A Comparison of the Role of Two Blue-green Algae in THM and HAA Formation

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Abstract The contribution of two blue-green algae species, *Anabaena flos-aquae* and *Microcystis aeruginosa*, to the formation of trihalomethanes (THMs) and haloacetic acids (HAAs) was investigated. The experiments examined the formation potential of these disinfection by-products (DBPs) from both algae cells and extracellular organic matter (EOM) during four algal growth phases. Algal cells and EOM of *Anabaena* and *Microcystis* exhibited a high potential for DBP formation. Yields of total THMs (TTHM) and total HAAs (THAA) were closely related to the growth phase. Reactivity of EOM from *Anabaena* was slightly higher than corresponding cells, while the opposite result was found for *Microcystis*. Specific DBP yields (yield/unit C) of *Anabaena* were in the range of 2-11μmol/mmol C for TTHM and 2-17μmol/mmol C for THAA, while those of *Microcystis* were slightly higher. With regard to the distributions of individual THM and HAA compounds, differences were observed between the algae species and also between cells and EOM. The presence of bromide shifted the dominant compounds from HAAs to THMs.

Keywords algae; *anabaena flos-aquae*; *microcystis aeruginosa;* disinfection byproducts; trihalomethanes; haloacetic acids.

INTRODUCTION

Algae are ubiquitous in rivers, reservoirs and lakes. During algal blooming seasons, the increase of algae cells and their excreted metabolic substances may cause a series of problems for water treatment: (1) undesirable taste and odour; (2) potential toxicity concerns, particularly with blue-green algae which may excrete algal toxins; (3) interference by both algal cells and their metabolic substances with the coagulation process (Plummer and Edzwald, 2002; Takaara et al., 2007; Henderson et al., 2008); (4) contribution to total organic carbon and disinfection by-product (DBP) formation. Algae cells contain a wide range of organic nitrogen compounds, such as polysaccharides, proteins, peptides, amino sugars and traces of other organic acids. These materials will be excreted as metabolic substances during growth through diffusion driven by the equilibrium between intra- and extracellular concentration, often referred to as extracellular organic matter (EOM). The cell wall consists of cross-linked peptide chains of N-acetyglucosamine and N-acetylmuramic acids and contains other organic nitrogen compounds as well. The irreversible degradation of cell wall surface is considered to be another EOM material (Watt, 1966). EOM released by diffusion is mostly found during the exponential growth phase with low molecular weight intermediate products such as glycolic and amino acids, while EOM from senescent cells are those with high molecular weight products, such as polysaccharides, which occur often in the later growth phases of algae. All these organic compounds may contribute to DBP formation and particularly to prominent DBP species such as trihalomethanes (THMs) and haloacetic acids (HAAs) (Scully et al., 1988; Hureiki et al., 1994; Westerhoff and Mash, 2002). The potential role of algae (cells and EOM) in DBP formation has been considered in several studies in the past two decades (Wardlaw et al., 1991; Graham et al., 1998; Glezer et al., 1999; Plummer and Edzwald, 2001; Nguyen et al., 2005).

The formation of THMs varies according to algae species, growth phase and also the chlorination

conditions (e.g. pH, temperature, contact time). Under similar chlorination conditions (pH 7, 24h contact time, 20-24^{o}C), the reported yields of THMs from algal biomass range from 3.5 μg $CHCl_3$/mg TOC to 7.3 μg $CHCl_3$/mg TOC, and those from EOM were similar, ranging from 3.7 μg $CHCl_3$/mg TOC to 8.7 μg $CHCl_3$/mg TOC (Wardlaw et al., 1991). A difference was observed between algal biomass and EOM when extending the contact time (Plummer and Edzwald, 2001), partly due to the release of intracellular organic matter resulting from cell lysis.

There has been very little research to-date on the role of algae in HAA formation. HAA yield from EOM extracted from a green algae, *Senedesmus*, was 60 μg total HAA/mg TOC, and green algae have been argued to be the most productive in THM formation as compared to blue-green algae and diatoms (Nguyen et al., 2005). However, contradictory results were found in other research, where EOM extracted from blue-green algae was reported to be the most reactive, followed by EOM from diatoms and green algae (Plummer and Edzwald, 2001).

It is clear that the information on HAA formation from algae is insufficient, particularly the role of algal cells. Water utilities that apply pre-chlorination may cause the release of intracellular organic matter (IOM) from the disruption of algal cells, and this IOM can be a significant DBP precursor. The potential of both algal cells and extracellular organic matter (EOM) to form THMs and HAAs was investigated in this study. Two blue-green algal species, *Anabaena flos-aqua* and *Microcystis aeruginosa* were selected, as they are common species in UK surface waters. Also, blue-green algae are nitrogen fixers and liberate up to 45% of their fixed nitrogen as organic-N (Westerhoff and Mash, 2002), which may lead them to be significant contributors to THM and HAA formation. Thus, previous studies have indicated that chlorination of amino acids can form an unstable intermediate dichloroacetonitrile (DCAN), which will continue to react with chlorine to form both THMs and HAAs (Ueno et al., 1996; Reckhow et al., 2001). In addition, other organic-N

compounds such as proteins and amino sugars contain significant amount of di-HAA active sites (Croué et al., 2000; Hwang et al., 2001). In this paper several aspects will be discussed: (1) the difference between algal cells and EOM in total DBP formation, specific DBP yield (yield/unit C used) and DBP species distribution; (2) the influence of algal growth phase (3) the influence of algae species; (4) interactions between algal cells and EOM in DBP formation; and (5) the relative importance of bromide on total DBP formation and individual DBP species distribution in the presence of algae.

MATERIALS AND METHODS

Cultivation of Algae

Two axenic stock cultures of *Anabaena flos-aquae* and *Microcystis aeruginosa* were obtained from the Culture Collection of Algae and Protozoa (CCAP), Windermere, UK and Institut Pasteur, France, respectively. Both species are blue-green algae. *Anabaena flos-aquae* grows in long filaments of vegetative cells while *Microcystis aeruginosa* is usually observed as individual spherical cells.

