Impact of AOC and chlorine residual on regrowth of microbes in a model distribution system receiving UV-treated potable water

Wenjun Sun, Wenjun Liu, Lifeng Cui and Leibin Liu

ABSTRACT

The microbial ecology of distribution systems is complex and is influenced by a number of factors. This study assessed whether or not during drinking water treatments it is important to add a chemical disinfectant after ultraviolet (UV) disinfection in order to control the regrowth of microbes in distribution systems. Results showed that low UV doses did not impact assimilable organic carbon (AOC) significantly. No definite relationship between UV dose and steady-state biofilm heterotrophic (HPC) bacteria was evident. AOC and chlorine residual are two important factors affecting biofilm and suspended HPC growth in distribution systems. When the AOC concentration was 0, 5 and 10 μg AC/L (μg acetate-C/L), a chlorine residual was not necessary to keep the fluid HPC under 500 CFU/mL. When the AOC concentration reached between 100 and 200 μg AC/L, the regrowth of microbes in the model distribution system necessitated a chlorine residual of over 0.5 mg/L. At lower AOC levels (0, 10–20 μg AC/L), the AOC appeared to have a strong effect on controlling biofilm formation and suspended HPC concentration. In contrast, at higher AOC levels (50, 100 and 200 μg AC/L), a chlorine residual was indispensable when controlling the regrowth of HPC in the distribution systems. AOC and the required chlorine residual in these systems were interrelated.

Key words | assimilable organic carbon (AOC), chlorine residual, model distribution system, ultraviolet (UV) disinfection

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INTRODUCTION

An overarching goal for providing safe water is to disinfect water affordably and robustly for protection from traditional and emerging pathogens without creating more problems by the disinfection process itself (Shannon *et al.* 2008). Ultraviolet (UV) disinfection became a common technique for disinfecting drinking water because it was found effective against most microbes, almost without any formation of disinfection by-products (Chang *et al.* 1985; Craik *et al.* 2000; Zimmer *et al.* 2003; Hijnen *et al.* 2006); however, UV disinfection has no long-term residual effect in a distribution system. Potential pathogens do have the ability to survive, reproduce and to form biofilms under potable water conditions (Schwartz *et al.* 1998). Thus, most waterworks that have applied UV as the primary disinfectant usually add chemical disinfectants such as chlorine, chloramines or chlorine dioxide after UV treatment to sustain a disinfection effect in the distribution systems and to prevent overall regrowth of microbes.

There is a growing debate over the value of disinfectant residuals being required in distribution systems. Two schools of thought exist on the maintenance of disinfectant residuals. The North American school favours the maintenance of high residuals throughout the distribution system to maintain the regrowth of microbes in the distribution system. Microbiological safety within the context of regrowth is defined here as a steady-state suspended heterotrophic plate count (HPC) of less than 500 CFU/mL. Microbiological safety from the perspective of target

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pathogen reduction as measured by faecal coliform indicator counts has been addressed in other studies of UV disinfection (Sun & Liu 2009). The European school limits the use of disinfectant residuals in distribution systems and in some countries residuals are avoided altogether (Prevost *et al*. 2005). The complexity of distribution line safety is related to water quality parameters and pipe conditions as well as other factors, and raises questions of under what conditions and how much chemical disinfectant should be added after UV. These questions are difficult to answer because they involve microbial characteristics and processes in distribution systems that are very complex. Generally speaking, assimilable organic carbon (AOC) and chlorine residual are two of the important factors that impact regrowth of microbes in distribution systems, and these two parameters are controllable by selection of the water treatment process. This study focused mainly on the impact of these two factors on HPC levels in model distribution systems.

