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# Influence of Chlorine on Algae as Precursors for Trihalomethane and Haloacetic Acid Production

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**Abstract:** Two species of freshwater algae were isolated from Nile River water. These species represent the green algae (*Scenedesmus obliquus*) and the blue-green algae (*Anabaena flos-aquae*). Contribution of the algal cells of both species in the formation of trihalomethanes (THMs) and haloacetic acids (HAAs) during chlorination in the laboratory was investigated. The results showed that the predominant disinfection by-products (DBPs) generated were chloroform, monochloroacetic acid, dichloroacetic acid and trichloroacetic acid. The yields of all DBPs were insignificantly higher upon chlorination of *S. obliquus* than in case of *A. flos-aquae*. The amount of algal cells in both species (measured as chlorophyll "a" content) was significantly influenced on the formation of HAAs only. The concentrations of all DBPs were increased as the reaction time was extended. However, 60 % of the chloroform was formed during the first five minutes of the reaction, while 57 -71 % of HAAs was formed during the first 30 minutes of the reaction. Generally, the current study elucidated that the cellular biochemical composition of both algal species may have contribute as DBPs precursors.

**Key words:** Algae · Chlorination · THMs · HAAs · Chlorophyll "a"

## INTRODUCTION

Since trihalomethanes (THMs) were discovered in chlorinated water in the 1970's by Rook [1], haloacetic acids (HAAs) and other disinfection by-products (DBPs) have become a focus of attention in drinking water treatment. Trihalomethanes (THMs) and haloacetic acids (HAAs) are the two most prevalent classes of DBP, stringently regulated by the U.S. Environmental Protection Agency (USEPA) and the World Health Organization (WHO) due to their potential cancer risks [2,3]. Naturally occurring organic materials such as humic and fulvic acids serve as precursors for these byproducts [4]. Algal cells and their metabolites have been shown to be a precursor for DBPs [5-8]. However, DBPs production levels vary based on the algae species, algal growth phase and reaction conditions such as pH, temperature, contact time and chlorine concentration [6,9].

As compared to humic substances, algae can contribute significantly to the DBP precursors. Laboratory experiments with four algal species in different stages of growth showed that maximum chloroform yields from algal cultures exceeded yields from humates [9]. Additionally, algal biomass and extracellular organic matters could be potent precursors for THM and HAA formation and that DBP production varies with the algal species and their growth phase [10,11]. Moreover, it has been verified that algal biochemical composition plays an important role in determining DBP yields upon chlorination of the algae [12].

The objective of the present study focused on evaluating the contribution of algal cells in disinfection by-products (DBPs) formation upon chlorination. Two different algal groups namely green algae (*Scenedesmus obliquus*) and blue-green algae (*Anabaena flos-aquae*) were chlorinated in control laboratory conditions. The THMs and HAAs yields were determined to compare the variations in DBP formation from different algae groups.

## MATERIALS AND METHODS

The current experimental work herein follows a laboratory bench-scale model that has been carried out in Water Pollution Research Department of the National Research Centre of Egypt.

**Algal Culturing:** Two species of algae were isolated from Nile River water. These are the green algae *Scenedesmus obliquus (S. obliquus)* and the blue-green algae *Anabaena flos-aquae (A. flos-aquae)*. The isolated

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algae were recultivated under controlled laboratory conditions (algae grown in 1 L flasks at 23 ± 2°C under 2000 lux of illumination). *S. obliquus* was cultured in modified BG11 medium [13]. The modification was done by diluting the amount of sodium nitrate to the $5^{th}$ of its original concentration [14]. *A. flos-aquae* was cultured in modified Watanabe medium [15]. To ensure maximum concentration of algal cells, all experimental cultures were allowed to attain their late logarithmic phase which is the beginning of the first stage of stationary growth. Stationary growth was determined by measuring the concentration of algal chlorophyll "a" content according to APHA [16]. All experimental cultures were run in triplicates and the mean values were used.