Media preparation and cultivation procedures of both algal species were followed strictly with the instructions provided by the suppliers to achieve the optimal growth of algae. In brief, stock cultures of both species were firstly inoculated into an inorganic growth medium and incubated until the cell density indicated an optimal growth for further sub-culturing. Sub-cultured samples were placed in a shaking water bath for homogenous mixing, with temperature controlled at 20 ± 1^{o}C. Cool white fluorescent-light was provided for illumination in 12h light/12h dark cycles, and sufficient aeration was supplied. With each algae species, samples for different culture periods were run in batch without any replacement or replenishment of growth media. To prevent contamination,

111 the media used to culture both the algae species were sterilised by autoclaving and all operations
112 with algae culture were undertaken under air filter and sterile conditions.
113
114 Algal growth was monitored by measuring the concentration of chlorophyll-a. Two other
115 commonly used methods, namely optical density measurement and cell number counting, were also
116 conducted to confirm the results of the chlorophyll-a measurements. Methanol was used to extract
117 chlorophyll-a from the two species according to the method created by Papista et al. (2002), which
118 was slightly modified based on the ISO 10260 standard procedure (ISO, 1992). Due to the
119 difficulties in cell counting for *Anabaena*, this measurement method was only carried out on
120 *Microcystis*. Measurements of optical density at 730nm for OD_{730} and at 664nm and 750nm for
121 methanol extracts were all done by a Shimadzu UV-2401 spectrophotometer with a 1-cm cell.
122 Measurements were undertaken at least in duplicate to improve experimental accuracy.
123
124 **Separation of Cells and EOM**
125 To assess the contribution of algae to DBP formation over time, samples containing both algal cells
126 and EOM were removed from the growth flasks at certain intervals throughout their growth phase
127 and subjected to centrifugation. EOM was collected from the centrifugate after passing through a
128 0.45-μm Whatman membrane filter to remove any remaining cells. The separated cells from the
129 centrifugation were washed three times and re-suspended in de-ionised water. Separated cells,
130 EOM, as well as the original algae suspension before separation were transferred to 250ml amber
131 bottles for chlorination tests. Duplicate quantities of the cell suspensions, EOM aliquots and
132 original algae samples were taken for TOC determination (TOC analyser, Shimadzu Ltd, Japan).
133
134 **Chlorination and DBPs Analysis**

All algae samples were adjusted to pH 7 by HCl before chlorination and buffered with phosphate to maintain the pH. Excess chlorine was applied based on a chlorine demand test conducted beforehand to ensure a substantial residual of chlorine (≥0.5 mg/L) after a 7-day chlorination period (DBP formation potential). All chlorinated samples were stored head-space free at 21^{o}C in the dark for periods of 1 day and 7 days, in accordance with standard procedures (APHA, 1998). Bromide was also purposely spiked into some of the samples (6 μmol/L) to investigate the effect of bromide on DBP formation.

At the end of the chlorination period (either 1 day or 7 days), samples for THM analyses were collected head-space free in 40ml glass vials containing sodium thiosulphate quenching agent, while samples for HAA analyses were collected in vials with ammonia sulphate quenching agent. Residual chlorine was determined at the time of sample collection by using the DPD Standard Method 4500-Cl F (APHA, 1998) and pH was measured at the sampling times as well. The four chlorine- and bromine- containing THM compounds were extracted by liquid/liquid extraction with methyl tert-butyl ether (MtBE) and determined by gas chromatography and electron capture detection (GC/ECD) according to Standard Method 6232B (APHA, 1998) but with the minor modifications developed by Baribeau et al. (2005). The nine HAA (HAA_9) compounds were quantified by liquid/liquid extraction with MtBE, followed by derivatisation with acidic methanol and finally by GC/ECD analysis in accordance with USEPA Method 552.3 (USEPA, 2003). To avoid degradation of DBP species, all samples were processed within 3 days after collection. All analyses were carried out in duplicate. In general, molar concentration units are used throughout the paper to present the data of DBP yield and to assist in the interpretation of results. Occasionally mass concentration units are used to enable comparison of the results with other published findings.

159 The potential complication of NH_2Cl formation in the chlorination tests arising from the presence of
160 $(NH_4)_6Mo_7O_{24}•4H_2O$ in the algal growth medium was believed to be insignificant owing its low
161 concentration (1mg/L). In addition, the potential impact of the growth medium in terms of DBP
162 formation can be neglected since the yield of THM and HAA compounds produced by the medium
163 alone was found to be very low compared to those from samples with algae and EOM.
164
165 **RESULTS AND DISCUSSION**
166 **Algal growth**
167 Fig. 1 shows the relationship between chlorophyll-a, optical density at 730nm (OD_{730}) and DOC of
168 EOM for *Anabaena flos-aquae* and *Microcystis aeruginosa*. Changes in chlorophyll-a are
169 commonly used to distinguish the growth phases for blue-green algae. All four growth phases,
170 namely, lag, exponential, stationary and death phase, can be distinguished. The lag phase of
171 *Anabaena* and *Microcystis* lasted approximately 10 to 15 days, during which time barely any
172 changes were observed in both chlorophyll-a and OD_{730}. A dramatic increase in chlorophyll-a for
173 both species indicated the start of the exponential phase, which lasted until Day 25 and Day 29 for
174 *Anabaena* and *Microcystis*, respectively. For *Anabaena*, it was difficult to distinguish the transition
175 from the exponential phase to the stationary phase based solely on chlorophyll-a, since the colour
176 kept turning dark with culture time while the cell numbers seemed to stop increasing (based on
177 OD_{730} value). The death phase was believed to have started at some point beyond Day 34 for
178 *Anabaena* and Day 36 for *Microcystis*, when the pigment inside the cells began to fade resulting in
179 a decrease in chlorophyll-a.
180
181 The concentration of EOM excreted from both species increased steadily with culture age.
182 *Microcystis* produced a much greater amount of EOM compared to *Anabaena*, which reached 2.26
183 mg/L before the excretion of intracellular organic matter (IOM) from the autolysis of cells in the

death phase. Consistent with findings reported by Nguyen et al. (2005), a close linear relationship between chlorophyll-a and OD_{730} (R^2 = 0.97 for *Anabaena* and 0.98 for *Microcystis*) was observed. This suggests that OD_{730} can also be used as a parameter to indicate the growth of the two blue-green algae. However, no correlations were found between TOC and chlorophyll-a or OD_{730}.

