Formation of disinfection by-products during the monochloramination of co-existing *Microcystis aeruginosa* and *Cyclops* metabolites

Biyao Song, Xingbin Sun, Shusong Zhang, Yifeng Jiang and Jiameng Liu

ABSTRACT

Microcystis aeruginosa and *Cyclops* exist together in their natural states. The formation of carbonaceous disinfection by-products (C-DBPs), including trihalomethanes, haloacetic acids, chloral hydrate (CH), and haloketones, as well as nitrogenous DBPs, including trichloronitromethane (TCNM), and haloacetonitriles, was investigated with respect to co-existing *Microcystis aeruginosa* and *Cyclops* metabolites under different conditions. The reaction conditions (monochloramine dosage and reaction time) and water quality conditions (pH, temperature, Cl/N, and *Microcystis aeruginosa* density) were evaluated. The formation of 1,1,1-trichloro-2-propanone (1,1,1-TCP) and TCNM followed an increasing and then decreasing pattern with increased monochloramine dosage and prolonged reaction time. The formation of C-DBPs (e.g., TCM, CH, DCAA, and TCAA) increased with increasing monochloramine dosage and reaction time. The formation of CH, dichloroacetonitrile, 1,1-DCP, and 1,1,1-TCP increased first and then decreased. The formation of TCM increased with increasing pH value and temperature. Additionally, the Cl/N mass ratio affected the formation of DBPs, and as a whole, a lower Cl/N ratio led to a decrease in the concentrations of the five most common DBPs. When the density of *Microcystis aeruginosa* was 10^9 count/L, the formation of the tested DBPs reached a minimum.

Key words | *Cyclops*, disinfection by-products, *Microcystis aeruginosa*, monochloramination

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INTRODUCTION

Algae have come to the attention of the drinking water industry as a result of the continuing eutrophication of surface water supplies. In China, algal blooms have impacted several major water systems (e.g., Taihu Lake, Chaohu Lake, and Dianchi Lake) in recent years (China Daily 2007). *Microcystis aeruginosa* is a type of blue-green algae that is distributed all over the world and is present in high quantities and with a high frequency of occurrence in most of the eutrophic water bodies in China. During the period of the outbreak of *Microcystis aeruginosa* in summer, the monitoring results showed that the number of *Microcystis aeruginosa* could reach 10^9 count/L (Zhang *et al.* 2014). Algae and algal metabolites greatly impact the treatment of potable water by causing problems such as poor settling, filter clogging and breakthrough of sand filters by small algae, producing unpleasant tastes and odours, in addition to toxins, and increasing the microbial regrowth potential in distribution systems (Knappe *et al.* 2004). In addition, algal cells can serve as precursors of disinfection by-products (DBPs) during chlorination. Increased reaction time, chlorine dosage and temperature improved the formation of the relatively stable carbonaceous DBPs (C-DBPs) (e.g., trihalomethane (THM), haloacetic acid (HAA), and chloral hydrate (CH)) and trichloronitromethane (TCNM). The formation of dichloroacetonitrile (DCAN)

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followed an increasing and then decreasing pattern with prolonged reaction time and increased chlorine dosages (Fang *et al.* 2010a). Algogenic organic matter, including extracellular organic matter (EOM) and intracellular organic matter (IOM), was rich in organic nitrogen. The MW of organic carbon in EOM and IOM was low. IOM had a higher fraction of free amino acids but lower fractions of aliphatic amines than those of EOM. EOM formed fewer C-DBPs than did IOM. Most trichloro C-DBP yields, such as those of TCM, CH, and 1,1,1-TCP, in chloramination were much lower than those from chlorination. 1,1-DCP yields were higher in chloramination than those in chlorination (Fang *et al.* 2010b). During the chlorination of *Microcystis aeruginosa*, the concentrations of THMs and HNMs fluctuated with age. The formation of haloacetonitriles (HANs) during chlorination increased with longer culture ages. The formation of nitrogenous DBPs (N-DBPs), including DCAN and TCNM, did not follow the similar trends during chlorination of algal cells with increasing culture age (Yang *et al.* 2011).