**Chlorination Procedure:** A laboratory bench-scale model was carried out to estimate the formation of disinfection by-products (DBPs) during the chlorination process. The contribution of algal cells in formation of trihalomethanes (THMs) and haloacetic acids (HAAs) was evaluated upon chlorination. The stock chlorine solution was prepared by bubbling a pure chlorine gas (> 98 %) through de-ionized water and its concentration was determined by iodometric method whereas the chlorine residues were measured by the DPD colorimetric method according to APHA [16]. The chlorination experiments were conducted in batch mode using a series of 1 L TFE-lined screw cap amber glass bottles (reactor). To prepare algal samples for the test, chlorophyll "a" contents were determined for each algal culture. The volume of algal solutions were inoculated to the reactor and diluted by de-ionized water with media to produce 10 and 20 µg/l of chlorophyll "a" then the chlorine solution was dosed to obtain the desired concentration. Chlorine dosages of 5 and 10 mg/l were chosen to determine the formation of HAA and THM, respectively. The 1 L reactors were allowed to react under standardized pH 8 and temperature (20°C). The formation of THM was evaluated after reaction contact time of 5, 10, 20, 30, 60 and 120 minutes while the production of HAA was determined after reaction contact time of 30, 60, 120 and 240 minutes. A reagent blank sample bottle was prepared with each batch of samples by the same manner in absence of algal cells.

**DBPs Quantification:** Subsequent to each reaction period, the chlorinated samples were analyzed to determine the levels of THM and HAA. THM concentrations were determined by using simple liquid-liquid extraction gas chromatographic method (GC, EPA method 501.2) while HAA concentrations were determined by using micro liquid-liquid extraction gas chromatographic method (GC, EPA method 552.3).

A Varian 4000 gas chromatograph equipped with Varian auto-injector model CP 8410 and 30 meter CP-selected 624CB fused silica capillary column and an electron capture detector (ECD) was used for analysis of THM and HAA. Data acquisition and processing were controlled via Varian windows based MS workstation version 6.6 data system. Concentrations of THM and HAA were calculated by using external standard calibration method. A certified mixture standards of THM (chloroform ($CHCl_3$), bromodichloromethane, dibromochloromethane and bromoform) and HAA {monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromochloroacetic acid (BCAA) and dibromoacetic acid (DBAA)} obtained from Suppelco Inc. were used. High purity (99.99 %) grade gases were used. Helium was used as a carrier gas, while nitrogen was used as make-up gas. All sample extracts including standards and blanks were injected in splitless mode. The operating conditions of the gas chromatograph were as follow:

For THMs

- Injector temperature was 200°C isothermally in splitless mode.
- ECD temperature was 270°C isothermally.
- Carrier gas flow was 1 mL/minute and make-up gas flow was 25 mL/minute.
- Capillary column temperature program was: start with 104ºC for 3 minutes, ramp 40ºC/minute to 160°C and holding for 2.6 minutes.

For HAAs

- Injector temperature was 210°C isothermally in splitless mode.
- ECD temperature was 290°C isothermally.
- Carrier gas flow was 1 mL/minute and make-up gas flow was 25 mL/minute.
- Capillary column temperature program was: start with 40ºC, ramp 25ºC/minute to 85, ramp 10ºC/minute to 175ºC, ramp 10º C/minute to 205ºC.

**Quality Control and Data Analysis:** For quality control and quality assurance, minimum duplicate samples were analyzed. A procedural blank and the certified standards

were analyzed routinely with each batch of samples. The percentage recovery of the method was calculated by spiking de-ionized water with known concentrations of both THM and HAA standards in separate and analyzed routinely with the samples. The average recoveries varied between 85 ± 4% for trihalomethane compounds and 90 ± 4% for haloacetic acid compounds, but not applied in calculation the concentrations of these compounds in the samples. The detection limits of the methods were verified and carefully noticed. The linearity response of ECD for trihalomethane and haloacetic acid compounds, in area count, was observed. It was showed that the ECD was responding linearly for all compounds during the current study. Mean concentrations of individual compounds in addition to sum of compounds were calculated. Standard error (± SE) was used as statistical measurement to verify the accuracy of the results. Simple t-test and analysis of variance (ANOVA) were used to determine the statistical variation among the chlorination experiment conditions.

## RESULTS AND DISCUSSION

The results showed that the predominant DBPs formed were $CHCl_3$, MCAA, DCAA and TCAA. The brominated species of halomethanes and haloacetic acids were not generated during this experiment. The yields of $CHCl_3$, MCAA, DCAA and TCAA upon chlorination of the algal cells of both species are presented in Tables 1 and 2. As evident in Table 1, the $CHCl_3$ exhibited higher yields with *S. obliquus* than with *A. flos-aquae* but without statistical significance. The amount of algal cells in both species measured as chlorophyll "a" content (10 and 20 µg/l) had no significance effect on the formation of chloroform ($P > 0.05$). In a previous study [17] no statistical significant correlation has been observed between chlorophyll "a" concentration and THM formation. It has been observed that concentrations of the chloroform were increased as the reaction time extended, but 60% of the chloroform was formed during the first 5 minutes of the reaction. Assuming that the chlorophyll "a" contents in both species are equal (as the design of the current experiment), the chloroform production could be dependent on the variation of the cellular biochemical composition (protein, lipids and carbohydrate) between *S. obliquus* (green algae) and *A. flos-aquae* (blue-green algae). The relative percentage of the cellular biochemical composition in different algal groups may have a major influence as DBP precursors [18,19].

