TRIHALOMETHANE FORMATION POTENTIAL OF ALGAL EXTRACELLULAR PRODUCTS AND BIOMASS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Since 1974, hazardous organic chemicals in public drinking water supplies have received much attention. Foremost among the organic compounds of interest have been the trihalomethanes (THM), one of which is chloroform. After the discovery by Rook (1) that the trihalomethanes are produced by the chlorination process and are present in drinking water, research concerning various aspects of the problem, including identification of the organic precursors in the haloform reaction, has greatly accelerated. Rook postulated the natural humic substances as the most likely trihalomethane precursors. Recent discoveries, to be discussed later, have shown other naturally ocurring substances also may be important as precursors.

Algae are known to liberate a large number of organic compounds into the surrounding aquatic environment during normal growth (2). As algae are normal inhabitants of surface water supplies, it was postulated that they are contributors of THM precursors .

The objectives of this research were to determine, under laboratory conditions, whether species of the blue-green algae and green algae could contribute THM precursors, either in the form of extracellular products (ECP) or biomass, and, if so, whether there were variations in the extent of precursor formation during the various stages of the growth cycle (i.e. exponential, stationary, and death phases). Supplemental studies were conducted to determine whether one or more of 19 organic compounds selected from a list of those mentioned in the literature as known algal ECP's, could be identified as high yield THM-

precursors. Solutions of these were chlorinated after adjustment to pH 7.5 and, in some cases, pH 9.3, both pH values being representative of most natural and treated waters. A few additional studies were conducted to determine whether sediments containing algal remains and other organics from a highly eutrophic reservoir would yield THM's upon chlorination. In all studies, the precursor concentrations were measured in terms of the total organic carbon (TOC) content of the various solutions and suspensions.

II. LITERATURE REVIEW

This section includes a general review of the available literature concerning 1) trihalomethanes and 2) algal extracellular products as potential precursors in the haloform reaction. Also included is a brief discussion of synthesis and release of extracellular products, as these functions may be significant in the occurrence and type of product found, both in algal culture, and in the aquatic environment where algae are growing prolifically. The literature review has been divided into two major topical areas: "Trihalomethanes" and "Algae and Their Metabolites".

TRIHALOMETHANES

Background Information

Early work: The first major publication concerning the presence of the trihalomethanes (compounds of methane with three hydrogen atoms replaced by halogen atoms) in drinking water was released by Rook (1) of The Netherlands in 1974. He found that trihalomethanes, especially chloroform, were formed by the chlorination of natural waters. Similar results were reported by Bellar et al. (3) in the United States, who found that the concentration of chloroform increased significantly as water passed through the water treatment process and that the chlorination of surface waters yielded higher concentrations than the chlorination of ground waters. The authors of both reports concluded that the formation of the trihalomethanes (THM's) was the result of the chlorination process.

About the same time, the Environmental Protection Agency (EPA)

released its report (4) on the presence of organic compounds in the water supply of the City of New Orleans and a possible connection with the high incidence of cancer in that city. This report and those by Rook and Bellar et al. received immediate attention because chloroform was, at that time, suspected of being carcinogenic (5). EPA announced its plan for a nationwide survey to determine the extent of the problem.

Legislation and national surveys. In December 1974, the Safe Water Drinking Act (PL93-523) was passed by Congress. Section 1442(a)(9) of PL93-523 directed the EPA administrator to "conduct a comprehensive study of public water supplies and drinking water sources to determine the nature, extent, sources of, and means of control of contamination by chemicals or other substances suspected of being carcinogenic".

On December 18, 1974, EPA initiated the National Organics Reconnaissance Survey (NORS) of 80 cities whose water supplies represented a variety of sources and treatment processes (6). The results of this survey confirmed the earlier reports that THM's are formed by chlorination and showed the problem to be widespread. Partial results of this survey are given in Tables 1 and 2 where it can be seen that chloroform was found in the highest concentration. The highest levels of the THM's were found in cities using surface waters as the water source. Similar data were found in another study of 83 cities conducted by Region V of the EPA (8). More recently, EPA completed the National Organics Monitoring Survey (NOMS), a year long survey in 113 cities of 20 organic chemicals, including the four trihalomethanes (9)

In June 1977, EPA's Interim Primary Drinking Water Regulations (10) became effective. This document did not include Maximum Contaminant

TABLE 1. Raw Water Analysis from the National Organics Reconnaissance Survey (After EPA, ref. 7)

Compound	Number of Locations Detected	Range of Concentrations (ppb)
None Detected	30	-
Chloroform	45	0.1-0.9*
Bromodichloromethane	6	0.2-0.8*
Dibromochloromethane	0	*
Bromoform	0	-
1,2-Dichloroethane	11	0.2-3
Carbon Tetrachloride	4	2-4

*One additional location received raw water prechlorinated by a nearby industry. This water contained 16 ppb of chloroform, 11 ppb bromodichloromethane, and 3 ppb dibromochloromethane.

TABLE 2. Finished Water Analysis from the National Organics Reconnaissance Survey (After EPA, ref. 7).

Compound	Number of Locations Detected	Range of Concentrations (ppb)
Chloroform	79	0.1-311
Bromodichloromethane	76	1.8-116
Dibromochloromethane	70	0.4-100
Bromoform	25	1.0-92
Carbon Tetrachloride	10	2.0-3
1,2-Dichloroethane	26	0.2-6

Levels (MCL's) for most organic chemicals including chloroform because of inadequate information on the health effects and suitable treatment procedures (11). However, the revised Primary Drinking Water Regulations, scheduled to become effective in March 1979, are to contain either MCL's for THM's or to require suitable treatment techniques to remove them or prevent their formation. The revised regulations will be based on the recommendations of the National Academy of Sciences (NAS) scientific review panel that recently completed a study for EPA on the health effects of suspected toxic substances (12). The NAS recommendations list chloroform as an animal carcinogen but point out that "effects in animals, properly qualified, are applicable to man." Regulations for MCL's are now being drafted by EPA, and a standard of 100 ppb total trihalomethanes for communities of greater than 75,000 is being proposed (13).

The Haloform Reaction

Organic compounds can undergo several types of reactions with hypohalous acids one of which the classical haloform reaction, is generally accepted as being the reaction involved in the formation of trihalomethanes during potable water treatment (14, 15, 16). Morris (14) described the haloform reaction as one "which occurs generally in alkaline, aqueous solution with organic compounds containing the acetyl group (CH_3-$\overset{O}{\overset{\|}{C}}$-) or with structures such as (CH_3CHOH-) that may be oxidized to the acetyl group." The overall reactions may be written (14):

$$CH_3COR + 3HOX \longrightarrow CX_3COR + 3H_2O \qquad [1]$$

$$CX_3COR + H_2O \longrightarrow CHX_3 + RCOOH \qquad [2]$$

The reaction involves initial dissociation of a hydrogen and addition of the positive halogen to the resulting carbanion. Dissociation and addition are repeated until the methyl group is fully halogenated, which is then displaced by nucleophlic base attack and adds a hydrogen to yield the trihalomethane. The entire mechanism is written (14):

$$RCOCH_3 \longrightarrow RC(O^-)=CH_2 + H^+ \qquad [3]$$

$$RC(O^-)=CH_2 + HOX \longrightarrow RCOCH_2X + OH^- \qquad [4]$$

$$RCOCH_2X \longrightarrow RC(O^-)=CHX + H^+ \qquad [5]$$

$$RC(O^-)=CHX + HOX \longrightarrow RCOCHX_2 + OH^- \qquad [6]$$

$$RCOCHX_2 \longrightarrow R\text{-}C(O^-)=CX_2 + H^+ \qquad [7]$$

$$RC(O^-)=CX_2 + HOX \longrightarrow RCOCX_3 + OH^- \qquad [8]$$

$$RCOCX_3 + OH^- \longrightarrow RCOOH + CX_3^- \qquad [9]$$

$$CX_3^- + H^+ \longrightarrow CHX_3 \qquad [10]$$

The initial enolization (reaction 3) is the rate-limiting step so that the entire sequence occurs at the same rate as the enol formation.

When hypochlorous acid (HOCl) is involved, the product will be chloroform. However, in most surveys and experiments using natural waters, bromoform and mixed bromo- and chloro- substituted methanes were found. Room (1) explained that the brominated species resulted from the formation of hypobromous acid (HOBr) from the oxidation of any bromide ions

(Br^-) in the water by HOCl and subsequent reactions of HOBr with the organic material.

In the presence of HOCl and HOBr, the relative concentrations of the chlorinated and brominated species will depend upon the rate at which each adds to the methyl group, as well as upon the relative concentration of each acid. Carbon tetrachloride (CCl_4) is not a product of the haloform reaction (16) and its presence is most likely of industrial origin (17).

Effects of the reactant concentrations in the haloform reaction: According to the generalized chemical equation:

$$[A]+[B] \longrightarrow [C]+[D] \qquad [11]$$

the concentration of organic precursor and chlorine (A and B) will determine the amount of chloroform produced. The importance of the precursor concentration has been demonstrated by several investigators (3, 6, 16). Symons et al. (6) concluded that the organic level of the source water is probably the major factor affecting the trihalomethane concentration if the chlorine is not exhausted.

A fairly linear relationship between total organic carbon (TOC) and trihalomethane concentration has been reported (5, 15), and Rook (16) found the relationship to be linear up to 250 mg/l TOC (Fig. 1). However, Hoehn et al. (18), in a study of seasonal variations in THM concentrations, could find no direct correlation between THM concentration and the concentration of either TOC or carbon chloroform extract (CCE). They suggested that precursor type may be more significant than precursor concentration.