Zooplankton of the genus *Cyclops* were observed to propagate excessively in waters that are eutrophiedas, a result of water pollution, especially in reservoirs and fresh lakes that serve as drinking water sources (Sun *et al.* 2013b). When *Cyclops* are fully developed, they have a body length of 2.0 millimetres and can be observed by the naked eye causing consumers to have an unsanitary view of the water. In addition, *Cyclops* may become a disease transmission medium as the host of pathogenic parasites. *Cyclops* are large in size compared with algae, bacteria, and other microorganisms, which suggests that the *Cyclops* biomass of amino acids, protein, fat, and other organic matter has greater potential to form DBPs during chlorination (Liu & Fu 2010). Thus, determining how the metabolites produced by these organisms affect water safety and contribute to the production of DBPs is interesting. In addition, *Cyclops* have strong motility and can easily penetrate filters to enter water supply systems (Cui *et al.* 2002; Lin *et al.* 2007). Abundant *Cyclops* not only pollute the ecological balance but also pose a considerable threat to human health. Many factors have been extensively studied to examine their effects on DBPs formation potential (DBPsFP) during disinfection, including the reaction time, pH, temperature, disinfectant concentration, and precursor properties. The formation of stable THMs and HAAs increased with increasing reaction time and chlorine dosage (Sun *et al.* 2013b). The formation of DCAN, and TCNM increased and then decreased with increasing chlorine dosages, followed by a continuous decrease with prolonged reaction time (Sun *et al.* 2013b). 1,1-DCP and 1,1,1-trichloro-2-propanone (1,1,1-TCP) increased with increasing chlorine dosage. 1,1-DCP increased continuously with increasing reaction time, while 1,1,1-TCP decreased with increasing reaction time (Sun *et al.* 2013b). The pH affected DBP formation differently, with TCM increasing and with DCAA, TCAA, DCAN, and 1,1,1-TCP decreasing (Sun *et al.* 2013b). TCM, DCAA, and TCAA could accumulate to their respective stable values with a progressive elevation in reaction time and monochloramine concentration. The 1,1,1-TCP content decreased correspondingly with a continuous increase in the reaction time. The formation of CH, TCNM, 1,1,1-TCP, and DCAA initially increased and then decreased with increasing monochloramine doses. A higher temperature resulted in a decrease of CH, DCAN, 1,1-DCP, 1,1,1-TCP, DCAA, and TCAA concentrations. DCAA, TCAA, CH, and 1,1-DCP decreased continuously with increasing pH (Sun *et al.* 2014).

Chloramine was first used as a disinfectant in water treatment in 1971. Chloramination exceeds the parameters of chlorination in the following three aspects: chloramine has good stability and can sterilize water for long periods, chloramine can slow down the chlorination of humus by free chlorine, and chloramine has a slightly corrosive effect on the pipe network. The monochloramination of chironomid larvae metabolite without generating high levels of most DBPs has been reported (Sun *et al.* 2013a). TCM is generally considered to be a non-genotoxic carcinogen, whose mechanism of action involves cytotoxicity and regenerative cell proliferation (IARC 1999). TCAA can cause bacterial mutation. DCAA has a carcinogenic effect on rodents, and an higher hereditary than TCAA. With respect to adverse effects, DCAN leads to mutagenicity in bacterial assays (Oliver 1983), and some of these DBPs (e.g., HANs, HNMs, and HAcAms) have significantly higher cytotoxicity and genotoxicity than the regulated THMs and HAAs (Richardson *et al.* 2007). Moreover, 1,1-DCP and 1,1,1-TCP have carcinogenic and mutational effects on rats (Bull & Robinson 1986).

Consistent efforts have been made to determine the identities and toxicities of various DBP species and their

groups, especially those of THMs, HAAs, and HANs to model their formation and to control their occurrence. Currently, no studies have investigated the monochloramination of co-existing *Microcystis aeruginosa* and *Cyclops* metabolites. The objective of this research was to evaluate the formation of selected DBPs during the monochloramination of co-existing *Microcystis aeruginosa* and *Cyclops* metabolites, with the dissolution evaluated under various conditions, including the monochloramine dosage, reaction time, pH, temperature, Cl/N, and *Microcystis aeruginosa* density. The concentration of the co-existing *Microcystis aeruginosa* and *Cyclops* metabolites in solution was measured as the total organic carbon (TOC).

MATERIALS AND METHODS

Chemicals

Deionized water was used to prepare all solutions. Methanol, acetone and methyl tert-butyl ether (MTBE) were all HPLC grade. The monochloramine solution (500 mg/L) was freshly prepared by mixing a free chlorine solution with an ammonium chloride (NH_4Cl) solution at an initial Cl/N mass ratio of 4/1. Buffer solutions at pH 5, 6, 7, 8, 9, and 10 were prepared with phosphate salts (Tianjin Chemical Plant, China). Calibration standards, internal standards, and surrogate standards for the analyses of THM, nine HAAs (HAA_9), HAN, CH, and TCNM were obtained from Supelco.

Microcystis aeruginosa

Microcystis aeruginosa (blue-green algae, Collection No. HB909) was cultured in 250 mL flasks containing 150 mL of BG11 media under a fluorescent lamp with an automated light/dark cycle of 12 h/12 h in an incubator at 23 °C (LRH-250A, TaiHong, China). *Microcystis aeruginosa* was cultured for 20 days under these conditions.