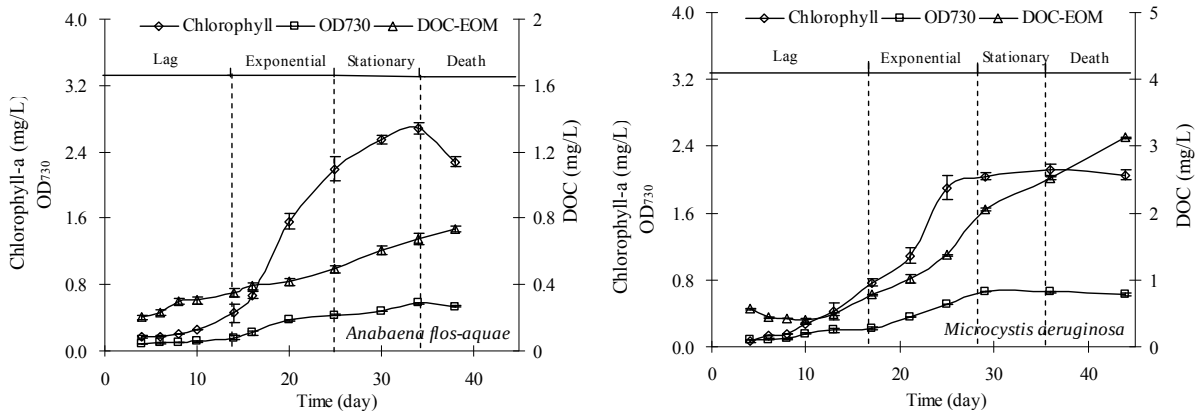


Fig. 1. Growth curves for *Anabaena flos-aquae* and *Microcystis aeruginosa*.

Total DBP Formation from Cells and EOM

Fig. 2 shows that the total THM (TTHM) and total HAA (THAA) yield produced by *Microcystis* (cells and EOM) varied with growth age. During the lag phase, both the TTHM and THAA yield remained constant, with a slight increase at the beginning of the exponential phase. The yield fluctuated at the end of the exponential phase, then steadily increased in the stationary phase. The maximum yield of TTHM and THAA (without bromide spike) in cell samples of *Microcystis* was 1.41 μmol/L in the exponential phase and 3.06 μmol/L in the later stationary phase. In the death phase, THAA formation from the cells and EOM decreased, while TTHM produced by EOM was increased. A similar trend was found for *Anabaena* (Huang et al., 2008), which suggests that IOM released due the autolysis of cells in later growth phases may favour THM formation over HAA formation.

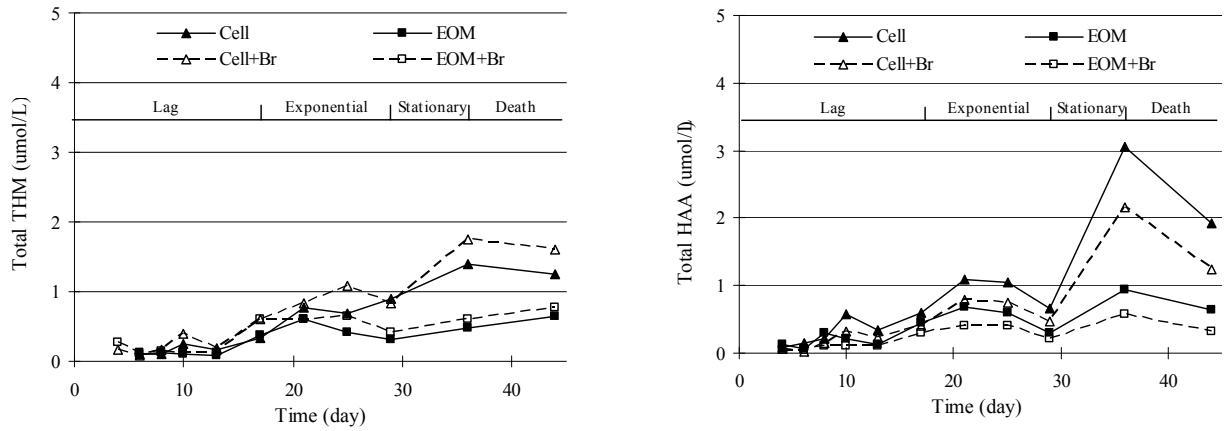
For both algae species, cells exhibited a higher productivity in THM and HAA formation as compared to their corresponding EOM, as was also found with other algae species (Wachter, 1982; Graham et al., 1998; Plummer and Edzwald, 2001). This implies that treatment to physically remove algal cells (without rupture) can be a more effective way to control DBP formation, while on the other hand pre-treatment such as pre-ozonation and pre-chlorination, which may cause cell breakage and the release of IOM, should be avoided if possible.

