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Effect of disinfectant residual on the interaction between bacterial growth and assimilable organic carbon in a drinking water distribution system

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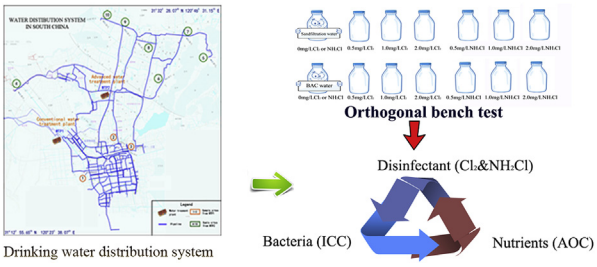
HIGHLIGHTS

- Only with chlorine less than 0.15 mg/L, ICC was significantly correlated to AOC.
- Cl_2 above 0.15 mg/L or NH_2Cl above 0.4 mg/L was associated with low ICC proportion.
- Chloramine tended to cause lower AOC level and intact cells proportions.
- ICC was found to be limited when AOC was less than 135 μg/L.
- Particles control and use of chloramine were proper ways to limit bacterial growth.

GRAPHICAL ABSTRACT



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ABSTRACT

Public health is threatened by deteriorated water quality due to bacterial regrowth and uncontrolled growth-related problems in drinking water distribution systems (DWDSs). To investigate the scope of this problem, a two-year field study was conducted in south China. The amount of assimilable organic carbon (AOC), total cell concentrations (TCC), and intact cell concentrations (ICC) of water samples were determined by flow cytometry. The results indicated that ICC was significantly correlated to AOC concentration when the chlorine concentration was less than 0.15 mg/L, and ICC was lower at chlorine concentrations greater than 0.15 mg/L, suggesting that free chlorine level had effect on AOC and ICC. To further analyze the effect of disinfectant on AOC and bacterial growth, we designed an orthogonal experiment with different dosages of two commonly used disinfectants, chlorine and chloramine. The results demonstrated that high concentrations of free chlorine (> 0.15 mg/L) and chloramine (> 0.4 mg/L) were associated with relatively low proportions of intact cells and cultivable bacteria. Compared with chlorine, chloramine tended to cause lower AOC level and intact cells, likely because the chlorinated disinfection byproducts (DBPs) were more easily absorbed by bacteria than the chloraminated DBPs. Based on the statistical analysis of 240 water samples, ICC was limited when AOC concentration was less than 135 μg/L, while temperature and the number of small-size particles showed positive effects on ICC

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($P < 0.05$). We conclude that the use of chloramine and controlling particle numbers should be suitable strategies to limit bacterial regrowth.

1. Introduction

With rapid economic development and population growth, water shortages and water pollution problems have become much severe (Liu et al., 2013; Wang et al., 2014a; Prest et al., 2016). The final goal of water treatment is to achieve good quality drinking water at the taps of customers, not just at the time of processing at the water treatment plant (WTP). After water distribution, water at the consumers' taps had a lower quality than the treated water at the WTP (Liu et al., 2013; Schwake et al., 2016), indicating the development of contamination during distribution. Drinking water distribution systems (DWDSs) must act as protective barriers to prevent contamination and bacterial regrowth as the treated water travels to the customer. The World Health Organization stated that 'Water entering the distribution system must be microbiologically safe and ideally should also be biologically stable.' in 2006 (Lautenschlager et al., 2013). There is general consensus that the term 'biological stability' in this context refers to the concept of maintaining microbial water quality from the WTP to the point of consumption (Lai et al., 2006; Lou et al., 2012). Therefore, assessment of the biological stability of drinking water and identification of factors that alter microbial growth are required to enable efforts to maintain biological safety in DWDS.

Bacterial regrowth in DWDS can lead to proliferation of opportunistic pathogens or the corrosion of infrastructure (Wang et al., 2014b; Ashbolt, 2015). Huang found a high diversity of bacterial species and a number of opportunistic pathogens in some tap water samples (Huang et al., 2014). Water quality in a pipeline can be seriously impacted by different factors such as the presence of different disinfectants (free chlorine or chloramines), hydraulic retention time (typically 1–3 days), or the pipe material (PVC, iron, and cement surfaces) (Masters et al., 2015; Völker et al., 2015), particles(Proctor and Hammes, 2015; Liu et al., 2016) and biofilms(Ling et al., 2016). Prest found that in addition to the type and concentrations of available substrates, the environment, bacterial community structure, and bacteria concentration were essential parameters in competition processes regulating bacterial growth (Prest et al., 2016). Gillespie pointed out that microbiological water quality was mainly affected by the presence of disinfectant residuals in DWDSs (Gillespie et al., 2014b). To limit bacterial regrowth, many countries distribute drinking water with low levels of disinfectant. In China, the recommended dosages of chlorine and chloramine for usage in a WTP are 0.3–4.0 and 0.5–3.0 mg/L, respectively (Wu et al., 2015). However, disinfectants can potentially select microbes that are resistant to oxidants (Proctor and Hammes, 2015). A number of bacteria, such as *E. coli*, *Enterococcus,* and *Salmonella*, can enter into a viable, but non-culturable (VBNC) state during the disinfection processes. These VBNC bacteria may resuscitate when optimal conditions are restored, such as when the concentration of disinfectant is lowered, which can then allow bacterial regrowth (Mustapha et al., 2015; Zhang et al., 2015). The disinfectant concentration can greatly affect bacteria growth and biological stability in DWDSs (Zhang et al., 2016). However, it remains unknown how the presence of disinfectant can affect the relationship between AOC and bacteria. Overall, improved understanding of bacterial interactions in distribution systems and the impact of environmental conditions is needed for better control strategies to minimize bacterial regrowth during drinking water production and distribution.

Here, we present a comprehensive study to address the interactions among AOC, disinfectants (free chlorine, monochloramine), and bacteria. A two-year study of a DWDS was conducted in which biological evaluation indexes (assimilable organic carbon (AOC), total cell concentration (TCC), and intact cell concentration (ICC)) were measured. Additionally, an orthogonal experimental test was designed to study how the disinfectant types and concentrations altered the relationship between AOC concentration and ICC. Both ICC and TCC, including VBNC bacteria, were detected by flow cytometry in drinking water samples. To determine the best strategies to control bacterial regrowth, statistical analysis was conducted to examine the key factors affecting bacterial regrowth in a real DWDS. The results of this study suggest guidelines to control the potential health risks of bacterial regrowth in drinking water.

2. Materials and methods

2.1. Characteristics of sampling sites

The region of sampling is illustrated in Fig. 1. Each sampling site received water from one of two treatment plants, WTP1 or WTP2. WTP1 used a conventional water treatment process and WTP2 used an advanced water treatment process (ozone combined with biological activated carbon). The ten sampling sites in DWDS used nine year old ductile iron pipes. The average water velocity ranged from 0.03 m/s to 0.31 m/s. The characteristics of the sampling sites are shown in Table 1.

2.2. Preparation of carbon free materials

Carbon-free bottles and vials were prepared by the method of Hammes (Hammes et al., 2010). Briefly, all bottles, vials, and Teflon-coated screw caps were first washed with a common detergent and then rinsed three times with deionized water. The glassware was then submerged overnight in 0.2 N HCl and subsequently rinsed with deionized water again and then air-dried. Finally, the bottles and vials were baked in a muffle furnace at 500 °C for at least 6 h.

