Pergamon

Wat. Sci. Tech. Vol. 37, No. 2, pp. 83–89, 1998.

Printed in Great Britain.
0273–1223/98 $19·00 + 0·00

PII: S0273-1223(98)00013-4

THE SIGNIFICANCE OF ALGAE AS TRIHALOMETHANE PRECURSORS

Nigel J. D. Graham, Valerie E. Wardlaw, Roger Perry and Jia-Qian Jiang

Department of Civil Engineering, Imperial College of Science, Technology and Medicine, London SW7 2BU, UK

ABSTRACT

The objective of this study was to assess the relative importance of algae and algal derived organic precursors in the generation of Trihalomethane compounds (THMs). Laboratory tests have been carried out using cultures of two common algal species dominating natural reservoir populations to assess the importance of species, growth phase and biomass versus Extracellular product (ECP) in THM production. The results of the study showed that THMFP of algae cells and ECP increased with culture age. THM formation from cells was more than double that from ECPs for *Asterionella formosa* and *Anabaena flos-aquae*. It is predicted that typical blooms in a reservoir of either species could produce a substantial fraction of the THM's formed during chlorination. © 1998 IAWQ. Published by Elsevier Science Ltd

KEYWORDS

Algae; *anabaena*; *asterionella*; biomass; chlorination; extracellular product; trihalomethanes; water treatment.

INTRODUCTION

It has been speculated that algal biomass and extracellular products (ECP) can act as Trihalomethane (THM) precursors and, especially for source waters from eutrophic reservoirs, cause elevated levels of THMs at certain times of the year. Consequently, an assessment of the relative importance of algal biomass and ECP released during growth as THM precursors is required to predict levels of THMs and to help evaluate water treatment options.

Preliminary research into the role of algae in the production of THMs indicates that algae are equally potent as precursors as humic and fulvic acids. A full review of this has been published (Wardlaw *et al.*, 1991). THM formation from algal biomass and ECP is dependent on pH, chlorine to carbon ratio, contact time, and temperature, and increases with increasing values of these parameters. Great variation in THM yields (as C-$CHCL_3$/TOC) from biomass and ECPs between species and growth phase are reported in the literature (eg. Briley *et al.*, 1980). It is difficult to draw conclusions from published data due to the contradiction in results for the same species between different researchers, and few comprehensive surveys including both biomass and dissolved organic products throughout the life cycle. Experimental conditions also vary considerably between researchers. Finally, the role of bacteria in modifying algal extracellular products to reduce or enhance THM production has not been investigated previously.

The general aim of this project was to examine the role of algae and algal derived organic compounds as precursors in the production of THMs. Specific objectives included :

1. Correlation of data from reservoir source waters and corresponding final treated waters to relate algal populations with THM production.
2. Examination of the production of THMs from the chlorination of filtered and unfiltered reservoir water samples, linked with data on TOC and chlorophyll *a* to assess the importance of algal biomass in natural waters.
3. Experimentation with laboratory cultures of bloom-forming algae to assess the relative contribution of algal biomass versus ECP to THM formation, at different growth phases under standard conditions. Culturing of two types of algae, a Diatom and a Blue green, to reveal any species variation in yields.
4. Monitoring of the bacterial contamination of algal cultures during growth and culturing of predominant bacteria to determine their role in modifying algal derived precursors.

