standard spread plate techniques to determine heterotrophic plate counts (HPC) following incubation at 22°C ± 1°C for 7 d.

2.3.3. Biofilm morphology analysis

The surface morphology of the biofilm on the coupon was analyzed using scanning electron microscopy (SEM, SU-8010, Hitachi, Japan), which was performed at a coarse vacuum with an accelerating voltage of 5 kV. Three-dimensional structure was analyzed using Image-Pro Plus 6.0 based on SEM.

2.3.4. Biofilm microbial community analysis

Metagenomic sequencing was performed to analyze the bacterial community present in biofilm, which included DeoxyriboNucleic Acid (DNA) extraction, polymerase chain reaction (PCR) amplification, library construction and sequencing of metagenomics. Total genomic DNA was extracted from the biofilm using E.Z.N.A. Soil DNA Kit (Omega Bio-tek, USA) and the Qubit 2.0 DNA Kit (Life Technologies, USA) was used to quantify DNA precisely. The V3-V4 regions of 16S ribosomal ribonucleic acid (rRNA) were amplified by PCR with primer set 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) [16]. The resulting library was sequenced on a MiSeq Sequencing instrument by Sangon Biotech Co. Ltd., (Shanghai, China).

The reads from the original DNA fragments were merged using FLASH (version 1.2.3), and the quality filtering was performed using quantitative insights into microbial

ecology (Qiime, version 1.8.0). Operational taxonomic units (OTUs) were clustered with 97% similarity using Usearch (version 5.2.236) and chimeric sequences were identified and removed using Uchime (version 4.2.40). The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (version 2.12). All the high-quality sequences data have been deposited in sequence read archive database at National Center for Biotechnology Information (NCBI) under bio project number PRJNA508479.

3. Results and discussion

3.1. Microbial inactivation efficiency

3.1.1. Suspended bacteria in water

The disinfection effect of chlorine alone and chlorine-UV on suspended bacteria in domestic hot water is shown in Fig. 2. Chlorine-UV had a strong disinfection effect. The inactivation efficiency of the total number of bacteria, *E. coli* and HPC by the chlorine-UV disinfection at 1 min was 2.30, 2.56, and 2.28 lg, respectively, which increased by 2.19, 2.50 and 2.24 lg compared to chlorine alone for the same sampling time. The inactivation efficiency of simultaneous UV and chlorine against the total number of bacteria, *E. coli*, and HPC were higher than the sum of stand-alone UV and chlorine at the same doses, indicating that the enhancement was synergistic (Fig. 2d). The radicals formed in the simultaneous presence of chlorine and UV irradiation may be responsible for the damage to bacteria [17].

The disinfection effect of chlorine alone was initially relatively poor, resulting in a shoulder or leg occurring between 5–10 min, indicating that sufficient exposure time was required for chlorine disinfection alone. After this lag, the inactivation efficiency increased rapidly, reaching 3.30, 3.33 and 3.12 lg for a total number of bacteria, *E. coli* and HPC at 60 min, which decreased by 0.14, 0.23 and 0.11 lg

