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Mixed Algal Population and *Scenedesmus* sp. as Trihalomethane Precursors

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Conventional water treatment involves, in most cases, the use of chlorine for the control of microflora including bacteria and algae. Chlorine reacts with several organic compounds to yield trihalomethanes (THMs) and other non-volatile chlorinated organics, NVCO, (Rook 1976; Morris and Baum 1978).

The presence of such chlorinated organics in drinking water has raised much concern because of their carcinogenic and / or mutagenic properties (USEPA 1983; WHO 1984).

Chlorination of raw surface water, loaded with algae and their extracellular materials, tends to increase the problems of THMs formation in drinking water (Briely et al 1979; Larson and Rockwell 1979; Johnson and Jensen 1986). The present study aimed to evaluate the role of natural mixed algal population in the formation of THMs, NVCO, and to assess the yield of such chlorinated by-products which are formed at various growth phases of the green alga *Scenedesmus sp.*

MATERIALS AND MEHTODS

Mixed algae were isolated from Nile River water samples collected near Cairo. Algae, in an aliquot of 30 L of the river water, were collected using Sedgwick-Rafter net and concentrated by Sedgwick Rafter funnels containing white sand as the filtering medium (APHA 1985). Algae retained by the sand were resuspended in organic-free distilled water and the volume of the algal suspension was made up to 1 L. A suitable aliquot of the algal suspension (100 mL) was treated with chlorine at pH 8.0 and room temperature (25 °C ± 2). Chlorine solution was prepared by bubbling chlorine gas (>98%) through bi-distilled water. The concentration of this stock solution was determined by the iodometric method whereas residues of chlorine were measured by the DPD colorimetric

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method (APHA 1985). Chlorine was dosed to yield a final concentration of 5 mg/L relative to the original volume of water sampled. The concentration of chlorophyll a [Chl (a)] was determined according to the standard procedure given by APHA (1985). Experiments and measurements were run in triplicates and the mean values were recorded.

Formation of THMs and NVCO was assessed at contact times of 1, 2 and 3 hr. Sodium thiosulphate (0.2 g/100 mL) was added by the end of each contact time investigated to terminate the chlorination reaction. Liquid-liquid extraction of THMs and NVCO was run using n-pentane (APHA 1985). An aliquot of 10 mL of the chlorinated samples were carefully introduced into a clean extraction flask containing 2 mL of n-pentane. The flask was stoppered, shaked vigorously for 1 min and let stand for 1 min. The pentane phase was separated and a volume of 3 μL was injected into the GC unit.

Scenedesmus sp. were isolated from the mixed concentrated algal biomass using a dissecting microscope and cultivated in nutrient media recommended for algal bioassay by APHA (1985), until a heavy algal growth was obtained. Algal culture showed that the exponential growth phase was reached after 3 d of incubation whereas the stationary phase was attained after 8 d. Aliquots of 50 mL of the algal cultures representing both growth phases were treated with 5 and 10 mg/L chlorine. THMs and NVCO were determined after each contact time of 1, 2 and 3 hr.

GLC was used for measuring the concentration of THMs and NVCO. Analyses were run using a Perkin Elmer GC, model 8320, equipped with Ni^{63} electron capture detector. The GC was fitted with a conventional stainless steel column (2 m length and 0.3 cm I.D) and a data station. The column was packed with 4% OV-101 and 6% OV-210 on 80/100 chromosorb W. The injector and detector temperatures were 230°C and 250°C, respectively. The column temperature was programmed as follows: isothermal at 60°C for 7 min to elute THMs, temperature was then raised to 180°C, at a rate of 20°C/min. and kept isothermal for 10 min for separation of NVCO. Nitrogen was the carrier gas at a flow rate of 30 mL/min.

Chloroform ($CHCl_3$), dichlorobromomethane ($CHCl_2$ Br), chloro-dibromomethane (CHCl Br_2) and Bromoform ($CHBr_3$) of high purity (>99%, Aldrich Chem. Co) were used for the preparation of standard solutions. The reference compounds were dissolved in n-pentane, and used for the identification and quantification of THMs. Percentage recoveries of the aqueous solutions amounted to 92.9, 88.2, 88.1 and 97.7, respectively. No attempt was made to identify NVCO which were measured in terms of $CHCl_3$ relative to the area given by its standard solution. The detection

limits for chloroform and bromohaloforms were 0.8 and 0.9 μg/L, respectively.

Results of THMs and NVCO were subjected to statistical analysis according to the method of analysis of variance and the value of least significant difference (L.S.D.) was calculated to compare the means (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

Total trihalomethanes (TTHMs), yielded on chlorination of mixed algal population, were found to increase as the contact time was extended to 3 hr (Table 1). The increase in TTHMs values is mostly attributed to the increase in $CHCl_3$ which constituted the dominant THMs species at all contact times investigated. Statistical analysis revealed that differences in $CHCl_3$ values are significant as indicated by the L.S.D. value (Table 1). Variations in $CHCl_2Br$ levels reached on chlorination were also significant at all contact times studied. However, $CHCl_2Br$ only presented 27% up to 29% of the TTHMs yielded on chlorination. $CHClBr_2$ was the minor derivative of THM species (Table 1). Extending the reaction time more than 2 hr has no significant effect on the formation of both $CHCl\ Br_2$ and NVCO. Briely *et al.* (1984) previously reported that on chlorination of several algal species the concentrations of the formed bromine containing THMs were very low. In the present study $CHBr_3$ was not detected.

