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Formation of disinfection byproducts from chlor(am)ination of algal organic matter

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ABSTRACT

Algal cells and extracellular organic matter (EOM) of two algae species, *Microcystis aeruginosa* (blue-green algae) and *Chlorella vulgaris* (green algae), were characterized. The low specific UV absorbance (SUVA) values of EOM and cells from both algae species indicated the very hydrophilic nature of algal materials. Fluorescence excitation–emission matrix showed that algal EOM and cells were enriched with protein-like and soluble microbial by-product-like matters. The formation potential of a variety of disinfection by-products (DBPs) during chlorination and chloramination of algal cells and EOM were evaluated. Algal cells and EOM of *Microcystis* and *Chlorella* exhibited a high potential for DBP formation. Yields of total DBPs varied with the algae cultivation age. Cellular materials contributed more to DBP formation than EOM. The presence of bromide led to higher concentrations of total trihalomethanes (THMs), haloacetonitriles (HANs), and halonitromethanes (HNMs). Bromide also shifted the DBPs to brominated ones. Bromine incorporation was higher in HNMs than in THMs and HANs. Compared to natural organic matter, algae under bloom seasons can contribute significantly to the DBP precursor pool.

1. Introduction

Algal bloom has become a world-wide problem. In fresh water bodies, algal cells and their excreted metabolic substances may cause a series of problems during water treatment processes: (1) generating undesirable taste and odor; (2) poor settling and plugging the filters; (3) releasing algal toxins; (4) contributing to the formation of disinfection by-products (DBPs). Among the series of problems caused by algae, the role of algae in the formation of DBPs has been evaluated in several studies [1–7].

DBP exposure may be also associated with miscarriages or reduced birth weight, a public health risk that is under active investigation [8–13]. Additional health problems from DBP exposure may include rectal and colon cancers, kidney and spleen disorders, immune system problems and neurotoxic effects [14]. Wang et al. [9] found that chlorine–chloramine treatment could decrease the cancer risk of THMs and ozone treatment could decrease the cancer risk of both THMs and HAAs. To obtain better drinking water quality, DBPs should be controlled effectively. To protect human health, the Stage II D/DBP rule in the United States set the maximum contamination levels (MCLs) for total trihalomethanes (TTHM) at 80 μg/L and five haloacetic acids at 60 μg/L on local running annual averages [15].

The formation of regulated DBPs, including trihalomethanes (THMs) and haloacetic acids (HAAs), during chlorination of algal cells and extracellular organic matter (EOM) has been investigated. Their formation varied with the algae species, algae growth phases, and chlorination conditions [1,2,16,17]. Wardlaw et al. [1] summarized that the reported yields of THMs from algal biomass were in the range of 0.35–0.73 μmol/mmol C and those from EOM were in the range of 0.37–0.87 μmol/mmol C under similar chlorination conditions (pH 7, 20–24 °C, 24 h reaction time). Huang et al. [18] compared DBP formation potential from two blue-green algae species with and without bromide and found that specific DBP yields of *Anabaena* ranged from 2 to 11 μmol/mmol C for total THM and from 2 to 17 μmol/mmol C for total HAA and those for *Microcystis* was slightly higher under chlorination conditions of pH 7, 21 °C, and 7-day reaction. Algal suspensions were also found to form haloacetonitriles (HANs) [19,20].

Algae are the major sources of dissolved organic nitrogen (DON) in the natural water [21]. DON can be transformed to nitrogen containing DBPs (N-DBPs) during chlorination or chloramination. There is a concern that non-regulated DBPs, such as N-DBPs, are more toxic than the regulated THMs and HAAs [4,14], but the relevant research is very limited. A recent study [6] found that chlorination of *Microcystis aeruginosa* cells

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generated cyanogens chloride, HANs, and halonitromethanes (HNMs). Chlorination of algal cells of higher organic nitrogen content generated higher concentrations of N-DBPs (e.g., dichloroacetonitrile and trichloronitromethane) than chlorination of natural organic matter (NOM). The formation of DBPs from algae was affected by contact time, chlorine dosages, pH, temperature, ammonia concentrations and algae growth ages [7]. However, most of study does not address the effect of bromide, specially its effect on N-DBPs. Moreover, the studies on DBP formation from algae mainly focus on chlorination and chloramination is only evaluated in one study [6].

Clearly, the information on the formation of DBPs from algae is insufficient. The aims of this work were to (1) evaluate the characteristics of algal cells and extracellular organic matter at different growth phases, and (2) investigate DBPs generated during chlorination and chloramination of algal cells and EOM with/without bromide. Two algae species, *M. aeruginosa* (blue algae) and *C. vulgaris* (green algae), were included.

2. Materials and methods

2.1. Chemicals and materials

A stock of free chlorine solution was prepared from 5% sodium hypochlorite (NaOCl) (from Sigma), diluted to 1000 mg/L as Cl_2 and stored in an aluminum foil-covered glass stoppered flask. Monochloramine (NH_2Cl) solutions were prepared daily by reacting equal volumes of ammonium chloride and sodium hypochlorite solutions at a weight ratio of 4 mg/L Cl_2 to 1 mg/L N–NH_4^+. The resulting solutions were standardized by DPD/FAS titration [22]. A mixed standard containing HANs and haloketones (HKs), a THM mixture standard, chloral hydrate, and internal and surrogate standards were obtained from Supelco. Nine HNM standards were obtained from Orchid Cellmark (Canada).

2.2. Algae cultivation procedures

Axenic cultures of *C. vulgaris* (green algae, Collection No. FACHB-31) and *M. aeruginosa* (blue-green algae, Collection No. FACHB-905) were obtained from the Culture Collection of Algae at the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Both species were grown using Jaworski's Media at 25 °C and a 12 h light/12 h dark cycle in an incubator (GXZ-280B, Ningbo, China). The receipt of Jaworski's Media is listed in Table S1 in the Supplementary material. For each algae specie, samples for different culture periods were run in batch without any replacement or replenishment of growth media. The number of algal cells at different periods was measured with a photomicroscope (CX31, Olympus).

2.3. Separation of cells and EOM

Cell suspension at certain growth intervals was centrifuged to separate algae cell and EOM. The supernatant was filtered with 0.45 μm Whatman membrane filter, hereafter referred to as EOM solution. The deposited algal cells in the centrifuge tube were collected and washed with ultrapure water, followed by two cycles of centrifugation and supernatant removal, and resuspended in 200 mL water, hereafter referred to as algal cell solution. Both EOM and algae cell solutions were taken for analysis of total organic carbon (TOC), UV absorbance at 254 nm (UV254), and fluorescence.