2.3. General parameters

Chlorine and water temperature were determined immediately at sampling sites, and chlorine residuals were neutralized after water samples were transported back to the lab. Turbidity, DOC, UV_{254}, pH, ammonia, total particles count, AOC concentration, TCC, and ICC were detected for all samples. The examined indicators and laboratory equipment are shown in Table 2.

2.4. Biological stability detection method

TCC and ICC analysis: TCC and ICC were performed by flow cytometry, according to van der Wielen's study (van der Kooij and Veenendaal, 2013). Briefly, for measurements of TCC, SYBR green (Life) was diluted with dimethyl sulphoxide (DMSO) to a working stock concentration of 100 ×, and 5 μL were added to sample

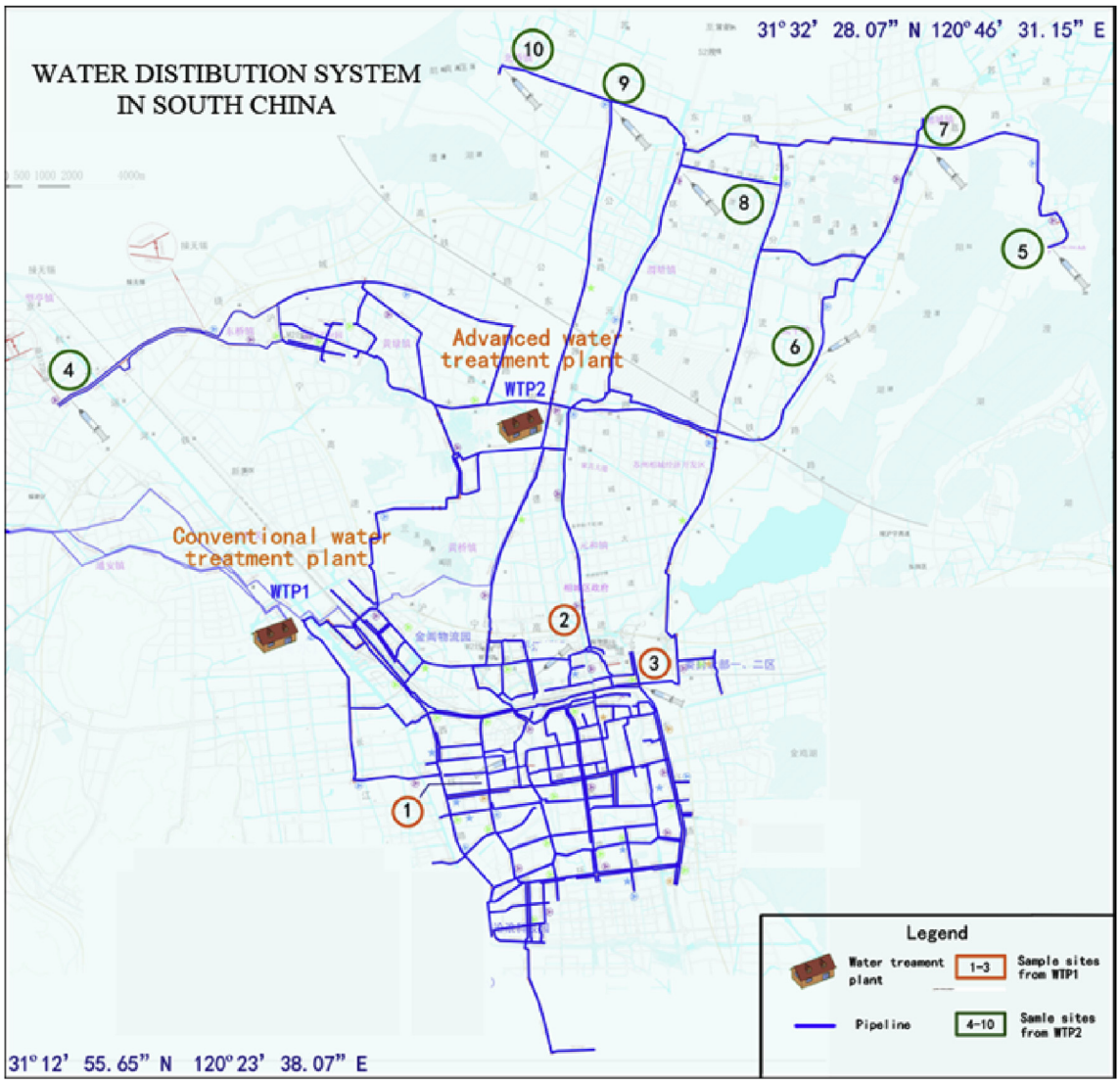

Fig. 1. Sampling sites of DWDS in south China.

Table 1
Characteristics of each sampling sites.

Sample sites	Water treatment plant	Water distribution distance (m)	Pipe age (years)	Water velocity (m/s) (average/minimum/maximum)	Pipe material
1	WTP1	10614	9	0.23/0.15/0.3	Ductile iron
2	WTP1	9047	9	0.03/0.01/0.04	Ductile iron
3	WTP1	12065	9	0.19/0.14/0.24	Ductile iron
4	WTP2	18826	9	0.17/0.1/0.22	Ductile iron
5	WTP2	26119	9	0.04/0.02/0.05	Ductile iron
6	WTP2	12001	9	0.19/0.1/0.28	Ductile iron
7	WTP2	19168	9	0.23/0.1/0.34	Ductile iron
8	WTP2	10667	9	0.05/0.01/0.11	Ductile iron
9	WTP2	11018	9	0.22/0.11/0.3	Ductile iron
10	WTP2	14236	9	0.31/0.15/0.47	Ductile iron

volumes of 500 μL. For measurements of intact cell concentrations, 1 mL of the water sample was incubated with 10 μL SYBR green (100 × dilution of a 10,000 × concentrate) and 10 μL propidium iodide (50 g/mL) (Life) and stained for 15 min at room temperature. Subsequently, membrane-intact and membrane-disrupted cells were counted on a FACSCalibur flow cytometer (BD). Results were expressed in cells per milliliter (cells/mL).

AOC-analysis: The concentration of assimilable organic carbon (AOC) significantly affects the microbiological stability of drinking water, but the conventional bioassay is time-consuming and labor-intensive. We used the assay described by Hammes et al. (2007). This approach allowed for the detection of inactive and/or unculturable microorganisms. Briefly, chlorinated samples were firstly neutralized with 100 μL thiosulphate stock solution. The water samples were collected into a 40 mL sample tube and were heated at 70 °C for 0.5 h within 6 h after sampling to destroy any vegetative

Table 2
Test indexes and analytical instruments.

