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Project Summary

Alternative Water Disinfection Schemes for Reduced Trihalomethane Formation *Volume II. Algae as Precursors for Trihalomethanes in Chlorinated Drinking Water*

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Three species of algae (*Anabaena cylindrica, Scenedesmus quadricauda,* and *Pediastrum boryanum*) were investigated for their trihalomethane (THM) formation potential in water treated with chlorine. Algae were cultured and the cells (algal biomass) were separated from the extracellular products (ECPs) at several points along the normal growth curves of each species for a separate study of their contributions as THM precursors. The cells were resuspended in organic- and chlorine-demand-free water, and the cells and ECPs were then separately dosed with three chlorine concentrations. The THMs formed after 1 and 24 hr of chlorine contact time were analyzed by the gas-sparging technique and gas chromatography. For each point examined along the growth curve, growth was monitored by both cell counts and fluorometric assay of chlorophyll-a. Correlation of the algae growth period and THM production was observed. Furthermore, significant levels of THM were produced from both the ECPs and the isolated algal cells of all three species when dosed with chlorine. As expected, the THM levels formed were related to the free chlorine residual and to the TOC levels observed. These findings suggest that THMs may be partially reduced by observation and control of the natural phytoplankton communities in the water sources for domestic water supplies.

This Project Summary was developed by EPA's Municipal Environmental Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Chlorination of surface waters generally results in the formation of trihalomethanes (THMs). These compounds result from a reaction or series of reactions of chlorine with natural organic precursor material present in the source water. The exact nature of the precursor material is not clear, though humic and fulvic acids and algae have been implicated as contributors. Chloroform ($CHCl_3$) is the THM found in greatest quantities in finished waters, but dibromochloromethane ($CHBr_2Cl$), bromodichloromethane ($CHBrCl_2$), and bromoform ($CHBr_3$) are commonly found in varying quantities as

well. The U.S. Environmental Protection Agency (EPA) has established a maximum contaminant level of 0.10 mg/L for THMs in finished waters for communities with populations of 10,000 or more. Attempts to reduce total trihalomethanes (TTHMs) below the established levels in an economical way are hampered by the lack of a complete understanding of the nature of the THM precursors. Identification of THM precursors would allow establishment of techniques to remove them selectively.

The purpose of this study was to examine a class of potential THM precursors in surface waters. Specifically, investigations were made of the contribution of algal cells and extracellular metabolic products (ECPs) from algae to the production of THMs. This information may be useful for developing alternative disinfection procedures that can allow reduced levels of THMs in finished water and still maintain effective water disinfection.

Experimental Procedures

To learn the extent to which algal cells and ECPs might contribute to THM formation, three algal species were grown in the laboratory under controlled conditions. The cells were separated from the ECPs, each fraction was chlorinated, and THM concentrations were measured. The latter were compared with algal cell concentrations in the original suspension to indicate the ability of algae to cause THM problems.

The algal species chosen for study were nonaxenic *Anabaena cylindrica, Pediastrum boryanum,* and *Scenedesmus quadricauda.* These species were selected for study because they are easy to grow in the laboratory and because they are abundant in South Central Texas lakes. The algae were grown using Bold* basal pH 6.6 medium cultured in Erlemeyer flasks with metal snap-on caps that permitted some air flow to the cultures without allowing contamination. The cultures were further aerated by frequent shaking. The algae were subjected to 16 hr of light and 8 hr of dark each day on a constant schedule with a light source that ranged from 275 to 450 foot-candles. The temperature for algal growth was maintained between 23° and 25°C.

For each experiment in this study, algae from a stock culture with a known number of cells/mL were inoculated into 2 L of freshly prepared medium to make a final dilution of approximately 10^3 cells/mL. The algae were then cultured until the growth phase desired for THM analysis was reached.

The growth phases desired for THM precursor studies were determined by cell count techniques and chlorophyll-a measurements. These growth-monitoring techniques allowed algal growth curves to be generated. From these curves, the times chosen for THM precursor examination were selected with the intention of encompassing the five basic growth phases (lag, early exponential, late exponential, early stationary, and late stationary) of each species employed. The death phase was not examined. The times chosen for experimentation along the growth curve of *Anabaena* were days 0, 2, 4, 7, 10, 14, and 21. Day 0 encompassed the brief, if any, lag phase; days 2, 4, and 7 were during the exponential growth phase, and day 10 was transitional between the late exponential and early stationary phases. Days 14 and 21 were during the stationary phase. Similarly, for *Pediastrum,* days 0, 2, 7, 10, 15, 21, and 28 were chosen for study, and *Scenedesmus* experiments were conducted on days 0, 2, 4, 8, 11, 15, and 21 of the growth cycle.