2.4. DBP formation potential tests

All samples were buffered to pH 7.2 with phosphate buffer (10 mM) before chlorination/chloramination to maintain the pH. For chlorination, excessive chlorine was applied to ensure a residual of at least 0.5 mg/L after incubation for a 3-day period. All chlorinated samples were stored head-space free in the dark at room temperature (22 ± 1 °C). Bromide (1 mg/L) was spiked in some of the samples to evaluate the effect of bromide on DBP formation. Chloramination tests were conducted in the same manners as those of chlorination except that preformed monochloramine was added to the solutions. After 3 days, samples were quenched with sodium thiosulfate and extracted immediately with methyl tert-butyl ether (MTBE). The extracts were subjected to gas chromatograph for DBP analysis.

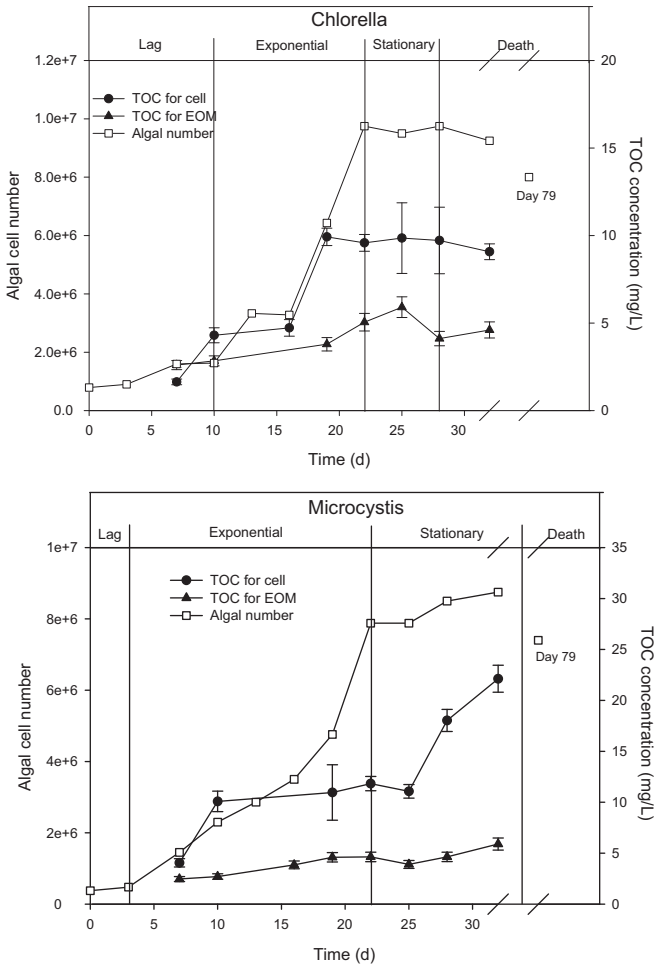

Fig. 1. The growth curves for *Chlorella* and *Microcystis*.

2.5. Analytical methods

Chlorine was measured by DPD/FAS titration [22]. Analyses of THMs, HANs, HNMs, HKs, and chloral hydrate were carried out with a gas chromatograph (Agilent 7890) with an electron capture detector (ECD), based on the USEPA Method 551.1 [23]. The column used was an HP-5 fused silica capillary column (30 m × 0.25 mm I.D. with 0.25 μm film thickness, J&W Scientific). The GC temperature program consisted of an initial temperature of 35 °C for 6 min, ramping to 100 °C at 10 °C/min and holding for 5 min, ramping to 200 °C at 20 °C/min and holding for 2 min.

TOC was analyzed on a TOC analyzer (TOC-V_{CPH}, Shimadzu). UV absorbance at 254 nm was analyzed using a UV/vis spectrophotometer (754PC, Shimadzu). Fluorescence excitation–emission matrix (EEM) measurements were conducted using a Hitachii F-4500 spectrometer. The spectrometer used a xenon excitation source, and slits were set to 10 nm for both excitation

and emission. To obtain fluorescence EEMs, excitation wavelengths were incremented from 200 to 400 nm at 5 nm steps; for each excitation wavelength, the emission was detected from 290 to 500 nm at 5 nm steps. All samples were diluted to a final DOC concentration of 1 mg/L with 0.01 M KCl. The EEM figures were drawn with SigmaPlot 2001 with 20 contour lines.

3. Results and discussion

3.1. Algal growth

Fig. 1 shows the relationships between algal cell numbers, TOC concentrations of EOM and cell solutions for *M. aeruginosa* and *C. vulgaris*. Changes in algal cell numbers are used to distinguish the four growth phases: lag, exponential, stationary, and death phase. The lag phase of *Microcystis* and *Chlorella* lasted approximately 3 and 10 days, respectively, during which time no significant changes were observed. The exponential phase was from Day 3 to Day 22 for *Microcystis* and from Day 10 to Day 22 for *Chlorella.* Dramatic increases in algal cell numbers were found. The stationary phase of *Chlorella* was from Day 22 to Day 28, after which the cell numbers began to decrease and the death phase started. For *Microcystis,* it was hard to distinguish the transition from the stationary phase to the death phase since the algal cell numbers were not counted until Day 79.

The TOC concentrations of EOM increased with culture age for both algae specie and they were 5.9 and 4.6 mg/L for *Microcystis* and *Chlorella*, respectively at Day 32. The TOC concentrations of cells increased to 22 and 9 mg/L for *Microcystis* and *Chlorella*, respectively at Day 32. Specific UV absorption at 254 nm (SUVA), an indicator of the hydrophobicity and aromaticity of aquatic humic substances [24,25] of water, is determined by dividing UV254 (in m^{-1}) by its concentration of organic carbon (in mg/L). SUVA values for cell and EOM of both species are listed in Table S2 in the Supplementary material. The highest SUVA values of EOM solutions were 1.6 and 1.5 L mg^{-1} m^{-1} for *Chlorella* at Day 28 and Day 32. The average SUVA value before Day 28 was 0.7 L mg^{-1} m^{-1}. Similar trends were found for *Microcystis*. SUVA values were 1.2 and 1.0 L mg^{-1} m^{-1} at Day 28 and Day 32 and the average value was 0.5 L mg^{-1} m^{-1} before Day 28. But the SUVA values for cell solutions were around 1.1 L mg^{-1} m^{-1} for both species at different culture ages. The low SUVA values indicated the very hydrophilic nature of algal materials [25,26].