Test indexes	Analytical Instruments
Turbidity	HACH 2100P Turbidity
UV_{254}	UV-2550 Spectrophotometer
DOC	OC-VCPH Total Organic Carbon Analyzer
Temperature	Mercury thermometer
pH	HACH LA-pH10 acid estimation apparatus;
COD_{Mn}	Standard acidic potassium permanganate method
Ammonia	UV-2550 Spectrophotometer
Total Particle count	Particle Counter
Total/Intact cell concentrations	Flow Cytometry

cells. After pasteurization, tubes were allowed to cool to room temperature. *Pseudomonas fluorescens* strain P17 was inoculated into the water samples to a final concentration of 10000 cells/mL. The water samples were incubated at 25 °C for 3 days and then cell concentrations were counted. Subsequently, the same water sample was heated at 70 °C for 0.5 h to kill P17 and then was inoculated with the Spirillum NOX strain. The water samples were then incubated at 25 °C for 4 days and the cell concentrations were counted. The AOC concentration was calculated by comparing the cell concentrations and the yield coefficient. The experimental yield values from acetate standards were 4.6 × 107 cells/μg of acetate-C for P17 and 1.1 × 107 cells/μg of acetate-C for NOX.

2.5. Data presentation and calculations

Pearson correlation statistics were used to evaluate significant correlations among available substrates, bacteria concentrations, and environment factors. The experimental data was regressed using Origin 9.1 and SPSS version 18.0. The correlation was regarded as significant for P values below 0.05.

2.6. Bench test

An orthogonal experimental bench test was designed to study how disinfectant types and concentrations affected the relationship of AOC concentration and live bacteria. Water samples were acquired from WTP2 after sand filtration or after BAC. Before sample collection, 15 min flushing was performed and then a 2 L sample was collected. As shown in Fig. S1 and Table S1, free chlorine or chloramine was added to samples at concentrations of 0 mg/L, 0.5 mg/L, 1.0 mg/L, and 2.0 mg/L. The samples were then incubated at 25 °C in the dark, and we then measured TCC, ICC, disinfectant residual, and AOC level at different time (30 min, day 1–10, day 14, and day 28–30). The water quality of the samples was assessed and the measured parameters are shown in Table 3.

Table 3
Water quality of different water samples.

Test indexes	Sand filtration water	BAC water
pH	7.4 ± 0.2	7.3 ± 0.2
COD_{Mn} (mg/L)	1.98 ± 0.32	1.7 ± 0.16
Ammonia (mg/L)	0.03 ± 0.01	0.03 ± 0.0.01
Nitrite (mg/L)	0.001 ± 0.001	0.002 ± 0.001
Nitrate (mg/L)	1.62 ± 0.20	1.65 ± 0.15
TOC (mg/L)	3.162 ± 0.24	2.845 ± 0.12
UV_{254} (cm^{-1})	0.034 ± 0.006	0.025 ± 0.005
Intact cell count (cells/mL)	(6.00 ± 0.28)*10^5	(1.30 ± 0.08) *10^5

3. Results and discussion

3.1. Variation of biological stability and factors affecting biological stability in DWDS

As shown in Fig. 2, the AOC concentration of ten sampling points varied between 25.96 and 429.60 μg/L from January 2014 to December 2015, and the level was higher in winter than in summer. A decrease in the AOC concentration was observed in May, likely because of changes in microbial activity due to changes of TOC concentration in raw water. The main reason lies in the TOC concentration which rose along with the temperature. The TOC concentration of raw water and treated water were higher in winter than in summer. Given that AOC partially belongs to TOC, the AOC concentration of the water samples in DWDS was high in winter and low in summer as well. The ICC ranged from 10 cells/mL to 5.11 × 10^4 cells/mL. As illustrated in Fig. 2, ICC was high in summer and low in winter, while chlorine concentration showed the opposite trend, which implied that ICC was negatively correlated to chlorine concentration. The bacteria might have high metabolism and grow fast due to rapid chlorine depletion and more suitable temperature for growth in the summer. Overall, the chlorine concentration played an important role on the presence of bacteria in DWDS.

3.2. Different free chlorine level imposed different effects on AOC concentration and ICC

As shown in Fig. S2, the AOC concentration increased as the chlorine residual increased ($n = 240$, $P < 0.05$), mainly due to reaction between chlorine and macromolecules of organic matter (Świetlik et al., 2004). Chlorine concentration and ICC showed a negative correlation ($n = 240$, $P < 0.01$). The average ICC level with chlorine concentration lower than 0.15 mg/L was 5.5 times higher than that with chlorine higher than 0.15 mg/L, which demonstrated the effect of high chlorine concentration to suppress bacterial growth.

The ICC level had no significant relationship with AOC for all samples. However, considering disinfectant residuals, ICC was positively correlated to AOC concentration ($n = 118$, $P < 0.05$) when chlorine concertation was less than 0.15 mg/L (Fig. 3), due to the prompting effect of AOC on bacterial growth. However, the relationship between AOC and ICC was not significant ($n = 122$, $P > 0.05$), when chlorine was more than 0.15 mg/L. The main reason for the correlation was that chlorine suppressed bacteria growth. HPC concentration was reported to be higher in samples with chlorine residual <0.3 mg/L than in those with residual >0.3 mg/L (Francisque et al., 2009). With a large amount of biological stability data obtained from DWDS, additional analysis of bacterial regrowth and biological stability with different level of chlorine residuals is required.