Related studies by Pozos (2004) and Giese (2002) used two parallel model distribution systems to investigate the impact of UV disinfection on water distribution system biofilms, as well as their microbial community compositions. Results demonstrated that UV disinfection did not change community composition and biofilm densities in the model distribution system. Lehtola *et al.* (2003) found that a low-pressure UV system could not significantly impact AOC and phosphorus, the main nutrients for biofilm in distribution systems. Momba *et al*. (1998) studied the impact of UV disinfection on biofilm cell densities and found that UV disinfection alone has no obvious difference on steady-state biofilm densities compared with the non-disinfected system. The results are in agreement with this study. A few studies reported on water distribution system biofilms using chemical disinfectants after UV. Dykstra *et al.* (2007) evaluated the synergistic effects of UV and secondary disinfectants on water quality in a model distribution system. The results suggested that UV treatment prior to chemical disinfection enhances microbial control in distribution systems, and that the use of UV can be effective in reducing the residual disinfectant concentration necessary for microbial control, and thereby reducing the potential formation of a disinfectant by-product in the system. Helbling & Vanbriesen (2008) compared the efficacy of chlorine (Cl_2) to monochloramine (NH_2Cl) with and without post-treatment with UV for heterotrophic bacteria (HPC) control. Data presented suggest that treatment with Cl_2 alone was the most effective disinfection strategy against suspended HPC in influent and effluent samples, and also against attached HPC. Chlorine with or without post-UV treatment was more effective than monochloramine in removing suspended and attached HPC.

The goal of this study was to estimate the role of AOC and chlorine residual in controlling regrowth of microbes in model distribution systems following UV application. Specific objectives are aimed at characterizing: (1) the impact of UV on AOC and biostability of model distribution systems; (2) the independent impact of AOC and chlorine residual on the regrowth of microbes in model distribution systems; and (3) the synergistic effects of AOC and chlorine residual on the regrowth of microbes in model distribution systems.

MATERIALS AND METHODS

UV disinfection system and water quality

The current study used the ground water supplied to Tsinghua University as the influent to the UV reactor. Table 1 shows the characterized parameters of the influent.

The influent was irradiated with a low-pressure UV lamp (254 nm) in an annular UV reactor. The delivered UV dose was determined using a bioassay of male-specific coliphage

Table 1 | Water quality of influents

Parameter	Value
pH	7.2–7.8
UV absorbance at 254 nm (cm^{-1})	0.01–0.02
UV transmittance[a]	95–98%
Turbidity (NTU)	0.1–0.2
TOC (mg L^{-1})	0.6–0.8
AOC (μg acetate-C equivalents (μg AC)/L)	64 ± 7
Temperature (°C)	15–17

[a]UV transmittance is the measured value between a known UV light source (@ 254 nm) and what is measured by a calibrated detector through a 1 cm thick sample of the water to be treated.

Table 2 | Parameters of UV reactor

Lamp power (W)	Lamp efficiency (%)	Reactor length (cm)	Lamp number	The MS2 bioassay dose (mJ/cm^2)
43	30	49.5	1	43

(MS2, ATCC #15597-B1) and a collimated beam test, as described by Bolton & Linden (2003) and Braunstein *et al.* (1996). No decrease in dose was observed over the duration of the work, indicating no significant ageing of UV lamps. The main parameters of the UV reactor are indicated in Table 2.

The experimental system used in this study is shown in Figure 1. Water was pumped by a centrifugal pump through a ball valve to the UV disinfection unit from a storage tank. Sodium hypochlorite was added after UV disinfection. Flow passed into the model distribution system (a biofilm annular reactor, BAR) through a static mixer. For different experimental runs, the AOC and chlorine residual were adjusted to certain values. The chlorine residual was measured twice per day. The chlorine was continuously added into the BAR at a certain rate and measured twice per day to keep a constant residual. AOC concentration was chosen at 0, 5, 10, 20, 50, 100 and 200 μg AC/L. Lower AOC water (5, 10, 20 and 50 μg AC/L) was obtained by mixing ground water from Tsinghua University with Ultrapure water, and higher AOC was attained by proportionally adding sodium acetate into the ground water. A brief description of this experiment was described by Sun *et al.* (2009). Ultrapure water was used as the 0 control for AOC testing.

Model distribution systems

BARs were used to simulate a potable water distribution system. These reactors were used in numerous studies investigating biofilm growth in both pure cultures and mixed cultures under potable water conditions (Butterfield *et al.* 2002). The reactors, consisting of an outer stationary cylinder and an inner rotating cylinder, were operated to simulate the shear in a 0.40 m pipe with a flow of 1.0 m/s (BioSurface Technologies Corp. 1998). Each reactor contained 20 stainless steel coupons. The reactors were shielded from light to preclude any photo reactivation.