The TOC concentration may not be a valid indication of haloform

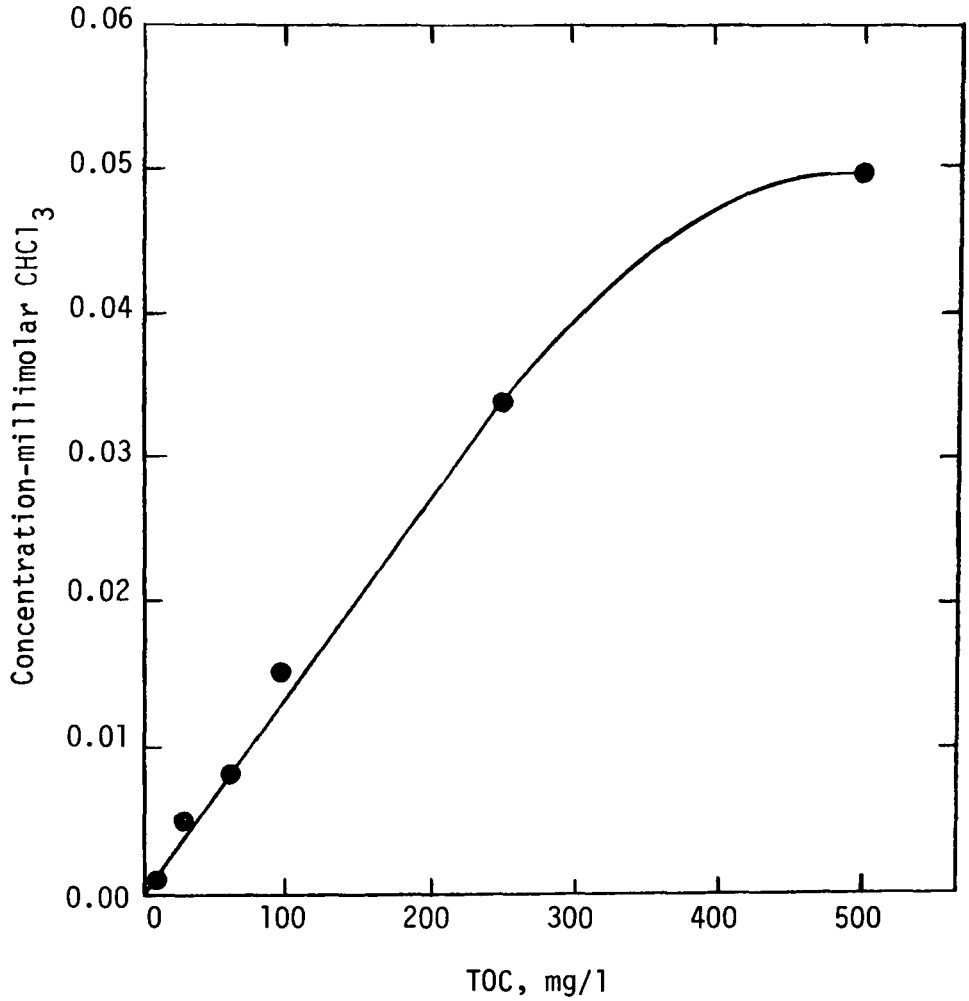


FIGURE 1. Haloform reaction with increasing fulvic acid precursor (expressed as TOC); chlorine concentration 885 mg/l, 10°C, 4 hr. (after Rook, ref. 16).

precursor concentrations because many waters contain a TOC fraction that does not enter the haloform reaction. Hoehn et al. (18) found that while the TOC concentration varied only slightly in a water supply over a one year period, the THM concentration showed considerable variation.

As yet a truly reliable method of measuring precursor concentration has not been found. Symons et al. (6) evaluated alternative measurements, including UV absorption, emission fluorescence scan (EmFS) and rapid fluorometric method (RFM), but could find no correlation between these parameters and non-volatile TOC. The CCE method has also been proposed as a possible measurement parameter (19). Symons et al. (6) found some correlation between CCE and non-volatile TOC (NVTOC) but concluded that NVTOC was the more useful of the two in estimating the haloform precursor concentration.

The concentration of chloroform produced can be increased by increasing the chlorine dose, but the relationship does not appear to be linear (6, 16, 20). Rook (16) demonstrated that even small doses of chlorine (0.5 mg/l) could yield measureable amounts of chloroform. Goode (21) found a better correlation between chloroform and chlorine concentrations than with between chloroform and precursor concentration. Chloride demand or chlorine consumed may be more significant than chlorine dose. Good correlations between chloroform concentration and chlorine demand have been demonstrated (15, 17, 19). Morris and Baum (15) reported that chlorine demand can be used in determining the number of carbon-chlorine bonds produced and concluded that the total chlorinated bond level may be more important when assessing the health risk than the total trihalomethane concentration. Chlorine demand is useful in deter-

mining the formation of chlorinated intermediates, which will utlimately yield chloroform (15, 20).

It should be noted that chlorine must be in the form of free chlorine for chloroform production. Combined chlorine (as chloramines) or chlorine dioxide do not react with organic material to any significant degree to produce chloroform (22, 23, 24).

Effects of contact time: The haloform reaction has been shown to be time dependent (16, 22). There is a rapid formation of chloroform from the moment of contact and most of the chlorine is consumed within a few hours (16, 22, 25). However, the reaction will continue at a slower rate for up to 24 hours or more (20, 22, 25). The significance of this continuing reaction, even though at a slow rate, is that the reaction can continue to occur in the distribution system, giving the consumer a higher concentration than is measured leaving the treatment plant (26, 27, 28). Harms and Looyenga (26) actually observed this phenomenon in the distribution system of Huron, South Dakota. A good discussion of the various THM concentrations that can be measured in the distribution system and the significance of each is given by Stevens and Symons (27).

Effects of pH and temperature: In general, the rate of the haloform reaction can be increased by increasing pH (15, 16, 25). Rook (16) explained that increasing the pH enhances enolization, which produces more reactive sites on the precursor, but Stevens et al. (25) suggested that increasing the pH also may cause many low molecular weight compounds that are reactive only at higher pH's to enter the haloform reaction.

Morris and Baum (15) demonstrated that the chlorine demand was less at higher pH's but the yields of chloroform were approximately the same

as were observed when the reaction was conducted at lower pH's. The only exception was in the case of ploracetophenone, which Morris and Baum found to be more reactive at pH 7 than at pH 11.

Stevens et al. (25) pointed out that the effect of pH is of special importance to treatment plants that practice lime softening. Harms and Looyenga (26) were able to reduce the chloroform concentrations 75 per cent merely by moving the point of chlorination to the recarbonation basin. However, Morris and Baum (15) suggested that the point of chlorination is not important, that the chlorination of organic compounds proceeds at low as well as high pH values, and that only the final hydrolysis of the trihalogenated hydrocarbon requires an elevated pH.

As with most chemical reactions, the haloform reaction proceeds more quickly at higher temperatures (16, 25). Stevens et al. (25) suggested that this may be significant in seasonal fluctuations of THM levels, but Hoehn and Randall (29) in a two year study found that the THM concentrations were not consistently higher in warm weather and concluded that seasonal variations are determined by a variety of factors. The real impact of temperature in affecting haloform concentrations in finished drinking water lies in the fact that the reaction rate will be slower in colder water and less haloform will be produced before the consumer drinks the water in winter than in summer, provided the time in the distribution system is less than approximately 24 hours (29).

Precursor Compounds

Rook (1) was the first to postulate that the naturally occurring humic substances in surface waters were precursors for THM's. He demon-

strated that the chlorination of peat extracts and the waters taken from the peaty region of a lake caused the formation of THM's. In subsequent work (16), Rook showed that raw water did not lose the potential for THM formation even after it was stored for several days, a fact that supports the theory that there exists a biologically resistant precursor of THM's. He also demonstrated that chloroform could be produced from compounds like 1,3-cyclohexandione, resorcinol, and other dihydroxy aromatic compounds that may be constituents of the humates.

The humic substances,-- the humic acid, fulvic acid, and humins-- are now accepted as major THM precursors. Direct relationships between the concentration of fulvic and humic acid and THM concentrations have been demonstrated experimentally, and the THM yields from these substances reflect those found upon chlorination of natural waters (16, 20 25).

Particulate matter contributes significantly to THM formation (25). A relationship between turbidity and chloroform concentrations has been reported (30) and removal of particulates, by settling or filtration prior to chlorination repeatedly has been proven to reduce the concentration (6, 21, 25, 31). Stevens et al. (25) explained that reduced THM's in clarified water may be due to the elimination of the particulates themselves or to some soluble component that is associated with the particulate, perhaps by adsorption. They suggested that the humic substances are more likely to be associated with particulates than the more simple precursor compounds such as the methyl ketones.

Although the humic compounds are known to be major THM precursors, Stevens et al. (25) pointed out that the precursors are probably found in a mixture of humic substances and simpler compounds that contain the

acetyl group. Morris and Baum (15) indicated that chemical structures that can react as ketones have the potential for haloform formation, and they listed some structures that form carbanions (Figure 2). Furthermore, they demonstrated experimentally that representatives of the acetogen family and compounds containing the pyrrole ring, which are widely distributed in nature (e.g. chlorophyll), do produce chloroform upon chlorination. These compounds and the corresponding yields are given in Table 3. All the compounds listed produced chloroform under some conditions.

Hoehn et al. (17) found a definite correlation between THM concentration and chlorophyll-a concentrations in reservoir water (Figure 3). This finding was corroborated by Morris and Baum's (15) report that chloroform could be produced by the chlorination of chlorophyll (Table 3). The authors of both reports suggested the importance of algal biomass and extracellular product as potential THM precursor material in drinking water supplies.