At the chosen time, a 270-mL aliquot was removed from the 2-L experimental culture. From this aliquot, 20 mL was used for growth-monitoring measurements done by cell enumeration and fluorometric assay of the chlorophyll-a. Duplicate fluorometric chlorophyll-a measurements and cell counts were made on the algae each time THM experiments were performed.

The chlorophyll-a measurement was made using a fluorometer with a 5-60 excitation filter and a 2-28 emission filter. Chlorophyll-a was extracted as described in Standard Methods (*Standard Methods for the Examination of Water and Wastewater,* 14th edition; 1975; Amer. Public Health Assoc., Amer. Water Works Assoc., Water Pollution Control Federation; pp. 1030-1032) for the spectrophotometric procedure. A calibration curve for the fluorometer was constructed using commercially obtained chlorophyll-a. The calibration curve obtained had a correlation coefficient (R^2) of 0.993 determined by regression analysis. Reference samples were prepared by extraction in the same manner without algae, and these readings were subtracted from the fluorescence readings before chlorophyll-a calculations. Replicate measurements were performed on each organism.

Cell count measurements were made along the algal growth curves using a mechanical counter with an aperture tube of 100-μm diameter. The instrument was calibrated using pollen and glass spheres of known volumes, and the optimal sensitivity settings for measuring cell counts for each algal species were determined. Commercially obtained electrolyte solution was used for making the cell dilutions and corresponding control dilutions. The reference reading values made using medium and diluent alone were subtracted before cell count calculation. The algae were dispersed by vigorous shaking with glass beads before cell counts were performed.

After the 20-mL volume had been removed for the growth measurement procedures, the remaining 250 mL was used for the THM precursor experiments. The cells and their corresponding extracellular products were separated by centrifugation at 2400 to 2600 rpm (1050 to 1250 x g) for 20 min. The ECPs and medium (supernatant) were then poured off and vacuum filtered using a 0.45-μm (47-mm) membrane filter. The filtered ECPs were transferred to a clean flask and refrigerated at 4°C in the dark overnight or until chlorine addition. Before chlorination, the ECPs were warmed to room temperature. The pellet formed after centrifugation (algal biomass) was washed twice with 0.9 percent sterile sodium chloride, resuspended, and recentrifuged. The supernatant was then discarded. The washed cells were resuspended in 250 mL of organic-free, chlorine-demand-free water. Approximately 10^{-4} moles of phosphate buffer (filtered through a 0.45-μm membrane filter) were added to the resuspended cells for pH control. The resuspended cell or ECP aliquots were then divided into three 80-mL samples, measured for pH and temperature, and immediately dosed with the desired amount of chlorine (for the ECPs, 7, 24, 33 mg/L; and for the cells, 1.5 and 5.9 mg/L). After 1 hr of chlorine contact time, the pH, temperature, and chlorine residuals (free and total) were again measured in duplicate. Samples were obtained for TOC, TKN, and NH_3-N determinations. A THM sample (1-hr contact time) was collected in a vial containing 0.3 to 1.0 mL of 0.01 N sodium thiosulfate (according to chlorine dose) to quench the reaction (instantaneous THM) and sealed with a Teflon-faced septum that allowed no headspace. A second THM sample was stored at room temperature in a Teflon-sealed vial with no headspace for 24 hr and then quenched with sodium

*Mention of trade names or commercial products does not constitute endorsement for use.

thiosulfate (terminal THM). After the sodium thiosulfate addition, the THM samples were all stored at 4°C until analysis. All vials were warmed to room temperature before THM analysis. THM samples were measured using the gas-sparging technique developed by Bellar and Lichtenberg (Bellar, T. A., and J. J. Lichtenberg; 1974; Determining Volatile Organics at Microgram-per-Litre Levels by Gas Chromatography; J. Amer. Water Works Assoc., 66(12):739) and all analyses were performed on a Varian 3700 gas chromatograph equipped with a flame ionization detector system and a reporting integrator.