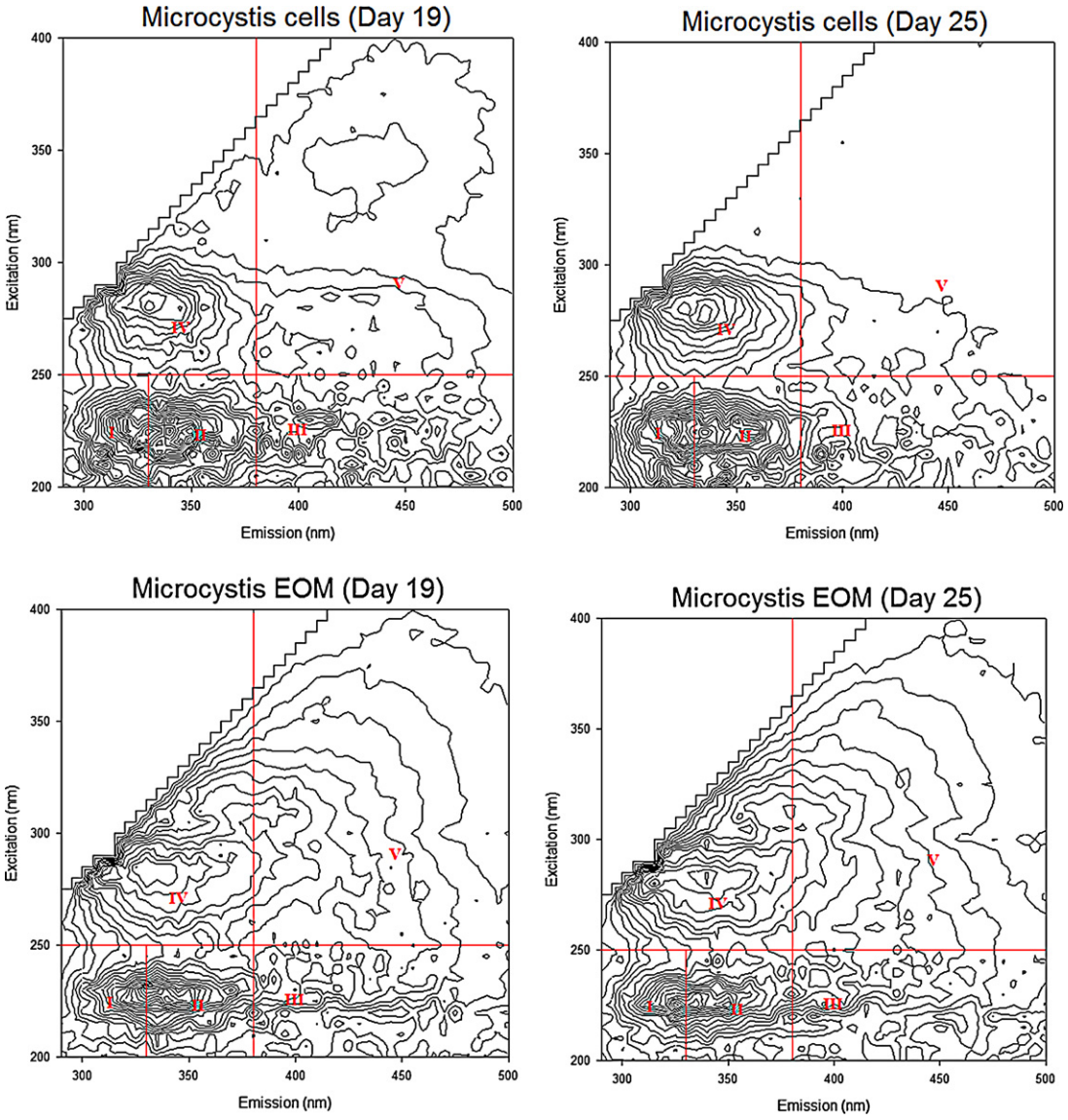

Fig. 2. Fluorescence EEMs of EOM and cells of *Chorella* and *Microcysitis* at cultivation Day of 19 and 25.