ALGAE AND THEIR METABOLITES

Importance in Water Supplies and General Growth Characteristics

Algae in water supplies: Algae are common to all waters exposed to sunlight and are known to be troublesome organisms in drinking water supplies. The most common problems associated with them are taste-and-odor production and clogging of filters. However, they also are known to alter alkalinity, color, pH, and turbidity and have been associated with fish kills by significantly lowering the oxygen content of the water at night (32, 33). Algal biomass and their extracellular products

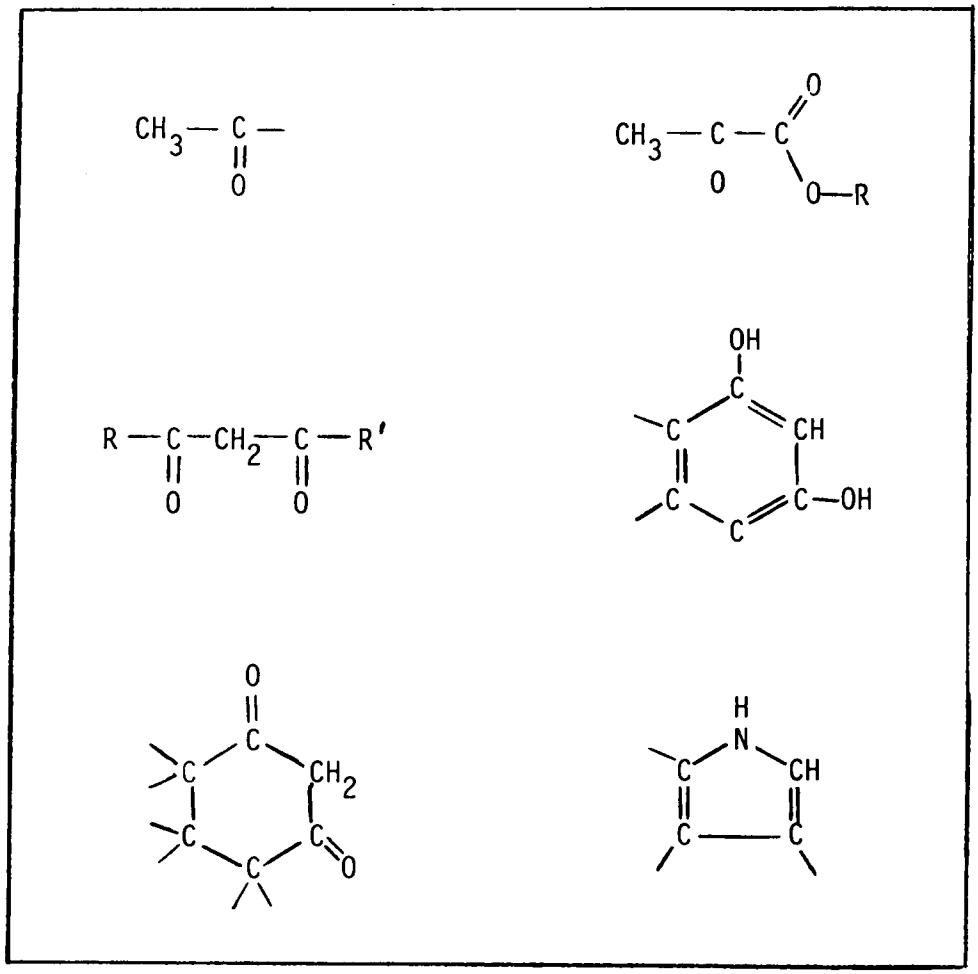


FIGURE 2. Structures that form carbanions (after Morris and Baum, ref. 15).

TABLE 3. Some Potential Precursors of Chloroform in Water Chlorination

(after Morris and Baum, ref. 15)

Compound	Theor Molar D. R.	Molar Ratio Used	Obs. Molar D. R.	pH	Time (hours)	% Molar Yield, $CHCl_3$
Orcinol	15	18	17	7.2, 9.2	25	38, 70
Ferulic Acid	20	24	23	9.9	25	58
Phloroacetophenone	15	9	8	7	4	50
		18	17	6, 9, 11	48	96-100
Vanillin	16	28	12	9.2	24	22
		19	18	10	48	42
O-Vanillin	16	22	-	5.6-11	62	30
Springaldehyde	18	18	8.6	9.2	24	22
		28	26	9.2	60	17
Pyrrole	9.5	13.6	12	10	24	30
Tryptophan	25	20	13	7.5	7	18
		25.2	25	6-11	47	100
Proline	11.5	16.5	15	6.6-11	25	30
Hydroxyproline	10.5	28	26	6.0-10	170	110
Uracil	7	8.2	8.2	6.5-11	25	100
Chlorophyll	-	23 (by wt.)	20 (by wt.)	9.2	100	15.5 (by wt.)

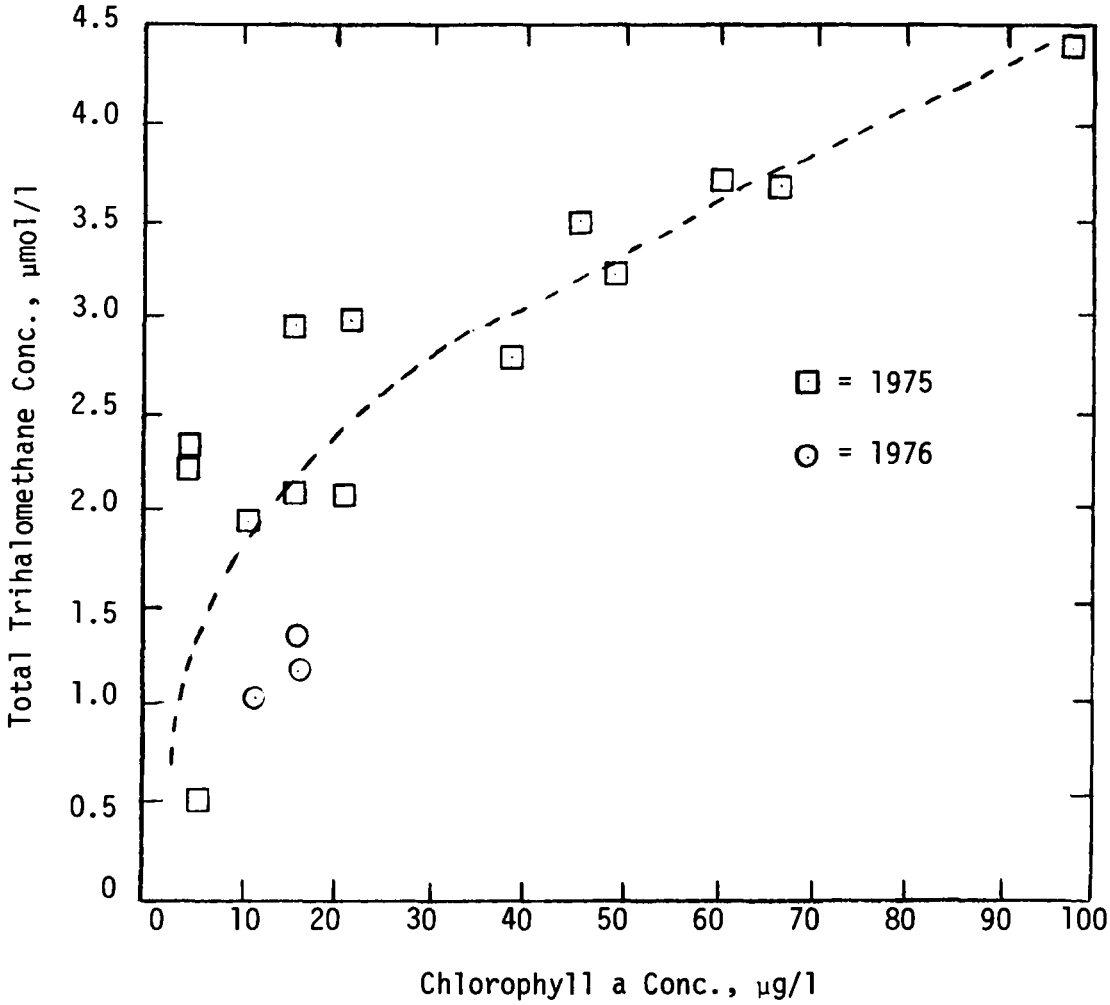


FIGURE 3. Variations in finished-water trihalomethane concentrations with chlorophyll-a concentrations in the Occoquan Reservoir (after Hoehn et. al, ref. 17).

(ECP) have even been implicated in contributing to the eutrophication of waters by increasing the organic content (34, 35). These will be discussed in more detail in a subsequent section.

The blue-green algae have received considerable attention as the major nuisance algae. They proliferate in waters where conditions are often unfavorable for most other types. They most often are associated with taste and odor problems, although some species of the green algae and diatoms also are known to produce malodorous substances (32, 33, 34). The blue-green algae also are of importance because some species produce substances that are toxic to animals (34).

Nutritive requirements: Obviously it would be impractical here to review in detail the specific nutritive requirements of all the major types of algae; however, a few general comments can be made. Algal growth can be supported by a medium containing inorganic salts including the macronutrients and a few trace elements. In addition to carbon, hydrogen, and oxygen, the major nutritional requirements include nitrogen, phosphorus, sodium, potassium, magnesium, calcium, and sulfur (33, 36, 37, 38). The diatoms also require silicon (37). The major source of carbon is carbon dioxide, but many species can utilize organic carbon as a supplementary source and some species of the blue-green and green algae are capable of heterotrophic growth in diminished light or in the dark (35, 36, 39).

Algal growth cycles: Normal growth of algae consists of the following phases: lag, exponential (log), stationary, and death. The duration of these phases is different for the different algae and the general conditions under which they are grown (36, 37).

Many algae, especially the blue-greens, are able to tolerate a relatively wide variation in temperature. Most are found in neutral to alkaline waters (36, 37).

Extracellular Products of Algae

Healthy, actively growing algal cultures are known to release metabolic products into the surrounding environment (2, 40, 41, 42). Up to 50 percent (or more if conditions are favorable) of the carbon fixed by a population can be released (43).