The specific molar yield of DBPs, expressed as μmol/mmol C, is normally used to indicate the reactivity of organic matter with chlorine, thereby allowing comparison between different types of organic matter and their significance in DBP formation. Contrary to earlier findings for *Anabaena* (Huang et al., 2008), the specific yield of both THMs and HAAs from the cells of *Microcystis* was about 2-3 times greater than that from EOM throughout the growth phases (Fig. 3). In the absence of bromide, the average value of specific yield produced by cells and EOM of *Microcystis* was 5.76 and 3.47 μmol/mmol C, respectively, for THMs, and 9.73 and 4.61 μmol/mmol C, respectively, for HAAs. Similar levels of THMs were observed in *Anabaena* samples containing either cells or EOM. However, the specific yield of HAAs produced by *Anabaena* was slightly lower compared to *Microcystis*, perhaps due to its lower hydrophobic characteristics and HAA precursor content in general (Liang and Singer, 2003; Hua and Reckhow, 2007).

In contrast to the total yield observed with both algae species, the specific DBP yield was much less influenced by growth phase (Fig. 3). In the case of *Microcystis*, a peak in the specific yield of THMs and HAAs was observed at the end of the lag phase, but the pattern of yield was quite different for *Anabaena* which gave a greater specific yield in the later growth phases. Overall, for both algae under all the conditions investigated, the THM specific yield was ≤14 μmol/mmol C, and the HAA specific yield was ≤ 24 μmol/mmol C.

228

229 Potential interactions between cells and EOM were also investigated. The numerical sum of the
230 DBPs formed individually by cells and EOM was compared with the yield produced by the two
231 together. An antagonistic effect was observed with both algae species, although it was less apparent
232 for *Microcystis*. With regard to individual DBPs, the interaction between cells and EOM had more
233 of an impact on THM formation than HAA formation (Fig. 4a). Several reasons may help to explain
234 the observed antagonistic effect. Firstly, cell debris may serve as an adsorbent for THMs and HAAs
235 in chlorinated samples containing both cells and EOM. THMs are relatively hydrophobic and may
236 be more readily adsorbed by cell material than the more hydrophilic HAAs, thereby explaining the
237 greater apparent antagonistic effect for THMs than HAAs. Secondly, there may be interactive
238 scavenging of THM or HAA intermediate species produced during chlorination leading to a
239 consequent reduction in the final compounds, or interactions between intermediate compounds that
240 react with the cells and EOM leading to other (non-THM/non-HAA) DBP compounds. Similar
241 antagonistic effects between substances with different chemical properties and polarity have also
242 been reported in other studies (Kanokkantapong et al., 2006). Finally, the extent of cell breakage
243 resulting in the release of organic matter to react with chlorine, which mainly depends on cell
244 morphology and cell-to-chlorine ratio (Plummer and Edzwald, 2002), may also be responsible for
245 the antagonistic effect. As shown in Fig. 4b, the antagonistic effect was much less obvious in the
246 results corresponding to a 1-day chlorination period, in which the samples still had a high chlorine-
247 to-cell ratio.



248

249 **Fig. 2.** Total THMFP and HAAFP for *Microcystis* cells and EOM (pH 7, 21°C, 7 days).

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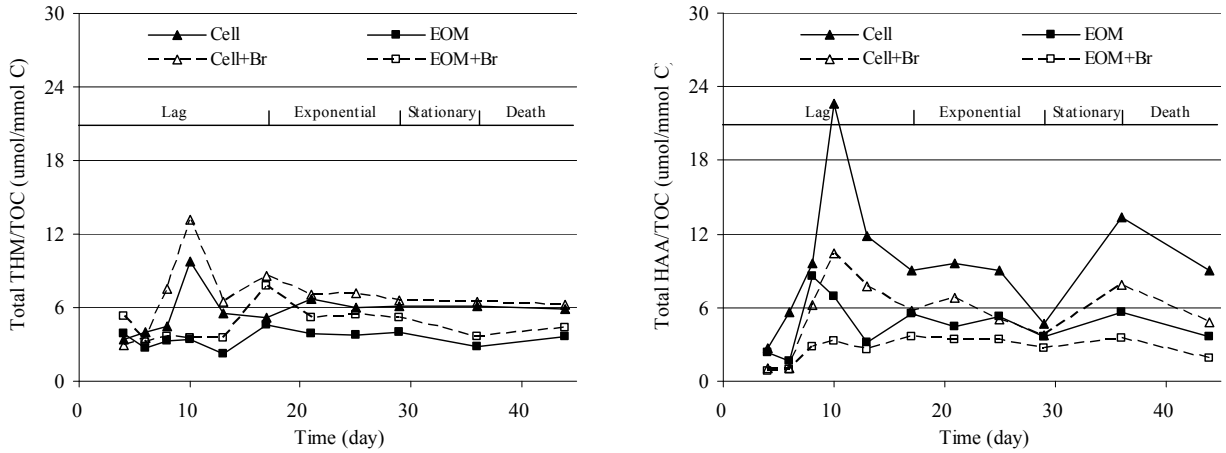

251

252 **Fig. 3.** Specific total THMFP and HAAFP for *Microcystis* cells and EOM (pH 7, 21°C, 7 days).