Cyclops

Cyclops was initially collected from the Mopanshan Reservoir, which is the largest tributary of the Harbin and main drinking water resource. *Cyclops* was cultured in aerated 10 L glass aquaria filled with raw water from the reservoir. The aquaria were maintained at a constant temperature (23 °C) and exposed to a consistent photoperiod (12 h light/12 h dark). *Cyclops* was cultured for 2 days under these conditions.

Sample preparation

During the period of the outbreak of *Microcystis aeruginosa* and *Cyclops* in summer, the monitoring results showed that the number of *Microcystis aeruginosa* could reach 10^9 count/L, while *Cyclops* reached 200 count/L. For this study, 10^9 count *Microcystis aeruginosa* and two hundred active *Cyclops* were cultured in aerated 25 L glass aquaria filled with deionized water. The aquaria were maintained at a constant temperature (23 °C) and exposed to a consistent photoperiod (14 h light/10 h dark). The samples were cultured for 3 days under these conditions, and then they were centrifuged to obtain the upper liquid. One liter of deionized water was added, and the sample suspensions were filtered through a 0.45 μm membrane, the filtrate was analyzed within a week. The TOC concentration was measured based on standards that were prepared by diluting reagents to 5 mg/L. The dissolved organic carbon (DOC) concentration was unchanged.

Analytical methods

The TOC was analyzed on a TOC analyzer (TOC-VCPH, Shimadzu). The monochloramine concentration was measured by DPD/FAS titration (Standard Methods 4500-Cl 1998). Analyses of selected DBPs were carried out on a gas chromatography (GC) (Agilent 7890) with an electron capture detector (ECD), following USEPA methods 551.1 (USEPA 1995) and 552.3 (USEPA 2003). The THM, HAN, haloketone (HK), CH, and TCNM concentrations were measured by a liquid-liquid extraction procedure using MTBE and acid methanol, according to USEPA method 551.1 (USEPA 1995). The column used was an HP-5 fused silica capillary column (30 mm × 0.25 mm I.D. with 0.25 mm film thickness). The GC-ECD operating conditions were as follows: detector temperature 290 °C; injector temperature, 200 °C; injection volume 1 mL; and temperature

program, 35 °C for 5 min, ramped to 75 °C at 10 °C/min, held for 5 min, then ramped to 100 °C at 10 °C/min, and then held for 2 min.

For DCAA and TCAA analysis, the samples were pretreated with an extraction/derivatization procedure using MTBE and acidic methanol, according to USEPA Method 552.3 (USEPA 2003). The column used was an HP-5 fused silica capillary column (30 mm × 0.25 mm I.D. with 0.25 mm film thickness). The injector, ECD and GC oven temperature programs for compounds other than HAA9 were as follows: injector temperature, 200 °C; ECD temperature, 290 °C; and oven temperature program, 35 °C for 9 min, ramping to 40 °C at 2 °C/min, holding for 8 min, ramping to 80 °C at 20 °C/min, ramping to 160 °C at 40 °C/min and holding for 4 min.

The GC conditions for HAAs analysis were as follows: injector temperature, 210 °C; ECD temperature, 290 °C; oven temperature program, 30 °C for 20 min, ramping to 40 °C at 1 °C/min, ramping to 205 °C at 20 °C/min and holding for 4 min.

Experimental procedures

The stock solution containing the co-existing *Microcystis aeruginosa* and *Cyclops* metabolites was diluted with deionized water to prepare a testing solution with 5 mg/L as TOC. Monochloramine was added at 10 mg/L to the solution (5 mg/L as TOC), which was buffered at pH 7.0 at a room temperature of 23 ± 1 °C as the baseline condition. The reaction was terminated by Na_2SO_3 prior to DBP extraction. The influencing factors and their tested levels were as follows: monochloramine concentration (2, 4, 8, 10, 20 mg/L as Cl_2), reaction time (6, 12, 24, 48, and 72 h), pH (5, 6, 7, 8, 9, and 10), temperature (10, 20, and 30 °C), Cl/N ratio (1/0, 20/1, 5/1, and 5/4), and *Microcystis aeruginosa* density (10^8, 10^9, and 10^{10} count/L).

RESULTS AND DISCUSSION

Effect of monochloramine dosage

Figure 1 shows the formation of DBPs upon treatment with different concentrations of monochloramine at pH 7.0.