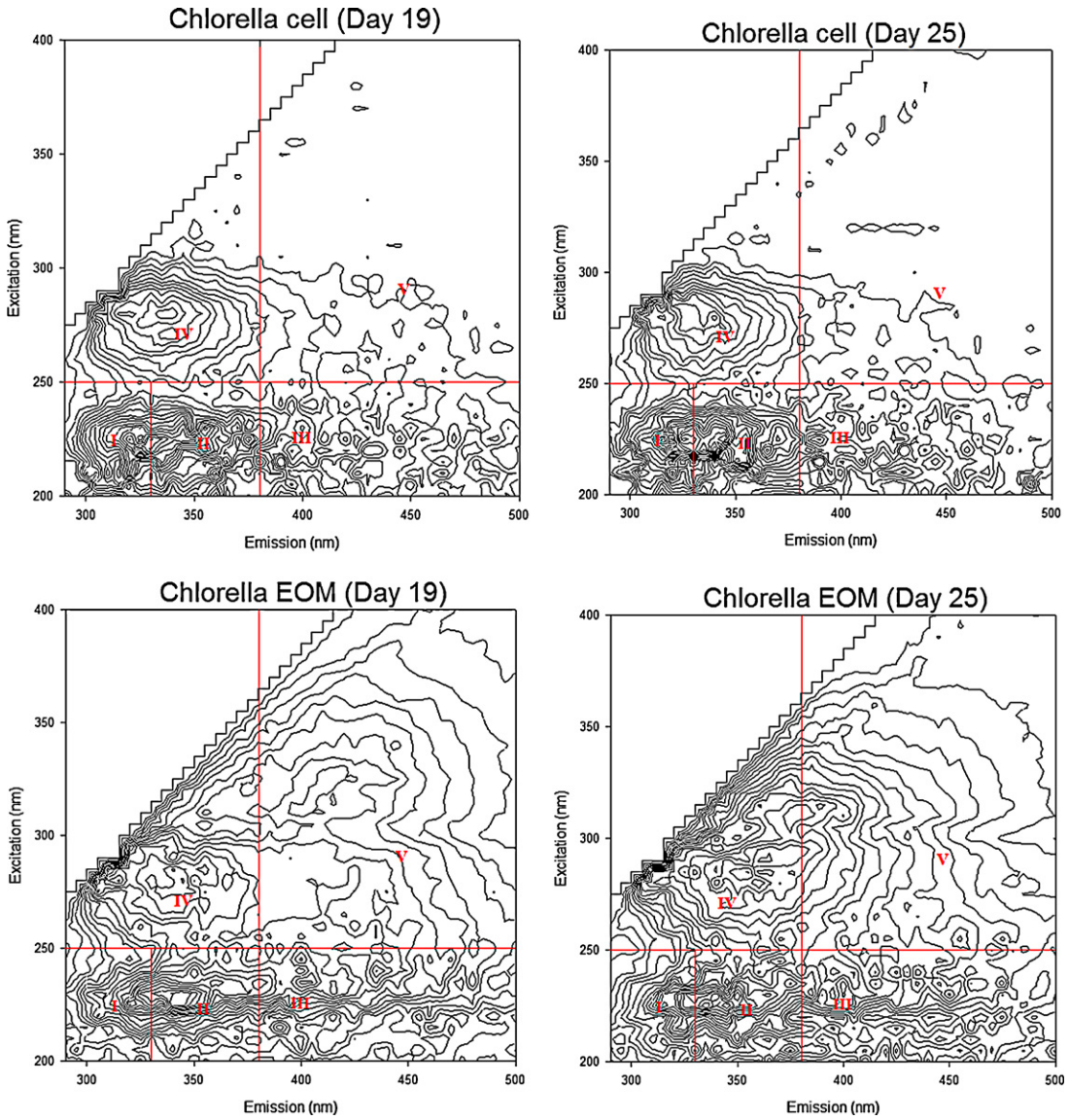

Fig. 2. (*Continued*).

Selected fluorescence EEMs of EOM and algal cells at the exponential phase (Day 19) and the stationary phase (Day 25) are displayed in Fig. 2. EEM peaks have been associated with humic-like, tyrosine-like, tryptophan-like, or phenol-like organic compounds [27–29]. The EEM figures are operationally defined into five regions based on fluorescence of model compounds: aromatic protein (regions I and II, $\lambda_{ex} < 250$ nm, $\lambda_{em} < 350$ nm), fulvic acid-like (the region III, $\lambda_{ex} < 250$ nm, $\lambda_{em} > 350$ nm), soluble microbial by-product-like, including tyrosine-, tryptophan- and protein-like components (the region IV, 250 nm $\lambda_{ex} < 280$ nm, $\lambda_{em} < 380$ nm), and humic acid-like (the region V, $\lambda_{ex} > 280$ nm, $\lambda_{em} > 380$ nm) regions [30]. As shown in Fig. 2, EEMs of *Microcystis* and *Chlorella* share similarities. For example, both had peaks maxima at $\lambda_{excitation} = 280$ nm and $\lambda_{emission} = 330$ nm and $\lambda_{excitation} = 225$ nm and $\lambda_{emission} = 335$ nm. Protein-like and soluble microbial by-product-like rather than humic/fulvic acid-like fluorescence dominated in all EEMs. Humic/fulvic acid-like fluorophores were also found in some of the EEMs for algal cells, such as *Microcystis* at Day 19. EOM for both algal species has fluorescence in locations attributable to humic/fulvic-like substances. Based on the calculations proposed by Chen et al. [30], the percent fluorescence response values ($P_{i,n}$) for combined region I and II were over 60% for *Microcystis* cells at all culture age and they were around 50% for EOM of *Microcystis* (shown in Fig. S1 in the Supplementary material). It indicates that EOM has more humic/fulvic-like substances than cells. A fluorescence index (FI) was calculated for all samples as the ratio of fluorescence intensity at emission wavelength of 450–500 nm when the excitation wavelength is 370 nm. The values were shown in Table S2 in the Supplementary material. The FI ratios in the range of 1.5–2.9 are consistent with those of algal derived materials obtained in other studies [5].

3.2. DBP formation

3.2.1. Total DBP formation

Fig. 3 shows the formation of total DBPs produced by *Chlorella* during chlorination. Great increases were found from the start of exponential phase for all tested DBPs and the yields varied afterwards. Concentrations of total THMs decreased at the end of exponential phase. Total HANs increased and total HNMs remained constant. The presence of bromide increased the total concentrations of THMs, HANs, and HNMs. Concentrations of chloral hydrate and 1,1,1-trichloro-2-propanone (1,1,1-TCP) decreased and their brominated counterparts were not analyzed due to the shortage

