Evaluation of *Anabaena flos-aquae* as a precursor for trihalomethane and haloacetic acid formation

J. Huang, N. Graham, M. R. Templeton, Y. Zhang, C. Collins and M. Nieuwenhuijsen

ABSTRACT

This paper summarizes an investigation of a common blue–green algae species, *Anabaena flos-aquae*, as a precursor substrate in the formation of trihalomethane (THM) and haloacetic acid (HAA) compounds during chlorination. The algae were cultured under controlled and axenic conditions throughout all four growth phases and samples taken during these phases were subjected to chlorination to determine disinfection byproduct (DBP) formation potentials. Algal cells and extracted extracellular organic matter (EOM) of *Anabaena* showed a comparable ability to form THM and HAA compounds as humic and fulvic acids. Overall yields of total THM (4) and HAA (9) compounds were closely related to the growth phase, with peak formation in the late exponential-stationary phases. Specific (normalized) DBP yields (yield/unit C) were in the range of 2–11 μmol/mmol C for TTHM and 2–17 μmol/mmol C for THAA. The presence of bromide appeared to increase TTHM formation and decrease THAA formation, thereby leading to a shift in the DBP species from HAA to THM compounds. The distribution of HAA species varied with growth phase. Monochloroacetic acid was found to be the dominant HAA species during the lag and early exponential phases, and a prominent compound in the later growth phases.

Key words | algae, anabaena flos-aquae, byproducts, disinfection, haloacetic acids, trihalomethanes

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INTRODUCTION

Algae and their excreted metabolic products, extracellular organic matter (EOM), contribute to the pool of natural organic matter (NOM) present in source waters and are particularly associated with eutrophic lakes and reservoirs. The presence of algal cells and EOM is known to cause adverse effects on drinking water quality, such as taste and odour and disinfection by-product formation, and on the operation of water treatment processes, where they can cause an increase in coagulant dosing and rapid filter clogging. In the UK the seasonal development of algal blooms is managed by a combination of reservoir operations (eg. hydraulic mixing and phosphorus removal) and pre-treatment typically involving pre-oxidation by either chlorine or ozone. The latter has the undesirable effect either of producing chlorination by-products directly, or indirectly by releasing soluble intracellular organic matter that is available subsequently as a disinfection by-product (DBP) precursor for reaction during the final chlorination stage.

The formation of DBP compounds from algae has been the subject of various studies in the past two decades, which have focused almost exclusively on the formation of trihalomethane (THM) compounds. The results from these studies have shown that both algal cells and EOM are significant precursors in THM formation during chlorination (Wardlaw *et al.* 1991; Graham *et al.* 1998; Nguyen *et al.* 2005). However, the THM concentrations reported in these studies were shown to depend on the algae species, the algal growth phase, and the chlorination conditions

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(e.g. pH, temperature, contact time). Under similar chlorination conditions (pH 7, 24 h contact time, 20–24°C), yields of THM from algal biomass ranged 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). Extending the contact time causes an increase in THM yield (Plummer & Edzwald 2001), partly because of the release of intracellular organic matter resulting from cell lysis. By comparing three different algae species (diatom, green algae and blue–green algae), Nguyen *et al.* (2005) found that green algae is the most productive in THM yield. However, in other research EOM extracted from blue–green algae was reported to be the most reactive, followed by EOM from the diatom and green algae (Plummer & Edzwald 2001).

While many fundamental studies of haloacetic acid (HAA) precursors have concentrated on NOM in general and the significance of chemically separated organic fractions, such as hydrophilic and hydrophobic acids and bases (e.g. Liang & Singer 2003), very little work has been reported to-date on the specific role of algae. The potential for algae to be important HAA precursors has been investigated in this project and the specific algal species, *Anabaena flos-aquae*, was chosen for study since it is found widely in UK surface waters and is believed to be a contributor to the production of THMs. In this paper we summarise the results of laboratory chlorination tests undertaken to investigate the significance of *Anabaena flos-aquae* as a precursor for THM and HAA formation. The study has considered the following aspects: (a) the comparative reactivity of algal cells and EOM in terms of total DBP yield, specific DBP yield (yield/unit C used) and the distribution of DBP compounds; (b) the influence of algal growth phase; (c) interactions between cells and EOM (whether synergistic or antagonistic); (d) the effect of background bromide on the formation of DBP species and the distribution of individual DBP compounds.