A microprocedure for residual chlorine determinations (free and total) was developed because of the sample size available for analysis. The leuco crystal violet technique for chlorine residual was modified to use a 5-mL sample (a total of 10 mL for both free and total chlorine) in both the presence and absence of the Bold basal medium. The reference technique was amperometric titration. The absorbance of 592 nm of the leuco crystal violet color development was monitored. Calibration curves were constructed for free and total available chlorine residual determinations in both chlorine-demand-free water and in the Bold basal medium. Chlorine-demand-free water was used in the resuspension of the algal cells for THM precursor studies, and the Bold basal medium was used for the extracellular product THM studies. The free and total chlorine residuals were measured after a 1-hr chlorine contact time for both the water and the medium calibration curves. The calibration curves obtained for the chlorine-demand-free water had correlation coefficients (R^2) of 0.995 and 0.991, respectively.

Results

Increasing cell count was directly related to the amount of chlorophyll-a produced by the cells. Linear regression analyses for each algal species were obtained by averaging each day's chlorophyll-a replicate measurements and comparing these with the average of that day's cell count replicate measurements. These analyses yielded correlation coefficients (R^2) for chlorophyll-a to cell count of 0.952 for *Anabaena,* 0.955 for *Scenedesmus,* and 0.989 for *Pediastrum.* The three species exhibited different growth curve shapes and growth rates.

After chlorine was added to the cell and ECP test samples of *Anabaena,* THM production was monitored at contact times of 1 and 24 hr (Table 1). The pH for all measured data points ranged between 6.5 and 7.4 for the ECPs and 6.4 and 7.6 for the cells. The temperature ranged between 21° and 24°C for all *Anabaena* measurements. Typically, TTHM production increased slightly and then fell as the stationary phase of growth was reached. The free chlorine residual was low during the early portions of the growth curve, indicating a high chlorine demand. After 6 days, however, a free residual persisted, indicating a change in the type and/or degree of ECP produced. THM production increased with an ill-defined maximum near the late exponential growth phase, followed by a slight decrease in amount of THM. This type of pattern was also observed for the other chlorine doses. The drop off in THM level was not due to chlorine limitations alone, since a free residual (0.4 mg/L) persisted for the 32.5-mg/L dose and a similar decrease was noted. Results of chlorinating the isolated *Anabaena* cells paralleled those observed for the ECPs. A free chlorine residual was maintained throughout the growth cycle except in the case of the lowest dose (at days in the growth cycle greater than 10).

Table 1. Comparison of THM Production from Three Species (ECPs and Cells)

Day in Growth Cycle (days)	Contact Time (hr)	Chlorine Dose	THM, µg/L Anabaena Cells	Anabaena ECPs	Pediastrum Cells	Pediastrum ECPs	Scenedesmus Cells	Scenedesmus ECPs
7*	1	Low	315.6	29.6	252.1	944.6	167.4	397.9
	1	High	392.8	45.4	424.0	466.1	<0.1	37.8
	24	Low	408.6	64.6	97.3	120.8	333.1	321.5
	24	Medium	400.3	258.9	343.4	124.7+	359.7	782.8
21	1	Medium	138.0	73.1	481.8	157.1	<0.1	47.3
	1	High	392.8	149.5	31.9	7.1	<0.1	13.9
	24	Low	308.3	57.2	119.6	285.6	<0.1	553.0
	24	Medium	549.2	497.0+	59.7	333.3	<0.1	539.7

**Eight days for* Scenedesmus.

+Only high chlorine dose data available.

The *Scenedesmus* cells exhibited a pH range of 6.2 to 7.4, and the pH of the ECPs ranged from 6.4 to 7.0. The temperature for all *Scenedesmus* measurements ranged from 24° to 25°C. The *Scenedesmus* cells after both 1 and 24 hr of chlorine contact time showed high chloroform production immediately after initiation of the experiment. After the initially high levels, the chloroform levels dropped until approximately day 4 of growth, at which time a peak in chloroform production was observed (days 4 to 10). After this peak, the chloroform levels dropped to less than the detection limits toward the last day examined along the growth curve (day 21). A comparison of 7- to 8-day versus 21-day THMFP values appears in Table 2. The *Scenedesmus* ECPs at a 1-hr contact time produced chloroform levels in generally the same pattern as that described for the cells. For the *Scenedesmus* ECPs after 24 hr of chlorine contact time, maximum chloroform production occurred somewhat later in the growth curve (approximately days 11 to 21) than that observed for the cells of the ECPs at the 1-hr contact time.