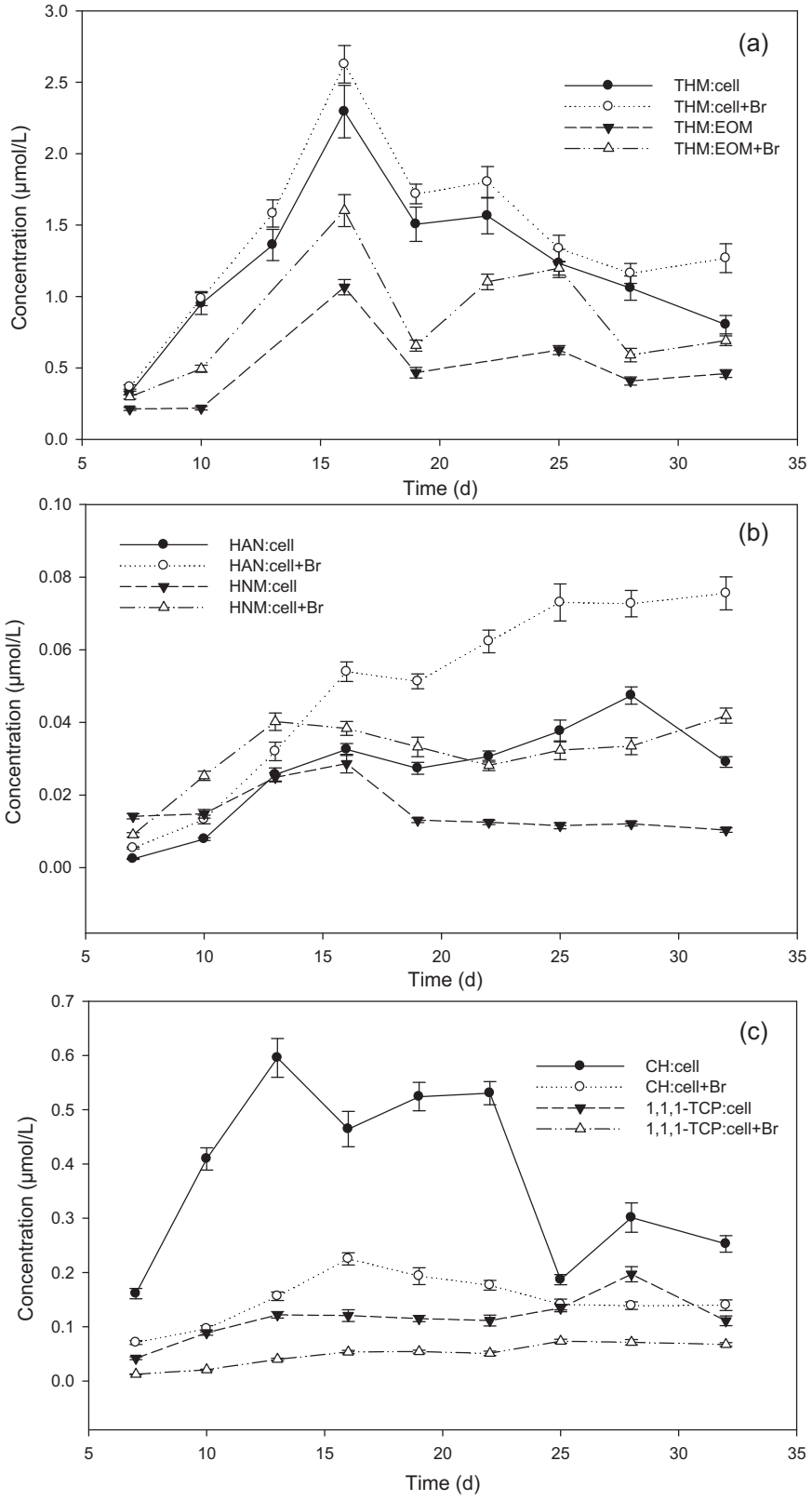

Fig. 3. The formation potential of DBPs during chlorination of *Chlorella* EOM and cells in the absence and presence of bromide (pH 7.2, 3-day reaction, 22 ± 1 °C).

of standards. Fig. 4 shows the formation of total DBPs produced from *Microcystis* during chlorination. Concentrations of THMs and HNMs fluctuated with age. The formation of HANs during chlorination increased with longer culture ages. The results of THM yields showed some inconsistency with a previous study [18]. THM generated during chlorination of *Microcystis* at the stationary growth phase was higher than those generated during the exponential growth phase. It could be due to the different chlorination schemes.

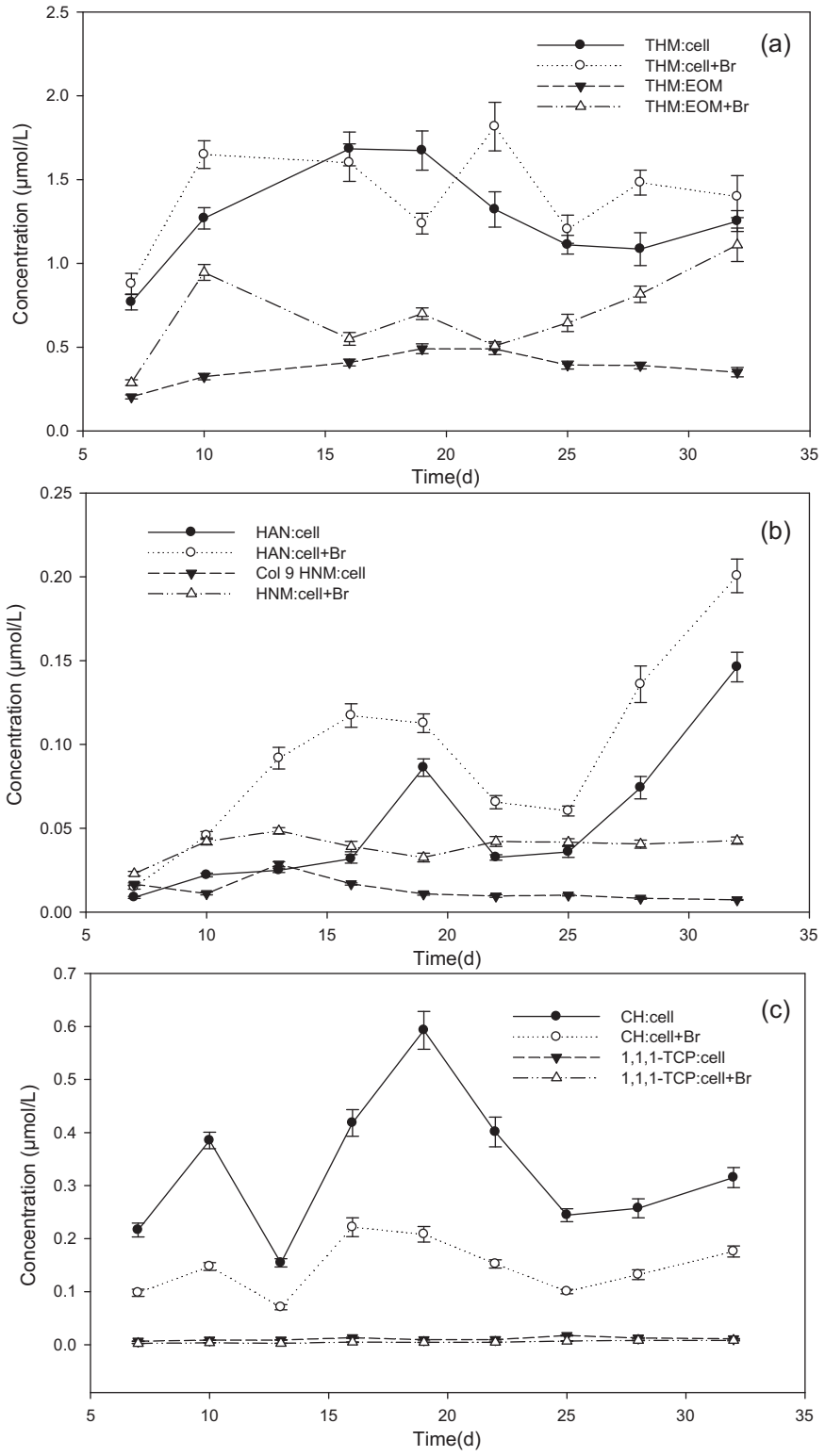

Fig. 4. The formation potential of DBPs during chlorination of *Microcystis* EOM and cells in the absence and presence of bromide (pH 7.2, 3-day reaction, 22 ± 1 °C).