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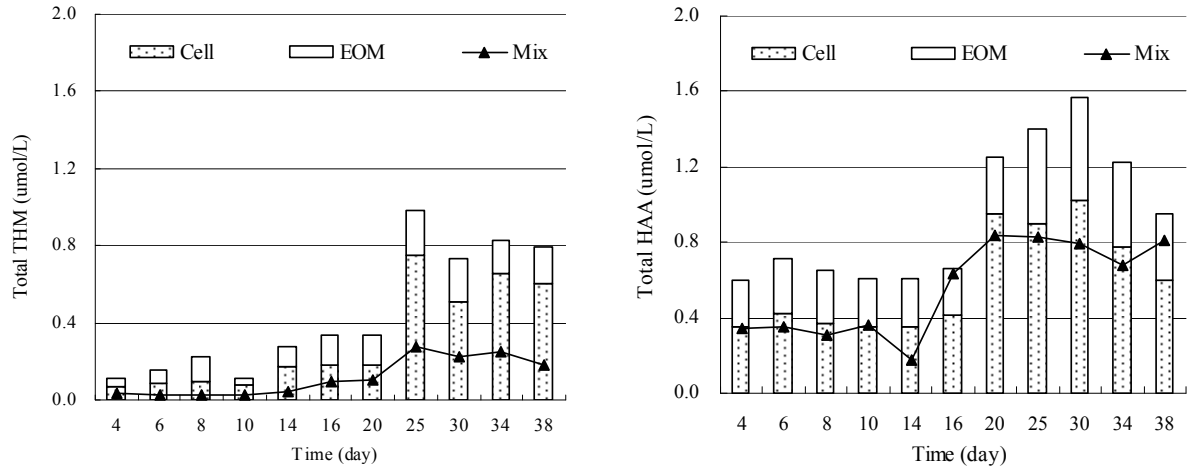

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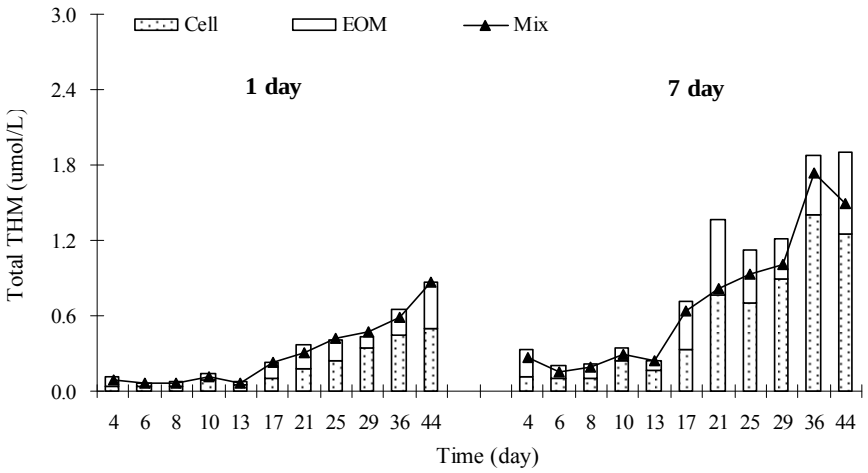
255 (a)



256

257 (b)

258 **Fig. 4.** Interaction effects between cells and EOM in DBP formation: (a) TTHM-FP and THAA-FP
259 for *Anabaena*; (b) TTHM formation (1 day and 7 days) for *Microcystis*.

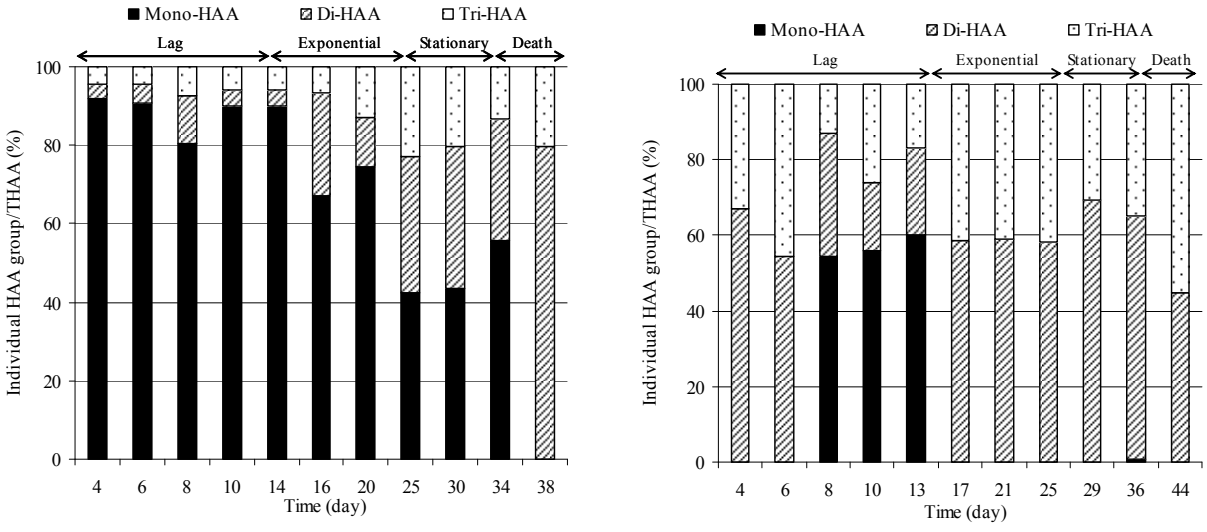
260

261 **HAA Speciation from Cells and EOM**

262 Apart from the total yield of DBPs, differences were also observed in the distribution of individual
263 DBPs (mainly the HAA speciation) for cells versus EOM. Mono-HAA was the predominant species
264 produced by both cells and EOM of *Anabaena* in the early growth phase (lag and exponential
265 phase) (Fig. 5a). However, higher halogenated species became dominant when the growth phase
266 progressed into the stationary phase, which was especially prominent in cell samples. For
267 *Microcystis*, mono-HAA appeared only at the transition between the lag and exponential growth
268 phases. The average ratio of tri-HAA to di-HAA was 1.2 umol/umol for cells and 0.66 umol/umol
269 for EOM, which are comparable to the results of Nguyen et al. (2005) and Plummer and Edzwald
270 (2001). The dissimilarity existing in individual HAA species distribution from the two algae species
271 may be attributed to differences in the composition of individual algogenic organic matter (AOM),
272 including both IOM and EOM.