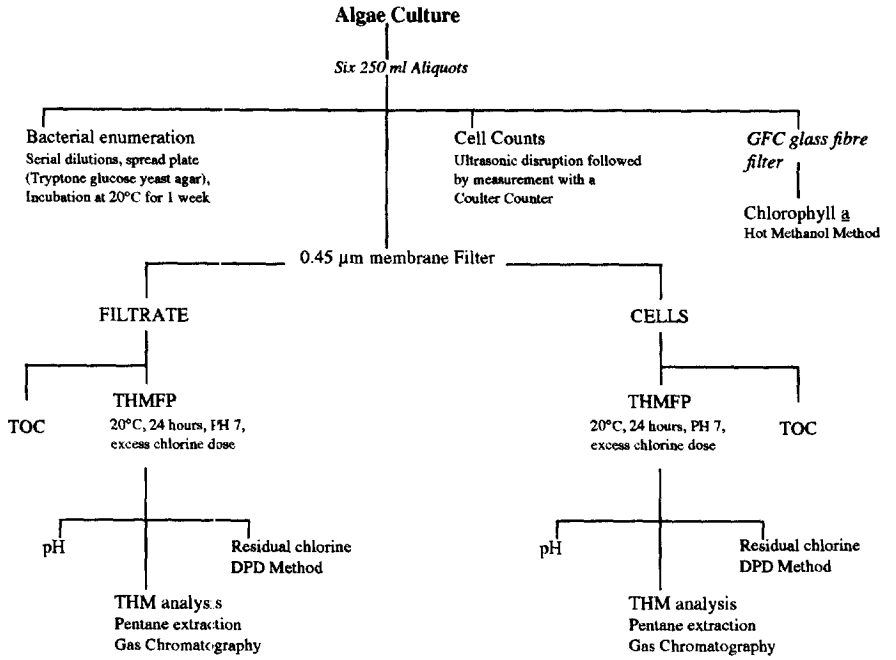
METHODOLOGY

Water quality data from three reservoirs and corresponding water treatment works in the South-East of England were obtained from relevant Water Authority records. These included Total Organic Carbon (TOC), temperature, pH and chlorophyll *a* from raw water and Trihalomethanes, pH, TOC and chlorine residual from the final water. Such reservoirs suffer from excess algal development due to their eutrophic nature. Prechlorination is practised to aid removal of algal cells during coagulation/sedimentation and filtration. The biological and chemical characteristics of the reservoir water were correlated with final water THM levels to reveal any relationships between algal populations and THM formation.

During the period from February 1990 to November 1990 water samples were collected from one reservoir at one meter depth on a weekly basis and used in chlorination experiments to examine THM formation from actual algal populations. Replicate filtered (GFC) and unfiltered samples were dosed with excess chlorine and incubated in the dark at 10°C for four hours and four days (representing finished water ex-works and consumer tap levels respectively).

Stock cultures of *Asterionella formosa* and *Anabaena flos-aquae* obtained from the Culture Collection of Algae and Protozoa, Windermere, were grown in duplicate under controlled conditions of temperature (17°C), illumination (16 hours light / 8 dark), and water saturated aeration. Stock cultures were non axenic, but further contamination was controlled by air filters and sterile technique. Algal cell numbers were measured on a Coulter counter Model TAII using a 100 µm aperture tube. Chlorophyll *a* was extracted under SCA Standard Methods (Standing Committee of Analysts, 1980) and measured on a Pye Unicam SP8-100 ultraviolet Spectrophotometer. The TOC of algal cultures and filtrates was analysed by injection into a Dohrman DC80 Carbon analyser with a modified high temperature furnace. Samples of filtered and unfiltered algal cultures were dosed with excess chlorine and phosphate buffer then incubated at 20°C in the dark for 24 hours following AWWA Standard Methods (Clesceri *et al.*, 1989). Free residual chlorine was measured on a Wallace and Tiernan Residometer. Bacterial contamination of algal cultures was assessed using serial dilutions plated onto Tryptone glucose yeast agar and incubated for a week at 20°C. Two types of bacteria predominated, a Flavobacterium and a Pseudomonas type. These two types were isolated and grown on agar plates to generate material which was suspended in algal growth medium and used in THMFP experiments. The TOC, colony forming units and absorbance (540 nm) of suspensions were measured.

The experimental protocol is summarised below.



RESULTS

Data from the reservoir and treatment works for a period covering two years revealed a seasonal variation in levels of THMs found in the finished water. Similar variations were observed in the concentration of chlorophyll *a*, denoting algal populations in the reservoir. A definite correlation between chlorophyll *a* and TTHM of final water was established (r = 0.76, n=20). A multiple linear regression of chlorophyll *a* temperature and TOC explained 72% of the variation in THMs.

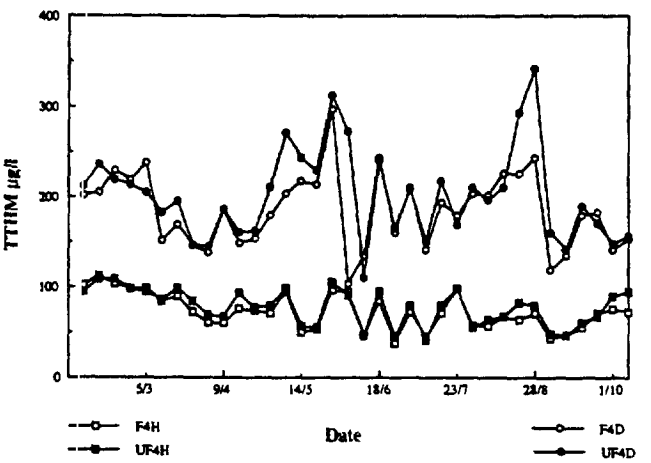


Figure 1. Four hour and four day Trihalomethane Formation Potentials from Filtered and Unfiltered water reservoir samples (Means of two Replicates, 10°C, 20 mg/l chlorine dose).