Fogg (40) described ECP as a "soluble substance liberated from intact living cells and excluding soluble substances set free on injury or by decomposition of dead cells". He defined two types of ECP (2, 40): Type I products are "metabolic intermediates, usually of low molecular weight, for which there is quasi-equilibrium between intra- and extracellular concentrations, so that the amount liberated varies according to metabolic activity, the presence of consumers, and other factors," and type II are "end products of metabolism, usually of high molecular weight, the liberation of which does not depend on an equilibrium and which are more or less proportional to the amount of growth".

Glycolic acid and the glycolate pathway: Glycolic acid ($CH_2OHCOOH$) is the algal extracellular metabolite that has been studied most extensively. It is regarded as a type I compound (2). Most algae, both freshwater and marine species, excrete at least some glycolate (43, 44). It is now recognized to be a major photosynthetic product (2, 43, 45) and has been isolated from natural waters (46).

Although the biochemical origin of glycolic acid is uncertain, it

is generally accepted as being derived from a two-carbon fragment produced by oxidative cleavage of ribulose-1,5-diphosphate, the CO_2 acceptor in the Calvin-Benson carbon fixation cycle (47, 48, 49, 50). The glycolate pathway, as it is presently accepted, is given in Figure 4 (49). Synthesis appears to be dependent on the availability of light and oxygen, and it can be synthesized in the absence of inorganic carbon (51).

In many algal species, glycolic acid may be further metabolized to glyoxylic acid, then to glycine and serine that eventually enter the tricarboxylic acid cycle (49, 52, 53). However, some species of algae appear to be incapable of further metabolism and liberate all that is synthesized (52). Hess and Tolbert (52) suggested that glycolate is released by algae that lack the enzyme required for further metabolism. Other evidence indicates Hess and Tolbert's hypothesis does not always hold true, as some strains of Chlorella pyrenoidosa, which are known to liberate glycolate, have also been shown to reassimilate it (35, 53, 54).

The amount of glycolic acid that is released appears to be controlled by the relative intracellular and extracellular concentrations (2, 37). Although excretion occurs throughout the growth cycle, maximum excretion occurs during the early stages of growth (47, 55, 56, 57), possibly to establish an equilibrium with the external concentration (2, 37).

The cell membranes of some species of the green algae (2, 37) and the blue-green algae (53) appear to be permeable to glycolic acid, and it can be reabsorbed if the culture is CO_2 limited (58).

Other algae-produced organic acids and volatile substances: Healthy

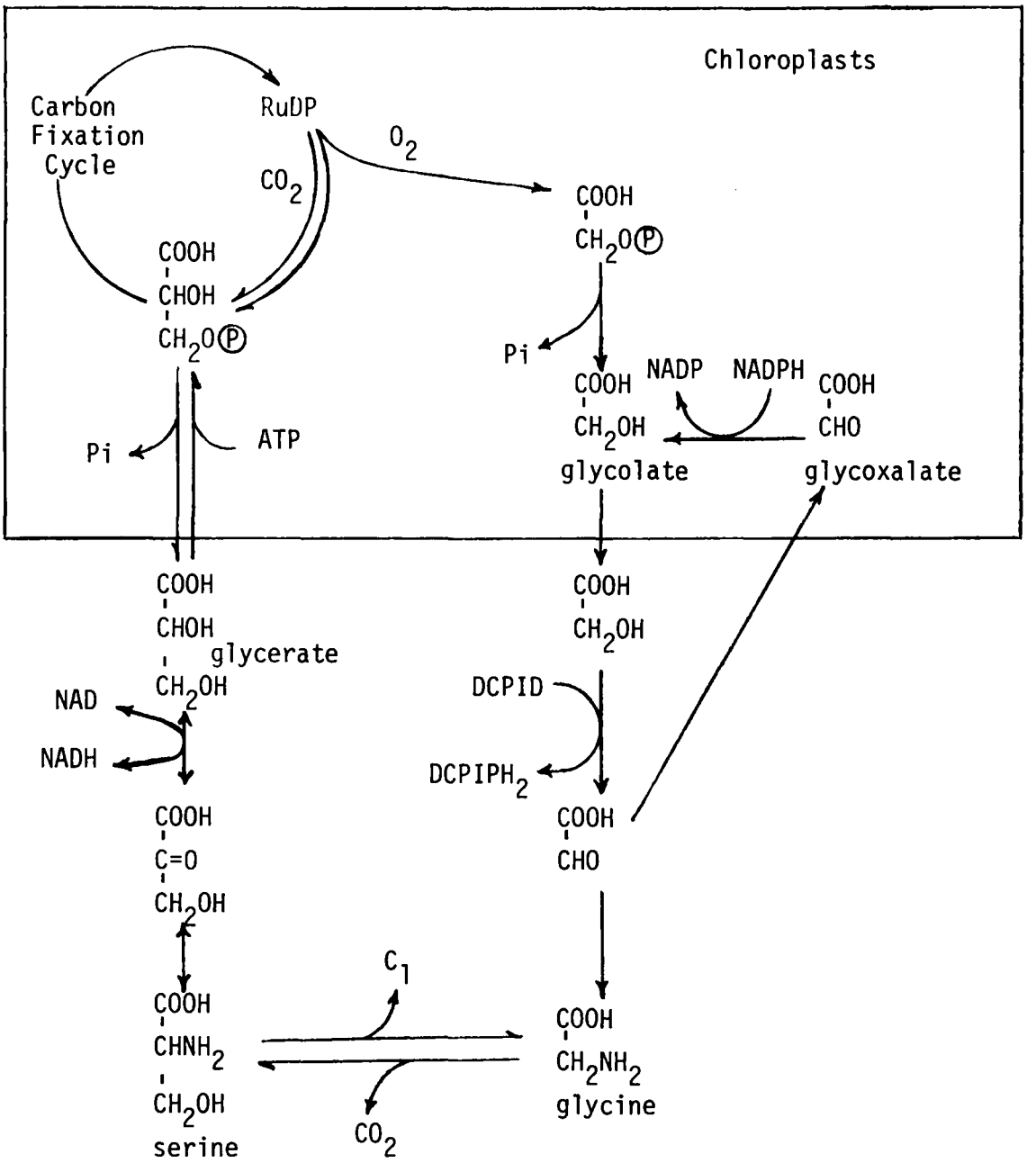


FIGURE 4. The glycolate pathway (after Tolbert, ref. 49). RUDP, ribulose - 1,5 diphosphate; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide dinucleotide phosphate, DCPIP, dichlorophenol-indophenol.

cultures of algae are known to excrete organics acids other than glycolate. Formic, acetic, lactic, pyruvic, a-ketoglutaric, and acetoacetic acids are reported to be liberated by strains of C. pyrenoidosa, and glyoxylic acid was found in filtrates of C. vulgaris (56). Chang and Tolbert (59) found dividing cells of Ankistrodesmis sp. to liberate mesotartrate and isocitrate lactone. Badour and Waygood (60) reported mesoxalic acid semialdehyde to be a product of Gleomonas sp.

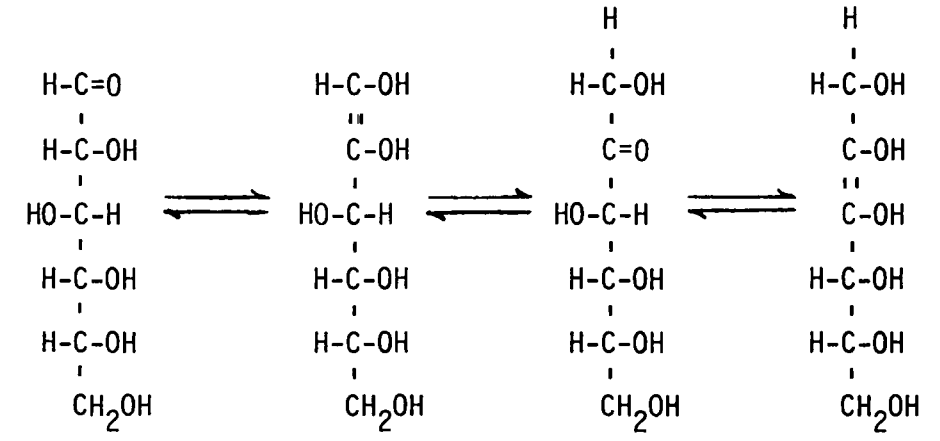
Algae liberate volatile substances, many of which are associated with taste-and-odor problems. The major volatile substances that have been reported are formaldehyde, acetaldehyde, methyl ethyl ketone (61, 62), furfuraldehyde, ethyl alcohol and acetone (63). While these single substances do not produce the typical noxious odors variously described as earthy, woody, musty, etc., they are volatile none-the-less and may contribute to off-flavors of drinking water. One compound, geosmin, has been identified as the compound producing the earthy odor in drinking water during algal blooms (64, 65, 66). Acetone, acetaldehyde, and ketones have been shown to be highly reactive in the haloform reaction (25). It is likely that many of the organic acids and other volatile compounds have the potential to form haloforms.

Carbohydrates: Simple sugars and polysaccharides are major end-products of metabolism and are liberated by a large number of algae (67, 68, 69, 70). Extracellular carbohydrates comprise as much as 40 percent of the total carbohydrate synthesized and generally account for 20-30 percent of the total carbon excreted by the cell (69, 70). Moore and Tisher (69, 70) reported glucose, arabinose, fucose, glucuronic acid, ribose, and xylose to be major constituents of the extracellular

polysaccharide fraction of Anabaena flos-aquae. Galactose, rhamnose and mannose also have been found to be produced by species of the green algae (44) and diatoms (71, 72).

The greatest synthesis of polysaccharide material occurs during the late log phase of growth (70), and extracellular polysaccharides are found more often in old cultures where growth is slow or has ceased (41). Much of the extracellular polysaccharide is associated with mucilage material (68) or mucilagenous stalks (72, 73).