273

In the early growth phase, EOM excreted from algae is mainly derived from a diffusion process driven by the equilibrium between intra- and extracellular concentrations (Nguyen et al., 2005) and is comprised of up to 90% polysaccharides and a small amount of protein, amino acids and other trace amounts of nitrogenous organic matter (Myklestad, 1995). The proportion of protein-related substances in EOM increases with time and usually reaches a maximum when IOM is released resulting from the autolysis of cells. The increasing proportion of proteinaceous material in EOM intensified the domination of di-HAA, as observed for both algae (Fig. 5), which is consistent with the suggestion that organic-N compounds contain active sites for di-HAA formation (Croué et al., 2000; Hwang et al., 2001). As compared to other algae species, *Microcystis* also produced a large amount of tri-HAA, especially in cell samples, in which tri-HAA accounted for nearly 60% of the total HAA formation. This may be attributed to the high hydrophobicity of algogenetic organic matter produced by *Microcystis* (Choi et al., 2004; Henderson et al., 2008). A sharp increase in the tri-HAA ratio was observed in EOM samples during the death phase, which suggests that intracellular organic matter (IOM) from decaying cells of *Microcystis* can be a significant tri-HAA precursor. The reason for the appearance of a high proportion of mono-HAA from both algae species during the early growth phase is not clear, but polysaccharides, as the predominant metabolic substance, may be responsible for the production of low halogenated HAA species.



(a) (b)

Fig. 5. Distribution of HAA compound groups (with bromide spike) from EOM of (a) *Anabaena* and (b) *Microcystis*.

Impact of bromide on DBP formation

Greater concentrations of HAAs compared to THMs were observed for both algae species in the absence of bromide as (Fig. 6), which is different from earlier findings obtained with green algae (Nguyen et al., 2005). Blue-green algae are nitrogen fixers which can excrete up to 45% of the total fixed nitrogen as organic-N, which supports the formation of HAA over THM and di-HAA over tri-HAA when in a relatively high ratio to DOC (C/N < 15) (Westerhoff and Mash, 2002). Nevertheless, in the presence of bromide the DBP species shift from HAAs to THMs. This is consistent with the theory that bromide is more effectively incorporated into low UV-absorbing, low molecular weight and hydrophilic fractions, since more than 70% of AOM are hydrophilic (Choi et al., 2004; Henderson et al., 2008). With regard to total DBP yield (THM and HAA), however, no significant change was evident in algae samples with a bromide spike compared to those without bromide; this was also reported by an earlier study of chlorination tests carried out on raw water under different bromide levels (Hua et al., 2006).

The degree of bromine incorporation, on the other hand, varies from species to species and also changes with growth phase due to the alteration in AOM components (Fig. 7). To examine the degree of bromine substitution in DBP species, the bromine incorporation factor, n' (Symons et al., 1996), was calculated. It is defined as follows:

For THMs: $n' = THMBr_3\ (\mu mol/L) / TTHM\ (\mu mol/L)$

where $THMBr_3 = [CHCl_2Br] + 2[CHClBr_2] + 3[CHBr_3]$

For HAAs: $n' = HAABr_6$ (μmol/L) / THAA (μmol/L)

where $HAABr_6$ = [MBAA] + [BCAA] + [BDCAA] + 2[DBAA] + 2[CDBAA] + 3[TBAA]

and MBAA – monobromoacetic acid; BCAA – bromochloroacetic acid; BDCAA – bromodichloroacetic acid; DBAA – dibromoacetic acid; CDBAA – chlorodibromoacetic acid; TBAA – tribromoacetic acid

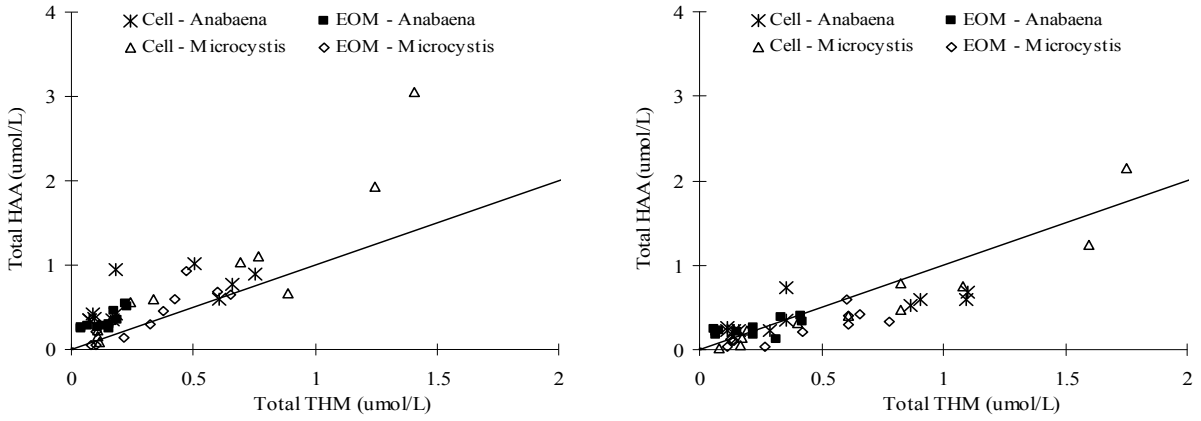
During the lag phase, a similar amount of bromide was incorporated into precursor material from cells and EOM to form HAAs and THMs (Fig. 7). With increasing culture age, less bromide active sites were available to form brominated THMs, whereas in *Anabaena* some sites favoured HAA formation. A decrease in bromide incorporation into THMs with time was especially obvious for *Microcystis*, which might be due to the decrease in its hydrophilic content with culture age (Henderson et al., 2008). When the growth phase of both algae species progressed to the death phase, bromine incorporation appeared to become more active again.

In terms of THM speciation, bromodichloromethane and dibromochloromethane were the two predominant THM species produced from cells and EOM of *Anabaena*, ranging from 65%-75% of total THM yield throughout the culture time. *Microcystis* produced a similar proportion of the two THM species during the lag and exponential phase, however the proportion of chloroform formed from cells increased from 22% to 38% after the growth phase progressed to the stationary phase, while a distinct decrease occurred for the two higher brominated species ($CHBr_2Cl$ and $CHBr_3$). Changes in THM species distribution with culture age were less dramatic for the EOM of *Microcystis*.