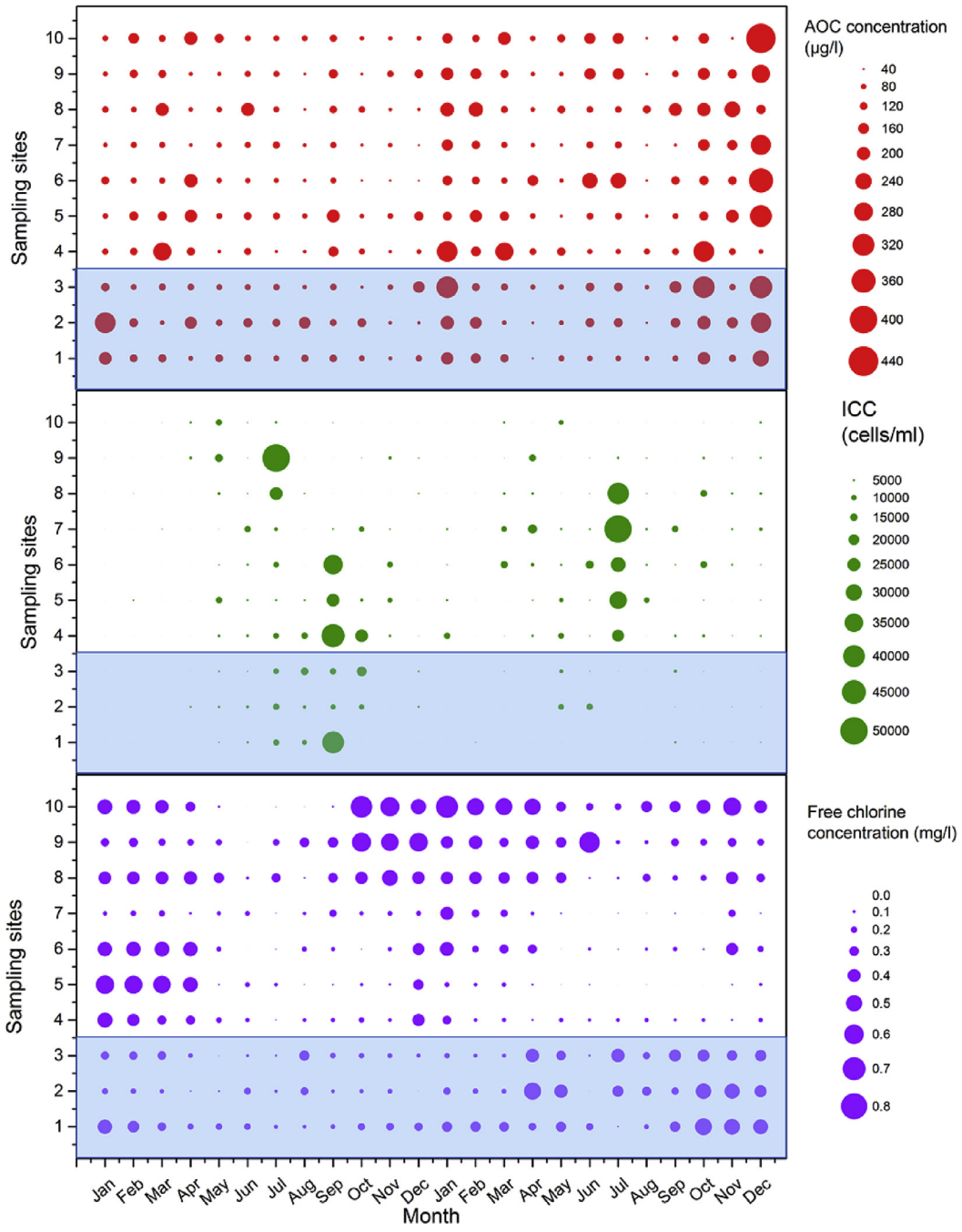

Fig. 2. Variation of different biological stability evaluation indexes and free chlorine concentration in DWDS for samples collected over two years. The blue shading indicates samples from the conventional water treatment plant, WTP1). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Disinfectant effect on the interaction between intact cells and AOC in DWDS

To more carefully evaluate the relationships between intact cells and AOC with varying amount of disinfectants, an experiment was designed and performed. Water samples were collected from two water treatment processes (sand filtration and BAC) to represent water samples with different nutrient content (Fig. S3 & Fig.S4) and different biomass.

As shown in Fig. 4, AOC level increased dramatically and accounted for almost 300 μg/L after the first 30 min and then began to decay in the first phase due to the interaction between organic carbon and chlorine. The disinfectant concentrations decreased throughout the experiment due to the rapid reaction of the disinfectant with organic compounds and other reducing substances in water. Bacteria showed almost no regrowth in this phase. In the second phase, ICC increased gradually when the chlorine level was less than about 0.15 mg/L or when chloramine was less than about 0.4 mg/L, which implied that bacteria regrew with low disinfectant residual. The AOC decreased sharply when the bacteria began to grow. At higher initial disinfectant dosages, bacteria could regrow after a longer time. In the last phase, the ICC and AOC became stable. The relationship of AOC and intact cells was essentially determined by the concentration of disinfectants. For BAC water

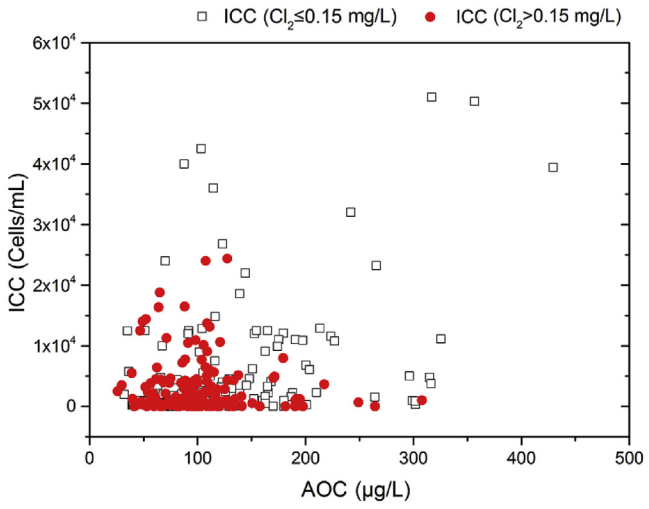

Fig. 3. Effect of chlorine residuals on AOC concentration versus ICC in DWDS.

with 2 mg/L chlorine residual, the bacteria did not regrow until the 30th day with a high chlorine concentration (more than 0.7 mg/L), because low organic carbon can interact with chlorine.

As illustrated in Fig. 5, compared with the chloraminated system, the high chlorine residual (more than 0.15 mg/L) assured substantially lower proportions of intact cells than chloramine disinfectant. However, a low chlorine concentration (less than 0.15 mg/L) correlated with a higher proportion of intact cells than samples with chloramine disinfectant. A threshold was around 0.15 mg/L for free chlorine, while the threshold for chloramine was around 0.4 mg/L. The reason for the suppression of bacteria by high disinfectant and bacterial regrowth with low disinfectant are owing to oxidizability of disinfectant and membrane integrity of bacteria (Lin S, 1984; Dodd M C, 2005; Dodd, 2012). When chlorine level is high, chlorine reacts primarily with constituents of bacterial cell walls (e.g., amino acid side chains in peptidoglycan or membrane-bound proteins), and HClO can penetrate into the bacterial cytoplasm to react with nucleic acids (Dodd, 2012). In contrast, with low HOCl in the solution, there is little inactivation of bacteria. As a result, viable bacteria can use available nutrients to regrow. Some bacterial species are induced by the presence of the disinfectant to enter a viable but non-cultivable state (VBNC) (I., 2008; Zhang et al., 2015). We found that bacteria could be detected by flow cytometry but not by culture methods after disinfectant had been added for 30 min. However, when disinfectant was low (chlorine less than 0.15 mg/L or chloramine less than 0.4 mg/L) the bacteria was detected by traditional culture methods. In the VBNC state, resuscitation occurred upon removal of the inducing stress, restoring culturability (Ayrapetyan et al., 2015). Thus, bacterial regrowth occurred when the chlorine concentration was low.

As shown in Figs. 6 and 7, the initial AOC level with chlorine was about 30% higher than that with chloramine and about 10% higher at the end of the experiment. The AOC level was higher in samples with chlorine than that in samples with chloramine throughout the experiment, which suggest that chlorinated DBPs might be used by bacteria for growth. Chlorinated water samples had lower ICC than chloraminated water in the first three days, but much higher ICC at the end of the experiment. The ICC of chloraminated water tended to be lower than that of chlorinated water, which was inconsistent with a previous report that chloramine suppressed regrowth more efficiently (Gillespie et al., 2014a). We found that the initial ICC was more than 1000 cells/mL for each sample. Especially for samples subjected to chloramine treatment, cultivable microorganisms were isolated. However, many microbial species cannot yet be culturable in the laboratory due to unknown culture requirements or because the selective conditions of the culture method are too stringent for growth. An interesting finding was that the ICC of chlorinated samples exceeded that of the raw water without disinfectant in the last period of the experiment, which implied chlorinated DBPs could be absorbed by bacteria and promote bacterial regrowth. Oppositely, no matter how much chloramine was added, the ICC level of disinfected samples did not exceed that of the raw water without disinfectant. Previous studies have converged on two points that the free chlorine produces a higher level of total organic chlorine (TOCl) and bromine (TOBr) than the chloramine in the presence of bromide dose (Hua and Reckhow, 2007; Richardson et al., 2007). And that the transformation among DBP analogues in chlorination plays a significant role in the formation and decomposition of halogenated DBPs while the influences imposed by decomposition reactions of chlorinated disinfection byproducts (Cl-DBPs), and brominated disinfection byproducts (Br-DBPs) by HOCl, HOBr, chloramines, or bromamines on chloramination could be neglected (Zhu and Zhang, 2016). Hydrolysis was found to be the only pathway that could cause a significant decay in total DBP generation during chloramination. Thus, the final DBPs produced by chlorine and chloramine disinfection was different. Cl-DBPs and Br-DBPs and their decomposition products by the substitution with HOCl/ClO− and/or HOBr/BrO− or hydrolysis in chlorination might be more easily used by bacteria than those in chloramination. The long contact time might result in the formation of aromatic and nitrogenous Br-DBPs, iodoform and total organic iodine (TOI), which are relatively stable and accumulated in the presence of monochloramine, bromochloramine, bromamines, and trace levels of free chlorine in chloramination (Zhai et al., 2014). These products had cytotoxic and genotoxic which cannot be easily used by bacteria. Overall, chloramine was found to be more efficient in controlling bacteria regrowth in drinking water.