Monosaccharides and carbohydrates containing a free sugar group tautomerize and form an enol salt (called enediol) under alkaline conditions (74). Under weakly alkaline conditions 1-2 enediols are formed and under stronger alkali 2-3 and 3-4 enediols can also be formed. The formation of 1-2 enediols and 2-3 enediols can be written as follows (74):



The enediols often are unstable and break at the double bond, forming a mixture of smaller molecular weight compounds such as formaldehyde glycolic aldehyde, and glyceric aldehyde, all of which are capable of

further enolization. The possibility of the extracellular carbohydrate fraction providing trihalomethane precursors cannot be ignored.

Nitrogenous compounds: Large quantities of nitrogenous substances often are liberated by the blue-green algae (75, 76). However, other types of algae also release some (44). Fogg and Westlake (75) reported that from 5 to 50 percent of the nitrogen assimilated by Anabaena cylindrica is excreted in polypeptide form. It appears that the nitrogenous compounds excreted by the blue-greens are not those associated with nitrogen fixation (2). Liberation of nitrogenous substances tends to follow growth and to increase with culture age (76, 77). Among the amino acids which have been isolated from the culture medium of the blue-green algae are leucine, serine, alanine, aspartic acid, and valine (76, 77).

Morris and Baum (15) demonstrated that the amino acids tryptophan, proline, and hydroxyproline had the potential for haloform formation (Table 3). Other nitrogenous compounds probably act as precursors in the haloform reaction.

Other compounds: A variety of other compounds have been found in the medium of growing algal cultures. Aaronson et al. (77) reported a large number of vitamins, including biotin, ascorbate, B_6, B_{12}, nicotinate, riboflavin and thiamine to be liberated by Ochromonas danica. Reports of lipids (77), protoporphyrin IX (78), enzymes (73), growth stimulatory and inhibitory substances (71, 79, 80), and toxins (2, 42) can be found in the literature.

Characteristics of ECP release in algae cultures: In general, early growth is characterized by excretion of low molecular weight

compounds, especially glycolic acid (43, 46, 57). As the culture ages, excretion of the larger molecular weight compounds becomes more pronounced (57). The total amount of excreted carbon in the medium increases with growth and is related to the amount of carbon fixed (81, 82, 83). However the percent release per cell is greater during early growth (42, 43, 47).

Excretion is favored by conditions which inhibit photosynthesis (45). In culture, maximum excretion occurs at light intensities sufficiently high to inhibit photosynthesis (2, 40, 43). Release may also be increased by low light intensities and by total darkness, but not to the extent as is observed during periods of very high light intensity (2, 42, 43).

The concentrations of CO_2 and O_2 in air have been shown to markedly influence algal excretion (84, 85, 86). Miller (85) cultured algae and supplied CO_2 by bubbling air with varying percentages of CO_2 and O_2 and observed the changes in ECP release. He found that an O_2 concentration of at least 17 percent was required for release and release was inhibited by a CO_2 concentration of greater than 1.5 percent.

The amount of release per cell is known to be inversely related to population density (2, 42, 83) and is favored by high pH (87) and inorganic nutrient deficiency (43, 50).

Environmental characteristics: Algae tend to liberate more organic material <u>in situ</u> than in laboratory culture (44, 58, 67). Conditions such as high O_2-low CO_2 concentrations, inhibiting light intensities, and low population densities, all of which favor release, are often found in natural waters.

Release in the aquatic environment becomes proportionally less as biomass and primary productivity increase (2, 50). Anderson and Zeutschel (88) reported the release by marine phytoplankton to range from one percent for the most eutrophic waters to 49 percent for oligotrophic waters. Berman (83) found the amount of release per cell of freshwater phytoplankton to be minimum at the depth of maximum productivity and to increase at the surface or at depth.

Variation of ECP release with depth have been reported by several investigators and are probably due to a variety of interacting factors (2, 42, 83). As much as 95 percent of the carbon fixed may be released at the surface on very sunny days when the extent of release at lower depths is 10 percent or less (42, 43, 47). Choi (89) reported that release by marine phytoplankton increases with depth; however, Wallen and Geen (90) found a decrease in the percent release at depth. Wallen and Geen also reported a shift in the type of extracellular product, from low molecular weight compounds to proteins and polysaccharides, and they concluded that the variations are due to changes in the spectral quality of light with depth in the body of water.

Utilization of organics by algae and bacteria: The organics liberated by algae can serve as nutrients for algae and bacteria. Many species of the blue-green and green algae are capable of photoassimilation of exogenous organic carbon (36, 42, 91, 92), although these nutrients usually cannot serve as the sole carbon source (36, 39). Assimilation of organic compounds by algae is usually increased when low light intensities prevail (35, 36, 39) and often occurs more readily in the dark than in the light (93).

Heterotrophic growth of bacteria can be supported by the extracellular metabolites of algae (94, 95, 96, 97). Bauld and Brock (96) reported that up to 9 percent of the carbon liberated by algae is assimilated by bacteria in natural systems. Organic acids and free sugars can be metabolized by bacteria to produce larger molecular weight compounds (57, 94). Belly et al. (95) also found the growth of bacteria and fungi to be supported by compounds released from dead algal cells.

Uptake of glycolate in lakes was found to be highest in the epilimnion during the summer and to decrease with depth (2) and a correlation exists between algal excretion and rates of glycolate uptake by bacteria.

In natural environments, it appears that most types of algae compete poorly with bacteria for organic compounds. However, the blue-green algae have been found to be competitive for the uptake of glycolate (53).

It should be mentioned that several organic compounds are produced by microbial decomposition of algal cells. Cranwell (98) found n-alkanes, alkenes, normal and branched cyclic alkanoic acids and alkenoic and hydroxy acids in the sediments as products of microbial decay of the algae Ceratium hirundinella.

It can be seen that much is known about the individual subjects of haloform production during drinking water treatment and of carbon excretion by algae. However, no reports have been found in the literature that discuss the role of algal ECP or biomass as potential THM precursors. It was this subject that the study described in this thesis addressed.

III. MATERIALS AND METHODS

The experimental procedures used in this study were designed to examine the extent of formation of chloroform by chlorination of the extracellular product (ECP) and biomass of algae at various times during their growth cycles. Four species, representing the blue-green and green algae, were inoculated into separate flasks containing an inorganic medium and were incubated in continuous light and at constant temperature. At various times during the growth cycle, samples were taken from each flask and the cells enumerated. The cells then were separated from the medium by centrifugation and filtration. The filtrate containing the ECP and the cell fractions resuspended in glass-distilled water, were then chlorinated and analyzed for trihalomethanes (THM's) and total organic carbon (TOC).

In later studies, solutions of single compounds, representing some of the known ECP of algae were also chlorinated and analyzed for THM's and TOC in an attempt to identify one or more of the high-haloform yielding precursors. Other studies involved the chlorination of sediments to determine whether the remains of algal cells and other organic debris were significant THM-precursors.

Algae

Four species of algae were selected for this study: two green algae--*Chlorella pyrenoidosa* and *Scenedesmus quadricauda* and two blue-green algae--*Anabaena flos-aquae* and two strains of *Oscillatoria tenuis*. These strains were selected because they are known to be taste-and-odor

producing algae (32) and have been found in the Occoquan Reservoir (38).

Anabaena flos-aquae (No. 1444), S. quadricauda (No. 76), and O. tenuis (No. 1566) were obtained from the Texas Culture Collection, Austin, Texas. Chlorella pyrenoidosa was obtained from Dr. B. C. Parker of the Biology Department, VPI&SU and the second culture of O. tenuis, which will be designated as "GLET" to distinguish it from No. 1566, was obtained from Dr. R. C. Hoehn of the Department of Civil Engineering, VPI&SU. The latter was isolated from the Garza-Little Elm Reservoir near Denton, Texas during a seige of "earthy" odor, and this strain did produce the characteristic odor. Oscillatoria tenuis (No. 1566) did not produce the earthy odor, which is supposed to be characteristic of the species. All algae were maintained in a medium of inorganic salts and trace elements which will be described later in this section.

Glassware Preparation

All glassware, with the exception of the 50 ml sample bottles (hypovials), was washed first in standard laboratory detergent, then rinsed, washed again in 20 percent hydrochloric acid (HCl), and rinsed three times in glass distilled water. This procedure removed residues that might have been toxic to the algae. Finally, the glassware was rinsed with acetone to remove any organic remaining residue and dried overnight at 103°C to evaporate completely all acetone. The glassware was then covered with aluminum foil until ready for use.

The 50-ml sample bottles were cleaned by simply rinsing them thoroughly with acetone. They were then dried overnight in a 103°C oven. These were capped with aluminum foil and stored in a desiccator. Some

of the sample bottles were capped with aluminum foil and fired in a muffle furnace at 550°C for 20 minutes. Upon removal from the furnace, the bottles were placed in a 103°C oven for 20-30 minutes, to slow the cooling process, then stored in a desiccator. The cleaning procedure for the bottles was not as rigorous as for the other glassware because they were not used for the culturing of the algae, only for containing the samples for THM analysis.

Media Preparation

The water used in this study was glass distilled and stored in a covered glass container. Several alternative procedures were evaluated for preparing a water supply free of organics that might serve as THM precursors; however, distillation in an all glass unit provided a water of low organic content and this method was selected because it required less handling of the water that could result in contamination. The alternative methods are described in Appendix Table A-1 and the resulting THM yields upon chlorination are given in Appendix Table A-2.

The algal culture medium was a modification of that described by Davis (99). The composition of the stock solutions used in the preparation of the medium is shown in Table 4. The original medium contained EDTA to chelate the trace metals and prevent their precipitation. However, it was necessary to delete it from the medium because it reacted with chlorine to yield large concentrations of chloroform (Appendix Table A-3). In addition, the compounds used in preparation of the media were contaminated with organic carbon (Appendix Table A-4).