Compared to THMs, the characteristics of precursors have more of an impact on HAA species distribution with bromine incorporation. No MBAA was found with *Anabaena* throughout the growth phases, while TBAA produced by cells only appeared in the stationary phase and by EOM

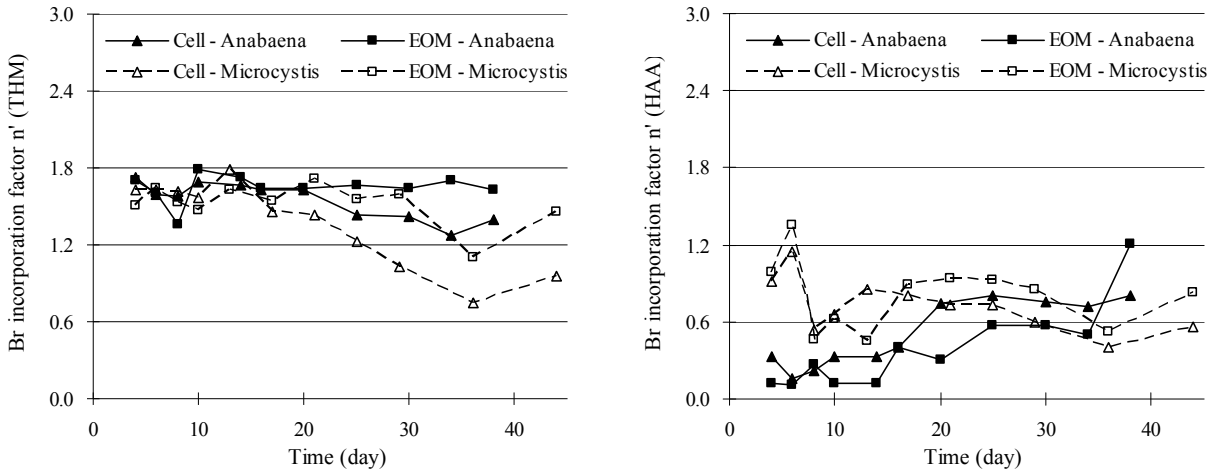
in the exponential phase (Fig. 8). DBAA, BCAA and BDCAA were the three dominant brominated HAA species formed from *Anabaena*; however, BCAA was not detected until the mid-exponential phase and BDCAA was prominent in the stationary phase.

Bromine incorporation seemed more extensive with AOM from *Microcystis* in the earlier growth phase as compared to *Anabaena*. Among the bromine incorporated HAA compounds, BDCAA, DBAA and CDBAA, were the three principal species observed with both cells and EOM of *Microcystis* in the exponential phase, accounting for more than 40% of total HAA formation (Fig. 8). However, bromine incorporation weakened once the growth phase progressed to the stationary phase, resulting in a sharp decrease in all brominated HAA species, especially those with a higher degree of bromine incorporation. A small amount of TBAA was produced in the exponential phase but was absent later. In contrast, MBAA did not appear until the stationary phase for *Microcystis*.



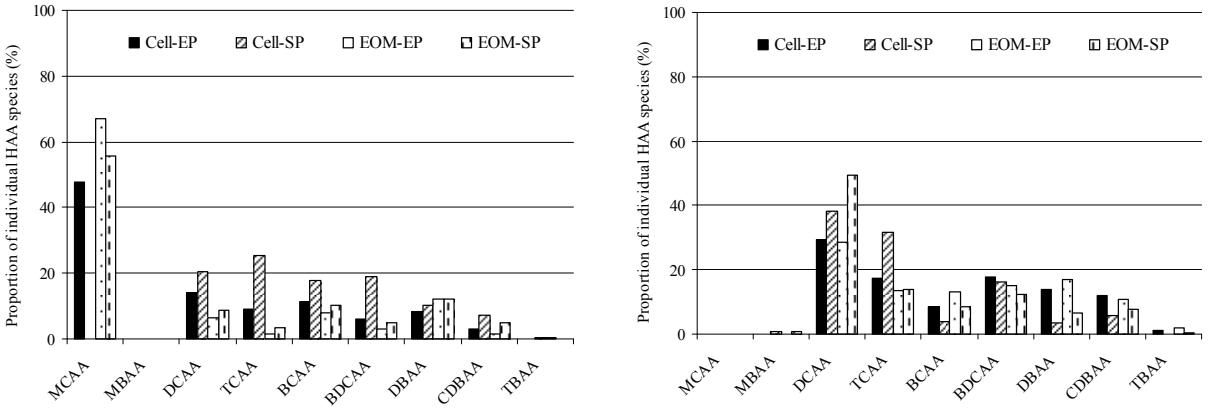
(a) (b)

Fig. 6. Total THM yield versus total HAA yield, (a) without bromide, and (b) with bromide (6 μmol/L). (diagonal line represents THM:HAA yield as 1:1)



(a) (b)

Fig. 7. Br incorporation factor as a function of algal growth phase for (a) THMs and (b) HAAs.



(a) (b)

Fig. 8. Individual HAA compound distribution in samples of (a) *Anabaena*, and (b) *Microcystis* (Day 16 and Day 34 were selected as the representative exponential phase and stationary phase, respectively, for *Anabaena*; Day 17 and Day 36, respectively, for *Microcystis*; EP: Exponential Phase; SP: Stationary Phase).