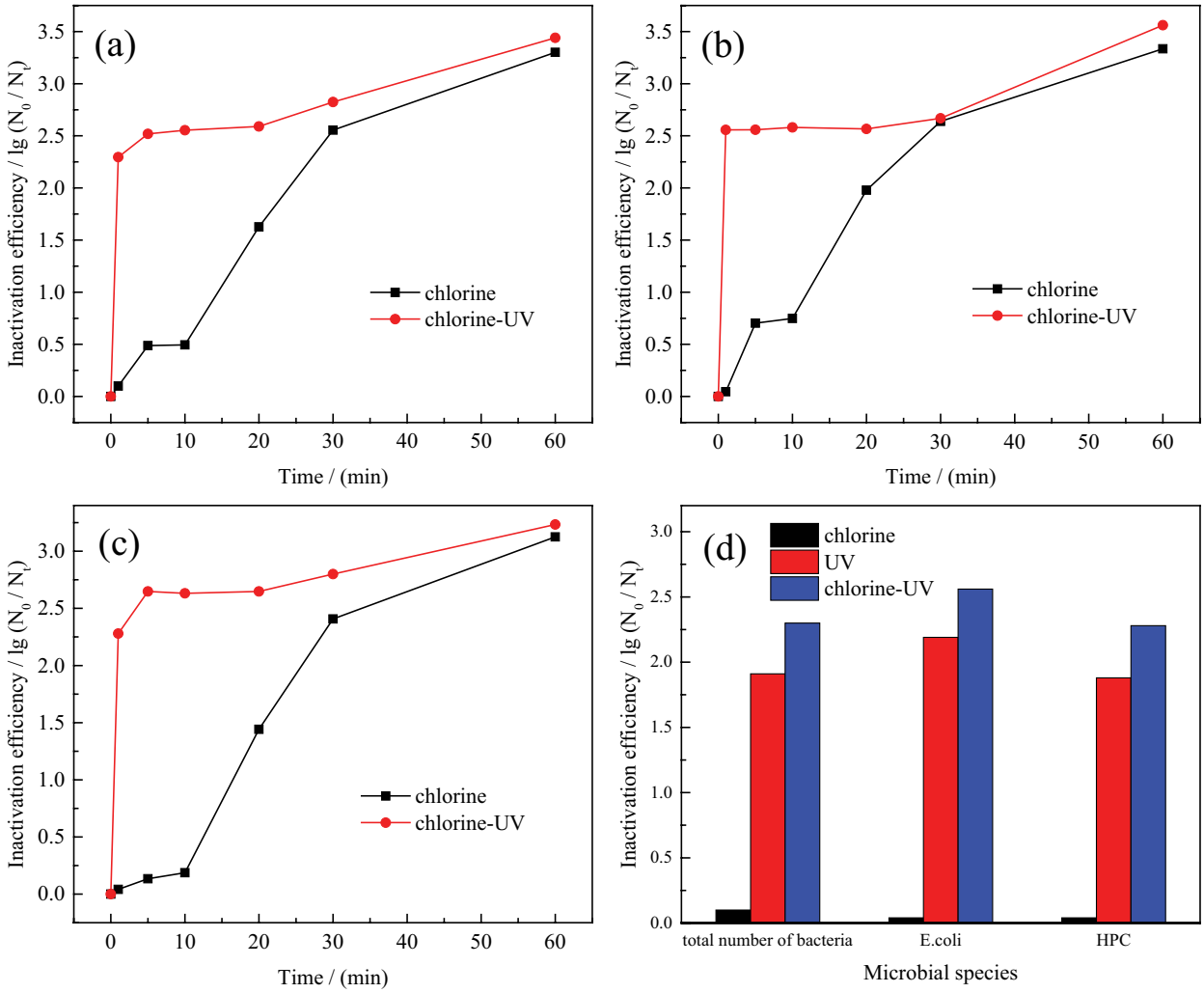

Fig. 2. Inactivation effect of suspended bacteria in domestic hot water by chlorine and chlorine-UV disinfection. (a) total number of bacteria, (b) *E. coli*, (c) HPC, (d) the inactivation efficiency of chlorine, UV and chlorine-UV disinfection after 1 min. (Before disinfection, total number of bacteria = 8.80×10^4 CFU/mL, *E. coli* = 8.00×10^4 CFU/ mL, HPC = 1.20×10^5 CFU/mL. Chlorine concentration = 0.3 mg/L, UV wavelength = 253.7 nm. Mean of duplicates were recorded, standard deviations less than 5% were not shown).

compared with chlorine-UV disinfection. It can be seen that chlorine was a good disinfectant for suspended bacteria, but required relatively long contact time.

Overall, chlorine-UV disinfection resulted in higher inactivation efficiency of suspended bacteria than chlorine disinfection alone, which was similar to the results observed by Rand et al. [18]. It has been demonstrated that by combining with UV, the effectiveness of chemical disinfectants could be enhanced to inactivate *E. coli*, coliphage MS2 and other microorganisms [19]. The results showed that chlorine-UV for secondary disinfection of domestic hot water combined the advantage of the high efficiency of UV and sustainability of chlorine so that it can inactivate suspended bacteria in a short time and effectively offset the shortcomings of chlorine disinfection alone. It had a good control effect on domestic hot water pollution even in the extreme case of biofilm shedding severely and fully guarantees the biosafety of water quality.

A water temperature of the DHWS usually varies between 45°C–60°C. The effect of temperature on chlorine-UV disinfection is mainly reflected in two aspects: on the one hand, the increase of water temperature can accelerate the decay of chlorine, and weaken the disinfection effect of chlorine to a certain extent; on the other hand, the high-temperature hot water has the function of thermal disinfection, which can improve the inactivation efficiency of bacteria. By comparing the inactivation efficiency of chlorine-UV disinfection on suspended bacteria at the different temperatures (Fig. S1), we found that the inactivation efficiency at 60°C was slightly higher than that of 45°C, and the temperature had no significant effect on chlorine decay. It indicates that the use of chlorine-UV for secondary disinfection in domestic hot water with high temperature could slightly improve the inactivation effect of suspension bacteria due to the addition of thermal disinfection function.

3.1.2. Attached bacteria in biofilm

The disinfection effect of chlorine alone and chlorine-UV on the attached bacteria in biofilm is shown in Fig. 3. As expected, chlorine resulted in a lower inactivation efficiency of the attached bacteria compared to suspended bacteria. The inactivation efficiency at 120 min was 0.67 lg for the total number of bacteria, 0.32 lg for *E. coli* and 1.54 lg for HPC. The concentration of chlorine decreased from 0.3 to 0.06 mg/L during this time (Fig. S2), which consumed mainly by the oxidation of bulk bacteria, biofilm bacteria, and biofilm extracellular polymeric substances. From thereon, there was no further obvious disinfection effect due to the low concentration of available chlorine. It can be inferred that the residual chlorine in domestic hot water cannot effectively inactivate bacteria in the biofilm.

Similarly to suspended bacteria, chlorine-UV disinfection performed better on attached bacteria, with inactivation efficiency of the total number of bacteria, *E. coli* and HPC increased by 0.49, 0.74, and 0.73 lg after 1 min compared to chlorine alone, which confirmed the high efficiency of UV. Chlorine-UV disinfection was considerably less effective in killing biofilm-associated bacteria, and there was no obvious synergistic effect between chlorine and UV (Fig. 3d). This can be explained by the fact that bacteria deep down in biofilms are protected, as they are not in direct contact with the disinfectants [4]. It can be seen that adding low concentration chlorine in water combined with short-term UV disinfection can also inactivate biofilm bacteria to a certain extent, but the inactivation efficiency was significantly lower than that of suspended bacteria. Periodic shock disinfection (adding high concentration disinfectant) of the DHWS can be considered to inactivate the biofilm on the pipeline wall.