The results in Table 2 revealed that the formation of all compounds of HAAs were insignificantly higher with *S. obliquus* than with *A. flos-aquae*. Generally, the DCAA and TCAA yield with both algae species were significantly much higher than MCAA ($P < 0.05$). The ratios of TCAA/DCAA ranged between 0.86 and 0.98, reflecting that both algal species might contain slightly high levels of organic compounds possessing high DCAA formation potential than TCAA, such results are in agreement with that in a previous report [20].

Conversely, variation in the amount of algal cells in both species measured as chlorophyll "a" concentration (10 and 20 µg/l) had significant influence on the formation of HAA compounds ($P < 0.05$). The levels of THAAs formed with *A. flos-aquae* (10 µg/l chlorophyll content) ranged between 17.18 and 24.96 µg/l, while these levels ranged between 26.51 and 37.24 µg/l in case of 20 µg/l chlorophyll "a" content. On the other hand, the concentrations of THAAs formed with *S. obliquus* (10 µg/l chlorophyll "a" content ranged between 17.86 and 27.1 µg/l, while these concentrations ranged between 27.17 and 47.75 µg/l in case of 20 µg/l chlorophyll "a" content. As the trend in case of chloroform formation, the yield of HAA compounds increased as the reaction time was extended, but from 57 to 71% of THAAs was formed during the first 30 minutes of the reaction. It seems that the chlorophyll "a" content may play an important role in HAA formation, because as the concentration of chlorophyll "a" was increased, the HAAs yield has significantly elevated ($P<0.05$) with both algae species.

Table 1: Average yields of chloroform (µg/l, ± SE) on chlorination* of various algal species based on chlorophyll "a" contents

| | Contact time (min) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 20 | 30 | 60 | 120 | 5 | 10 | 20 | 30 | 60 | 120 |
| Chlorophyll "a" µg/l | *Anabaena flos-aquae* (blue-green algae) | | | | | | *Scenedesmus obliquus* (green algae) | | | | | |
| 10 | 19.04 ± 0.06 | 19.54 ± 0.38 | 20.99 ± 0.02 | 21.41 ± 0.50 | 25.98 ± 0.34 | 31.11 ± 0.59 | 19.80 ± 1.95 | 20.38 ± 1.58 | 21.10 ± 0.25 | 22.15 ± 0.95 | 26.66 ± 0.44 | 32.90 ± 1.95 |
| 20 | 23.36 ± 0.24 | 24.44 ± 0.26 | 24.90 ± 0.74 | 28.29 ± 0.44 | 32.86 ± 1.03 | 38.95 ± 0.49 | 24.00 ± 1.40 | 24.62 ± 1.78 | 25.16 ± 0.40 | 29.51 ± 0.97 | 34.43 ± 0.75 | 39.10 ± 0.03 |

*Chlorine dose was 10 mg/l

Table 2: Average yields of haloacetic acid species (µg/l, ± SE) on chlorination* of various algal species based on chlorophyll "a" contents