Simulated chlorine disinfection of the reservoir water samples yielded a range of THMs with an overall trend of increasing concentrations from late winter to summer (Figure 1). Four day Total THM concentrations (mean = 202 µg/l), representing the time that treated water is in the distribution system between treatment plant and consumers tap, were on average over two times the four hour TTHM (mean =78 µg/l), representing final water ex works. Water samples were filtered before chlorination to remove algal cell biomass. The mean TTHMs generated in four hours from unfiltered samples was 78 µg/l compared with 73 µg/l from filtered samples. The mean TTHMs from four day incubation of unfiltered samples was 202 µg/l compared with 185 µg/l for filtered samples. Although the unfiltered water samples yielded on average higher TTHMs a student t-test did not reveal a statistically significant difference, suggesting that algal biomass does not contribute substantially to the formation of THM. There was no significant difference between the chlorine demand of filtered versus unfiltered water samples.

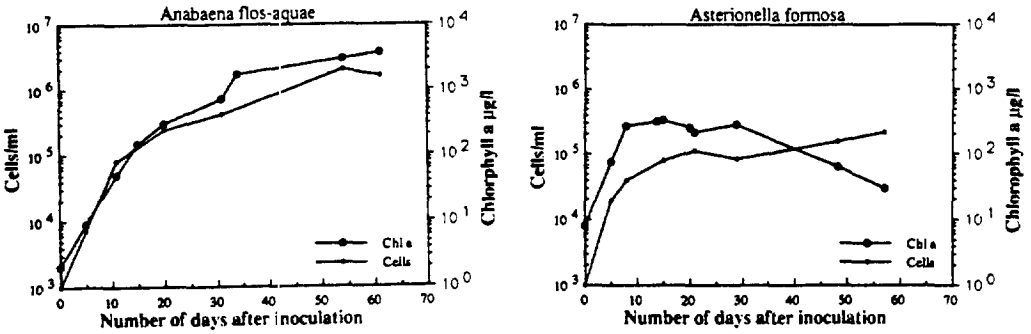


Figure 2. Growth curves of *Anabaena flos-aquae* and *Asterionella formosa* (means of three replicates of duplicate cultures).

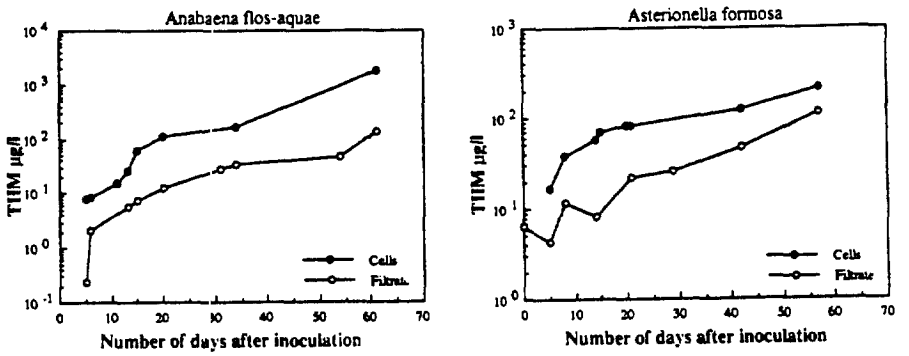


Figure 3. TTHMFP of Algal cells and filtrate (means of three replicates of duplicate cultures, 24 hours, 20°C, pH 7, excess chlorine dose).