Surprisingly, the formation of N-DBPs, including dichloroacetonitrile (DCAN) and trichloronitromethane (TCNM), did not follow the similar trends during chlorination of algal cells with increasing culture age. Formation of DCAN increased and that of TCNM remained relatively constant. Algae are enriched with organic nitrogen contents. Protein-like substances exist in algal cells, as indicated by the fluorescence EEMs in Fig. 2. Amino acids and aliphatic amines are also found in algal cells [6]. These organic-N compounds are known to react with chlorine to form N-DBPs. For example, glutamic acid and histidine were significant precursors of DCAN [31]. Methylamine and aspartic acid reacted with chlorine to form TCNM [32,33]. With increasing culture age, total nitrogen

contents increased [34], which may result in more precursors of N-DBPs. DCAN formation followed this trend. But generation of TCNM stayed relatively constant.

DBPs generated during chloramination are displayed in Figs. S2 and S3 in the Supplementary material. HAN generation during chloramination showed significant differences from that during chlorination. HANs concentrations fluctuated for both *Microcystis* and *Chlorella*. This could be due to the different precursors for HAN formation during chlorination and chloramination. Monochloramine, other than nitrogen contents in algae, could also contribute to the nitrogen source for HAN formation. A previous study using $^{15}NH_2Cl$ proved that nitrogen in DCAN originated from both monochloramine and organic nitrogenous compounds [32]. Compounds that do that contain nitrogen can also react with monochloramine to form DCAN. Therefore, different precursors could be the reason that led to difference in DCAN formation during chlorination and chloramination of algae at various culture age.

Risk assessment of the DBP may be an interesting topic to explore. But due to the formation potential conditions applied and the high algal concentrations used in this study, risk assessment is not evaluated here.

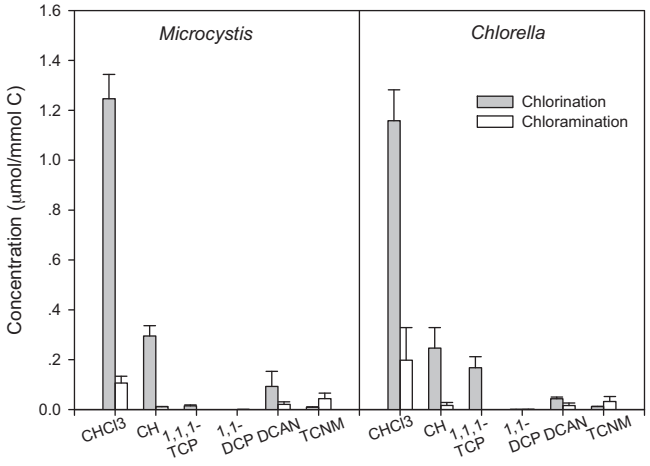

Fig. 5. The average values of specific DBP yield during chlorination and chloramination of *Microcystis* and *Chlorella* cells at the stationary growth phase (25 and 28 days of cultivation).

3.2.2. Comparison of Cl_2 and NH_2Cl

Fig. 5 displays the average values of specific DBP yields during chlorination and chloramination of algal cells at the stationary growth phase (Day 25 and Day 28) without the presence of bromide. During chlorination, chloroform, chloral hydrate and 1,1,1-TCP were the dominant species and chloroform was the dominant specie during chloramination. Concentrations of chloroform and chloral hydrate during chlorination of algal cells were much higher than those during chloramination, which were 5 and 15 times for *Chlorella* and were 11 and 26 times for *Microcystis*. DCAN concentrations during chlorination were 2–3 times higher than those generated during chloramination. TCNM formation showed the opposite trends and chloramination of algal cells generated larger amounts of TCNM than chlorination. Fang et al. [6] found similar results when chlorinating and chloraminating cells of *Microcystis*. The DBP results from chlorination and chloramination of algae followed the same trends as those from natural organic matter except TCNM [35]. Hu et al. [33] evaluated the formation potential of HNMs from raw and treated waters with a wide range of DOC and SUVA values and found that chlorination generated more HNMs than chloramination. Another study found similar formation of TCNM during chlorination and chloramination of NOM fractions [35]. Amine and amino acid, such as methylamine and aspartic acid, were precursors for TCNM formation [32,33]. Meanwhile, whether monochloramine contributes to the nitrogen source of TCNM remains unknown. The reaction mechanisms between disinfectants with the potential precursors of TCNM need further investigation.

3.2.3. Effect of bromide

Greater concentrations of total THMs, HANs, and HNMs were observed for both algae species in the presence of bromide, as shown in Figs. 3 and 4. Bromide also shifted the DBPs to brominated ones. The speciation of THMs, HANs, and HNMs during chlorination of *Chlorella* cells in the presence of bromide is shown in Fig. S4 in the Supplementary material. Four THMs, including $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$, were detected. Of the four HANs analyzed, bromochloroacetonitrile (BCAN), dibromoacetonitrile (DBAN), and DCAN were detected. Of the nine HNMs, TCNM, bromodichloronitromethane (BDCNM), dibromochloronitromethane (DBCNM) were found and DBCNM were the dominant specie with the presence of 1 mg/L bromide. No brominated haloacetaldehydes and haloketones were analyzed, but the formation of chlorinated counterparts, such as chloral hydrate and 1,1,1-TCP decreased. The formation of these DBPs varied with the increase

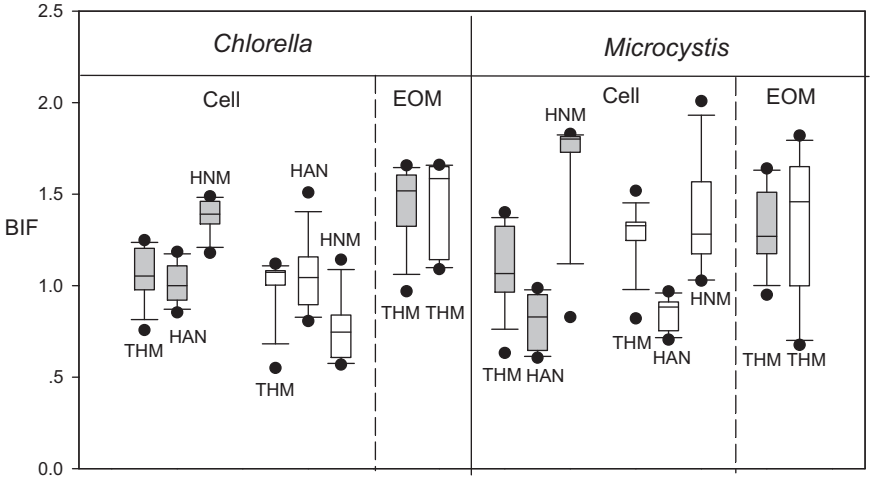

Fig. 6. Bromine incorporation in THM, HAN and HNM during chlorination and chloramination of *Chlorella* and *Microcystis* cell and EOM solutions. (Grey: chlorination, Clear: chloramination; The data obtained from 3, 7, 10, 13, 16, 19, 22, 25, 28 and 32 days of cultivation.)