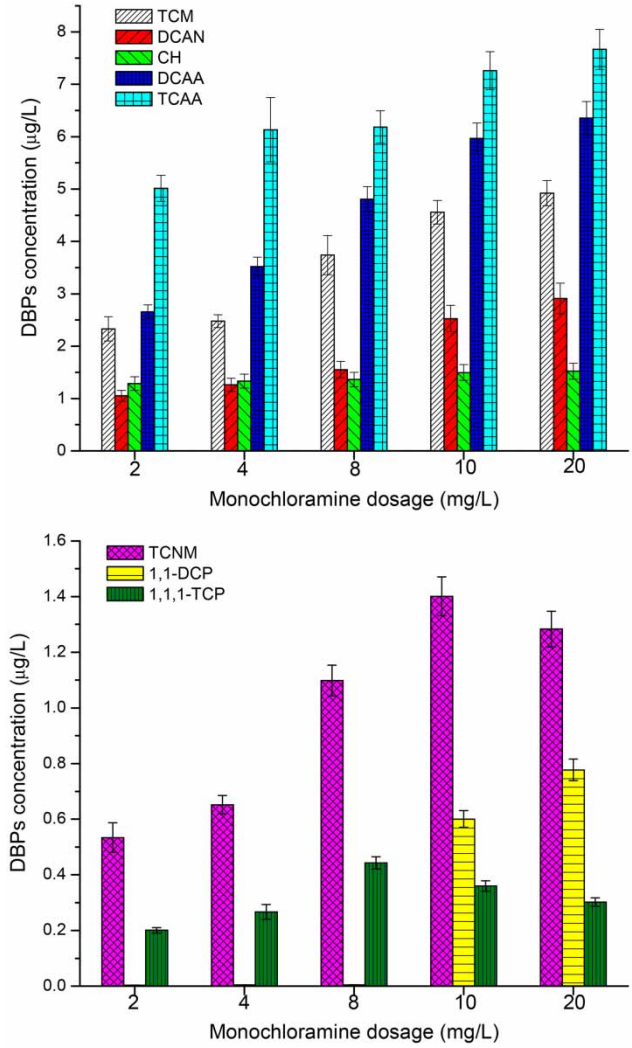

Figure 1 | Formation of DBPs as a function of the monochloramine concentration after 48 h chloramination of *Microcystis aeruginosa* mixed with *Cyclops* metabolite solution (5 mg/L as TOC) at pH 7.0 and a temperature of 23 ± 1 °C. The error bars represent the standard deviation of replicate measurements, $n = 2$.

Among the tested DBPs, the DCAA and TCAA concentrations were the highest, and those of 1,1-DCP and 1,1,1-TCP were the lowest. The yields of TCM, CH, DCAN, 1,1-DCP, DCAA, and TCAA increased with increasing monochloramine dosage, and the concentrations of TCNM and 1,1,1-TCP reached their maximum levels when the monochloramine dosage was increased from 8 to 10 mg/L, and decreased continuously with increasing monochloramine dosage. 1,1-DCP was practically undetectable at 0.003–0.006 μg/L when the monochloramine dosage was below 10 mg/L. The yields of unstable DBPs depend on their

formation and decomposition rates. The decomposition of 1,1-DCP due to oxidation or hydrolyzation faster than its formation at monochloramine dosages less 10 mg/L. 1,1-DCP is first oxidized to 1,1,1-TCP and then transformed into TCM, so 1,1,1-TCP first increased and then decreased with increasing monochloramine dosage.

Effect of reaction time

Figure 2 shows the time dependence of the formation of DBPs after the monochloramination of *Microcystis aeruginosa* metabolites mixed with *Cyclops* metabolites, with residual chlorine remaining at the end of the each test. The TCM, DCAA, and TCAA concentrations were highest among the tested DBPs, followed by CH. The concentrations of 1,1-DCP, and 1,1,1-TCP were low (0.007 μg/L, 0.056 μg/L at 72 h, respectively). The yields of TCM, CH, DCAN, DCAA, and TCAA increased with increasing reaction time, and the yields of TCNM, 1,1-DCP, and 1,1,1-TCP reached a maximum after 2 days and then decreased with increasing reaction time. In terms of the formation rates, TCM accumulated significantly at 48 h and the TCAA concentration rapidly increased after 48 h. The concentrations of CH and DCAA increased steadily. THMs, HAAs, and CH are all stable products (Xie 2001; Zhang & Minear 2002; Yang *et al.* 2007), so their concentrations increased with increasing reaction time. When the disinfectant and DBPsFP were sufficient, as the reaction time of the chloramine disinfection increased, 1,1-DCP and, 1,1,1-TCP could be generated. Simultaneously, 1,1-DCP and, 1,1,1-TCP can undergo the hydrolysis reaction by themselves. The precursor of 1,1-DCP could be easily converted to 1,1,1-TCP by oxidation, and 1,1-DCP ultimately hydrolyses to TCM and TCAA; therefore, the concentrations of 1,1-DCP and, 1,1,1-TCP decreased after 48 h. During the process of these reactions, the formation reaction of 1.1-DCP and, 1,1,1-TCP was severely influenced by the hydrolysis reaction, leading to low concentrations of 1,1-DCP, and 1,1,1-TCP. The DCAN concentration was observed to increase, which could be due to the stability of DCAN in monochloramine solutions (Yang *et al.* 2007).