It was obvious in the bench test that the proportion of intact cells could be significantly low (less than 10%) when chlorine concentration was above 0.15 mg/L. However, intact cells took a bigger proportion in DWDS (0.13–60.25%) (Fig. S5) than in the bench test (0.05–8.12%) (Fig. 5). Since there are many factors imposing impacts on the bacterial growth in DWDS, Materials accumulated in DWDS: four elements have been defined and studied 1) the bulk water; 2) the suspended solids which are particulate in the water; 3) the pipe surface with the associated material, e.g., biofilm, extracellular polymeric substance (EPS), scaling; and 4) the loose deposits which are particulate matter accumulated in the pipes (Liu et al., 2017). Growth of scales and biofilm conglomerates coupled with sorption of water chemicals and planktonic microorganisms have been increasingly recognized as underestimated contaminant sources in aging pipe networks of DWDS (Makris et al., 2014). The bacteria in conglomerates, which are recognized as particle associated bacteria, can be flushed into the tap water due to the unsteady flow rate. The conglomerates create a micro environment for bacteria growth and protect the bacteria from disinfectants, which prompts bacteria to use AOC and to regrow. Therefore, it is the existence of solid particles, biofilm, extracellular polymeric substance (EPS), scaling and loose deposits that gave rise to a higher proportion of live cells in DWDS than in batch test when chlorine concentration was above 0.15 mg/L.

3.4. Key factors affecting AOC concentration and ICC in actual DWDS

Minimizing undesirable microbial growth in the DWDS is currently achieved by ensuring low concentration of assimilable organic carbon (AOC) and other growth-rate limiting substrates

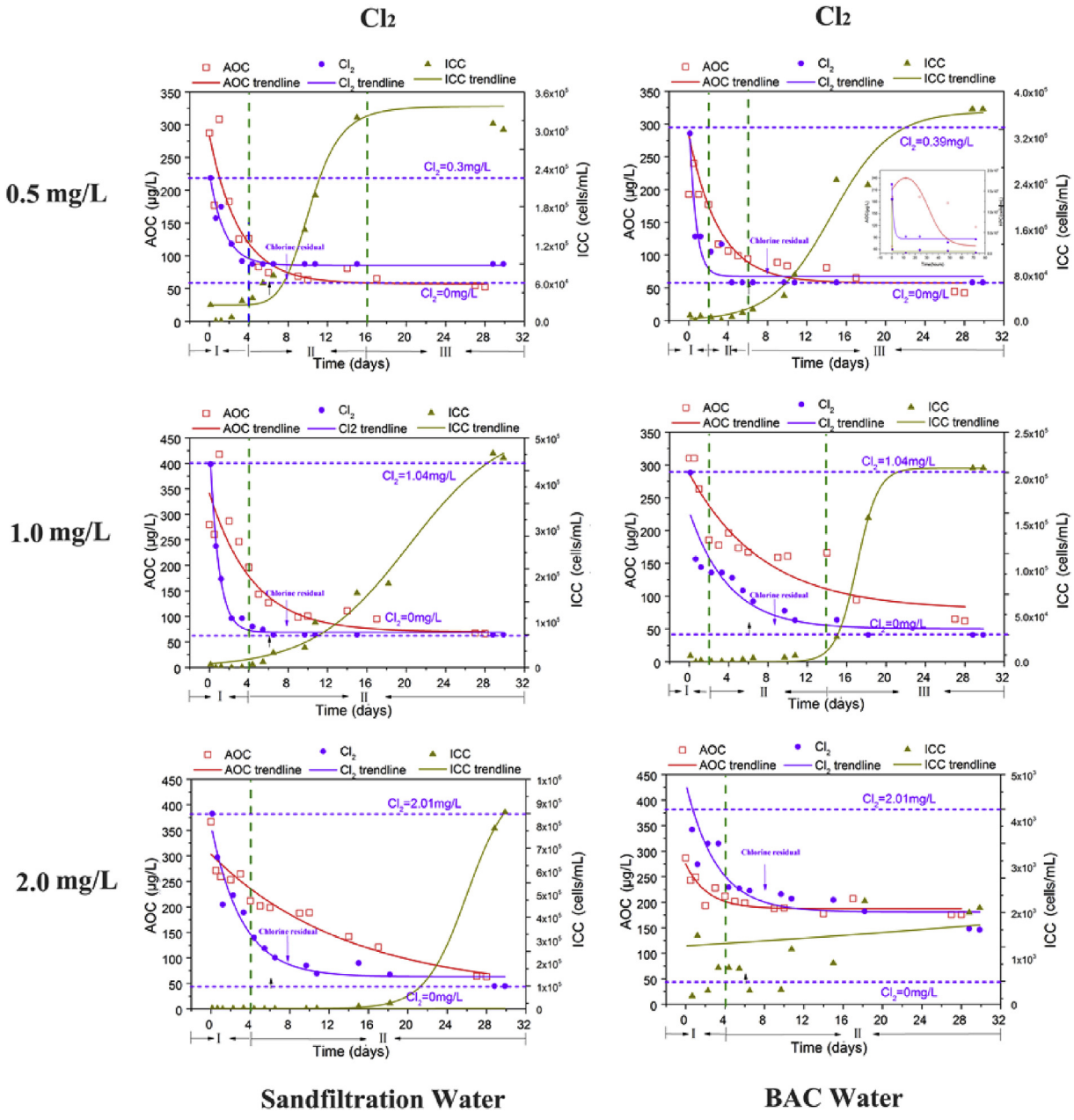

Fig. 4. Variation of biological stability of sand filtration water and BAC water with different amounts of chlorine and chloramine residuals in the bench test.