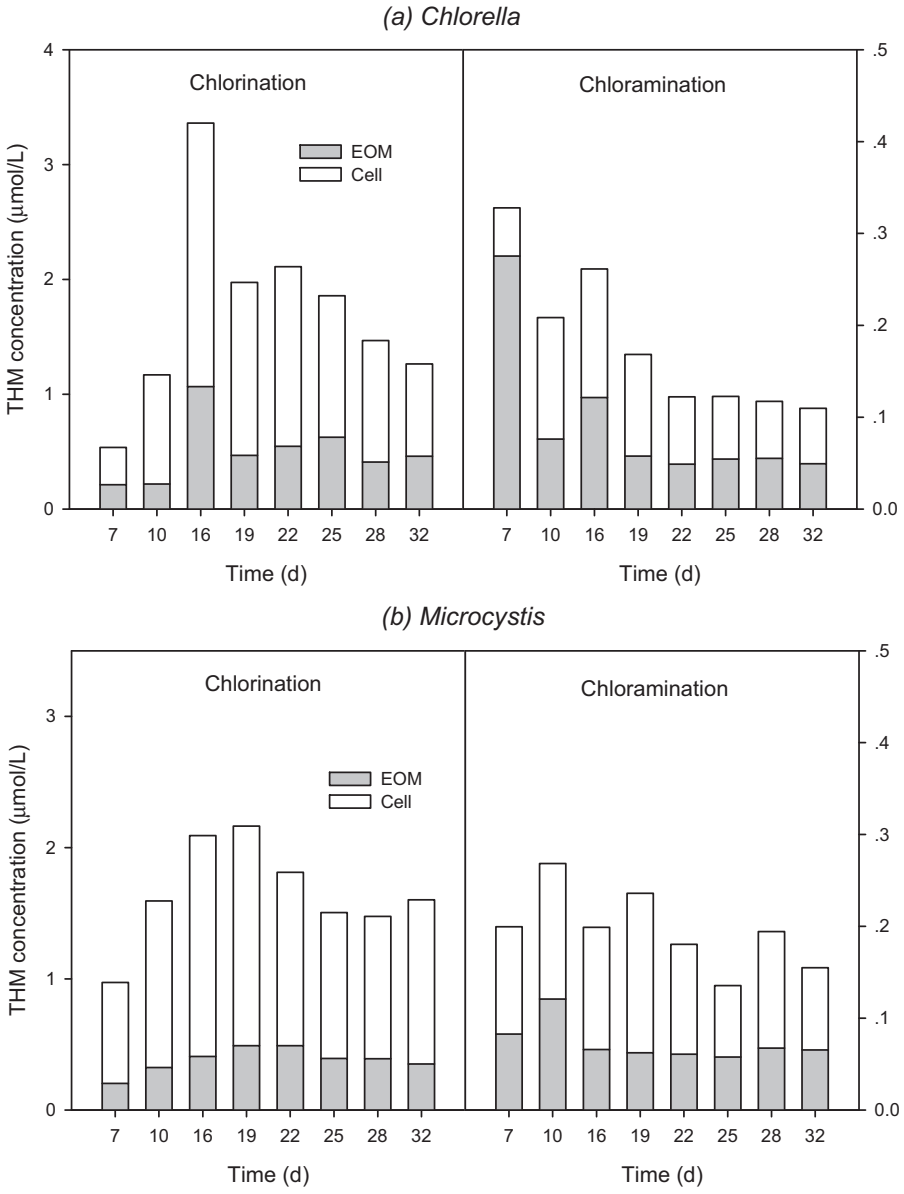

Fig. 7. Comparison of THM formation during chlorination and chloramination of algal cells and EOM solutions (pH 7.2, 3-day reaction, 22 ± 1 °C).

of culture age. The concentrations of all THM species increased steadily from Day 7 to Day 16 and decreased gradually from Day 22 on the start of stationary phase. The concentrations of HANs increased. HNM yields did not show significant variations after Day 16. The trend of the formation of DBPs with increasing culture age in the presence of bromide is similar as those without the presence of bromide.

Bromine incorporation factor (BIF) is often applied to indicate the degree of bromine incorporation, and is defined as follows:

$$\text{BIF} - \text{THM} = \frac{[\text{CHCl}_2\text{Br}] + 2[\text{CHClBr}_2] + 3[\text{CHBr}_3]}{\text{Total THM}} \quad (1)$$

$$\text{BIF} - \text{HAN} = \frac{[\text{BCAN}] + 2[\text{DBAN}]}{\text{Total HAN}} \quad (2)$$

For HNMs:

$$\text{BIF} - \text{HNM} = \frac{[\text{BNM}] + 2[\text{DBNM}] + 3[\text{TBNM}] + [\text{BCNM}] + [\text{BDCNM}] + 2[\text{DBCNM}]}{\text{Total HNM}} \quad (3)$$

BNM – bromonitromethane; DBNM – dibromonitromethane; TBNM – tribromonitromethane; BCNM – bromochloronitromethane.

All concentrations are in the unit of μmol/L.

Fig. 6 displays bromine incorporation in THMs, HANs and HNMs. The average BIF values for THMs, HANs, and HNMs generated during chlorination of *Chlorella* cells were 1.1, 1.0 and 1.4, respectively and those for *Microcystis* cells were 1.1, 0.8 and 1.7, respectively. Bromine incorporation in THMs was comparable to that in HANs for *Chlorella* and was higher than in HANs for *Microcystis*. Bromine incorporation in HNMs was much higher than that in THMs and HANs except the case of chloramination of *Chlorella* cells. The results are consistent with those obtained from natural waters. Hu et al. [33] compared bromine incorporation in HNMs and THMs generated from ozonation–chlorination of raw and treated water and found that more bromine was incorporated in HNMs than THMs. Obolensky and Singer [36] evaluated the halogen substitution patterns using Information Collection Rule data and

Table 1
Comparison of DBP formation from algae and NOM.