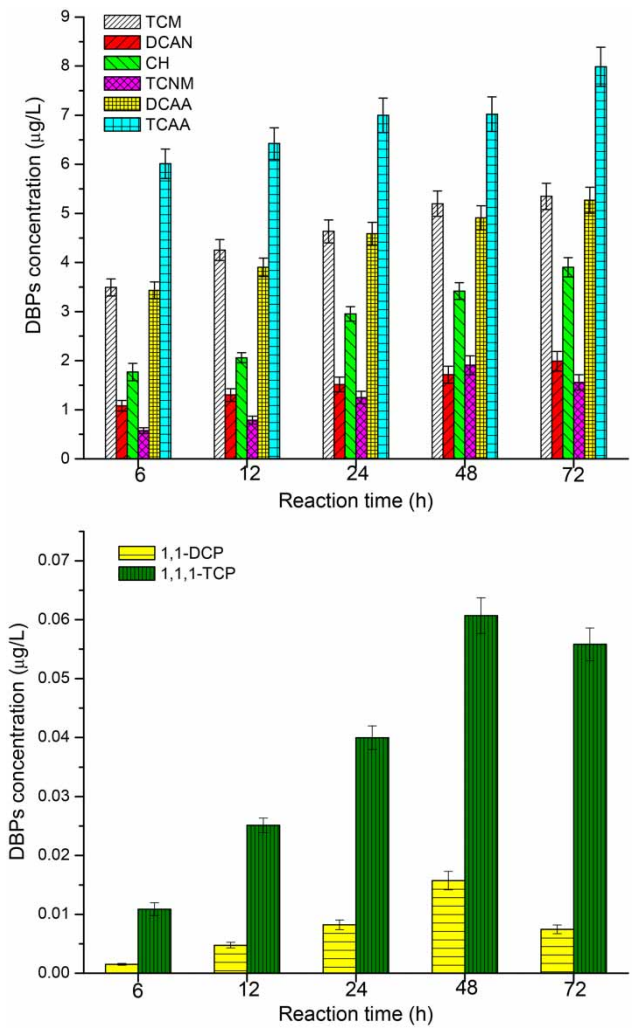

Figure 2 | Time-dependent formation of DBPs from the monochloramination of *Microcystis aeruginosa* mixed with *Cyclops* metabolite solution (5 mg/L as TOC) at 7.0; monochloramine concentration: 10 mg/L, temperature: 23 ± 1 °C. The error bars represent the standard deviation of replicate measurements, $n = 2$.

Effect of pH

Figure 3 shows the DBP concentrations after 48 h treatment with monochloramine at 10 mg/L under different pH values. The formation of TCM increased, and the amount of 1,1-DCP, and 1,1,1-TCP decreased continuously with increasing pH from 5 to 10. The formation of N-DBPs (e.g., DCAN, TCNM) increased with increasing pH from 5 to 7, but the DCAN, and TCNM levels significantly decreased at pH 8 and then remained stable, indicating that pH plays a role in the speciation of chloramines, monochloramine hydrolysis, and the stability of N-DBPs (e.g., DCAN, and TCNM) during monochloramination (Yang *et al.* 2007).

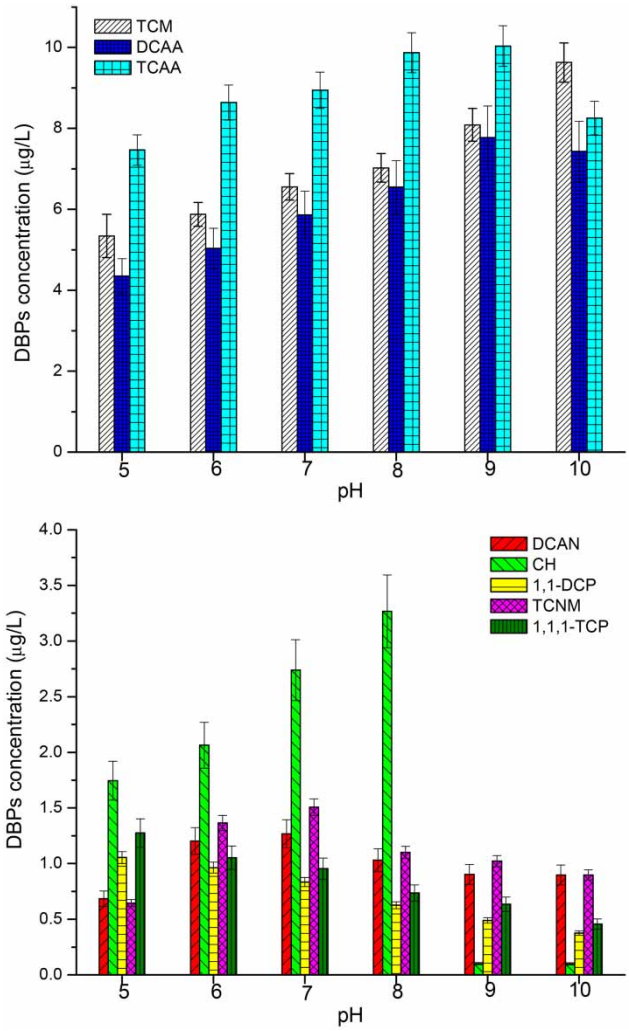

Figure 3 | The concentrations of DBPs and monochloramine using premixed monochloramine at 10 mg/L in TOC solution (5 mg/L) at different pH values after 48 h at 23 ± 1 °C. The error bars represent the standard deviation of replicate measurements, $n = 2$.