In this study, biofilm morphology changed obviously before and after disinfection. The biofilm of the untreated sample was thick and compact, and mainly formed a dense structure (Fig. S3a), which is conducive to the attachment and reproduction of bacteria. Following chlorine disinfection, the structure of the biofilm became relatively loose, and obvious holes appeared on the surface due to the shedding of biofilm (Fig. S3b). Lomander et al. [20] analyzed the percentage of biofilm coverage on a stainless steel surface and concluded that chlorine did kill biofilm. Biofilm exfoliation was most significant after chlorine-UV disinfection (Fig. S3c). The biofilm showed filamentous and reticular structure with large pores on the surface.

The three-dimensional structure of the biofilm obtained from the SEM is shown in Figs. S3d–f, which intuitively displayed the vertical direction of the biofilm structure. The original biofilm was relatively thick, indicated by the abundant red color. This structure changed after chlorine disinfection, although it was still quite compact. The red area decreased and the more yellow area appeared, indicating that biofilm exfoliated from the surface. After chlorine-UV disinfection, the biofilm structure was loose, and the thickness of biofilm was further decreased. The resulting image showed even less red and yellow areas, while green and blue parts were dominant, which indicated that the biofilm exfoliated severely. Adding disinfectant in domestic hot water for secondary disinfection affected the morphology of biofilm. Chlorine-UV disinfection reduced the biofilm thickness and had a more destructive effect on the biofilm structure compared with chlorine disinfection alone.

The biofilm has a strong adaptability to temperature, and it can be formed on the pipe wall of DHWS under fluctuating water temperature (45°C–60°C). Therefore, we did not further discuss the effect of temperature on the biofilm inactivation efficiency but focused on the variation of biofilm community structure and diversity after disinfection (section 3.2).

3.2. Biofilm bacterial diversity and community

The biofilm samples were analyzed using the high-throughput sequencing technology to investigate the characteristics of the bacterial community. The obtained valid Illumina reads for each biofilm sample ranged between 50976 and 51417. The sequences were assigned to 8,425 OTUs at a 97% sequence identity threshold for subsequent analysis. At least 2671 OTUs were observed in biofilm samples, suggesting that the microbial community was highly complex. The OTUs results and alpha diversity indices of bacterial community are shown in Table 1.

The Shannon diversity indices of samples already reached stable values at the sequencing depth used in this study, which means that most diversity had already been