	THM (μmol/mmol C)	DCAN (μmol/mmol C)	TCNM (μmol/mmol C)	CH (μmol/mmol C)	Chlorination conditions	References
Blue-green algae						
Anabaena flos-aquae						
-Cells	5.03				pH 7, 21 °C, 7-day reaction	Hoffman et al. [10]
-EOM	2.61				Stationary growth phase (Day 34)	
Microcystis aeruginosa						
-Cells	6.13				pH 7, 21 °C, 7-day reaction	Hoffman et al. [10]
-EOM	2.81				Stationary growth phase (Day 34)	
Oscillatoria sp.						
-Cells	2.61				20 °C, 3-day reaction	Ueno et al. [31]
Microcystis aeruginosa						
-Cells	2.41	0.46	0.04	0.87	pH 7, 21 ± 1 °C, 3-day reaction	Fang et al. [7]
-EOM	1.41	0.15	0.12	0.51	Stationary growth phase (Day 42)	
Microcystis aeruginosa (This study)						
(1)Cells	2.01	0.03	0.04	0.21	pH 7.2, 22 ± 1 °C, 3-day reaction	
(2)Cells	1.21	0.15	0.01	0.27	(1) Log growth phase (Day 13)	
(1)EOM	1.31				(2) Stationary growth phase (Day 25)	
(2)EOM	1.21					
Green algae						
Chlamydomonas sp.						
-Cells	3.42				20 °C, 3-day reaction	Hong et al. [38]
Scenedesmus quadricauda						
-EOM	2.21				pH 7, 21 °C, 7-day reactionLate log growth phase	Plummer and Edzwald [4]
Chlorella vulgaris (This study)						
(1)Cells	3.32	0.07	0.06	1.45	pH 7.2, 22 ± 1 °C, 3-day reaction	
(2)Cells	1.21	0.04	0.01	0.19	(1) Log growth phase (Day 13)	
(1)EOM	1.51				(2) Stationary growth phase (Day 25)	
(2)EOM	1.31					
Natural organic matter						
Suwannee River NOM	7.04	0.14	0.04	0.80	pH 7, 21 ± 1 °C, 3-day reaction	Fang et al. [7]

found that the median value of bromine incorporation (using the same definition as this study) in dihaloacetonitriles and trihalomethanes was 0.34 and 0.3, respectively. Variation of bromine incorporation with increasing culture age is shown in Fig. S4 in the Supplementary material. Bromine incorporation in THMs and HANs tended to decrease with increasing cultivation period during chlorination and that in HNMs stayed relatively constant.

Comparing EOM with algal cells, bromine incorporation in THMs during chlorination and chloramination of EOM was higher than that of cells. Median BIF in THMs generated during chlorination was 1.4 and 1.3 for EOM of *Chlorella* and *Microcystis*, respectively. BIF during chloramination of EOM was similar as that during chlorination. Bromine incorporation is found to be higher in waters with higher Br/DOC ratios [36]. The higher Br/DOC ratio in EOM solutions than cells could be one reason contributing to higher BIF in EOM. On the other hand, bromine appears to be more effectively incorporated into low UV-absorbing (i.e., low SUVA), low molecular weight (MW) and hydrophilic NOM fractions [37]. Compared to cell solutions (1.1 L mg^{-1} m^{-1} for both species), EOM solutions had lower SUVA values (0.7 L mg^{-1} m^{-1} for *Chlorella* and 0.5 L mg^{-1} m^{-1} for *Microcystis* on average before Day 28), which indicated more hydrophilic fractions. All above could lead to more bromine incorporation in EOM solutions.

3.2.4. THMs from cells and EOM

The potential impact of growth medium to DBP formation was evaluated. The yield of THM generated from growth medium alone was found to be very low compared to those EOM samples. But the yield of other DBPs from growth medium was observed. Therefore, for EOM solutions, only THM formation is considered.

The cellular materials had a higher production of chloroform during chlorination than EOM, as shown in Fig. 7. The cells accounted for 70% and 77% of the total chloroform during chlorination of *Chlorella* and *Microcystis*, respectively in the absence of bromide. The cells accounted for 58% and 63% during chloramination, with the remainder from EOM. The fractionation results of *Microcystis* are in agreement with the previous results [18]. Results from chlorination of other algal species, such as *Scenedesmus quadricauda* (green algae) and *Anabaena flos-aquae* (blue-green algae), had also found the higher fraction of DBP generation from algal biomass than the extracellular products [4,18]. The results suggested that coagulation or other techniques effective to remove intact algae cells is necessary to reduce the DBP formation from algae.

As mentioned previously, bromine incorporation in THMs during chlorination and chloramination of EOM was higher than that of cells. Besides the different characteristics between EOM and cells, another reason could be the higher Br/TOC values in EOM, as strong trends of increasing extents of bromine substitution with higher Br/TOC values has been reported [36].

3.2.5. Contribution of algae as precursor pool

To compare DBP formation from various sources of organic matter, including various algal species and natural organic matter, the results from this study together with other studies were normalized to the TOC concentration and are presented in Table 1. As shown in Table 1, NOM generated more chloroform than algae during chlorination. Formation of chloroform during chlorination of algae substances was in the range of 1.21–6.13 μmol/mmol C and that during chlorination of NOM was 7.04 μmol/mmol C. Formation of chloral hydrate during chlorination of algae ranged from 0.19 to 1.45 μmol/mmol C. DCAN and TCNM generated during chlorination of algae were comparable or higher than that from chlorination of NOM. This is related with the high organic nitrogen contents in algal materials. The findings of this study show that algae can contribute significantly to the DBP precursor pool, especially to the N-DBP precursors. Since the cellular materials were the major precursors to EOM, effective removal of intact algal cells prior to disinfection will minimize production of DBPs.

4. Conclusions

The low SUVA values of EOM and cell solutions from both algal species indicated the very hydrophilic nature of algal materials. Fluorescence EEMs showed that algal EOM and cells were enriched with protein-like and soluble microbial by-product-like matters. EOM had more humic/fulvic-like substances than cells.

Great increases in DBP formation were found from the start of the exponential phase for all tested DBPs and the yields varied afterwards during chlorination. The presence of bromide enhanced the total concentrations of THMs, HANs, and HNMs and shifted DBPs to more brominated ones. In general, bromine incorporation in HNMs was higher than in HANs and THMs. In comparison to EOM, cellular materials accounted for more fractions in DBP formation. Algae can contribute significantly to the DBP precursor pool. These results suggested that coagulation or other techniques effective to remove intact algae cells could minimize the DBP formation from algae.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.09.098.

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