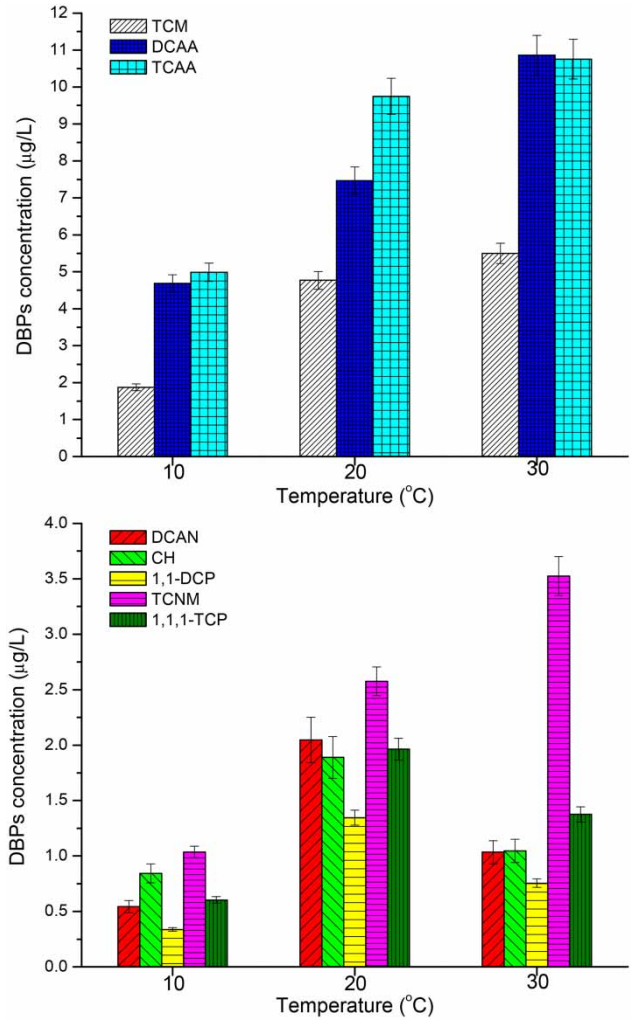

Figure 4 | DBP formation over 48 h due to chloramination as a function of temperature (adding 10 mg/L preformed monochloramine) of 5 mg/L TOC solutions at pH 7.0. The error bars represent the standard deviation of replicate measurements, $n = 2$.

The formation of DCAA and TCAA increased with increasing pH from 5 to 9, but when the pH was further increased to 10, the concentration of TCAA decreased. The concentration of CH increased with the pH from 5 to 8, but at pH 9 and above, CH was almost undetectable (detection limit of 0.001 μg/L). Monochloramine was the dominant chloramine at pH 7.5 or above, for which the sterilization ability is poor. The hydrolysis of monochloramine to form free chlorine was also affected by pH (Jolley & Carpenter 1983). The pH also affects the stability of unstable DBPs. DCAN, 1,1-DCP, and 1,1,1-TCP can be hydrolysed and decomposed in alkaline conditions (Yang *et al.* 2007), where the hydrolysis rate is accelerated by the increase in pH (Nikolaou *et al.* 2000). The hydrolysis rates of these unstable DBPs increase with increasing pH (Xie 2001).

Effect of temperature

Figure 4 shows the DBPs formation after 48 h monochloramination at three designated temperatures: 10, 20 and 30 °C. The formation of TCM, TCNM, DCAA, and TCAA were significantly enhanced when the temperature was increased from 10 to 30 °C, whereas the concentration of

CH, DCAN, 1,1-DCP, and 1,1,1-TCP first increased and then decreased again with the increase in temperature. The concentrations of C-DBPs (e.g., TCM, CH, 1,1-DCP, 1,1,1-TCP, DCAA, and TCAA) and N-DBPs (e.g., DCAN, TCNM) were quite low, the minimum concentration was 0.337 μg/L and the maximum concentration was 4.985 μg/L, when the temperature was 10 °C. At 20 and 30 °C, the DBP concentration increased obviously to a maximum of 13 μg/L. Algal blooms mostly occur in summer, because the high temperatures are beneficial to algal growth; meanwhile, the abundance of *Cyclops*, which feed on algae increase correspondingly, and all of these processes lead to an increased formation of DBPs. The DBP concentrations at different temperatures reflects the balance between their formation and decomposition rates. Therefore, increasing temperature is expected to accelerate decomposition reactions (e.g., hydrolysis), because these reactions are always endothermic (Reckhow & Singer 1985). At 30 °C, the decomposition rate with chloramine was enhanced, which resulted in a decrease in the relatively unstable DBPs, such as DCAN, 1,1-DCP, and 1,1,1-TCP (Nikolaou *et al.* 2000). In summary, temperature can fundamentally influence the decomposition of DBPsFP by affecting the interplay between the formation and decomposition rates.