(e.g. nitrogen, and phosphorus) and/or applying residual disinfectants such as chlorine or chloramine (Bautista-de los Santos et al., 2016). In our previous study, we found that when chlorine residuals were more than 0.15 mg/L, bacteria concentrations were suppressed by chlorine concentration. The inactivation rate of bacteria by chlorine disinfectant could be equal to, or exceed the growth rate of bacteria in the samples. Conversely, for chlorine residuals less than 0.15 mg/L, the AOC tended to promote microbial growth. To limit the AOC level to less than 135 μg/L may be a reasonable guideline for DWDS. According to LeChevallier et al.'s statistical analysis method (LeChevallier et al., 1996), AOC levels greater than 135 μg/L led to high levels of HPC when free chlorine residuals were less than 0.15 mg/L in water samples. The average $HPC_{AOC>135\,\mu g/L}$ was 2.89 times higher than the average $HPC_{AOC\leq135\,\mu g/L}$ when the chlorine level was more than 0.15 mg/l, suggesting that an AOC concentration less than 135 μg/L could limit HPC concentration. However, the HPC value only partially reflects bacteria growth in a real DWDS. To complement this data, ICC and TCC were detected using flow cytometry. As shown in section 3.2, the results were consistent with previous study. ICC was suppressed by chlorine at all concentrations, especially above 0.15 mg/L. ICC had no significant relationship with AOC for all samples. However, with classification of the chlorine level, the correlation between AOC and ICC in DWDS was weakly negatively correlated ($R = -0.10$; $P < 0.10$; $n = 122$) when chlorine concentrations were above 0.15 mg/L (Fig. 3). The ICC was positively correlated to AOC level ($R = 0.36$; $P < 0.01$; $n = 118$) for samples with a chlorine level lower than 0.15 mg/L, due to the promoting effect of AOC. Further, the average $ICC_{AOC>135\,\mu g/L}$/average $ICC_{AOC\leq135\,\mu g/L}$ was 3.41, and the average $AOC_{AOC>135\,\mu g/L}$/average $AOC_{AOC\leq135\,\mu g/L}$ was 2.41, indicating

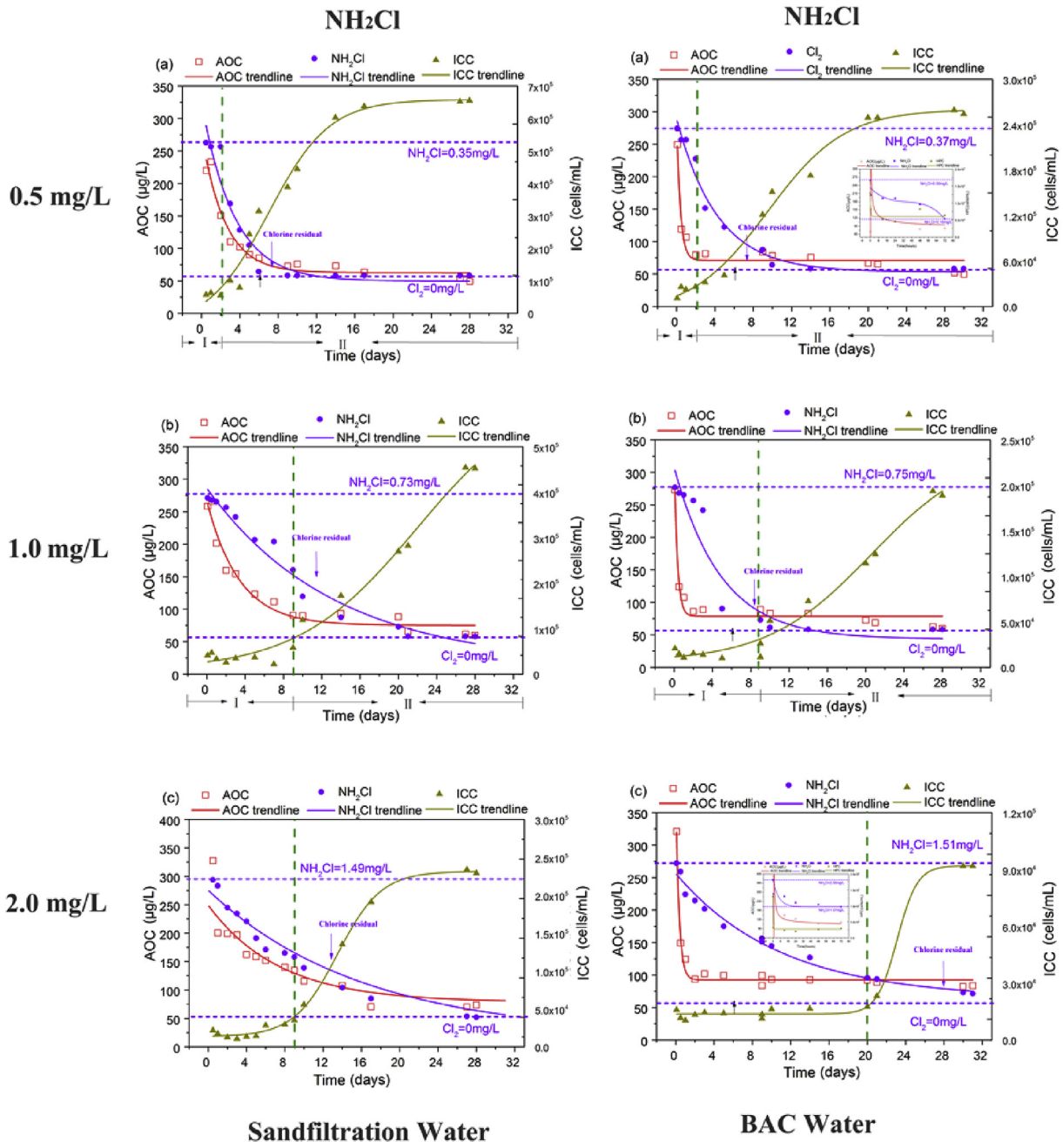

Fig. 4. (*continued*).

that controlling AOC less than 135 μg/L could also limit ICC in DWDS.

According to the statistical analysis of 240 samples in the two-year experiment presented in Table 4, AOC concentration was significantly correlated to TOC and temperature. ICC showed significant correlations with chlorine, temperature, and small size particles. Temperature is one of the most important environmental factors affecting microorganism growth (Ndiongue et al., 2005a, b; Diehl D L, 2010). The direct or indirect impact of water temperature is reflected in both physical and chemical conditions, such as water treatment process efficiency, microbial growth rate, and disinfection efficiency. Persson (Persson et al., 2007) reported that the specific respiratory activity of biofilter biomass is dependent on water temperature, and observed a crucial point between 8 °C and 13 °C for the respiratory activity of bacteria. Many studies have shown that at temperatures above 15 °C, microbial activity will increase (Ndiongue et al., 2005a, b). Fig. S6 demonstrates the annual change of ICC and AOC concentration with temperature. Statistical analysis showed that AOC concentration was negatively correlated with temperature ($R = -0.27$, $P < 0.01$, $n = 240$), which was mainly due to the lower microbial concentration in winter with low biological catabolism. However, in summer, the ICC in the DWDS was high and the AOC concentration was low. The results showed the ICC was positively correlated to temperature ($R = 0.29$, $P < 0.01$, $n = 240$). The average ICC with water temperature above 25 °C was 35.5 times higher than that under 10 °C. The ICC peaked at 31.7 °C, indicating that the water temperature was an important factor for microbial activity.