MATERIALS AND METHODS

Algae culturing

A stock culture of *Anabaena flos-aquae* was obtained from the Culture Collection of Algae and Protozoa (CCAP), Windermere, UK. *Anabaena flos-aquae*, is a blue–green algae commonly found in UK raw water reservoirs which cause water treatment and quality problems particularly during times of excessive growth. The stock culture was firstly inoculated into an inorganic growth medium and incubated until the cell density indicated an optimal growth for further sub-culture. Algae samples were sub-cultured into 250 mL conical flasks under air filter and sterile conditions, thereby preventing contamination. Sub-cultured samples were maintained in a shaking water bath under controlled conditions of temperature (20 ± 1°C), illumination (12 h light/ 12 h dark), and sufficient aeration.

Algal growth was monitored mainly by the concentration of chlorophyll-a, which was measured according to the ISO 10260 standard procedure (ISO 1992), modified according to the method of Papista (Papista *et al.* 2002); methanol was the extract agent used in this study. The optical density of extracts was measured at 664 and 750 nm using a Shimadzu UV-2401 spectrophotometer with a 1-cm cell. Nguyen *et al.* (2005) reported good linear correlations between optical density at 730 nm (OD_{730}) and dry algal biomass for three different algae species. Since the biomass in this study was too low to measure directly (dry weight), OD_{730} was used as a reference for monitoring algae growth. All measurements were undertaken at least in duplicate to improve experimental accuracy.

Cell and EOM separation

Based on the lifetime of the algae, tests were carried out using samples taken at intervals corresponding to different algae growth phases, up to 1.5 months. At each particular time, sample aliquots containing both algae cells and extracellular organic matter were removed from the flask for centrifugation. The centrifugate containing EOM was filtered through a 0.45 μm Whatman membrane filter and transferred into a 250 ml amber bottle for the subsequent chlorination tests. The separated cells from the centrifugation were washed three times and re-suspended in deionised water. Duplicate quantities of the cell suspensions, EOM aliquots and original algae sample were taken for TOC determination (TOC analyser: Shimadzu Ltd, Japan).

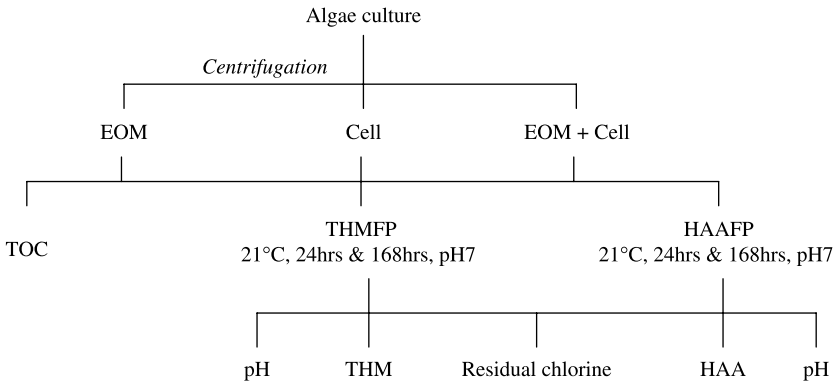

Figure 1 | Schematic of experimental protocol.

DBP determination

To assess the contribution of algal cells versus EOM in producing DBPs together with their possible interactive effect, measurements for THM and HAA formation were made on the cell suspensions and EOM aliquots separately, and on the original algae suspensions before separation. All the samples were dosed with excess chlorine, where the dosage was determined beforehand by undertaking a chlorine demand test to ensure that at the end of the chlorination period a free chlorine residual was still measurable (≥ 0.5 mg/L). Samples were buffered using phosphate and stored at 21°C in the dark for periods of 1 day and 7 days, in accordance with standard procedures (Standard Methods 1998); the 7-day results represent the DBP formation potential. To investigate the effect of bromide, some samples were purposely spiked with bromide ion (6 μmol/L). The DPD Standard Method 4500-Cl F (Standard Methods 1998) was followed in the measurement of residual free chlorine after chlorination. Residual free chlorine was quenched with sodium thiosulfate ($Na_2S_2O_3$) for the THM analyses and with ammonia sulfate ($(NH_4)_2SO_4$) for the HAA analyses. The four individual THM compounds were analysed by Standard Method 6232B (1998) but with minor modifications developed by Baribeau *et al.* (2006). The nine (chloro-bromo-) HAA compounds were determined according to USEPA Method 552.3 (USEPA 2003); all sample analyses were carried out in duplicate. A summary of the experimental protocol is shown schematically in Figure 1.