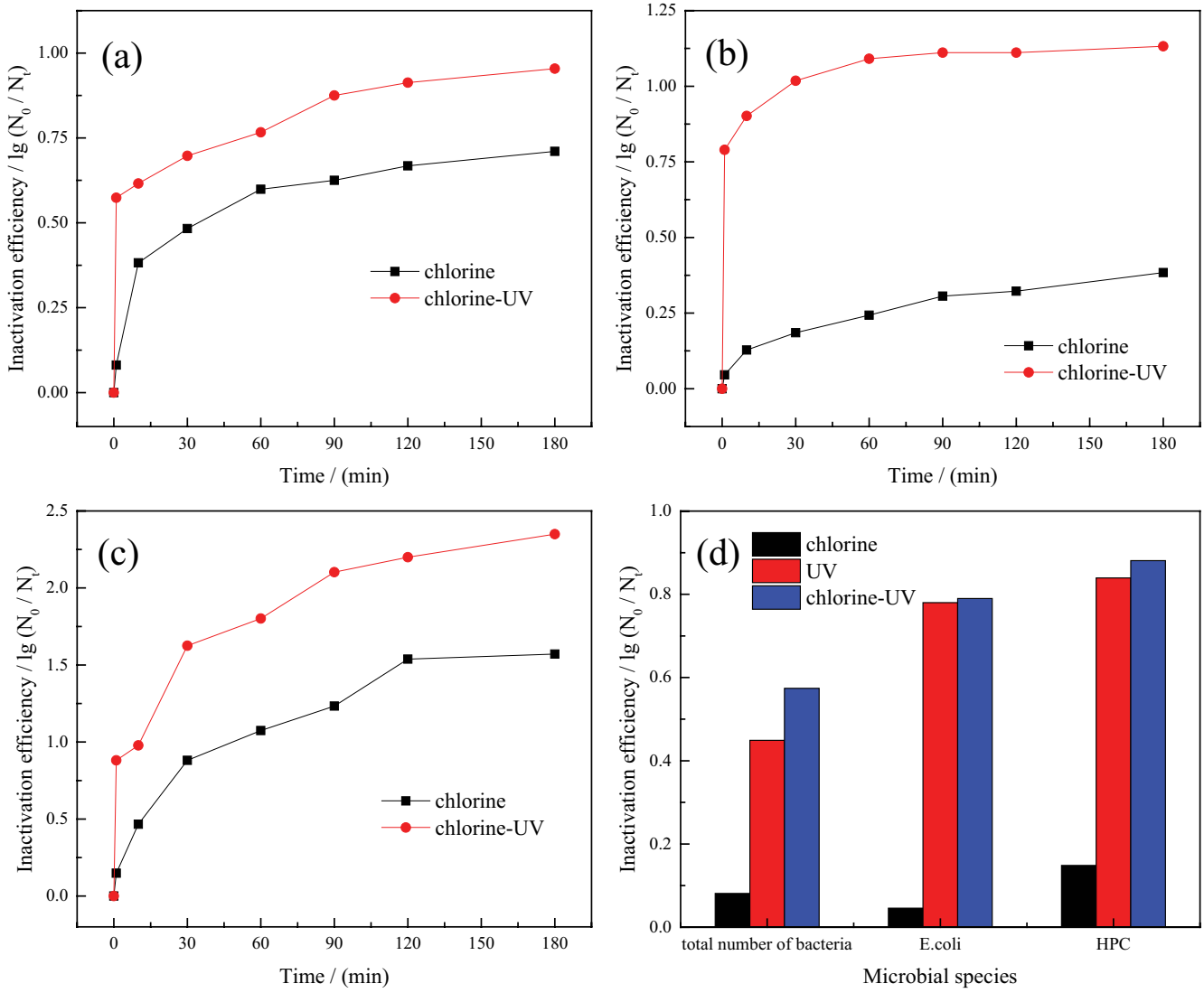

Fig. 3. Inactivation effect of microbes in biofilms in domestic hot water by chlorine and chlorine-UV disinfection. (a) total number of bacteria, (b) *E. coli*, (c) HPC, (d) the inactivation efficiency of chlorine, UV and their combined disinfection after 1 min. (Before disinfection, total number of bacteria = 1.59×10^3 CFU/cm^2, *E. coli* = 3.19×10^2 CFU/cm^2, HPC = 4.47×10^4 CFU/cm^2. Chlorine concentration = 0.3 mg/L, UV wavelength = 253.7 nm. Mean of duplicates were recorded, standard deviations less than 5% were not shown).

Table 1
Community diversity indices for biofilm samples

Sample ID	OTU	Shannon	Simpson	Coverage
Untreated	2,773	3.84	0.08	0.96
Chlorine	2,981	3.50	0.16	0.96
Chlorine-UV	2,671	3.27	0.16	0.96

captured although new phylotypes might be expected with additional sequencing (Fig. S4). Shannon index not only considered the species numbers but also considered species evenness. The Shannon index of biofilm samples ranged between 3.27 and 3.84. The untreated sample (3.84) had higher bacterial diversity than those disinfected by chlorine (3.50) and chlorine-UV (3.27), and this observation was also supported by the Simpson index. It indicated that disinfection can effectively reduce the diversity of the DHWS biofilm bacterial community, especially chlorine-UV disinfection. Moreover, Good's coverage revealed that the libraries represented the majority of bacterial 16S rRNA sequences presented in biofilm samples and covered the microbial diversity, with values of 0.96.

Of these high-quality sequences, 40 identified microbial phyla and 373 identified microbial genera were detected with the RDP classifier, which revealed a high level of microbial diversity in biofilm. The dominant phylum groups of each sample are shown in Fig. 4.

In this study, a total of 11 bacterial phyla were frequently identified in DHWS biofilm samples. *Proteobacteria* (57.42%) dominated in the untreated sample. That sample