The stock solutions (1 ml each) were added to 1-liter glass dis-

TABLE 4. Composition of Stock Solutions Used in the Preparation of the Algal Culture Medium. (1 ml stock per 1 ℓ glass-distilled water = final algal culture medium).

Constituent	Concentration (in glass-distilled water)
$Ca(NO_3)_2$	6.0 g/100 ml
$NaNO_3$	6.0 g/100 ml
K_2HPO_4	1.6 g/100 ml
$MgCl_2 \cdot 6H_2O$	10.0 g/100 ml
$MgSO_4$	2.0 g/100 ml
$NaHCO_3$	12.5 g/100 ml
Micronutrient Solution	
$ZnCl_2$	0.05 g/l
$MnCl_2 \cdot 4H_2O$	0.5 g/l
$CoCl_2 \cdot 6H_2O$	0.015 g/l
$CuCl_2 \cdot 2H_2O$	0.010 g/l
$Na_2MoO_4 \cdot 2H_2O$	0.10 g/l
H_3BO_3	1.0 g/l
$FeSO_4$	0.5 g/l

tilled water to make the culture medium. The medium was adjusted to pH 7.5-8.0 with 1 N NaOH or concentrated HCl and filtered through a glass fiber filter. It was then poured into the culture flasks, capped with aluminum foil and autoclave sterilized. Cheese cloth and cotton stoppers were not used because these could cause some organic contamination of the medium.

Determination of Cell Number and Growth Cycle

Before beginning this study, it was essential to become familiar with the growth characteristics of each algae and to establish a reliable method of quantifying the cells. Actively growing algal cells were inoculated into freshly prepared media and incubated at 24°C under continuous lighting from standard fluorescent bulbs (200-250 foot-candles). Initially (day 0), and each day thereafter, the flasks were shaken to disperse the algae and a small volume (5 ml), sufficient for use in determining optical density and cell counts, was removed. Because of the filamentous nature of the blue-green algae, it was necessary to treat the 5-ml samples by sonication for 30 seconds. Treatment for this period was more than adequate to insure that they were thoroughly dispersed before absorbance was determined. Absorbance readings were obtained with a Perkin-Elmer Double Beam Spectrophotometer at 680 nm, and cell counts of all the algae, except C. pyrenoidosa were made with the Sedgewick-Rafter counting chamber. A hemacytometer was used for counting the cells of C. pyrenoidosa because they were too small to be seen when using the Sedgewick-Rafter chamber. Single cells, not strands or chains of cells, were counted on A. flos-aquae and O. tenuis.

Standard curves for quantifying the cells were prepared for each algae. These are given in Appendix Figures A-1 to A-3. These curves were used throughout this study for estimating cell-population densities.

Culturing of the Algae for Experimentation

Approximately 10^3 cells were inoculated into 500 ml Erlenmeyer flasks containing 200 ml of freshly prepared medium. Nine flasks, each containing 200 ml of medium, were inoculated with each algal type, incubated under continuous fluorescent light, and were manually shaken daily to aerate the medium and disperse the algae.

Other cultures were inoculated and incubated undisturbed for about six weeks, then moved to the dark and allowed to die and decay. These cultures provided samples that were later chlorinated to determine THM yields from algae in the death phase.

Preparation of Metabolites and Cells for Chlorination

Algal cultures were sampled at various stages during their growth cycles so that yields of haloforms from the chlorinated medium and cell mass could be determined as functions of culture age.

Samples were taken initially on the first day of visible growth. Subsequent samples were taken at times representing intervals during the growth cycle that corresponded to the log and stationary phases of growth (as determined by spectrophotometer readings). Each culture was not necessarily analyzed on the same day. On each sampling date, the contents of one flask (200 ml) were sacrificed for the required analysis. The cell densities in each sample were determined prior to

separation of the ECP and cells.

Each sample was centrifuged (using a Fisher International Clinical Centrifuge) for 20 minutes. The supernatant, containing the ECP, was filtered through a glass-fiber filter to remove any cells not settled during centrifugation. The filtrate was poured into a clean bottle and covered with aluminum foil.

The cells were washed twice with glass-distilled water, then resuspended in approximately 10 ml distilled water and transferred to a clean test tube and capped.

All samples of filtrates and cell suspensions were frozen when collected and stored until the last sample, representing a complete growth cycle of each algae culture, was processed. All samples were thawed completely before chlorination. The cell suspensions were transferred to a 300 ml Erlenmeyer flask and diluted to 250 ml. Absorbance readings were then taken on the resuspended cells.

Approximately 50 ml were transferred from each sample of filtrate and cell suspension to a clean bottle to be used for TOC analysis by procedures to be described later. The remainder was chlorinated and sealed for haloform analysis.

Chlorination and Preparation for Haloform Analysis

A stock chlorine solution of approximately 6000 mg/l was prepared from a commercial hypochlorite (NaOCl) bleach the day samples were to be chlorinated. The concentration was determined using a Fisher-Porter Amperometric Titrator.

The ECP (filtrate) samples were dosed with 20 mg/l chlorine. The

resuspended cells were first macerated thoroughly with a Techmar SDT Tissumizer, then dosed with 50 mg/l chlorine. None of the samples were dechlorinated to insure that chlorine remained in sufficient quantities to allow the haloform reaction to go to completion.

Immediately following chlorination, the samples were carefully poured into a 50 ml sample bottle so that no bubbles would form. The bottle was filled in such a manner that a convex meniscus formed at the top. With forceps, a Teflon-coated septum was placed (Teflon-side down) on the top, displacing the excess sample. An aluminum cap was then placed on the neck of the bottle and crimped tightly with a crimping tool. The samples were free of headspace except for a small bubble that developed upon refrigeration. All samples were refrigerated until ready for shipping. Distilled water and the algae medium samples were chlorinated also with 20 mg/l chlorine and pepared by the same procedure to serve as controls.

The chlorinated samples were wrapped, placed in a styrofoam container and packed in ice for shipment (by air freight) to the analytical laboratories (either in California or Texas) where they were refrigerated upon arrival at the labs. Samples were analyzed for haloforms by the Bellar-Lichtenberg method (100) at California Analytical Laboratory, Sacramento, California or by the liquid-liquid extraction (LLE) method described by Henderson et. al (101) at North Texas State University in Denton, Texas.

To insure that sufficient chlorine had been added to cause the haloform reaction to go to completion, algae culture filtrates were collected at three different times during their growth cycle and chlor-

inated with varying doses to insure that the chlorine demand could be satisfied. Chlorine residuals were determined by amperometric titration after seven hours contact, and the results were used as a basis for determining the dose of chlorine to be applied to samples that were analyzed for haloforms. Seven hours was an arbitrary contact time selected on basis of convenience and on the assumption that the majority of the chlorine demand would be exerted in that period of time.

A diagram of the entire experimental procedure for the preparation of the algal cultures for haloform analysis is given in Figure 5.

Preparation of the Model Compounds

Certain algal extracellular metabolites, selected from those mentioned in the literature, were obtained in pure form and chlorinated to determine the extent of haloform yields.

Solutions were prepared so that the TOC concentrations were approximately 10 mg/l. Some samples were adjusted to pH 7.5 (a value representative of natural waters) with a phosphate buffer and some to pH 9.3 (representative of many treated waters following the softening process) with a carbonate buffer. Compounds having a greater chlorine demand, i.e. those containing nitrogen, were chlorinated with 30 mg/l chlorine and the remainder with 20 mg/l chlorine. For reasons explained previously the samples were not dechlorinated. The bottling and shipping procedures were the same as for the algae samples. Several of the solutions were checked for residual chlorine after two hours and found to have a residual of greater than 7.0 mg/l.

Preparation of Sediments

A limited number of experiments were conducted to determine the yields of haloforms from chlorinated sediments and associated water. Sediment samples were collected from two sites in the Occoquan Reservoir. The first (site 1) was located at the confluence of Bull Run and Occoquan Creek and the second (site 2) was located 11.5 miles downstream at the dam. Two carbon fractions from each sample were prepared: the soluble fraction, which is contained in the interstitial waters and the insoluble fraction associated with the particulates.

The soluble fraction was prepared as follows: the interstitial water was drained by gravity from the sediments through a small-mesh seive and clarified by filtration through a glass fiber filter. Fifty ml were removed for TOC analysis and the remainder chlorinated with 50 mg/l chlorine and sealed for haloform analysis. The insoluble fraction was prepared as follows: 100 g of wet sediments were suspended in 200 ml of glass-distilled water, 50 ml removed for TOC analysis, and the remainder chlorinated with 50 mg/l chlorine and sealed. Some of the larger sediment particles settled before the aliquot for TOC analysis was removed; therefore, the TOC concentrations represented only that which could be achieved by suspending the lighter sediment particles.

Dry weights of the sediments were determined by drying 100 g of each at 103°C for 72 hours until it appeared to be completely dry.