RESULTS AND DISCUSSION

Algal growth

The growth curve of *Anabaena flos-aquae* is shown in Figure 2 where the growth is represented in terms of the change in chlorophyll-a concentration, optical density at 730 nm (OD_{730}) and TOC. A close linear relationship between chlorophyll-a and OD_{730} ($R^2 = 0.97$) was found in this study, as reported by others (Nguyen *et al.* 2005). All four growth phases (lag, exponential, stationary and death) can be distinguished in the figure. The lag phase is believed to correspond to Day 0 to Day 10–15, during which there was very little change in both the chlorophyll-a concentrations and optical density values. After Day 15 a dramatic increase in chlorophyll-a indicated the start of the

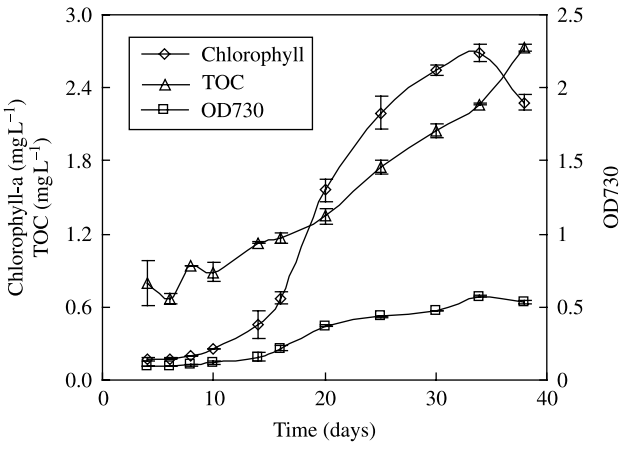

Figure 2 | Growth curve of *Anabaena flos-aquae*.

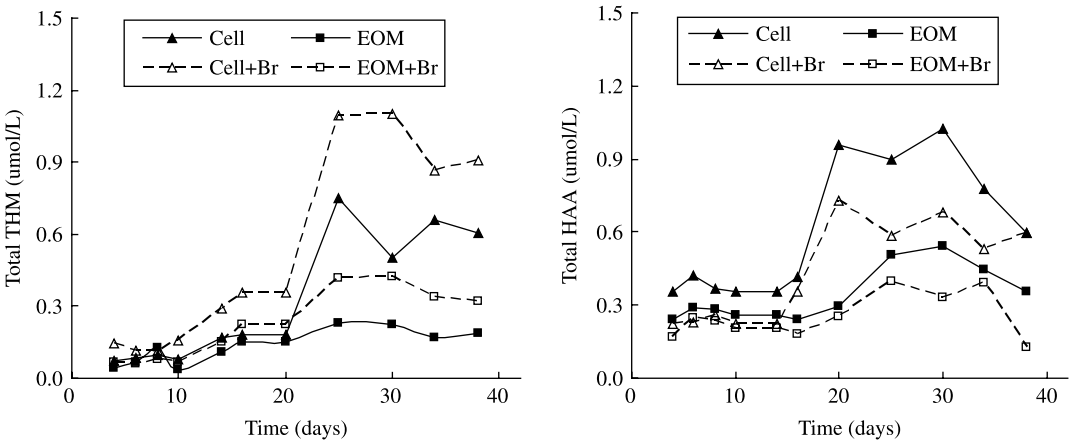

Figure 3 | Total THMFP and HAAFP for *Anabaena* cells and EOM (pH 7, 21°C).

exponential phase which lasted until approximately Day 25, when the declining rate of increase indicated the onset of the stationary phase. The death phase appeared to occur beyond Day 35 when the pigment inside the cells began to fade, and the chlorophyll-a and OD_{730} levels reduced. In contrast to the trend in chlorophyll-a and OD_{730} levels, the TOC concentration increased steadily through the first three growth phases, and rose sharply in the early death phase. The sharp increase in the TOC at the start of death phase might be explained by the autolysis of cells and consequent releasing of intracellular organic matter (IOM).

DBP formation from cells and EOM

Figure 3 shows the variation of total THM (TTHM) and total HAA (THAA) yield produced by cells and EOM with the culture age after 7-day chlorination (TTHMFP and THAAFP), yields of which are expressed in terms of molar concentration. The TTHM formation from cells dramatically increased during the exponential phase, with the concentrations increasing three-fold by the end of the exponential phase. In contrast there was a more modest (80%) increase in the yield produced by the EOM. TTHM levels from both cells and EOM fluctuated in the stationary phase, and then reduced at the beginning of the death phase. A similar trend was found for the THAA formation, but with the sharp increase in concentration occurring earlier in the exponential phase. The occurrence of the maximum formation of TTHM and THAA in the late exponential/stationary phases suggests that a high level of DBP formation can be expected during the algae blooming season, which is consistent with the results from previous surveys (e.g. Graham *et al.* 1998; Bukaveckas *et al.* 2007).