Effect of the Cl/N ratio

Figure 5 shows the formation of five typical DBPs after 48 h of chloramination under the baseline conditions with varied Cl/N mass ratios of 1/0, 20/1, 5/1, and 5/4. The Cl/N ratio was set as a variable and was controlled in the experiment. The N in Cl/N only represents the ammonia nitrogen formed from the preparation process of monochloramine disinfectant using hypochlorite (NaClO) and ammonium chloride (NH_4Cl) for adjustment. The organic nitrogen generated from algal biomass and highly concentrated nitrate in algae media was treated as invariants without further study and consideration. The yields of TCM, CH, DCAN, DCAA, and TCAA at an initial Cl:N ratio of 1:0 were higher than those of 20:1, 5:1, and 5:4, and the concentrations of five DBPs decreased as the mass ratio of Cl/N decreased. These trends follow the breakpoint chlorination principle. The conversion of free chlorine into monochloramine decreases the formation rates of most DBPs (Zhang *et al.* 2000).

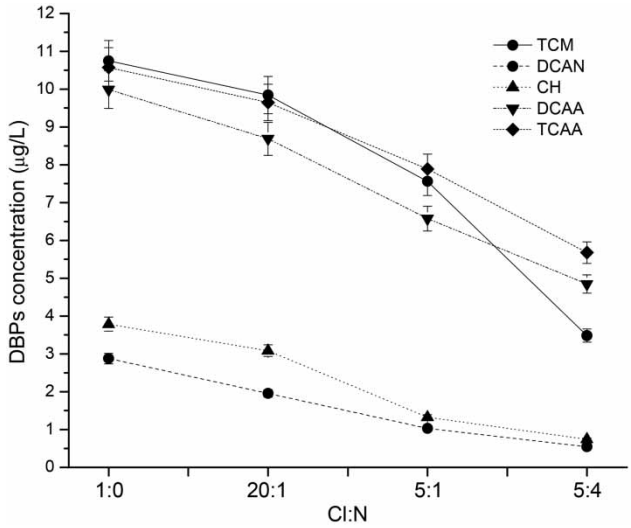

Figure 5 | Formation of DBPs as a function of the Cl/N ratio after 48 h chloramination of *Microcystis aeruginosa* mixed with *Cyclops* metabolite solution (5 mg/L as TOC) at pH 7.0 using a chlorine concentration of 10 mg/L at 23 ± 1 °C. The error bars represent the standard deviation of replicate measurements, $n = 2$.

Effect of *Microcystis aeruginosa* density

Figure 6 shows the DBP formation after 48 h of treatment with monochloramination at 10 mg/L under three designated *Microcystis aeruginosa* concentrations: 10^8, 10^9 and 10^{10} count/L. *Microcystis aeruginosa* was set as the variable, while the *Cyclops* amount of 200 count/L was unchanged. The results showed that TOC concentration was 15.53, 5.585, and 18.72 mg/L. When the density of *Microcystis aeruginosa* was changed, after 48 h, the DBPsFP and disinfectant were continuously consumed, while the formation reaction of 1,1-DCP and, 1,1,1-TCP was severely influenced by the hydrolysis reaction, resulting in low levels of 1,1-DCP and, 1,1,1-TCP. When the density of *Microcystis aeruginosa* was 10^{10} count/L, the formation of DBPs reached a maximum, and the concentration of the tested DBPs was 0.054–14.331 μg/L. When the density of *Microcystis aeruginosa* was 10^8 count/L, the DBP concentrations were 0.022–11.895 μg/L. When the density of *Microcystis aeruginosa* was 10^9 count/L, the DBP formation was at a minimum, and the