Analysis for Total Organic Carbon

All TOC analysis were conducted with the Dohrmann/Envirotech Organic Carbon Analyzer. Briefly, the operation of the instrument is as

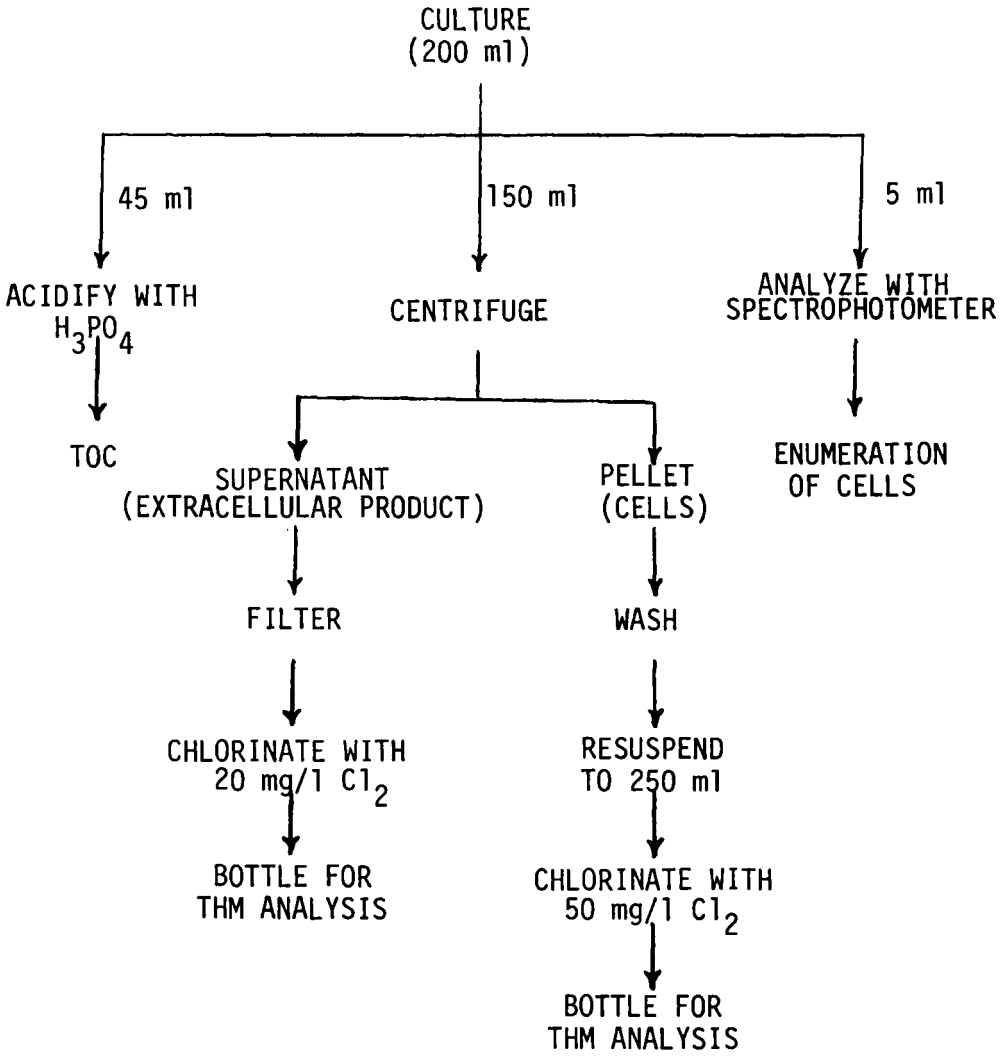


FIGURE 5. Experimental procedure for investigating the formation of trihalomethanes from chlorinated algal cultures.

follows: The instrument is comprised of two basic units, the DC-52 and the DC-54. The DC-52 is used for analysis of samples containing 10-2000 mg/l carbon. For organic carbon analysis, an acidified sample is injected onto an oxidizing agent and heated so that the organic carbon oxidizes to CO_2. The CO_2 is then swept (by helium carrier gas) into a hydrogen-enriched nickel catalyst pyrolysis zone where it is reduced to methane and is read as mg/l carbon by a flame ionization detector (FID). Inorganic carbon (HCO_3^-, $CO_3^=$) is eliminated prior to oxidation of the organic carbon by a carbonate bypass system. Total carbon (organic + inorganic) may also be analyzed on the DC-52 system by eliminating the bypass system step.

The DC-54 Analyzer System is used for samples containing less than 10 mg/l carbon. A solution of phosphoric acid (H_3PO_4) and potassium persulfate ($K_2S_2O_8$) is added to the sample and the sample is introduced into the instrument. The sample is purged with helium, which sweeps CO_2 and the purgeable organic carbon (POC), composed mainly of the volatile organic fraction, from the sample. The CO_2 is removed by a CO_2 scrubber and the POC fraction is carried to the pyrolysis zone, where it is reduced and is detected as described previously. The sample containing the nonpurgeable organic carbon (NPOC) fraction is then slowly transferred by a helium carrier past an ultraviolet reaction coil, which, aided by the persulfate solution, oxidizes the organic carbon to CO_2 which is swept into the pyrolysis zone. The DC-54 reads directly in µg/l carbon.

The precision of both the DC-52 and DC-54 is ±2 percent except at

the lower detection limits (less than 50 mg/l for the DC-52 and less than 500 µg/l for the DC-54), where the readings should reproduce within 1 mg/l carbon and 10 µg/l carbon, respectively.

An advantage of the DC-54 system is that a large volume (10 ml) is analyzed. This allows for a greater accuracy in the lower concentration range and less interference from contamination. For a detailed discussion of the operation and working conditions of the Dohrmann/Envirotech TOC Analyzer, consult the operations manuals (102, 103).

In this study, the samples for TOC analysis were acidified with 85 percent H_3PO_4 to pH 2 to stop microbial growth. The samples containing more than 10 mg/l carbon were analyzed with the DC-52 and samples containing less than 10 mg/l were analyzed with the DC-54. All cell suspensions were thoroughly mixed with a Techmar SDT Tissumizer immediately before the analysis. The model compound solutions were analyzed with the DC-54 system after diluting them 1:5 with glass-distilled water. Water blanks and the culture medium also were analyzed to determine baseline TOC concentrations.

The volatile (POC) organic carbon fraction can be analyzed on the DC-54 and an estimate of the volatile fraction can be read on the DC-52; however, for all samples in this study except one, the volatile fraction represented less than approximately 5 percent of the total and was not recorded separately.

All TOC analyses were repeated at least twice and data were cited as averages. The cell suspensions were mixed before each analysis. Many sample analyses, especially the cell suspensions, could not be reproduced within the stated precision range of the instrument. There-

fore these samples were analyzed several times and those clustering about some modal value were averaged (extreme values were considered not representative and were discarded).

The standards for calibrating the DC-52 and DC-54 were prepared from a potassium hydrogen phthalate solution as described in the operations manuals (102, 103) using water prepared in the same manner as that used in the experimental procedures.

Bacterial Analysis

The culture media harvested from old cultures of each algae were streaked on Standard Methods Agar and incubated at 35°C for three days to determine if they were contaminated with bacteria. No attempt was made to determine bacterial population densities during the algal growth cycles, however.

IV. RESULTS

The majority of the data collected during this study was intended to investigate the relationship between algal growth, characteristics of organic carbon excretion, and trihalomethane formation within the growth medium when chlorinated. Additional experiments were conducted with solutions of pure organic compounds, reported in the literature as extracellular metabolites of algae to determine the haloform yields when chlorine was added. It should be noted that during the presentation of the Results and Discussion sections that when chloroform concentrations are reported, they refer to those that were produced upon chlorination of the samples and should not be interpreted to mean those produced by the algae.

As was mentioned in the Methods section the medium was prepared without EDTA because uninoculated media containing it produced extremely high $CHCl_3$ concentrations (greater than 4000 µg/l). The results obtained during preliminary experiments involving algal cultures grown in media containing EDTA are given in Appendix Table A-5. The results of the preliminary studies, conducted to determine the approximate growth cycle of each algae, can be found in Appendix Figures A-4 to A-8.

Culture Age, Liberation of Organic Carbon, and Trihalomethane Formation

Results of the experiments concerning $CHCl_3$ formation from chlorination of the extracellular product (ECP) of the five algal species are given in Figures 6-10. Chloroform concentrations ranged from 100 µg/l for A. flos-aquae at 20 days of growth to 9600 µg/l for O. tenuis "GLET"