The flow rate through the BARs was 0.7 mL/min yielding a hydraulic retention time (HRT) of 24 h. The 24-h HRT was chosen to represent conditions in distribution systems (Camper 1995).

Estimation of biostability within model distribution systems

HPC and AOC were used as the main parameters to characterize the biostability of the model distribution system. Biofilm and bulk fluid samples were collected, separated, homogenized and analysed for HPCs using the method by Camper (1995). HPCs were enumerated on R2A agar, and incubated for 7 days at 25 °C. AOC was analysed via a modification (Miettinen *et al.* 1999; Liu *et al.* 2002) of the Van der Kooij method (Van der Kooij *et al.* 1982). The maximum growth of *Pseudomonas fluorescens* P17 (ATCC 49642) and *Spirillum* sp. strain NOX (ATCC 49643) was converted into the amount of AOC. Yield factors for the test strains were taken from standardization experiments and were 5×10^6–4×10^7 colony-forming units (CFU)/μg acetate for P17 and 2×10^7–1×10^8 CFU/μg acetate for NOX. Modifications included the addition of inorganic nutrients to ensure that only the AOC content restricted microbial growth, as suggested by Miettinen *et al.* (1999). In chlorinated water samples, residual chlorine was removed by the addition of 0.02 M sodium thiosulphate.

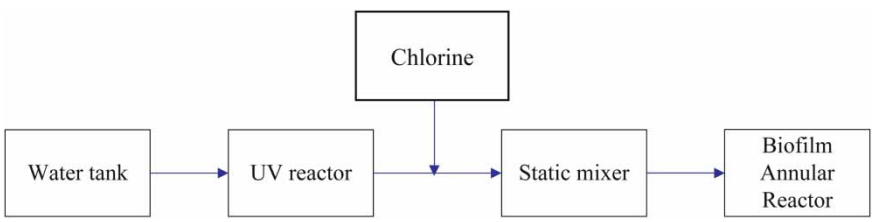

Figure 1 | Diagram of the experimental system.

Data analysis

Data analysis on the samples method involved two stages. First, a descriptive analysis was conducted based on observations on HPC distribution as a function of various parameters (UV dose, AOC and chlorine residual). Results obtained were used to guide the orientation of the second stage, which consisted of modelling simultaneous impacts of AOC and chlorine residual on HPC levels. The model is based on a multilevel analysis.

Results from the experiments were analysed using an analysis of variance (ANOVA) test and *t*-tests. Unless otherwise noted, the level of significance (α) for the values was less than 5%. For analytical purposes, all experimental factors were treated as qualitative variables.

RESULTS AND DISCUSSION

Impact of UV on AOC

Degradation of organic compounds by UV could potentially influence the availability of AOC in the water (Kulovaara 1996). UV irradiation could produce a minor effect on the content of organic acids which are potential microbial substrates and represent the smallest size in the molecular size distribution (Lehtola *et al.* 2003). In the Tsinghua source water used in this study, UV doses of 0–150 mJ/cm^2 did not impact AOC significantly (Figure 2).

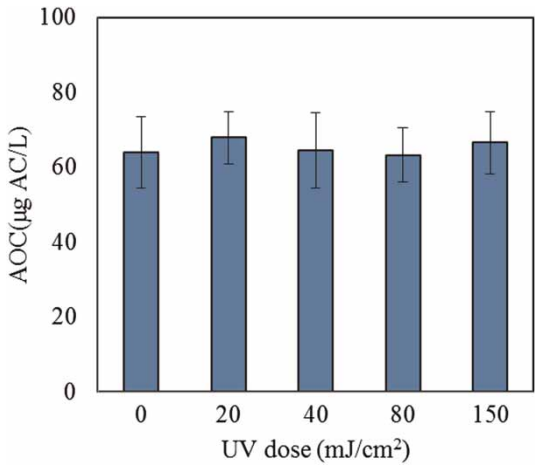

Figure 2 | Impact of UV on AOC concentration.