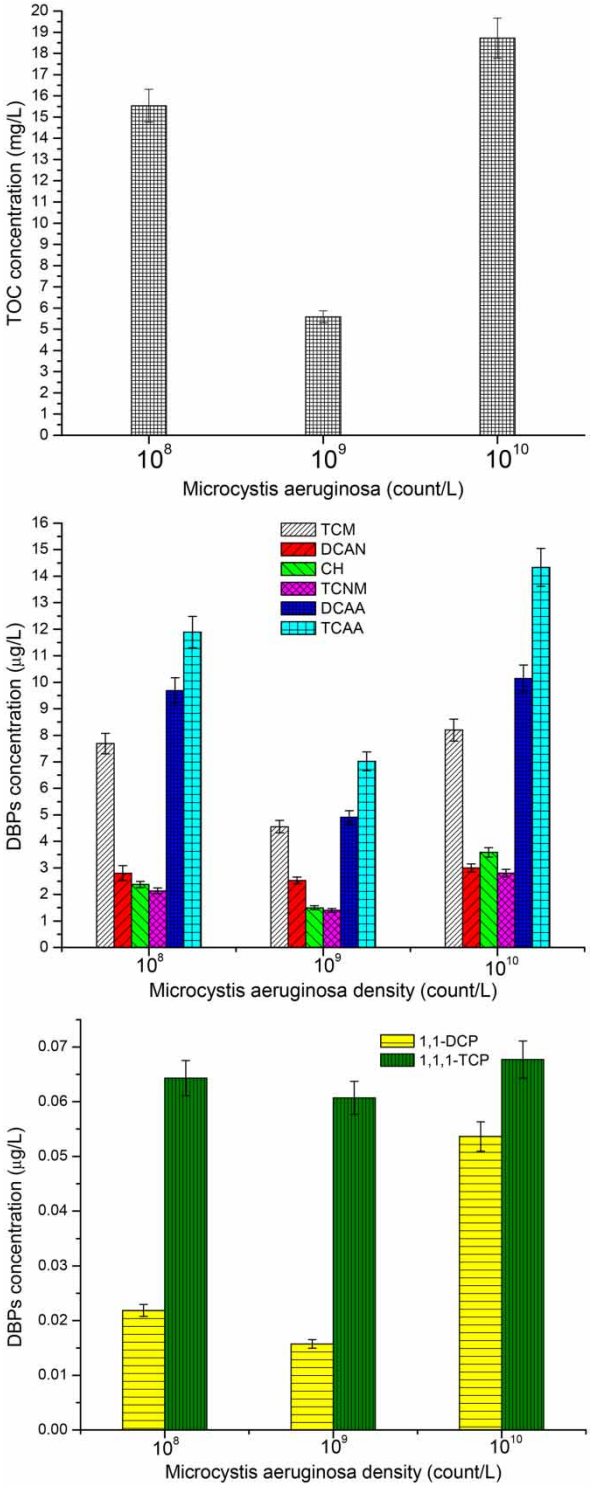

Figure 6 | Impact of *Microcystis aeruginosa* density on DBPsFP after 48 h of monochloramination of *Microcystis aeruginosa* mixed with *Cyclops* metabolite solution at pH 7.0; monochloramine concentration: 10 mg/L, temperature: 23 ± 1 °C. The error bars represent the standard deviation of replicate measurements, $n = 2$.

concentrations of 1,1-DCP was only 0.016 μg/L. The concentration of TOC was lowest when the *Microcystis aeruginosa* was 10^9 count/L. The TOC concentration is positively related to the formation of DBPs (Zhang & Sun 2015). Previous studies suggested that *Microcystis aeruginosa* and *Cyclops* exist in a co-dependent relationship, with *Cyclops* preying on *Microcystis aeruginosa*, decreasing its population and reducing its opportunity to release metabolites in aqueous solution. At the same time *Microcystis aeruginosa* preys on *Cyclops* metabolites, further reducing the concentration of the metabolites in aqueous solution. Consequently, *Microcystis aeruginosa* and *Cyclops* in dynamic equilibrium mutually influence the other's existence (Li *et al.* 2010), which may be one reason why the TOC concentration was at its lowest.

CONCLUSIONS

The co-existence of *Microcystis aeruginosa* and *Cyclops* metabolites could produce DBP precursors during monochloramine disinfection. Increasing reaction time and monochloramine dosage increased the formation of the relatively stable C-DBPs (e.g., THM, HAA, and CH). The formation of TCNM followed an increasing and then decreasing trend with prolonged reaction time and increased monochloramine dosage. The concentrations of DCAA, and TCAA were highest among all the DBPs (5–8 μg/L), and these compounds deserve further study. The pH affected the formation of DBPs in a different way. TCM was the only species that steadily increased in concentration with increasing pH, the HK concentrations varied in an opposite manner, and other DBPs had maximum concentrations at certain pH values. Higher temperatures enhanced TCM, TCNM, DCAA, and TCAA formation whereas the CH, DCAN, 1,1-DCP, and 1,1,1-TCP concentrations increased between 10 °C and 20 °C, followed by a decrease between 20 °C and 30 °C. The five most common DBPs decreased with a decreasing Cl/N mass ratio, indicating that a lower Cl/N mass ratio can effectively control the amounts of these five DBPs. Finally, the minimum DBP formation was observed from the data with a *Microcystis aeruginosa* density of 10^9 count/L.

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