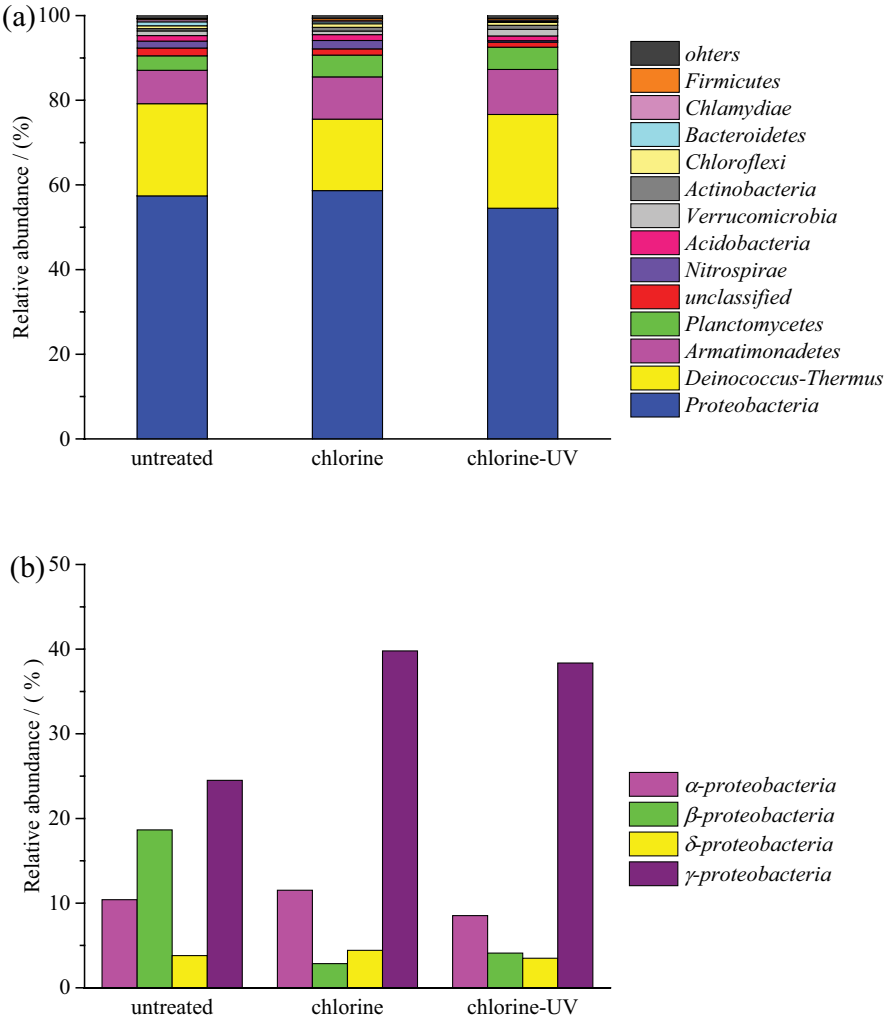

Fig. 4. (a) Taxonomic composition of bacterial communities at the phylum level and (b) *Proteobacteria* composition of samples at the class level. (Chlorine concentration = 0.3 mg/L, UV wavelength = 253.7 nm).

was also rich in members of the phylum *Deinococcus-Thermus* (21.77%), and, in order of decreasing abundance, members of *Armatimonadetes* (7.88%), *Planctomycetes* (3.43%), *Nitrospirae* (1.65%), *Acidobacteria* (1.29%) and *Verrucomicrobia* (1.09%) were detected, while other phyla were presented by less than 1% of the mapped reads.

After disinfection, the relative abundance of biofilm bacteria changed to some extent compared with the untreated sample, but *Proteobacteria* still predominated. Chlorine had the strongest impact on *Deinococcus-Thermus* whose relative abundance decreased with approximately 4.88%. *Proteobacteria* were relatively resistant to chlorine, resulting in a relative increase of 1.24% (as other phyla decreased stronger than *Proteobacteria* did). A high dose of chlorine has also been shown to increase the relative abundance of *Proteobacteria* in another study [21]. In the chlorine-UV treated sample, the relative abundance of *Proteobacteria* decreased by about 2.91%, and some other phyla such as *Nitrospirae*, *Acidobacteria*, *Bacteroidetes*, and *Chlamydiae* were also decreased to various degrees, while *Deinococcus-Thermus* slightly increased with about 0.36%. The relative increase of *Armatimonadetes* and *Planctomycetes* were observed in both treated samples (chlorine and chlorine-UV), indicating that these were more resistant than other phyla. It can be concluded that disinfection affected the DHWS biofilm bacterial community in different degrees, and there were noted differences in sensitivity between bacterial phyla. Compared with chlorine disinfection alone, chlorine-UV disinfection had a more significant impact on many phyla, especially *Proteobacteria*.

Proteobacteria was identified as the primary group of all samples in this study, which was usually dominated in the drinking water network [22–24]. Therefore, the community composition within the *Proteobacteria* phylum was further analyzed at the class level. We detected *α-Proteobacteria*, *β-Proteobacteria*, *δ-Proteobacteria*, and *γ-Proteobacteria* at variable levels (Fig. 4b), while the relative abundance of *ε-Proteobacteria* was so low that it could not be visualized in the figure. The dominant class was *γ-Proteobacteria* in all three samples, which was also dominant in undisinfected DWDS biofilms in a previous study [21]. Its relative abundance increased from 24.50% in the untreated sample to 39.79%

following chlorine disinfection and 38.36% in the chlorine-UV treated sample. The previous study showed that the proportion of γ-*Proteobacteria* increased in low or medium chlorine dosage, and decreased in high chlorine dosage [21]. It can be inferred that the increase of γ-*Proteobacteria* may be due to the low chlorine dosage. In contrast, the relative abundance of β-*Proteobacteria* decreased strongly, while the relative abundance before and after disinfection varied little for α-*Proteobacteria* and δ-*Proteobacteria* (Fig. 4b). It can be concluded that disinfection can influence the proportion of major components of the proteobacterial community in biofilm, and the chlorine-UV disinfection had the greatest effect on β-*Proteobacteria* and, to a lesser extent, α-*Proteobacteria*.

Further, at the genus level (Fig. 5a), the major genera of all samples were *Vulcaniibacterium* (20.81%–38.67%, members of the γ-*Proteobacteria*), *Meiothermus* (14.87%–21.52%, *Deinococcus-Thermus* members) and *Armatimonadetes_gp5* (7.74%–10.43%, members of the *Armatimonadetes*). The relative abundances of other individual genera were all less than 4%, and most of those were genera commonly found in freshwater and DWDS [25]. Compared with the untreated sample, chlorine reduced the relative abundance of *Meiothermus* and *Thermus* (both *Deinococcus-Thermus* members) with 4.10% and 0.69%, respectively. Both tested disinfection methods reduced the relative abundance of the genus *Sphingobium* (α-*Proteobacteria*), and of *Massilia*, *Methylophilus*, *Cupriavidus*, and *Acidovorax*, which all belong to the β-*Proteobacteria*. Their decrease was more evident in the chlorine-UV treated sample. The previous study found that *Methylophilus* dominated in samples before chlorination but almost disappeared after chlorination, which was in good agreement with the results of this paper; while there was a difference in the variation of *Acidovorax*, which may be due to different water quality and disinfectant dosage [26]. Also, after chlorine-UV disinfection, the relative abundance of *Elioraea* (α-*Proteobacteria*) and *Nitrospira* reduced about 0.32% and 1.29%, respectively. Overall, the changes at the genus level before and after disinfection were consistent with the changes found at the phylum level.