Impact of UV doses on biofilm in the model distribution system

A true biofilm steady state can never be achieved since selection is continually occurring, and slight changes in environmental conditions may favour the growth of different organisms (Boe-Hansen *et al.* 2002). The time required to reach a pseudo-steady state depends on environmental conditions and methods used to quantify the biofilm. LeChevallier (1990) reported that a stable biofilm population could develop on a pipe surface after two weeks of growth. Hallam *et al.* (2001) suggested that 21 days could be representative of the time necessary to grow a biofilm that had reached steady state. In the present study, a steady state was assumed only when relatively small changes in HPC numbers were measured in consecutive samplings. Under current experiment conditions, BAR systems may reach a steady state from 4 to 14 days, and thus 20 days was selected to represent an experimental cycle.

The four BAR systems were operated using different UV doses of 0, 10, 40 and 80 mJ/cm^2 and HPC monitored over time (Figure 3). Although UV was necessary to reduce the level of influent HPC to near-detection limit, no definite relationship between UV dose and biofilms HPC densities in the steady state was observed. Weak correlations between UV doses and microbial densities in the steady state could be explained by: the absence of a chlorine residual in the feed waters used in the distribution systems; variation in the HPC in feed water; and the possibility that steady state might not have been fully achieved in the case of high UV

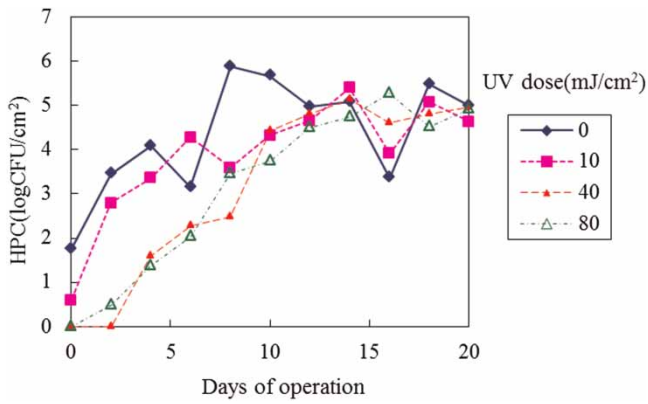

Figure 3 | Impact of AOC on biofilm HPC in the distribution system.

doses even after 20 days. At the initial stage of BAR operation, a lower influent under high UV dose obtained less biofilms on the coupons. When the system reached a steady state, the impact of microbial concentration of the influent was not as significant as perhaps AOC (see next section), which could support the growth of biofilm in the BAR and could not be changed by the UV doses used (see previous section).

The impact of AOC on the biofilm formation of BAR

Coupon biofilm densities of HPC for systems with different AOC concentrations are presented in Figure 4. All influents to the BARs were treated with a UV dose of 40 mJ/cm^2. The observation that the coupon biofilm HPC level in water with higher AOC required a shorter time to reach steady state compared with the biofilm HPCs in water with lower AOC is in agreement with other studies (Van der Kooij *et al.* 1995). The steady-state coupon biofilm densities of HPC for 0 μg AC/L AOC was 2.57 ± 0.67 log/cm^2, and increased with higher AOC levels.

Biofilm HPC numbers due to AOC differences ranged approximately from 1.8 (AOC = 0) to 5.5 log CFU/cm^2 (AOC = 100 μg AC/L) (Figure 5). These observations are in agreement with previous studies (Van der Kooij *et al.* 1995). Data presented by Van der Kooij *et al.* (1995) showed that the addition of acetate (10 μg AC/L) in drinking water with low levels of AOC (3.2 μg AC/L) could cause a strong increase (greater than 1 log CFU/cm^2) in the maximum HPC count on R2A. For UV disinfection alone, when AOC was controlled under 10 μg AC/L, the average

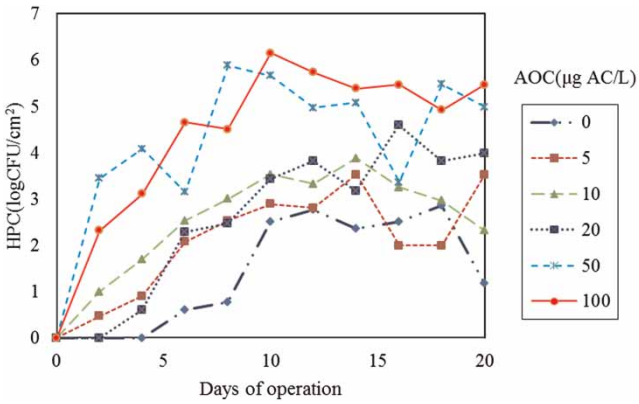

Figure 4 | Impact of AOC on the biofilm in the distribution system.