The *Pediastrum* cells had a pH range of 6.5 to 7, and the pH of the ECPs ranged from 6.5 to 7.9. For the *Pediastrum* cells and ECPs, the temperature ranged between 23° to 25°C. After both 1- and 24-hr contact times, the *Pediastrum* cells showed two chloroform production peaks. The chloroform levels were initially low during the beginning of the growth curve. Maximum chloroform production was reached between days 7 and 10 of growth. After day 10, the chloroform levels dropped and then began to increase again until the second chloroform maximum was reached between days 15 and 21. After the second peaks in chloroform production, the amount of chloroform dropped to low levels toward the latter part of the growth curve. Chloroform production from the *Pediastrum* ECPs for both chlorine contact times followed the same general pattern as that of the *Pediastrum* cells.

The chlorine doses employed for the *Scenedesmus* ECPs and cells were comparable and generally sufficient to maintain a residual at 1 hr of contact time. With both *Scenedesmus* cells and ECPs, a free chlorine residual was present for all but the lowest chlorine doses after 1 hr of chlorine contact time, even during the latter portions of the growth curve. Thus, the decline in chloroform levels toward

the end of the growth curves for the cells and the ECPs was not due to chlorine limitations. This result is general for all three species.

Discussion

The THM levels produced with the chlorination of algae depended on the species (*Anabaena, Pediastrum,* or *Scenedesmus),* the age of the culture, the chlorine dose, and the substance chlorinated cells versus ECPs). THM production was quite variable. Nevertheless, the results can be presented to facilitate comparison by calculating the THM formation potential (THMFP) of cells and ECPs per million algal cells in suspension. The THMFP ($\mu g/10^6$ cells) for the cells and ECPs of each of the algal species is listed in Table 2 for two time points in the growth cycle. The chlorine doses chosen for representation were 23.7 mg/L for the ECPs and 5.0 mg/L for the cells. The range of values depends on the algal species and the position of the algae in its growth cycle, with greater THM formation occurring in the exponential growth phase; but the values do not vary greatly between chlorine contact times of 1 and 24 hr. A bloom of these three species would produce 0.2 to 120 $\mu g/L$ THM per 10^6 cells. The ECPs produced by 10^6cells of the species studied would produce an additional 0.1 to 50 μg of THM. These values are approximate and vary with growth condition and chlorine dose, but algal sources can clearly contribute significantly to the overall THM production in a treatment facility.

The amount of algae-related THM precursor in water can be influenced by factors other than the number of algae present in the water. In an undisturbed natural water, both cells and ECPs would be present and would contribute to THM formation. In a water treated with algacide to control algal blooms, some cells would be present along with ECPs and a quantity of disintegrating cell material. At a water treatment plant that practiced coagulation and filtration for algae removal and then chlorinated the filtrate, the cells would be removed from the water before chlorination, but the ECPs would probably pass through the plant and react with chlorine to produce THMs.

The higher chlorine doses and the longer chlorine contact time (24 hr) for the cells and ECPs of both *Pediastrum* and *Scenedesmus* produced lower chloroform levels than expected (i.e., compared with the chloroform levels produced by the lower chlorine doses and by the 1-hr contact time). The reason for this observation has not been established, but possibly organohalide compounds other than haloforms were formed, given that these species had higher chlorine doses and a longer chlorine contact time, and may have different precursor compounds from *Anabaena.* These factors imply different mechanisms of action.

The concentrations of bromodichloromethane, dibromochloromethane, and bromoform for the *Pediastrum* and *Scenedesmus* cells and ECPs were generally below the detection limits, except for a few isolated instances in which the bromine-containing volatile concentrations were disparately high when compared with the chloroform levels. These results were probably caused by contaminants rather than by the compounds of interest. Thus for *Scenedesmus* and *Pediastrum,* only the chloroform levels were used in the data analysis.

The technique used for the *Anabaena* THM analyses gave no bromoform results, and the dibromochloromethane measurements were questionable. For this reason, the total THM data given here for *Anabaena* actually reflect the concentrations of chloroform and bromodichloromethane only.