The concentration of Chl(a) of the mixed algae decreased to 3 μg/L on chlorination and maintained this level at all contact times investigated. Chlorine consumed, however, showed a steady increase as the chlorination reaction was extended. Such results indicate that not only Chl(a), but also other cellular and extracellular products contributed to the increase in THM and NVCO contents. However, Hoehn *et al.* (1978) observed a correlation between Chl(a) and THM concentrations. Rook (1979) reported that chlorine first lyses algal cells followed by the production of THM precursors and intermediates. This may account for the progressive increase in THMs as the contact time was extended (Table 1).

Results given in Table 2 revealed that chlorine consumed by algal culture during the exponential phase was relatively higher, at a contact time of 3 hr and in presence of 5 mg/L chlorine. However, by increasing the applied chlorine dose to 10 mg/L, the values of chlorine consumed were higher for algae at the stationary growth phase.

The level of TTHMs and NVCO attained on chlorination of *Scenedesmus* culture, differed according to the dose of chlorine, contact time and

Table 1. Algae as precursors of THMs and NVCO.

Contact time, hr	Chl(a) μg/L	residual* Cl_2, mg/L	Concentration μg/L $CHCl_3$	$CHCl_2Br$	$CHClBr_2$	TTHMs	NVCO
0	27.0	5.00					
1	3.1	3.14	7.98 **(52.5%)	4.34 (28.6%)	2.85 (18.8%)	15.20	17.9
2	3.0	2.64	10.99 (52.5%)	6.16 (28.6%)	3.89 (18.8%)	21.03	20.8
3	2.98	2.50	22.73 (60.6%)	10.99 (29.3%)	3.81 (10.1%)	37.52	20.5
L.S.D. (5%)			1.47	0.33	0.56		0.88

* pH 8, Temperature 25°C ** (%)of THMs species.

Table 2. THMs and NVCO formation on chlorination of *Scenedesmus* algal cultures*.

Chlorine dose mg/L	contact time, hr	Chlorine consumed		TTHMs μg/L		NVCO μg/L	
		Expon. Phase	Station. Phase	Expon. Phase	Station. Phase	Expon. Phase	Station. Phase
5	1	1.44	2.58	8.02	5.41	2.10	1.68
	2	2.84	2.80	11.82	6.39	1.80	1.73
	3	3.76	2.90	16.43	7.79	2.34	1.83
10	1	1.00	5.40	16.89	18.81	7.94	9.78
	2	2.00	9.46	24.38	26.72	11.81	10.04
	3	3.00	9.70	28.27	51.61	13.54	12.46

*Chl (a) at exponential and stationary phases was 360 μg/L and 1260 μg/L, respectively.

growth phase (Table 2). In presence of the low chlorine dose, the yield of THMs and NVCO reached higher levels during the exponential growth phase. Hoehn *et al.* (1980) reported that the exponential growth phase of four algal species yielded greater amounts of THM precursors. Such a trend tends to explain the increase in chlorine consumed by algal cells at this growth phase. As the chlorine dose was increased to 10 mg/L, a corresponding increase in THMs yielded by algae, at both growth phases, was recorded. Maximum formation of THMs and NVCO was reached as chlorination was extended to 3 hr (Table 2). Statistical analysis of THMs and NVCO data is given in Table 3. Differences between the mean values of TTHMs yielded at the two growth phases were significant. Variations in TTHMs levels attained by the application of different chlorine doses were also significant. NVCO values given by the two chlorine treatments were significantly different as well as the NVCO attained at both growth phases in presence of 10 mL/L chlorine. The high level of THMs yielded by algal culture of the stationary phase, in

Table 3. L.S.D. values for THMs and NVCO yielded after 3 hr of chlorination.

Parameter	TTHMs μg/L		NVCO μg/L	
	Expon.phase	Station.phase	Expon.phase	Station.phase
Dose of Cl_2 5 mg/L	16.43	7.79	2.34	1.83
Dose of Cl_2 10 mg/L	28.27	51.61	13.54	12.46
L.S.D. (5%) for dose	1.65		0.62	
L.S.D. (5%) for growth phase	1.65		0.62	

presence of the high chlorine dose, may be attributed to the presence of chemically different metabolic products which yielded to chlorination by increasing the contact time.

Eutrophic water supplies are expected to contain different algal species at various growth phases. Consequently, they will yield significant amounts of THMs and NVCO on chlorination at water treatment plants. Removal of algal biomass by coagulation-flocculation operations will leave behind most of the extra-cellular products that act as THMs precursors (Hoehn *et al.* 1984).

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