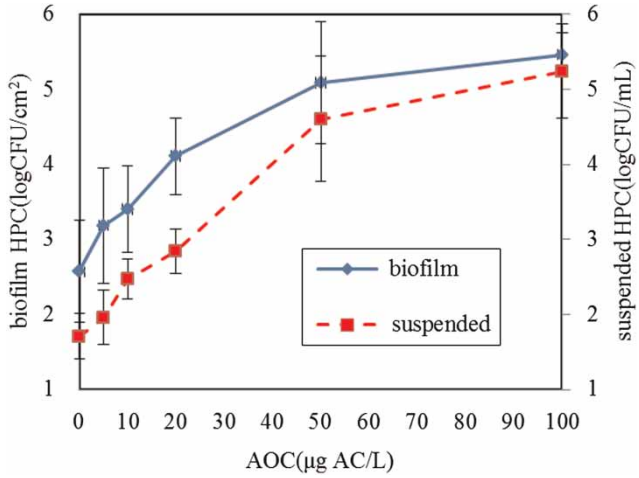

Figure 5 | Relationship of AOC and average steady-state HPC.

bulk fluid HPC could be kept controlled under 500 CFU/mL without a residual disinfectant. Thus, AOC was an effective controlling parameter for biofilm growth, but also a good indicator to estimate whether or not it is important to add chlorine into water after UV. Similarly, AOC could reflect the positive relationship of biofilm and suspended HPC. For higher AOC levels with more biofilm HPCs at the biofilm steady state, the difference between biofilm and suspended HPC values became smaller, suggesting that biofilm might have released microbes into the fluid under those conditions.

Relationship between HPC, AOC and chlorine residual

In Figure 6, the boundary dividing the grey section and the white section represents the combination of AOC and chlorine that together produced a suspended HPC concentration of 500 CFU/mL. The white section of the grid represents the AOC and chlorine combination giving suspended HPC less than 500 CFU/mL. The grey section reflects combinations when suspended HPC is more than 500 CFU/mL. When AOC was less than 15 μg AC/L, a chlorine residual was not required to maintain fluid HPC under 500 CFU/mL. However, when AOC was 20 and 50 μg AC/L, a chlorine residual of 0.04 and 0.36 mg/L, respectively, was required; and when AOC reached 100 and 200 μg AC/L, a chlorine residual of over 0.5 mg/L was required. For 100 and 200 μg AC/L AOC, there was no evident difference in demands for chlorine residual in the model distribution

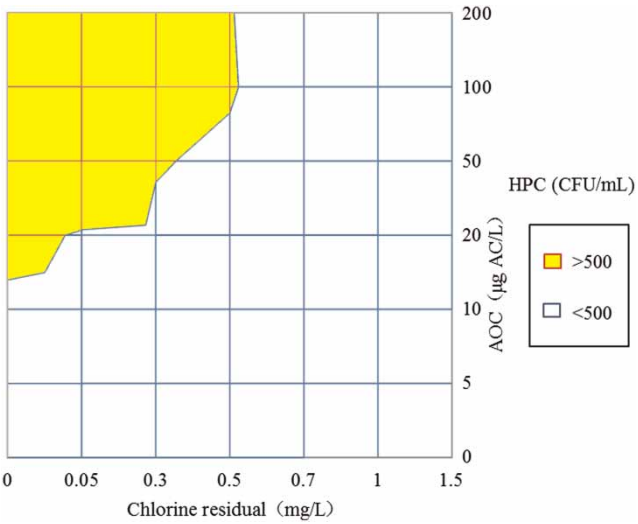

Figure 6 | Impact of AOC and chlorine residual on the suspended HPC of the distribution system.

systems. Low AOC appeared to have a strong impact on the control of suspended HPC. In contrast, at high AOC levels (100 and 200 μg AC/L), the AOC seemed to have more limited effects. Under this condition, a chlorine residual seemed to be indispensable in controlling suspended HPC.