The maximum concentrations of TTHM and THAA produced by the cells (without bromide spike) were 0.75 μmol/L and 1.03 μmol/L, respectively, which were two to three times more than that from EOM, regardless of the growth phase. The significance of cells as precursors in DBP formation compared to their corresponding EOM has also been observed by other researchers (e.g. Wachter 1982; Graham *et al.* 1998; Plummer & Edzwald 2001), which indicates that increasing the effectiveness of water treatment particularly for cell removal can have a large impact on reducing DBP formation. In terms of the *specific* molar yield of DBPs, expressed as μmol/mmol C, the differences in yield between the cells and EOM were much less and the influence of growth phase was less clear (Figure 4). The specific yield of DBP is often used to indicate the relative reactivity of organic matter with chlorine, thereby allowing comparisons to be made between different substrates and their importance as DBP precursors to be determined. From the results shown in Figure 4 it can be seen that EOM was slightly more productive than cells for TTHM during the lag and exponential phases, whereas the trend reversed for the stationary and death phases. For THAA the specific yield from EOM exceeded that from the cells throughout the lifetime of *Anabaena*, with one exception at the time of 20 days. The variability of the THAA values with growth phase for both cells and EOM meant that no clear conclusions can be drawn as to whether a particular growth phase is more

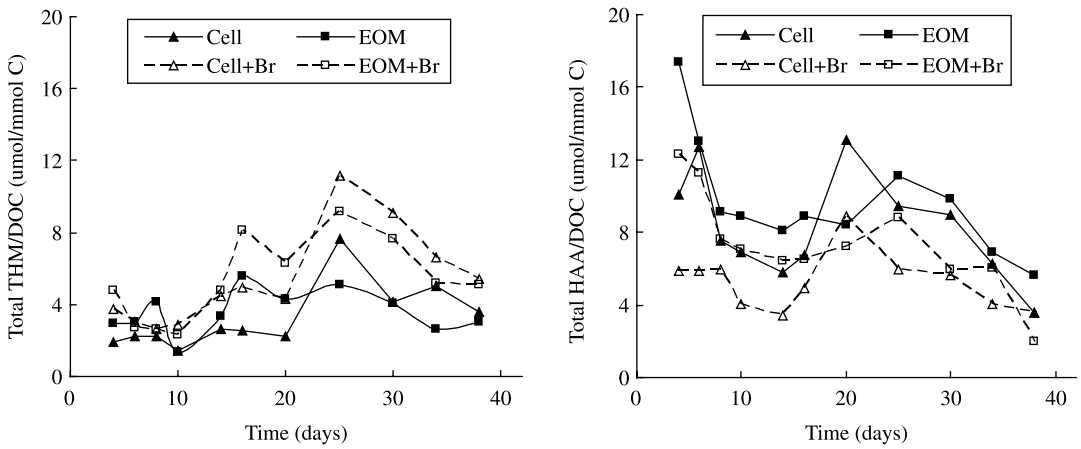

Figure 4 | Specific total THMFP and HAAFP for *Anabaena* cells and EOM (pH 7, 21°C).

important than another. However, the period between the late exponential phase and early death phase can be regarded as a particularly productive phase for DBP formation. A representative (average) value for the TTHM specific yield from EOM in the stationary phase of 3.2 μmol/mmol C, is comparable to the results reported in previous studies of blue–green algae of 1.8–6 μmol/mmol C (Wachter 1982; Graham *et al.* 1998; Plummer & Edzwald 2001; Nguyen *et al.* 2005). Coresponding values for the THAA specific yield from EOM in the stationary phase were in the range of 9–12 μmol/mmol C.

With regard to HAA formation, the cells and EOM behave differently in terms of the compound distribution as well as yield. During the lag and early exponential phases, mono-HAA was the predominant species (% of total molar concentration) for both cells and EOM (Figure 5). However, in the later exponential phase greater quantities of the higher halogen-incorporated species were produced by the cells, and this trend continued into the stationery and death phases where approximately equal amounts of di- and tri-HAA were produced, and there was no mono-HAA. In contrast, for EOM, mono-HAA was the predominant species (≥50%) throughout the growth phases (except well into the death phase), and the ratio of di-HAA to tri-HAA remained approximately constant.