Growth curves constructed from cell numbers and Chlorophyll *a* concentration generated by three replications of duplicate cultures of *Asterionella formosa* and *Anabaena flos-aquae* are shown in Figure 2. Day 0 represents the brief lag phase in both cultures. Exponential phase lasts until day 8 for *Asterionella formosa* and day 20 for *Anabaena flos-aquae*. Stationary phase ends at day 30 in *A. formosa* cultures and day 58 in *A. flos-aquae* cultures, followed by death phase. The TOC concentration of cells of *A. formosa* and *A. flos-aquae* mirrored the change in cell numbers. THM formation from cells and filtrates of both species increased with culture age (Figure 3). *A. flos-aquae* cells generated nearly ten times the concentration of TTHM formed by *A. formosa cells*, whereas TTHM from filtrates were of similar levels. These values reflect the maximum levels of THMs that could be generated by blooms of these species under the stated conditions. Yields of carbon incorporated into chloroform as a percentage of TOC (C- $CHCL_3$/TOC) generated from cells of both species exhibited a peak during exponential phase, decreasing during stationary

phase and then increasing during death phase. Yields from filtrates of *A. formosa* gradually increased during growth to a peak during death phase, whereas yields from *A. flos-aquae* filtrates remained low until death phase when they peaked. During exponential phase the yield from cells of *A. flos-aquae* was more than double the yield from *A. formosa* cells (Figure 4). Yields of TTHM from 10^6 cells of both species peaked during exponential and death phases. Yields from *A. formosa* filtrate increased with culture age, while *A. flos-aquae* filtrates peaked during exponential phase. Yields from 10^6 cells of *A. flos-aquae* were lower than from *A. formosa* cells possibly reflecting the disparity in cell size; *A. flos-aquae* cells are spheres of maximum diameter 10 μm and *A. formosa* are rods up to 100 μm long.

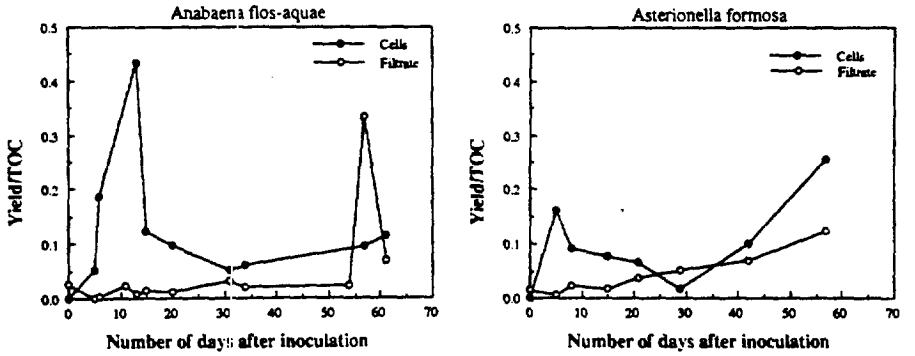


Figure 4. Yield of Chloroform as a percentage of TOC (C-$CHCl_3$/TOC mg per mg) of cells and filtrates (means of three replicates of duplicate cultures).

Bacterial contamination of algal cultures represented by colony forming units (c.f.u.) per ml were determined. Bacterial numbers peaked during early stationary phase, and rose again during death phase. The characteristics of bacterial suspensions were also investigated. Pseudomonas type bacteria had a lower TOC and THMFP than Flavobacterium type bacteria for similar numbers of colony forming units. Yields of THMs per unit TOC of the two bacterial types ranged from 0.089 to 0.254 (mean 0.166) for Flavobacterium type and 0.083 to 0.229 (mean 0.147) for Pseudomonas type.

SIGNIFICANCE OF THE RESULTS

Results from the chlorination of the reservoir samples suggest that algal biomass is not a significant precursor of THMs compared to levels generated from compounds such as humic and fulvic acids and products of planktonic metabolism which form the soluble organic pool. The generally low populations of algae in the reservoir could explain why no significant difference between THMs generated from filtered and unfiltered samples was found.

Algal culture experiments under controlled conditions showed that both algal cells and extracellular products can yield substantial levels of THMs upon chlorination. Levels of THMs produced from cells and filtrates of *Anabaena flos-aquae* and *Asterionella formosa* increased with age of culture and cells produced more than double the concentration of THMs than filtrates. THM concentrations produced from filtrates of the two species were of a comparable level, while concentrations from *Anabaena* cells rose to ten times the concentration produced by *Asterionella* cells. Increasing production of THMs with culture age is in part due to increasing TOC concentrations. However the TOC concentration of *Asterionella* filtrates remained constant throughout growth while yields of chloroform per unit TOC increased gradually indicating release of different types of organic compounds with differing ability to generate THMs. The TOC of *A. flos-aquae* filtrates did not vary during late stationary death phase, while yields of chloroform per unit TOC exhibited a substantial peak. This increasing yield could be due to the release of breakdown products of cell constituents due to cell lysis. *Asterionella* cultures do not exhibit this phenomenon, the yield from cells increases during death phase reflecting changes in intracellular compounds due to the breakdown of storage products to more chemically reactive compounds. The ability of cells to produce chloroform per unit TOC is greatest during