In this study, several genera were identified in the untreated sample that contains opportunistic pathogens, including *Sphingomonas*, *Legionella*, *Acinetobacter*, *Staphylococcus*, *Mycobacterium*, *Pseudomonas* and *Klebsiella*, which were listed by World Health Organization (WHO) [27]. Although their

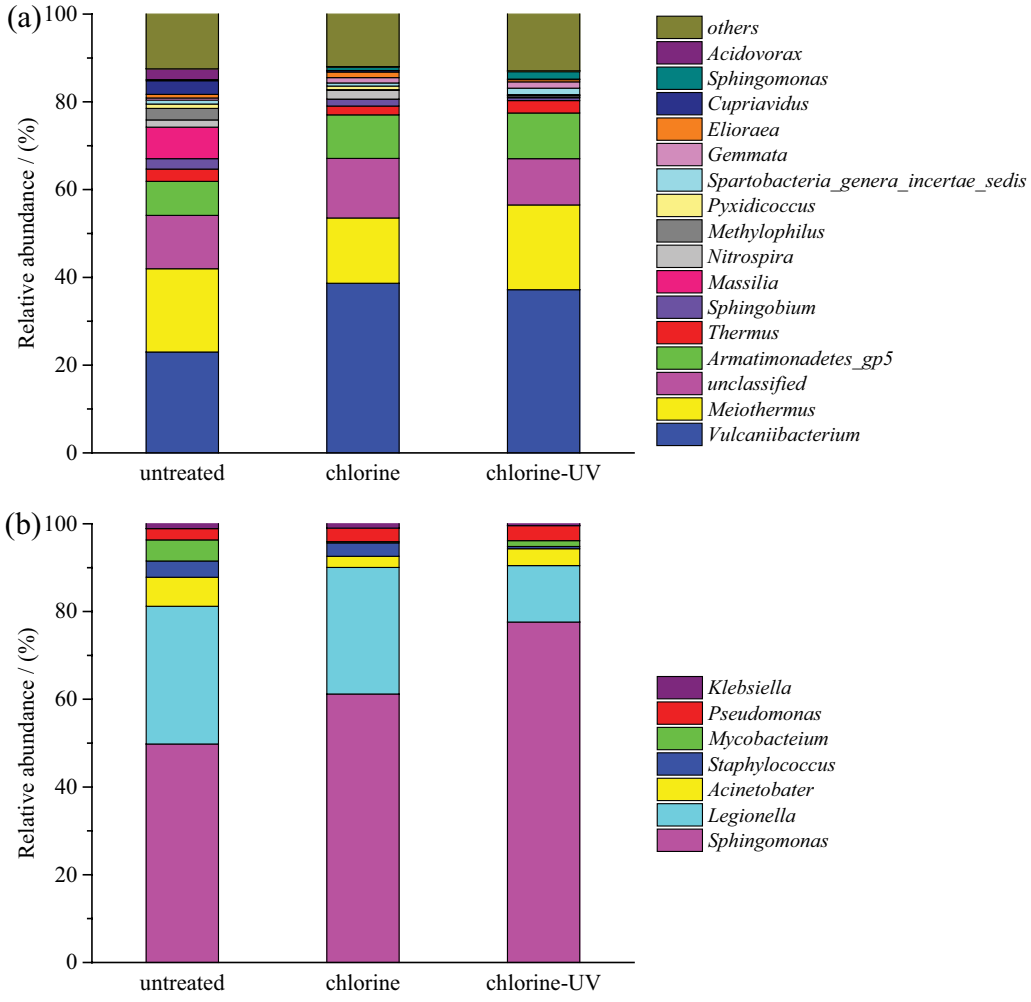

Fig. 5. (a) Taxonomic composition of bacterial communities and (b) opportunistic pathogens at the genus level. (Chlorine concentration = 0.3 mg/L, UV wavelength = 253.7 nm).

relative abundance was lower than that of the more abundant communities, and most species were not shown among the 15 dominant genera in Fig. 5a, they might still present biosafety risks. Environmental microorganisms like *Legionella*, *mycobacteria* or *Pseudomonas* adapted to oligotrophic aquatic conditions can persist over long periods in biofilm and possibly even multiply in these environments [28]. These genera were still detectable after disinfection, but their relative abundance has changed to a certain extent. As shown in Fig. 5b, both tested disinfection methods reduced the relative abundance of the *Legionella*, *Acinetobacter*, *Staphylococcus*, *Mycobacterium*, and *Klebsiella*. *Legionella* is an aquatic and ubiquitous Gram-negative bacteria detected in hot water plumbing systems and cooling towers, and human infection occurs when contaminated water aerosols are inhaled [14]. The relative abundance of *Legionella* in the chlorine-treated sample was higher than the chlorine-UV treated sample, and this phenomenon was also observed in *Staphylococcus* and *Klebsiella*. While the relative abundance of *Sphingomonas* increased after disinfection, this might be explained by the fact that *Sphingomonas* take less restriction on living conditions and have better resistance of residual chlorine, which allows them to grow in oligotrophic environments [29], and the proportion of *Pseudomonas* also increased slightly.