In precursor studies carried out using natural organic matter, several researchers have concluded that di- and tri-HAA compounds are produced from different precursors (Reckhow & Singer 1985; Reckhow *et al.* 1990; Liang & Singer 2003; Nikolaou *et al.* 2004). The former, di-HAA

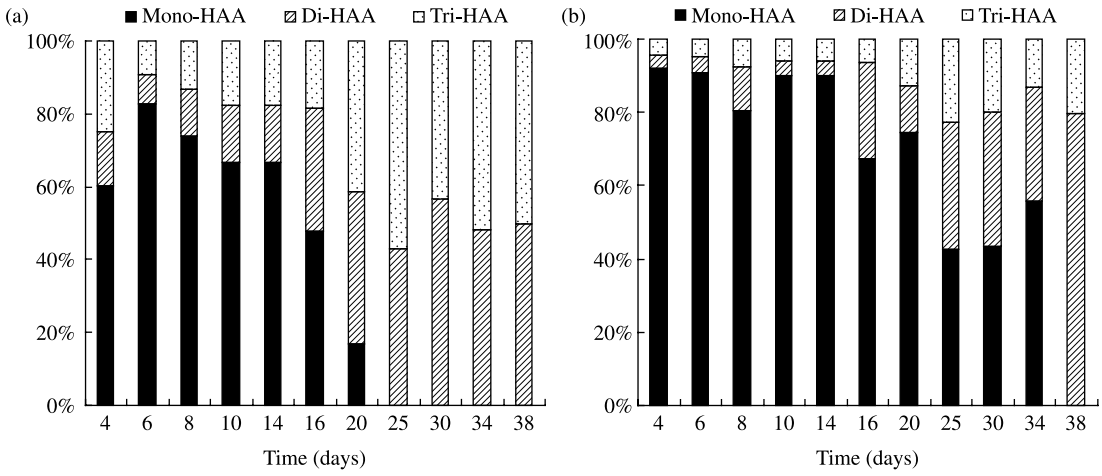

Figure 5 | Distribution of HAA compounds (with Br spike) from (a) cells and (b) EOM.

compounds, are suggested to be derived from more hydrophilic fractions, while the latter are more likely to be formed from hydrophobic and acidic fractions. The fundamental building and storage components of algal cells consist mainly of proteins, amino acids, polysaccharides, oligosaccharides, carbohydrates and other trace amounts of nitrogenous organic matter, which include the precursors of both di- and tri-HAA compounds. In contrast, polysaccharides and proteins, which are believed to be the precursors of di-HAA compounds, are the major components of EOM. This structural diversity found in cells and EOM most likely explains the observed difference in the distribution of HAA compounds produced. Thus, in the exponential growth period (e.g. Day 25), when there are large increases of protein, polysaccharides and carbohydrates developing inside the cells and being excreted as EOM, this leads to the predominance of di- and tri-HAA compounds, as discussed above. However, the reason for the high proportion of mono-HAA found for both cells and EOM during the early growth phase is not clear, since little is known about the nature of mono-HAA precursors.

By comparing the magnitude of DBP formation for samples containing both cells and EOM ('mix'), with the numerical sum of the DBPs formed individually by the separated cells and EOM, a clear and consistent antagonistic effect was apparent. This effect is especially pronounced in the case of TTHM formation than THAA formation (Figure 6). The reason for this is not clear but it suggests that during the chlorination of real waters containing both cells and EOM, there may be interactive scavenging of THM or HAA intermediate species leading to a consequent reduction in the final compounds, or interactions between intermediate compounds that react with the cells and EOM leading to other (non-THM/HAA) DBP compounds. Similar antagonistic effects occurring between substances with different chemical properties and polarity have also been reported in other studies (e.g. Kanokkantapong *et al.* 2006).

Impact of bromide on DBP formation

Figure 7 displays the comparative formation of TTHM versus THAA under conditions with or without a 6 μmol/L bromide spike, and for mixed and separate cells and EOM (all growth phases). It can be seen from the figure that in the absence of bromide HAA formation was clearly preferred over THM for cells and EOM, separately and mixed, whereas in the presence of bromide the formation of THAA and TTHM were approximately equivalent. As mentioned previously, *Anabaena* is composed mostly of hydrophilic substances and the results found here are in accordance with the theory that bromide is more effectively incorporated into low UV-absorbing, low molecular weight and hydrophilic fractions. However, with regard to total DBP yield (THM and HAA) no significant change was evident in algae samples with a bromide spike compared to those without bromide. This finding was also reported by an earlier study of chlorination tests carried out on raw water under different bromide levels (Hua *et al.* 2006).