The suspended HPC was strongly dependent on both AOC and chlorine residual. Free chlorine residual was required to reduce HPC levels, and for a given HPC level, the required chlorine residual decreased as AOC decreased. Chlorine residual might be more effective in controlling HPC with higher AOC levels. The system with higher AOC and lower chlorine residual showed a higher suspended HPC concentration.

From the results of reproducible double factor variance analysis (Table 3), AOC and chlorine residual manifested interrelated effects on suspended HPC. For chlorine residual and AOC, $F > F$ crit and p-value <0.05, indicating that both chlorine residual and AOC show significant impacts on the suspended HPC. F error $> F$ crit suggests that there is a synergistic effect between AOC and chlorine residual when controlling microbiological regrowth in drinking water distribution systems.

After achieving a chlorine residual in the effluent of reactors, biofilm HPC numbers dropped further as free chlorine residual increased. Hallam *et al.* (2001) suggested an exponential function to describe the variation of biofilm formation potential vis-à-vis free chlorine concentration. It was found that when the suspended HPC was expressed as log CFU/mL, an exponential regression could yield generally greater coefficients of determination than in linear regression. In the current experiment, results showed a very strong dependence of suspended HPC values on the chlorine residual since it combined the proposed exponential relationship with Equation (1) to develop a model that takes into account both AOC and chlorine residual (CR). From the results of two factor regression analysis (Table 4), the following formula is obtained:

$$\text{Log HPC} = 2.91 + 0.0068\text{AOC} - 2.20\text{CR} \quad (1)$$

The final suspended HPC concentration in the distribution system can be described as:

$$N = N_{\text{influent}} + N_{\text{detach}} + N_{\text{growth}} - N_{\text{disinfection}} \quad (2)$$

In Equation (2): N is the suspended HPC in the distribution system; N_{influent} is the influent HPC in the distribution system; N_{detach} is the HPC detached from the biofilm in the distribution system; N_{growth} is the growth of suspended HPC; $N_{\text{disinfection}}$ is the chlorination inactivation of suspended HPC.

In this equation, N_{growth} is related to AOC, water temperature (T), N_{influent}, retention time in the distribution

Table 3 | Results of correlation of HPC with AOC and chlorine residual

Differential source	SS	df	MS	*F*	*P*-value	*F* crit
Chlorine residual	1.81×10^{12}	6.00	3.01×10^{11}	23.85	3.23×10^{-17}	2.19
AOC	7.42×10^{11}	6.00	1.24×10^{11}	9.79	1.94×10^{-8}	2.19
Error	2.65×10^{12}	36.00	7.36×10^{10}	5.82	2×10^{-12}	1.54
Total	1.24×10^{12}	98.00	1.26×10^{10}			

Table 4 | Results of two factor regression analysis

	Coefficients	Standard error	t Stat	P-value	Lower 95.0%	Upper 95.0%
Intercept	2.91	0.13	21.91	5.54×10^{-26}	2.64	3.18
AOC	0.0067	0.0011	5.96	3.33×10^{-7}	0.0045	0.0090
CR	−2.20	0.15	−14.36	1.31×10^{-18}	−2.51	−1.90

line (t) and other factors:

$$N_{\text{growth}} = f(\text{AOC}, T, N_0, t \ldots) \quad (3)$$

$N_{\text{disinfection}}$ is related to (chlorine residual, water temperature (T), N_{influent}, retention time in the distribution line (t) and other factors:

$$N_{\text{disinfection}} = f(\text{CR}, T, N_0, t \ldots) \quad (4)$$

If other influencing factors are fixed, such as water temperature, N_{influent}, retention time and if detachment of biofilm is excluded, then Equation (2) can be described as:

$$N = k_1 + k_2 f(\text{AOC}) + k_3 f(\text{CR}) \quad (5)$$

In Equation (5), k_1, k_2, k_3 are the constants related to water qualities and distribution conditions.