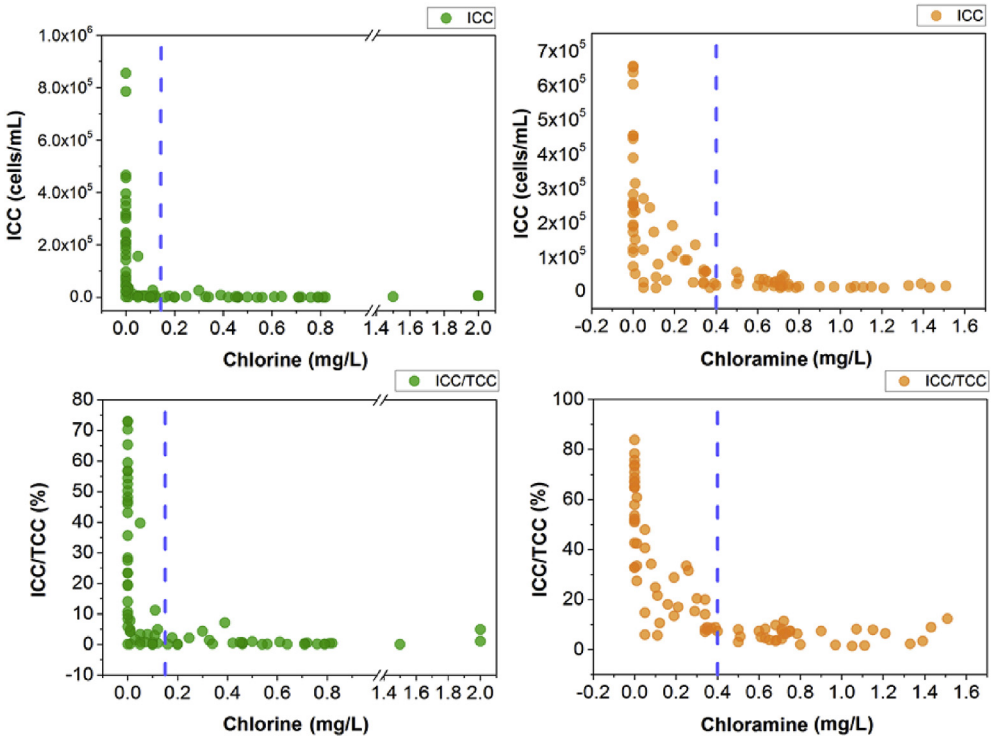

Fig. 5. Statistical analysis of orthogonal experiment results testing the effect of disinfectant concentrations on intact cells and the relative proportions of intact cells in chlorinated and chloraminated systems.

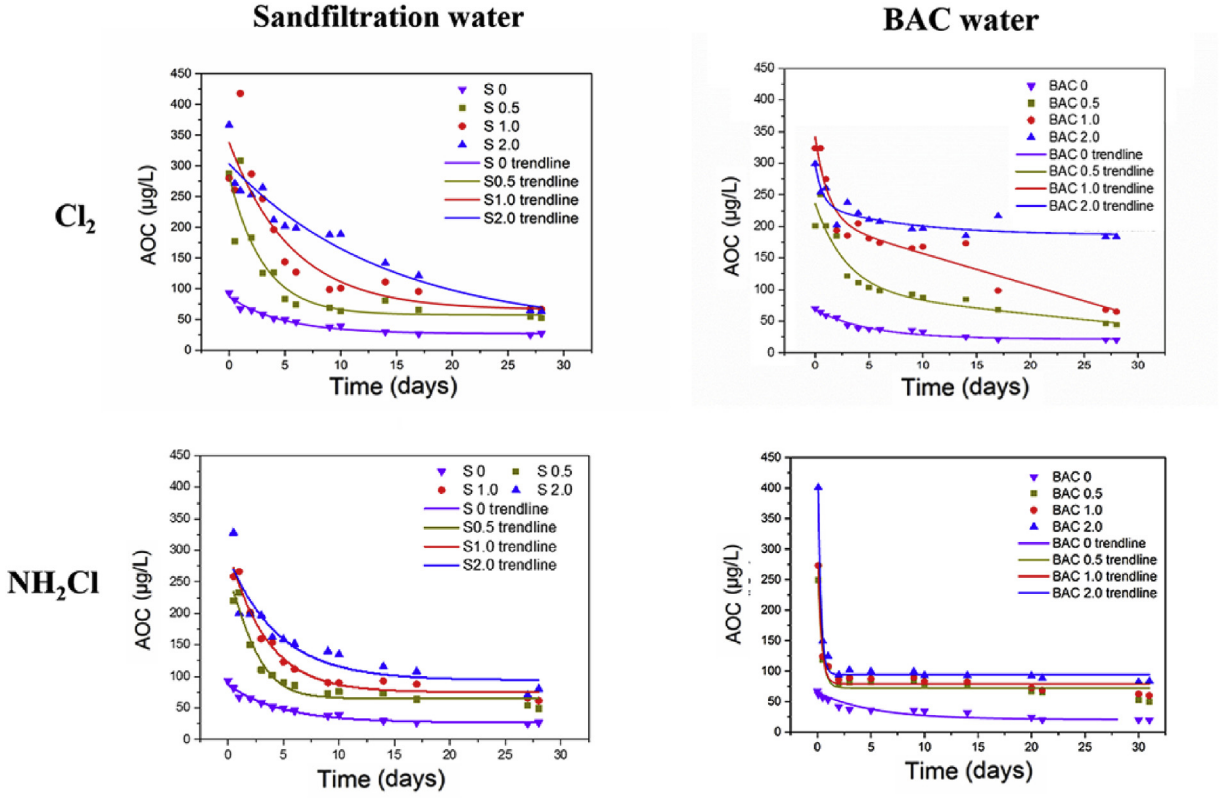

Fig. 6. Comparison of AOC variation of different water (sand filtration water and BAC water) with different dosages of disinfectant (chlorine and chloramine) during the 30-day bench test.

Particles in drinking water also greatly affected bacterial regrowth. A greater number of particles in the water increases the chance of collision of bacteria and particles. The water quality may be impacted during its distribution through piped networks due to