In addition to the change in the relative formation of DBP species, the addition of bromide also can cause a shift

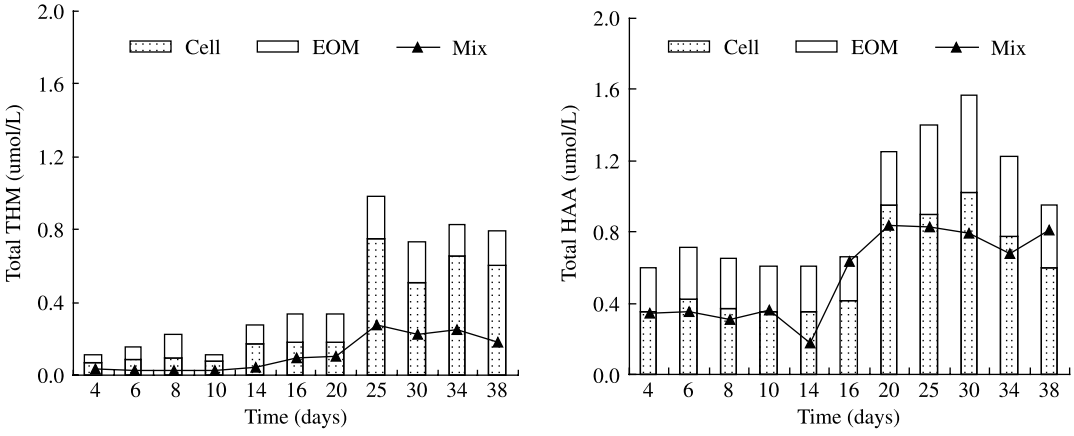

Figure 6 | Antagonistic effect between cells and EOM in TTHM and THAA formation.

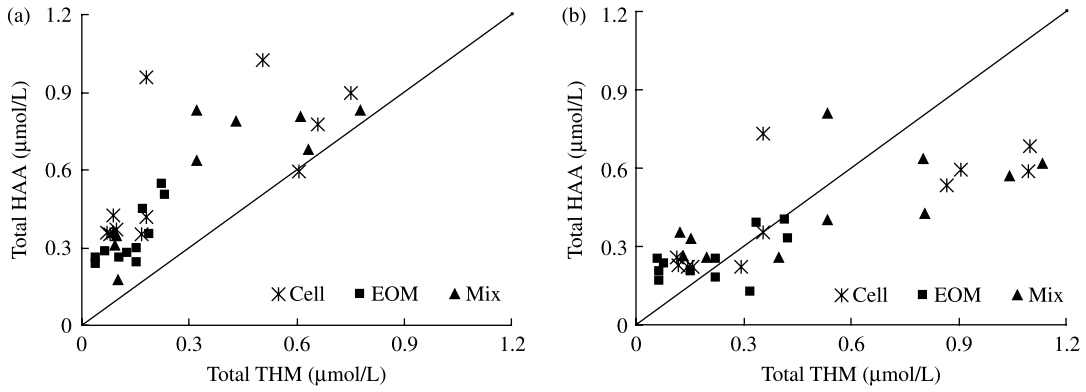

Figure 7 | TTHM yield versus THAA yield without (a), or with (b), bromide (6 μmol/L).

from chlorinated to more brominated compounds within each DBP group, where the degree of bromine incorporation depends on the ratio of bromide concentration to chlorine dose. A high but realistic ratio of Br^-/Cl_2 was applied in this study (e.g. ~60 mg/g), which ensures that bromide was competitive with chlorine in the reactions leading to DBP formation. All four THM compounds were present in samples with the bromide spike, while only seven species of HAA were observed (no monobromoacetic acid (MBAA) and tribromoacetic acid (TBAA)). Cells and EOM did not exhibit any significant difference in the distribution of THM formation. Bromodichloromethane and dibromochloromethane accounted for 70% of total THMs (in molar terms), with 20% bromoform and 10% chloroform in all growth phases. With the distribution of HAA species, differences were apparent between the cells and EOM, and also with the growth phase. During the lag and early exponential phases, dibromoacetic acid (DBAA) and bromodichloroacetic acid (BDCAA) were the two dominant bromine-containing HAA species produced by the cells. Bromochloroacetic acid (BCAA) appeared in the mid-exponential phase and increased along with other brominated species in later growth phases. The proportions of brominated and non-brominated HAA compounds formed from the cell samples in the stationary phase were approximately equal. In contrast, DBAA was the predominant brominated species produced by EOM regardless of the growth phase. In addition, EOM favoured the production of BCAA over BDCAA in the stationary phase. Table 1 summarises the change in the distribution of HAA species with three different growth phases (lag, exponential and stationary phase) for the cells and EOM.

Pivokonsky *et al.* (2006) reported that the proportion of protein-structured algogenic organic matter (AOM) in EOM from *Anabaena* increased with growth age, from 15% in early exponential phase to 40% in the later stationary phase. Therefore, the increasing ratio of brominated to chlorinated HAA species with growth age observed for EOM may indicate that nitrogenous organic matter favours bromine incorporation rather than chlorine.