By comparison with Equation (1), k_1, k_2 and k_3 can be assigned for the specific water quality and conditions of the model distribution system in this study. From the calculation results (Table 4), all t Stat values were above $t_{0.025,48} = 2.31$, so the degree of fit was high. From the above analysis, for the fixed water quality and model distribution system condition parameters, the suspended HPC in distribution systems can be described by:

$$N = 10^{k_1 + k_2\text{AOC} + K_3\text{CR}} \quad (6)$$

In this study, the high variability of HPC levels (Figures 3 and 4) suggests that factors potentially influencing HPC occurrence in the distribution system could be interrelated. For conditions with high AOC levels, for example, several phenomena may arise simultaneously: development of biofilm in the distribution system, release of bacteria from the biofilm, increase of organic matter from the biofilm, incremented turbidity, increased chlorine demand, and decreased free residual chlorine levels. Robust multivariate approaches for data analysis must be used to identify simultaneous effects of the above-mentioned variables and to develop models predicting HPC occurrence. For systems using UV disinfection, analysis of two of the most important factors (AOC and chlorine residual) can provide useful background information for understanding the biological pollutant issue in distribution systems.

Overall, microbial ecology of a distribution system is complex. It is related to a number of factors, such as temperature, retention time, pipe material, and flow pattern, among other factors. In this study, we fixed these parameters and mainly considered the impact of AOC and chlorine residual on biofilm and suspended HPC. The interrelated effect of all these factors would require further study.

From a practical perspective, there is the general realization that no individual disinfectant can solve all of the microbial and by-products problems and that a single disinfectant cannot meet most drinking water systems' needs. For example, adenovirus has a higher resistance to UV, but is sensitive to chlorine. *Cryptosporidium* oocysts, on the other hand, are resistant at any practical dose of chlorine, but are very sensitive to UV. Additionally, a certain level of chlorine residual in the distribution system is often considered indispensable for controlling the risk of external microbial contamination (e.g. from reduced pressure in the distribution lines). Multiple barrier disinfection paradigms such as UV combined with chlorine or chloramine are recommended for the safe disinfection and delivery of drinking water through distribution lines that are long and potentially compromised in integrity and/or pressure maintenance.

Although this study has shown that at low AOC levels, the HPC levels and biofilms can be kept under control without added chlorine following primary UV disinfection, more holistic thinking related to the sensitivities of different pathogens to different disinfectants and the potential need to address the integrity of distribution systems and/or

constancy of pressure in the distribution system, argues for us to evaluate the need and/or merits of a multiple-barrier protection strategy that includes a multiple-disinfectant strategy.

CONCLUSIONS

1. For UV doses less than 150 mJ/cm^2, UV did not significantly impact AOC. Although UV was necessary to reduce the influent HPC level to near the detection limit, no definite relationship between UV doses and steady-state biofilm HPCs was evident. Biofilm and suspended HPC in the model distribution system had a weak relationship to the influent of HPC in the disinfected water. Water quality conditions might have more evident effects on the microbial regrowth in the model distribution system.
2. AOC and chlorine residual are two important factors that significantly impact biofilm growth in the model distribution system. For the practice of UV disinfection alone, AOC impacted biofilm concentrations when AOC was low. Chlorine residual was shown to have a beneficial effect on HPC control when AOC was high.
3. AOC was an effective parameter in determining whether or not the addition of a chlorine residual was needed in model distribution systems when managing regrowth of microbes. When AOC concentration was under 10 μg AC/L, no chlorine residual was needed to keep the suspended HPC under 500 CFU/mL; however, when the AOC concentration was at 20 μg AC/L, a chlorine residual of 0.04 mg/L was required. When the AOC level reached 100 and 200 μg AC/L, a chlorine residual of over 0.5 mg/L was needed. At low AOC levels (<10 μg AC/L), AOC appeared to have a strong impact on controlling the biofilm. In contrast, at high AOC levels (50, 100 and 200 μg AC/L), AOC seemed to have a more limited effect. Compared with AOC, a chlorine residual seemed to be more effective in controlling biofilm formation.
4. The use of robust multivariate approaches for data analysis must be considered when identifying simultaneous effects of variables and during the development of models that could be used to predict HPC occurrence. For a fixed water quality and set of operational parameters for a model distribution system, the suspended HPC in the model distribution system can be described by:

$$N = 10^{k_1 + k_2 \mathrm{AOC} + K_3 \mathrm{CR}}$$

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