4. Conclusions

A safe and efficient secondary disinfection technique of domestic hot water using chlorine-UV compared to chlorine was systematically investigated, in terms of the inactivation efficiency of bacteria and its impact on the biofilm bacterial community. Chlorine-UV had a good disinfection effect on suspended bacteria in domestic hot water, and the inactivation efficiency of the total number of bacteria, *E. coli* and HPC reached 3.44, 3.56, and 3.23 lg, respectively. It can fully guarantee the biosafety of water quality even in the extreme case of biofilm shedding severely. Chlorine-UV disinfection reduced biofilm bacterial diversity and affected bacterial community structure in DHWS. The relative abundance of pathogenic bacteria, including *Legionella*, *Staphylococcus*, etc decreased after disinfection.

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Supplementary information

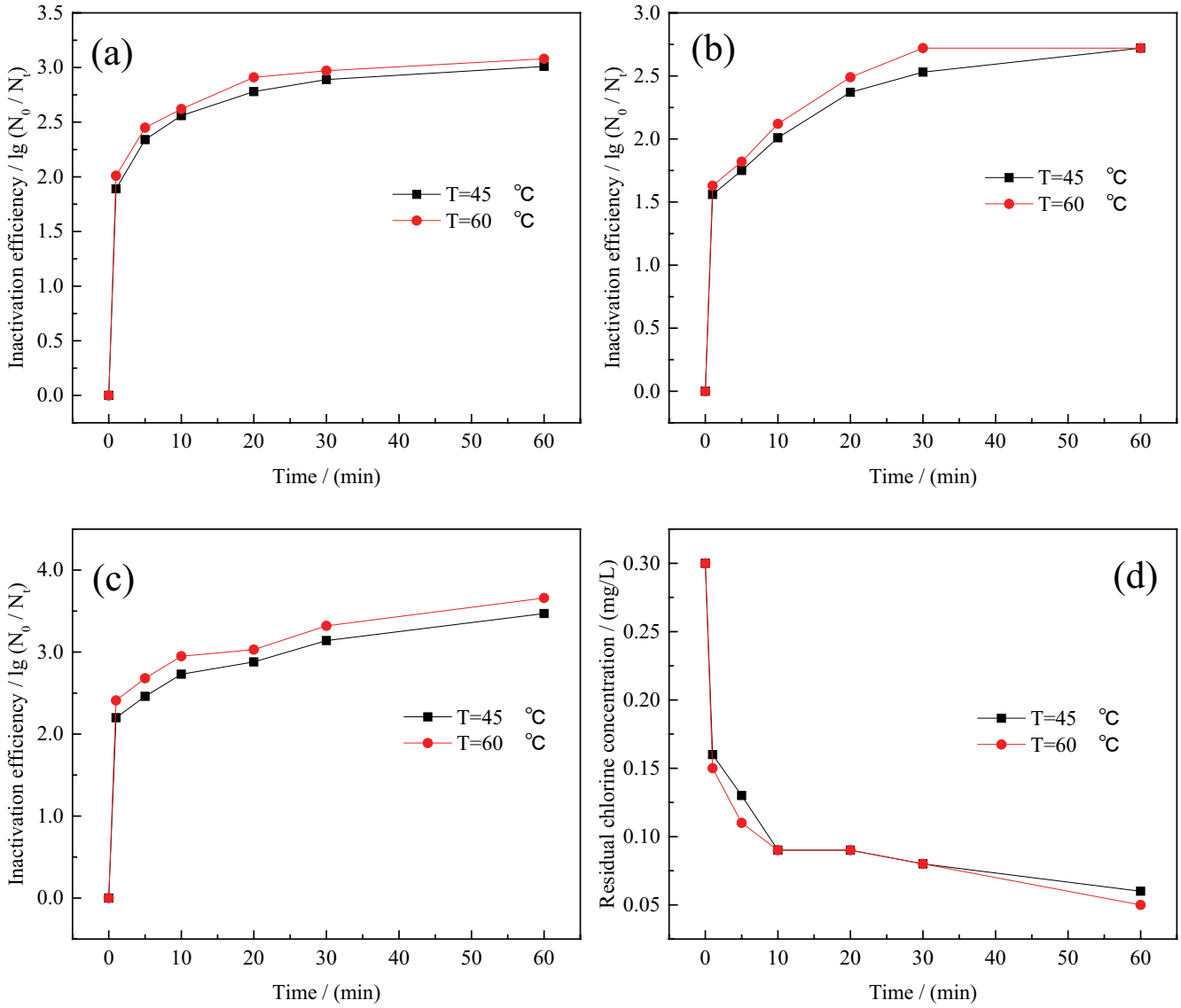

Fig. S1. Effect of temperature on inactivation of suspended bacteria in domestic hot water by chlorine-UV disinfection. (a) total number of bacteria, (b) *E. coli*, (c) HPC and (d) decay of chlorine at different temperatures during chlorin-UV disinfection. (Before disinfection, total number of bacteria = 1.20 × 10^3 CFU/mL, *E. coli* = 5.30 × 10^2 CFU/ mL, HPC = 2.70 × 10^4 CFU/mL. Chlorine concentration = 0.3 mg/L, UV wavelength = 253.7 nm. Mean of duplicates were recorded, standard deviations less than 5% were not shown).