Significance of *Anabaena* in DBP formation compared to other algae species

Other studies of the formation of DBPs from algae have been carried out recently, indicating that algae are increasingly recognised as an important contributor to the DBP precursor pool. However, the capability of algae

Table 1 | Distribution of HAA species with growth age for cells and EOM (% in molar units)

	Cells			EOM		
	Day 8	Day 16	Day 30	Day 8	Day 16	Day 30
MCAA	74	48	0	80	66	43
DCAA	7	14	24	4	6	11
TCAA	5	9	20	2	2	6
BCAA	0	12	21	0	8	13
DBAA	5	6	16	3	3	8
BDCAA	6	8	12	9	12	12
CDBAA	2	3	7	3	3	6

Table 2 | Comparison of DBP formation with different algal species

	TTHM (μg/mg DOC)		THAA (μg/mg DOC)	
	1 day	7 days	1 day	7 days
Anabaena flos-aquae – Cells	15.7	47.5	20.4	101.2
(in this study) – EOM	18.5	40.3	37.3	91.4
Anabaena flos-aquae – Cells	9.0	na*	na	na
(Graham *et al.* 1998) – EOM	3.0	na	na	na
Oscillatoria prolifera – Mix	na	30	na	na
Scenedesmus quadricauda – EOM (Nguyen *et al.* 2005)	na	63.3	na	57.7
Asterionella formosa – Cells	10.5	na	na	na
(Graham *et al.* 1998) – EOM	4.5	na	na	na
Cyclotella sp. (Plummer & Edzwald 2001) – Mix	17	45	37.5	93

*na – not available.

to form DBPs varies from species to species. To assess the relative significance of *Anabaena* in DBP formation during water treatment, the results in this study are compared with those reported for other algal species and are summarised in Table 2; these included the green algae *Scenedesmus quadricauda*, the blue–green algae *Oscillatoria prolifera*, and the two diatoms *Astrerionella formosa* and *Cyclotella*. As a basis for comparison, all results are normalised as yield per unit DOC (viz. *specific* yield). Since the chlorination conditions influence to a considerable extent the yield of DBP, the studies selected for the comparison employed similar chlorination conditions (pH 7, 20 ± 1°C and measurable chlorine residual at the end of chlorination). In addition, the results were from tests where the algae population was in its stationary phase of growth.

As can be seen in Table 2, the green algae *Scenedesmus quadricauda* appears to be equally reactive in producing THM and HAA compounds, while HAAs seem to be more readily formed with the blue–green algae and diatoms. In general terms the THM and HAA concentrations from the diatoms are similar than that from the blue–green algae, which are also comparable with the DBP formation from humic and fulvic acids (Nikolaou & Lekkas 2001). From our study it is clear that EOM is as reactive as cells in DBP formation. The implications of this for water treatment in practice are that pre-treatment processes which reduce the removal effectiveness of algal cells should be avoided, and particularly pre-oxidation, which disrupts the cells and releases intra-cellular organic matter. In particular pre-chlorination of algal source waters is of concern since it combines cell disruption with the simultaneous reaction of chlorine (at a relatively high dose) with cell-based and soluble (EOM) organic precursors. Thus, the approach to managing algae-related DBPs is to control the algal content of source waters and maximise the removal of algal cells/EOM prior to final chlorination.

CONCLUSIONS

This study has evaluated the role of *Anabaena flos-aquae* as a precursor of THM and HAA formation during chlorination. *Anabaena flos-aquae* is a blue–green algae commonly found in UK source waters and this has been studied as a representative algal species in order to examine particular aspects of DBP formation. Thus, in this paper we have considered the following: the comparative reactivity of algal cells and their soluble metabolic substances (EOM), the comparative yield of THM and HAA compounds, the effect of algal growth phase, and the influence of bromide.

The principal findings from this research are summarised as follows:

- Overall TTHM and THAA yield was closely related to the growth phase, suggesting a direct association with

biomass (cells and EOM). In contrast, the *specific* (normalised) yield was not clearly related to the growth phase.

- The absolute yield of TTHM and THAA from cells was substantially greater than that from EOM, while the *specific* yield was slightly greater from EOM than cells. Values for the specific yield were in the range of 2–11 μmol/mmol C for TTHM and 2–17 μmol/mmol C for THAA.
- There was some evidence of an antagonistic effect between cells and EOM in the chlorination reaction in terms of DBP formation.
- The presence of bromide appears to increase TTHM formation and decrease THAA formation, thereby leading to a shift in the DBP distribution from HAA to THM compounds.
- The distribution of HAA species varies with growth phase. For algal cells, mono-HAA is the predominant HAA species during the lag and early exponential phase, while di- and tri-HAA species predominate in the later growth phases. For EOM, mono-HAA is a major species throughout the growth phases up to the death phase.
- Among the nine HAA species, monobromoacetic acid (MBAA) and tribromoacetic acid (TBAA) were rarely detected at significant concentrations.
- A substantial increase in the proportion of brominated HAA compounds was found in later growth phases, suggesting that nitrogenous organic matter may be an important precursor for brominated HAA formation.