The results presented demonstrate that high THM concentrations are produced from both algal biomass and metabolites. The significance of these results suggests that THM may be partially reduced by observing and controlling the natural phytoplankton communities in the water source for domestic water supplies. Clearly, humic or fulvic precursors or both contribute to THM production upon chlorination; but this source can only account for a portion of the THMs produced. To date, no complete mass balance has been possible to determine all the precursor molecules that produce THMs. This problem is further complicated by the incomplete yields of THMs that model compounds produce. A significant amount of algal biomass is unlikely to survive through the coagulation and filtration steps of a well-operated water treatment plant; however, ECPs may persist if they are not destroyed by bacteria or removed in the coagulation process. Furthermore, algae can pass through the coagulation and filtration steps if they are present in large numbers or if the treatment plant is not being optimally managed. THM precursors (ECPs and cells as well as humic and fulvic acids) will be present at the point of prechlorination (chlorination before coagulation), a process used at many surface water treatment plants. For this reason, the presence of such materials may allow the THM levels to be greater than those formed if coagulation and filtration are performed before chlorination.

Table 2. *THMFP for the Three Algal Species (ECPs and Cells) with a Medium Chlorine Dose*

Time in Growth Cycle (days)	Contact Time (hr)	THMFP ($\mu g/10^6$ Cells) Anabaena Cells	Anabaena ECPs	Pediastrum Cells	Pediastrum ECPs	Scenedesmus Cells	Scenedesmus ECPs
7*	24	3.6	2.3	122.6	44.5	21.6	47.1
21	24	0.8	0.7	5.7	31.6	<.001	6.6
21	1	0.2	0.1	45.7	14.9	<.001	0.6

**Eight days for* Scenedesmus.

Conclusions

1. Significant THM levels (10 to 950 $\mu g/L$) were produced from both the resuspended cells and the ECPs at various stages of the growth curves of *Anabaena, Pediastrum,* and *Scenedesmus.* The *Anabaena* cells provided as many or more THMs as did the *Anabaena* ECPs. The *Scenedesmus* ECPs yielded more chloroform than the corresponding algal biomass. For *Pediastrum, the* chloroform provided by the ECPs upon chlorination was comparable to the chloroform levels provided by the *Pediastrum* cells. The THM concentrations from all three algal species were comparable with THM yields obtained in other studies evaluating humic and fulvic acids.

2. THM production from the *Anabaena* ECPs increased as the stationary growth phase was approached. The THM production from the *Anabaena* cells also followed this trend.

3. Chloroform production from the *Scenedesmus* cells and ECPs was initially somewhat high, then declined until the late exponential or early stationary growth phase, at

which point maximum chloroform production occurred.

4. The *Pediastrum* cells and ECPs provided two chloroform production peaks during the growth period examined. Both chloroform production peaks occurred during the period of growth considered to be the late exponential or early stationary growth phase.

5. THM production by the chlorination of *Anabaena, Pediastrum,* and *Scenedesmus* cells and ECPs was related to the total organic carbon (TOC) present, the cell count (growth phase), and the chlorine residual after 1 hr of chlorine contact time.

6. The ECP chlorine residuals of all three organisms increased with culture age, indicating a change in type and/or degree of ECPs produced. An alternative explanation is that a chlorine-demanding component of the medium may have been assimilated by the cells.

7. Because cell count is related to THM production and to chlorophyll-a concentration along the growth curve, a relationship between chlorophyll-a and THM production for the algal cells is inferred. The ECPs do not have significant levels of chlorophyll-a, indicating that because THMs are produced from both the cells and the ECPs, more than one THM precursor must be involved.

Recommendations

1. Since this study demonstrated that both algal biomass and metabolites from algae produce high THM concentrations, THM should be reduced by proper choice of the position of chlorination in a water treatment plant. Coagulation and filtration of surface waters should precede chlorination to reduce THM levels in finished waters. Such treatment before chlorination also reduces other THM precursors (organic and humic) that may be present.

2. Algal blooming should be monitored and controlled so that appropriate water treatment procedures can be adopted for the source water conditions. Prechlorination is an advantageous procedure at many water treatment plants and should not arbitrarily be abandoned. Monitoring algal conditions can help minimize THMs and simultaneously help maximize finished water quality through the proper choice of chlorination conditions.

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The complete report, entitled "Alternative Water Disinfection Schemes for Reduced Trihalomethane Formation: Volume II. Algae as Precursors for Trihalomethanes in Chlorinated Drinking Water," (Order No. PB 84-129 006; Cost: $11.50, subject to change) will be available only from:
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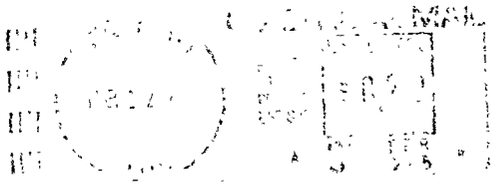

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