Implications of the results of this study

374 Within the experimental limitations of this study, the cells and EOM of two prominent blue-green
375 algae species have been shown to be significant THM and HAA precursors. As nitrogen fixers,
376 blue-green algae contain large amounts of organic-N compounds and exert a high chlorine demand,
377 thus decreasing the effectiveness of chlorine disinfection and leading to higher DBP formation. To
378 further understand the relative contribution of AOM to DBP formation and link it with available
379 information gained from other studies of natural organic matter (NOM), a comparison is made
380 between the two algae species in this study and information concerning two river sources: the South
381 Platte River and Suwannee River, located in the USA. The NOM of the South Platte River is
382 derived from both allochthonous aromatic and acid constituents and autochthonous contents from
383 phytoplankton and bacteria, while Suwannee River NOM is mainly derived from allochthonous
384 tannings and lignins, consisting of a large amount of humic and fulvic acids (Croué et al., 1999).
385 Table 1 shows that the specific yields of THM, DCAA and TCAA generated from the EOM of the
386 two algae species are comparable to those produced by hydrophilic acid and neutral fractions
387 isolated from the two river waters (Leenheer and Croué, 2003). However, slight differences exist
388 with regard to the capacities of EOM in producing THM, DCAA and TCAA from algae and NOM
389 fractions. This may be attributed to the existence of proteinaceous substances, accounting for 30%
390 of total AOM in the later stationary phase, which affects the dominance of HAAs relative to THMs,
391 and di-HAA to tri-HAA.
392
393 Algae cells have a much higher productivity in DBP formation as compared to EOM, the yield of
394 which is similar to that from hydrophobic fractions, especially those having high humic and fulvic
395 acid content. It can be deduced that N-enriched aromatic substances and other hydrophobic AOM
396 are mainly retained in cells, leading to a greater formation of TCAA over DCAA and THM. Hong
397 et al. (2008) reported that blue-green algal cells contained predominantly proteins (>50%),
398 carbohydrates and lipids, and showed that the specific HAA formation for a model algal-derived

399 protein (bovine serum albumin) was an order of magnitude greater than a model carbohydrate and
400 lipid. Since the formation of DBPs from cells can occur from the chlorination of intact cell wall or
401 lysing intracellular organic matter, it is difficult to confirm whether the cell wall is also a significant
402 DBP precursor. Overall, the findings in this study are consistent with those of Hong *et al*. (2008)
403 (for *Oscillatoria* sp.) that cells of blue-green algae may contribute as significantly to the DBP
404 precursor pool as humic and fulvic acids.
405
406 **Table 1.** Comparison of DBP formation from blue-green algae with river-derived NOM fractions

		C/N ratio (mmol/mmol)	Specific THMFP (μg/mg DOC)	Specific DCAAFP (μg/mg DOC)	Specific TCAAFP (μg/mg DOC)
Anabaena[*]	— Cells	na[**]	50	29	49
	— EOM	na	26	26	22
Microcystis[*]	— Cells	na	61	71	93
	— EOM	na	28	42	24
South Platter River, CO (Leenheer and Croué, 2003)					
Hydrophobic:	Acid	51.3	46	14	28
	Neutral	32.7	29	12	16
Transphilic:	Acid	21.0	39	14	21
	Neutral	4.7	25	20	12
Hydrophilic:	Acid	17.5	35	16	24
	Neutral	10.5	28	19	15
Suwannee River, GA (Leenheer and Croué, 2003)					
Hydrophobic:	Acid	81.7	55	25	59

	Neutral	54.8	51	24	51
Transphilic:	Acid	53.7	40	23	57
	Neutral	35.0	40	22	44
Hydrophilic:	Acid	39.7	36	22	36
	Neutral	17.5	23	22	26
	Base	9.3	29	39	31

*data was obtained when algae were in stationary phase with absence of bromide (Day 34 for *Anabaena* and Day 36 for *Microcystis*)

**na – not available

CONCLUSIONS

This has study examined the comparative contribution of two common UK blue-green algae, *Anabaena flos-aquae* and *Microcystis aeruginosa*, to the formation of THMs and HAAs during chlorination. The following summarises the key findings from this research:

- A close relationship was found between TTHM and THAA yield with growth phase and a direct association with biomass (cells and EOM). In contrast, no clear association was found for the *specific* yield (per unit carbon) with the growth phase.
- For both algae species, the absolute yield of TTHM and THAA from cells was substantially greater than that from EOM. However, the *specific* yield from EOM was slightly greater than cells for *Anabaena*, while the opposite trend was found for *Microcystis*.
- An antagonistic interaction between cells and EOM was observed for both algae species with regard to THM and HAA formation, though it is less apparent for *Microcystis* than *Anabaena*.
- The distribution of HAA compounds varies with algae species as well as growth phase. For *Anabaena* cells, mono-HAA is the predominant HAA species during the lag and early exponential phase, while di- and tri-HAA species dominate in the later growth phases; in EOM

samples mono-HAA is a major species throughout the growth phases up to the death phase. For *Microcystis*, mono-HAA only briefly appeared in the early exponential phase in samples of both cells and EOM. In cell samples, the proportion of tri-HAA was slightly higher than di-HAA, whereas di-HAA was dominant in EOM samples.

- The presence of bromide shifts the relative DBP speciation from HAAs to THMs.
- The degree of bromine incorporation changes with growth age. Higher bromine incorporation into THMs occurred at the early growth phase and decreased until the later stationary phase. A similar trend was found with *Microcystis* samples with regard to the bromination of HAAs, while the extent of bromine incorporation increased with the growth age in samples containing *Anabaena*.
- The behaviour of algal cells was similar to the hydrophobic fractions isolated from river waters in terms of reactivity to form DBPs, while the behaviour of algal EOM was similar to the hydrophilic fractions.
- The chlorination tests were conducted under standardized conditions to identify the maximum potential for by-product formation and to compare the DBP formation with the two algal species. Thus, the chlorination conditions used in this study are very different from those applied in practical water treatment processes (e.g. chlorine dose), and therefore may not reflect by-product formation under actual water treatment conditions.

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