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REFERENCES

Baribeau, H., Singer, P. C., Gullick, R. W., Williams, S. L., Williams, R. L. & Andrews, S. A. 2006 *Formation and Decay of Disinfection By-Products in the Distribution System*. AWWA Research Foundation/American Water Works Association/IWA Publishing, Washington DC, USA.

Bukaveckas, P. A., McGaha, D., Shostell, J. M., Schultz, R. & Jack, J. D. 2007 Internal and external sources of THM precursors in a midwestern reservoir. *J. Am. Water Works Assoc.* **99**(5), 127–136.

Graham, N. J. D., Wardlaw, V. E., Perry, R. & Jiang, J. Q. 1998 The significance of algae as trihalomethane precursors. *Water Sci. Technol.* **37**(2), 83–89.

Hua, G. H., Reckhow, D. A. & Kim, J. 2006 Effect of bromide and iodide ions on the formation and speciation of disinfection byproducts during chlorination. *Environ. Sci. Technol.* **40**(9), 3050–3056.

ISO 1992 Water quality – Measurement of biochemical parameters – Spectrometric determination of the chlorophyll-a concentration.

Kanokkantapong, V., Marhaba, T. F., Wattanachira, S., Panyapinyophol, B. & Pavasant, P. 2006 Interaction between organic species in the formation of haloacetic acids following disinfection. *J. Environ. Sci. Health Part a-Toxic/Hazard. Subst. Environ. Eng.* **41**(6), 1233–1248.

Liang, L. & Singer, P. C. 2003 Factors influencing the formation and relative distribution of haloacetic acids and trihalomethanes in drinking water. *Environ. Sci. Technol.* **37**(13), 2920–2928.

Nguyen, M. L., Westerhoff, P., Baker, L., Hu, Q., Esparza-Soto, M. & Sommerfeld, M. 2005 Characteristics and reactivity of algae-produced dissolved organic carbon. *J. Environ. Eng.-ASCE* **131**(11), 1574–1582.

Nikolaou, A. D. & Lekkas, T. D. 2001 The role of natural organic matter during formation of chlorination by-products: a review. *Acta Hydrochim. Hydrobiol.* **29**(2-3), 63–77.

Nikolaou, A. D., Golfinopoulos, S. K., Lekkas, T. D. & Kostopoulou, M. N. 2004 DBP levels in chlorinated drinking water: effect of humic substances. *Environ. Monit. Assess.* **93**(1-3), 301–319.

Papista, E., Acs, E. & Boddi, B. 2002 Chlorophyll-alpha determination with ethanol: a critical test. *SO – Hydrobiologia* **485**, 191–198.

Pivokonsky, M., Kloucek, O. & Pivokonska, L. 2006 Evaluation of the production, composition and aluminum and iron complexation of algogenic organic matter. *Water Res.* **40**(16), 3045–3052.

Plummer, J. D. & Edzwald, J. K. 2001 Effect of ozone on algae as precursors for trihalomethane and haloacetic acid production. *Environ. Sci. Technol.* **35**(18), 3661–3668.

Reckhow, D. A. & Singer, P. C. 1985 Mechanism of organic halide formation and implications with respect to preozonation. *Ann Arbor Science*. Mich, Ann Arbor, pp. 1229–1257.

Reckhow, D. A., Singer, P. C. & Malcolm, R. L. 1990 Chlorination of humic materials – by-product formation and chemical interpretations. *Environ. Sci. Technol.* **24**(11), 1655–1664.

Standard Methods for the Examination of Water and Wastewater, 1998 20th edition. American Public Health Association/ American Water Works Association/Water Environment Federation, Washington DC, USA.

USEPA. 2003. Method 552.3 – Determination of haloacetic acids and dalapon in drinking water by liquid–liquid microextraction, derivatization, and gas chromatography with electron capture detection. EPA 815-B-03-002.

Wachter, J. K. 1982 Characterisation of organohalide formation upon chlorination of algal extracellular matter. *Hygiene Dissertation*. Pittsburgh, PA., University of Pittsburgh. Sc.D.

Wardlaw, V. E., Perry, R. & Graham, N. J. D. 1991 The role of algae as trihalomethane precursors – a review. *J. Water Suppl. Res. Technol. – Aqua* **40**(6), 335–345.