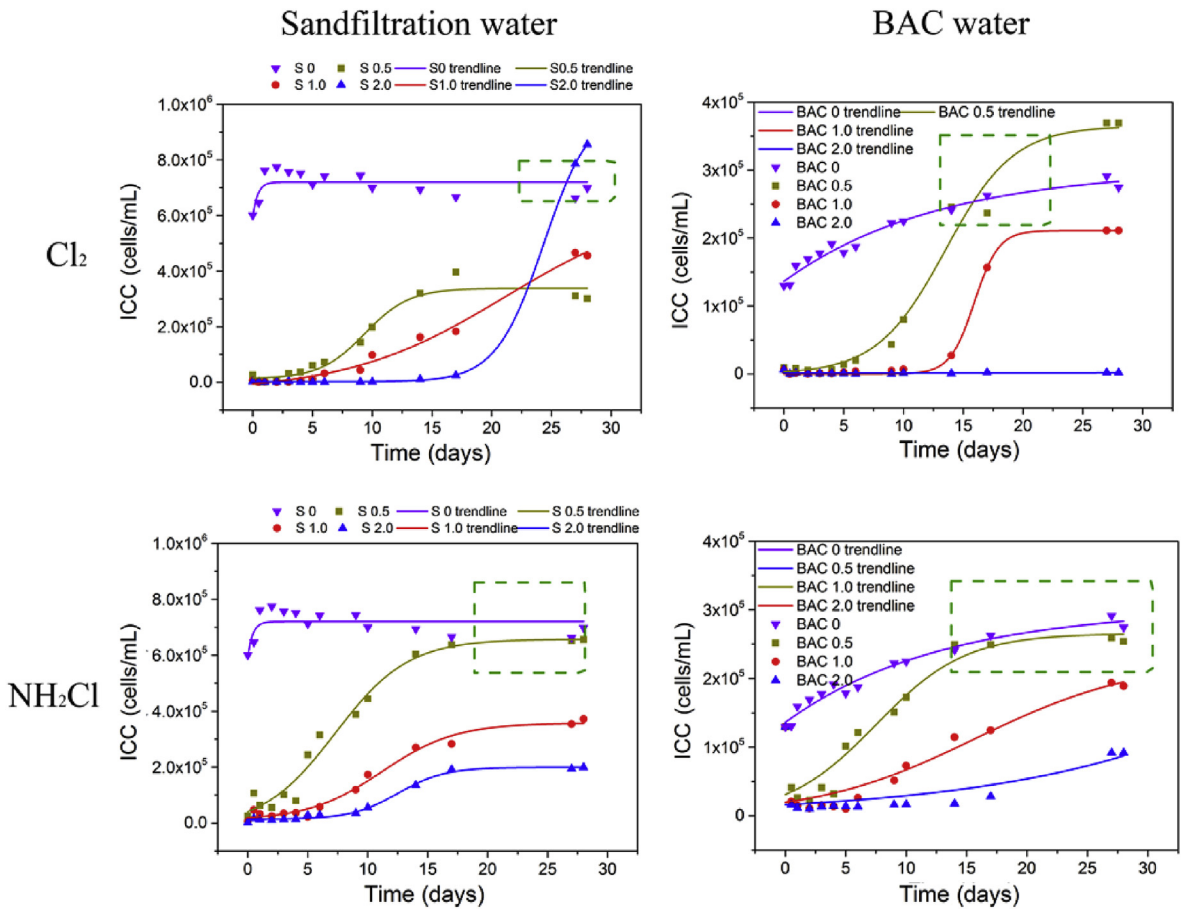

Fig. 7. Comparison of ICC variation of different water (sand filtration water and BAC water) with different dosages of disinfectant (chlorine or chloramine) for a 30-day bench test.

Table 4
Correlations of factors related to biological stability.

	AOC (μg/L)	ICC (Cells/mL)	Free chlorine (mg/L)	TOC (mg/L)	Turbidity (NTU)	Chemical Oxygen Demand COD (mg/L)	Ammonia (mg/L)	UV_{254}	Temperature (°C)	The total number of particles	Particle numbers (2~3 μm)
AOC (μg/L)	**1.00** *	−0.07	0.11	**0.20***	−0.07	−0.15	0.15	−0.13	**−0.27***	0.11	0.09
ICC (Cells/mL)	−0.07	**1.00** *	**−0.23***	0.04	0.07	0.15	0.05	−0.04	**0.29** *	0.06	**0.18***
Free chlorine (mg/L)	0.11	**−0.23** *	**1.00** *	−0.08	−0.22	−0.23	−0.16	0.04	**−0.43***	0.13	**0.21***
TOC (mg/L)	**0.20** *	0.04	−0.08	**1.00** *	−0.09	−0.14	−0.07	**−0.20***	**−0.18***	**−0.20***	−0.15
Turbidity (NTU)	−0.07	0.07	**−0.22***	−0.09	**1.00** *	**0.34***	0.07	0.05	−0.03	−0.01	−0.04
COD_{Mn} (mg/L)	−0.15	0.15	−0.23	−0.14	**0.34** *	**1.00** *	−0.03	**0.22***	**0.25** *	−0.01	−0.06
Ammonia (mg/L)	0.15	0.05	−0.16	−0.07	0.07	−0.03	**1.00** *	**−0.22** *	**0.25** *	−0.10	−0.14
UV_{254}	−0.13	−0.04	0.04	−0.20	0.05	**0.22** *	**−0.22***	**1.00***	0.04	0.14	**0.22***
Temperature (°C)	**−0.27** *	**0.29***	**−0.43***	**−0.18** *	−0.03	**0.25** *	**0.25***	0.04	**1.00** *	−0.05	**−0.22** *
The total number of particles	0.11	0.06	0.13	**−0.20** *	−0.01	−0.01	−0.10	0.14	−0.05	**1.00***	**0.88** *
Particle numbers (2~3 μm)	0.09	**0.18***	**0.21** *	−0.15	−0.04	−0.06	−0.14	**0.22***	**−0.22***	**0.88***	**1.00***

Numbers in bold and with a * indicate a significant correlation ($p < 0.05$) .

the processes such as pipe material release, biofilm formation and detachment, accumulation and resuspension of loose deposits in real DWDS, which can facilitate particle associated bacteria (Makris et al., 2014; Liu et al., 2017). Particles provide an environment for bacterial growth, thus promoting regrowth and reproduction of bacteria in the water. The distribution of different particle sizes is summarized in Fig. S7. The 2–5 μm particles accounted for the largest proportion, 75.69%–85.93%. Recent studies of the characteristics of particles in distribution systems (Vreeburg et al., 2008) and microbial ecology (Douterelo et al., 2013) revealed that particles act as bioreactors, alter sediment accumulation and release (Fabris et al., 2015). There was a positive correlation between the total particles in water and ICC (Fig. S8), but the linear fitting was only significant for small particles (2–5 μm) and ICC ($R = 0.18$, $P < 0.05$, $n = 240$) (Table 4), suggesting particles that are small and close to bacteria in size are easily adsorbed by bacteria.

Overall, the use of chloramine disinfectant, and the control of AOC concentration and the number of particles may be the effective way to limit bacterial regrowth.

4. Conclusion

The paper investigated the worldwide problem of unwanted or excessive bacterial growth in DWDS, which can cause deterioration of microbial water quality during storage and transport. AOC concentration, TCC, and ICC were measured as biological evaluation indexes in a DWDS in south China from January 2014 to December 2015. The results indicated that when chlorine was more than 0.15 mg/L, ICC was highly suppressed. ICC was significantly correlated to AOC concentration for chlorine levels less than 0.15 mg/L. The different free chlorine level imposed different impacts on AOC and intact cells. We designed an orthogonal bench test with different dosages (0, 0.5.1.0, or 2.0 mg/L) of chlorine of chloramine to more carefully evaluate the disinfectant effect on interactions between nutrient availability and bacteria. The results indicated that chloramine tended to result in low AOC level and low proportions of intact cells than chlorine. For both chlorinated and chloraminated systems, free chlorine concentrations above 0.15 mg/L and chloramine concentrations above 0.4 mg/L were associated with relatively low proportions of intact cells and cultivable bacteria. Low disinfectant levels correlated with substantially high percentages of intact cells. Compared with chlorine, chloramine treatment resulted in a lower level of AOC concentration and lower ICC. This is likely because chlorinated DBPs can be absorbed by bacteria more easily than chloramine byproducts. Thus, chloramine was more efficient in controlling bacteria regrowth than chlorine in drinking water. Statistical analysis was conducted to determine the best strategies to control bacterial regrowth in DWDS. The results showed that ICC was limited when AOC was less than 135 μg/L. Simultaneously, temperature and small-size particles imposed positive effects on ICC. Thus, the use of chloramine and the restriction of AOC concentration and small size particle number may be effective strategies to limit bacterial regrowth in DWDS.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.03.056.

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