exponential phase for both species of algae. This could reflect changes in intracellular products of cells actively dividing, compared with cells in stationary phase, the latter contain more complex carbohydrate storage products. Yields of Chloroform per unit TOC for cells ranged from 0 to 0.43 for *A. flos- aquae* and 0 to 0.25 for *A. formosa*. There are no references in the literature to yields from cells of either species, but yields from other species under the same experimental conditions ranged from 0.09 to 1.6. Yields from filtrates of *A. flos-aquae* ranged from 0.001 to 0.33 and from *A. formosa* 0.07 to 0.12. The only yield reported in the literature for *A. flos-aquae* filtrate, 0.07 during stationary phase, is of a comparable level. There are no reports of yields from *A. formosa*, but yields from other species ranged from 0.03 to 0.46. The generation of THMs from 10^6 cells of each species was highest during exponential and death phases. Based on these laboratory results it is predicted that a bloom of *Asterionella formosa* (10^5 cells/ml) could produce a maximum of 0.21 mg/l THM from cells and an additional 0.08 mg/l from ECPs, while blooms of *Anabaena flos-aquae* (10^6 cells/ml) could produce a maximum of 1.1 mg/l from cells and 0.2 mg/l from ECPs.

Bacterial populations in algal cultures peaked during stationary phase, a relationship often reported from natural plankton populations. Bacterial contamination of algal cultures could contribute up to 2 mg/l of TOC and 70 µg/l TTHM. Bacterial populations in the cultures were comparable to populations (10^4 to 10^6 cells/ml) in reservoirs reported in the literature (Hoehn *et al.*, 1984).

Algal cells and ECP can yield substantial levels of THMs upon reaction with chlorine, comparable with yields per unit TOC generated from humic and fulvic acids reported in the literature (0.14 to 0.8). Traditional water treatment processes will remove algal cells, although during blooms many treatment facilities experience difficulties due to algal penetration of filters. Algal ECPs are less likely to be removed by coagulation and filtration and will react during post-chlorination to produce THMs. If prechlorination is practised algal cells will break down, releasing substances which may also survive treatment. To prevent THM formation from algal derived material measures to reduce algal populations and prevent bloom formation in reservoirs should be implemented.

CONCLUSIONS

The principal conclusion of this work is that both algae and algal derived organics are important precursors in the production of THMs. This has significant implications for water supply systems using surface water sources and particularly those employing raw water reservoirs. Specific conclusions arising from the study are as follows.

1. A positive correlation between chlorophyll *a* levels in reservoir feed waters and TTHM levels in Treatment works final water was established.
2. Simple chlorination experiments on filtered and unfiltered reservoir water samples did not reveal statistically significant differences in TTHM levels produced. This was believed to be due partly to the low algal cell concentration prevailing in the reservoir during the period of sampling.
3. Significant levels of THM were produced from cells and filtrates of *Anabaena flos-aquae* and *Asterionella formosa* at various growth stages. Cells of both species produced double the THM concentrations produced by filtrates. Cells of *A. flos-aquae* produced ten times the TTHM levels produced by *A. formosa* cells, whereas levels produced by filtrates of both species were similar.
4. Yields of chloroform per unit TOC were highest from cells of both species during exponential and death phases. Yields from *A. formosa* filtrates increased with culture age, while yields from *A. Flos-aqua* exhibited a peak during death phase. Yields from cultures were of comparable levels with those published in the literature for other algal species and humic and fulvic acids.
5. Based on the laboratory results a bloom of *A. flos-aquae* could produce 1.1 mg/l of TTHM from cells and an additional 0.2 mg/l from ECPs. A bloom of *A. formosa* cells could produce 0.2 mg/l from cells and 0.08 from ECPs.
6. Bacteria associated with algae are themselves THM precursors but their contribution to overall THM formation is likely to be minor relative to that from algae.

7. To prevent substantial production of THMs from algae and algal derived products and generally minimise THM formation, algal populations in reservoirs should be controlled and prechlorination should be avoided (by using alternative disinfectants).

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the funding of this study by the Engineering and Physical Sciences Research Council (formerly SERC), UK.

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