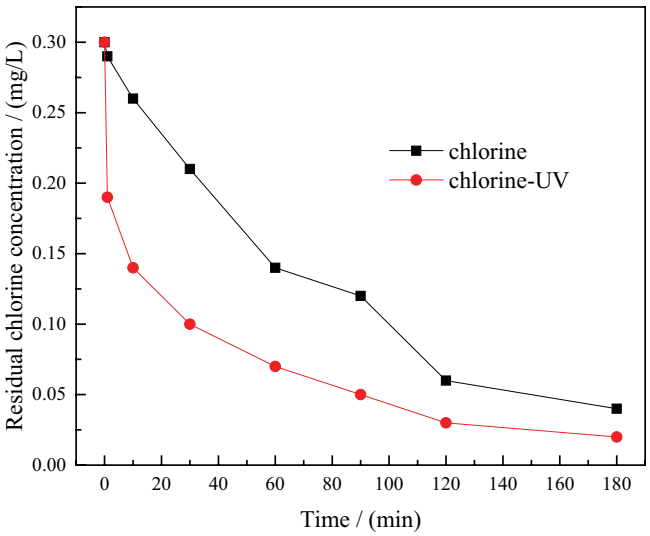

Fig. S2. Decay of chlorine during disinfection.

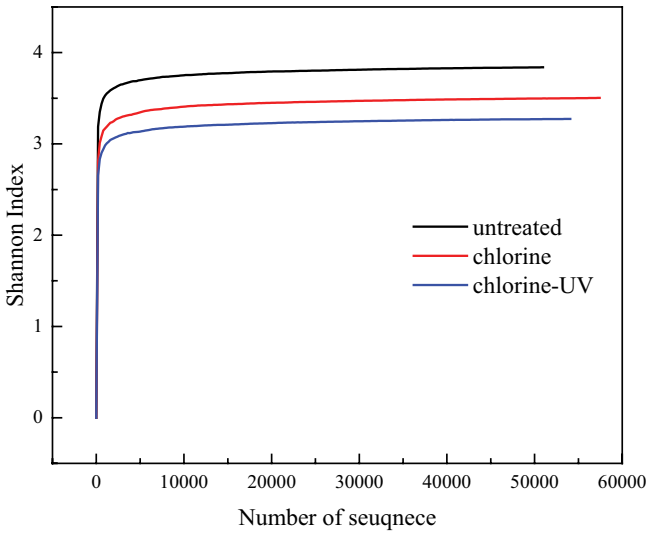

Fig. S4. Shannon-Wiener curves of biofilm bacterial communities.

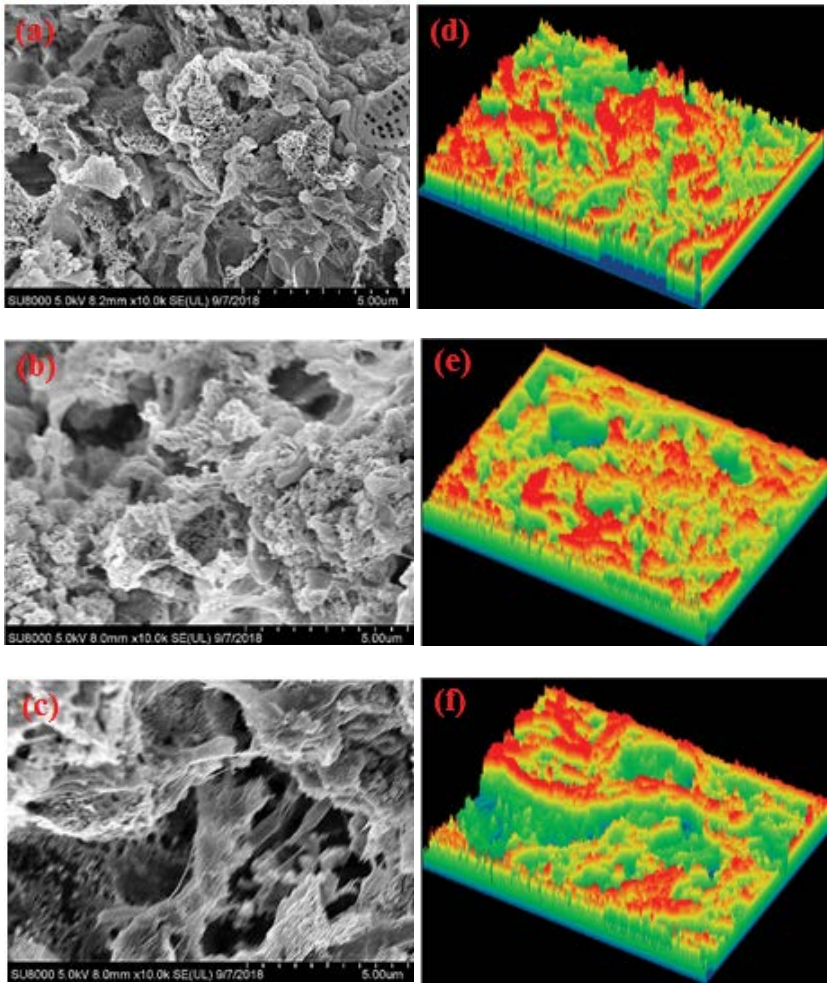

Fig. S3. SEM of biofilm: (a) untreated, (b) treated with chlorine, (c) treated with chlorine-UV; the three-dimensional structure of biofilm: (d) untreated, (e) treated with chlorine, (f) treated with chlorine-UV. (Red, yellow, green and blue in turn represent the vertical structure from the outermost to the innermost layer of the interface between the biofilm and the liquid phase. Red represents the biofilm structure near the liquid phase, yellow and green represent the middle layers of the biofilm and blue represents the biofilm structure near the coupon surface).