| | | Contact time (min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 30 | 60 | 120 | 240 | 30 | 60 | 120 | 240 |
| Chlorophyll "a" µg/l | HAA species | *Anabaena flos-aquae* (blue-green algae) | | | | *Scenedesmus obliquus* (green algae) | | | |
| 10 | MCAA | 0.43 ± 0.015 | 0.50 ± 1.49 | 0.73 ± 0.52 | 1.72 ± 0.06 | 0.66 ± 0.21 | 1.15 ± 0.40 | 1.55 ± 1.46 | 2.48 ± 2.15 |
| | DCAA | 8.52 ± 0.06 | 8.98 ± 0.32 | 10.46 ± 0.69 | 12.10 ± 0.23 | 8.79 ± 0.97 | 9.61 ± 2.67 | 11.31 ± 0.96 | 13.27 ± 0.90 |
| | TCAA | 8.24 ± 0.71 | 8.72 ± 2.16 | 10.05 ± 0.92 | 11.14 ± 0.80 | 8.50 ± 0.26 | 9.16 ± 0.53 | 10.97 ± 4.00 | 11.35 ± 2.52 |
| | THAA | 17.18 | 18.20 | 21.23 | 24.96 | 17.96 | 19.92 | 23.83 | 27.10 |
| 20 | MCAA | 0.93 ± 0.35 | 1.28 ± 0.34 | 1.42 ± 0.84 | 2.28 ± 0.17 | 0.99 ± 0.01 | 1.57 ± 3.70 | 2.30 ± 0.40 | 3.28 ± 1.15 |
| | DCAA | 13.12 ± 0.02 | 14.10 ± 0.05 | 15.87 ± 0.92 | 18.25 ± 0.37 | 13.66 ± 3.32 | 14.12 ± 2.88 | 17.12 ± 1.13 | 23.54 ± 0.47 |
| | TCAA | 12.46 ± 0.54 | 13.22 ± 0.56 | 15.48 ± 0.04 | 16.72 ± 1.67 | 12.52 ± 0.61 | 13.23 ± 4.43 | 15.52 ± 0.08 | 20.93 ± 1.67 |
| | THAA | 26.51 | 28.59 | 32.77 | 37.24 | 27.17 | 28.92 | 34.94 | 47.75 |

* Chlorine dose was 5 mg/l

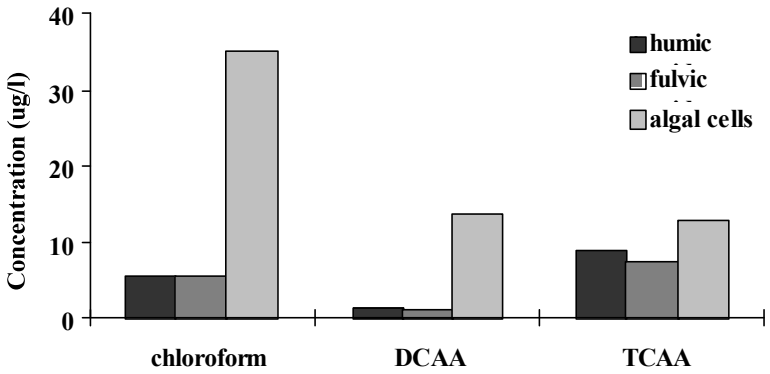


Fig. 1: Comparison of DBPs yields between algal cells and humic substances

It has been reported [21] that the organic components in the algae such as chlorophyll "a" might have high HAA formation potentials. In addition, variation in the HAAs yield between the two algal species may be attributed to the fact that *S. obliquus* contains both chlorophyll "a" and "b", whereas *A. flos-aquae* contains chlorophyll "a" only.

Humic and fulvic acids are usually regarded as the major DBP precursors in freshwater environment [4]. A comparison between the DBP formation upon chlorination of humic and fulvic acid [22] with that from algal cells of the current study has been carried out. Data from [22] were chosen for the comparison because they were generated under the similar chlorination conditions as the present experiment. It shows that the algal-derived organic matter of this study had higher potentials in producing chloroform, DCAA and TCAA than humic and fulvic acids of the other study (Fig. 1). A laboratory experiments with four algal species showed that maximum chloroform yields from algal cultures exceeded yields from humates [9]. However, the DCAA yield from algal cells of the present study (average of both species 13.69 µg/l) was much higher than humic acid (1.38 µg/l) and fulvic acid (1.2 µg/l). According to previous studies, it was proved that DCAA is a more potent carcinogen than chloroform and TCAA [2,23]. On the other hand, the average ratio of TCAA/DCAA derived from algal cells in the present study (0.95) was significantly lower ($P < 0.05$) than those of aquatic humic acid (6.38) and fulvic acid (6.15). These results were not expected since the aquatic humic

substances contain more aromatic carbon than algae deriver organic matter, in the same time these results can indicate that algae may be a significant contributor to DBP precursor, especially the most dangerous DCAA.

In conclusion, algal cells contribute significantly in the formation of DBPs such as trihalomethanes and haloacetic acids upon chlorination. The green algae species (*S. obliquus*) have influence on the formation of DBPs more than the blue-green algae (*A. flos-aquae*) which might be attributed to that *S. obliquus* contains both chlorophyll "a" and "b", whereas *A. flos-aquae* contains chlorophyll "a" only. The role of specific types of algal cellular biochemical composition such as proteins, lipids, carbohydrates and chlorophyll contents in contribution of DBPs formation during chlorination must be examined to elucidate the variances.

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