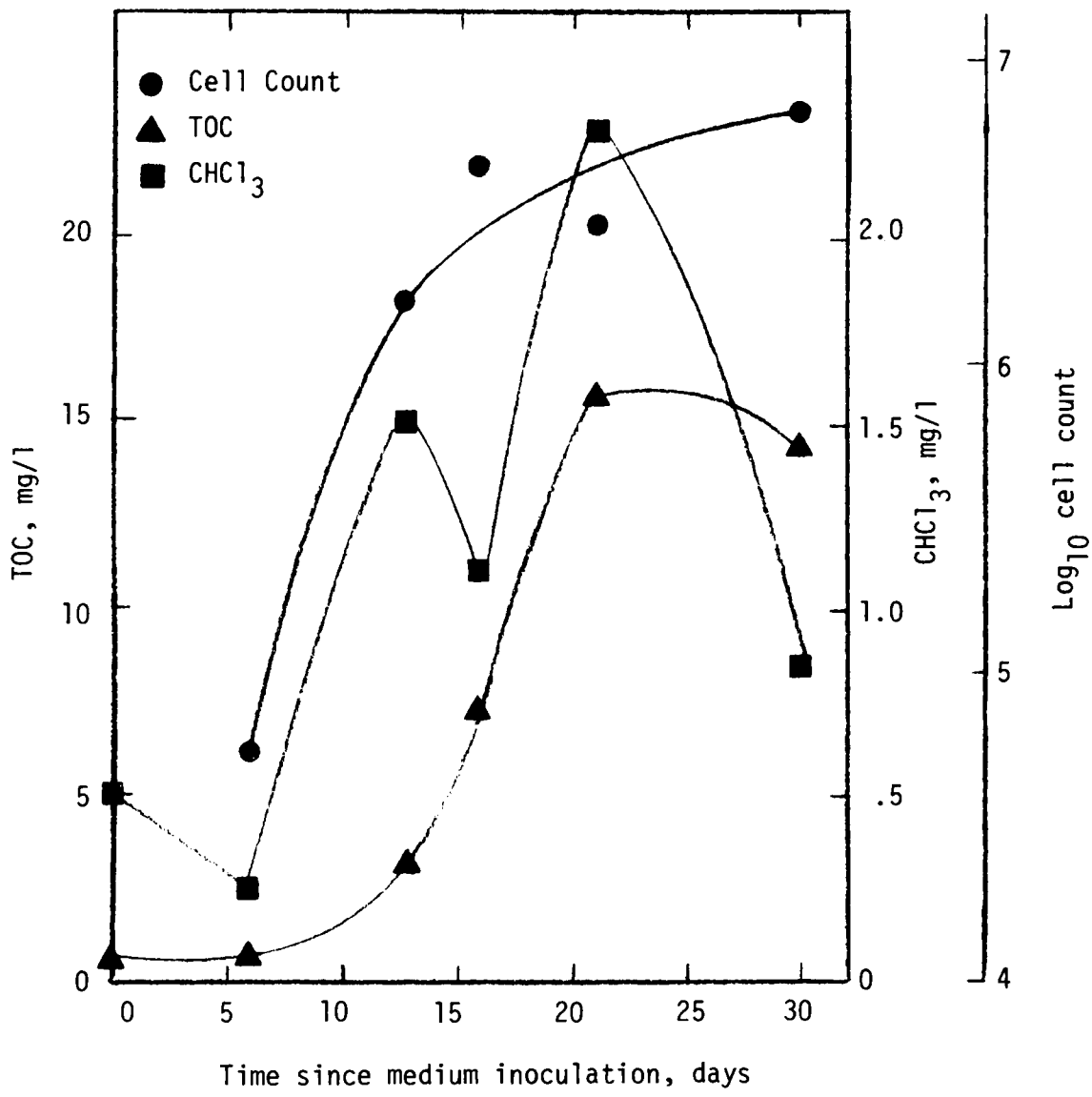


FIGURE 6. The relationships between growth of the green alga, Chlorella pyrenoidosa, the extracellular total organic carbon (TOC) present in the medium, and chloform ($CHCl_3$) produced by chlorinating the medium filtrates with 20 mg/l chlorine.

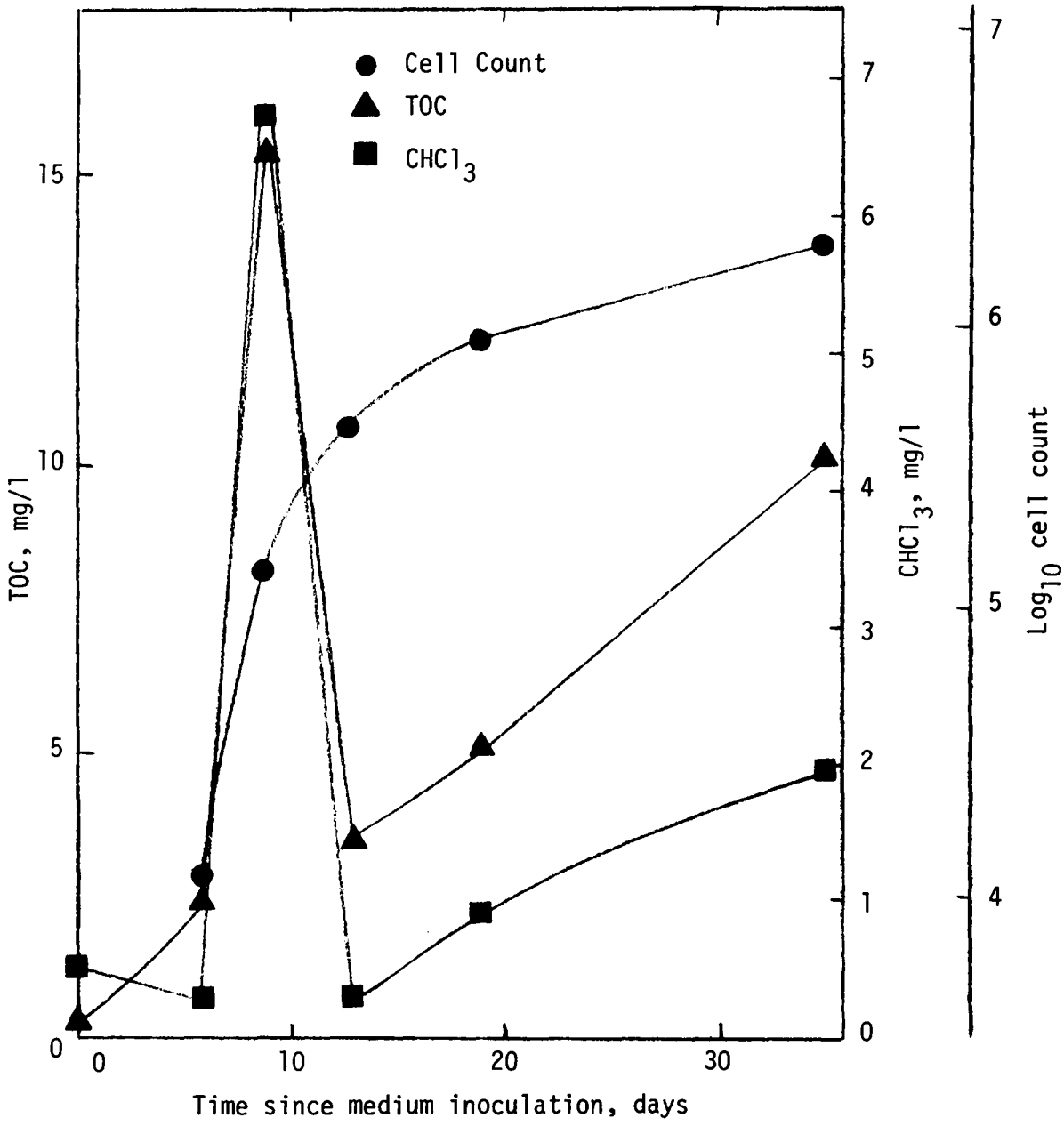


FIGURE 7. The relationship between growth of the green alga, Scenedesmus quadricauda, the extracellular total organic carbon (TOC) present in the medium, and chloroform ($CHCl_3$) produced by chlorinating the medium filtrates with 20 mg/l chlorine.

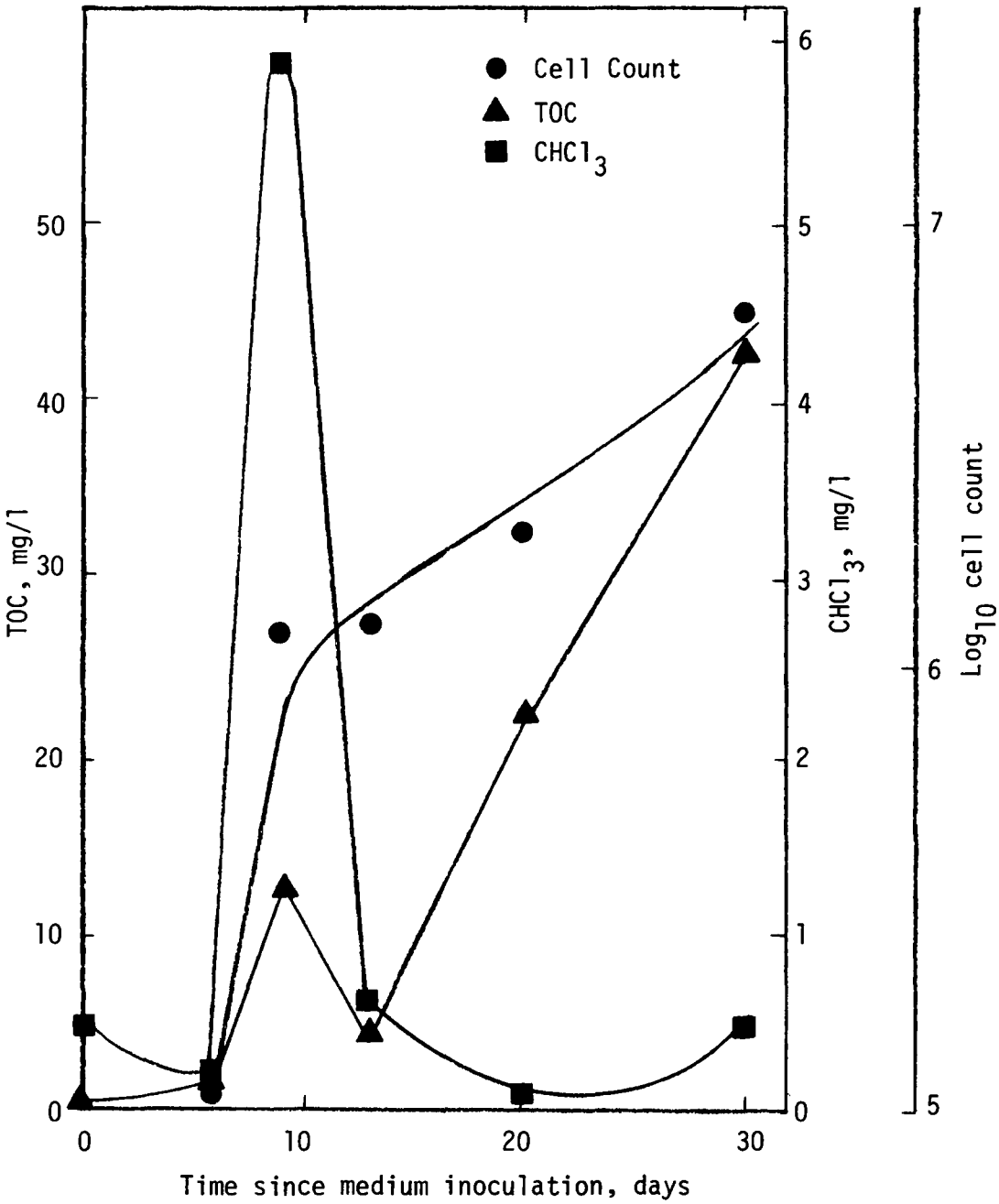


FIGURE 8. The relationship between growth of the blue-green alga, Anabaena flos-aquae, the extracellular total organic carbon (TOC) present in the medium, and chloroform ($CHCl_3$) produced by chlorinating the medium filtrates with 20 mg/l chlorine.

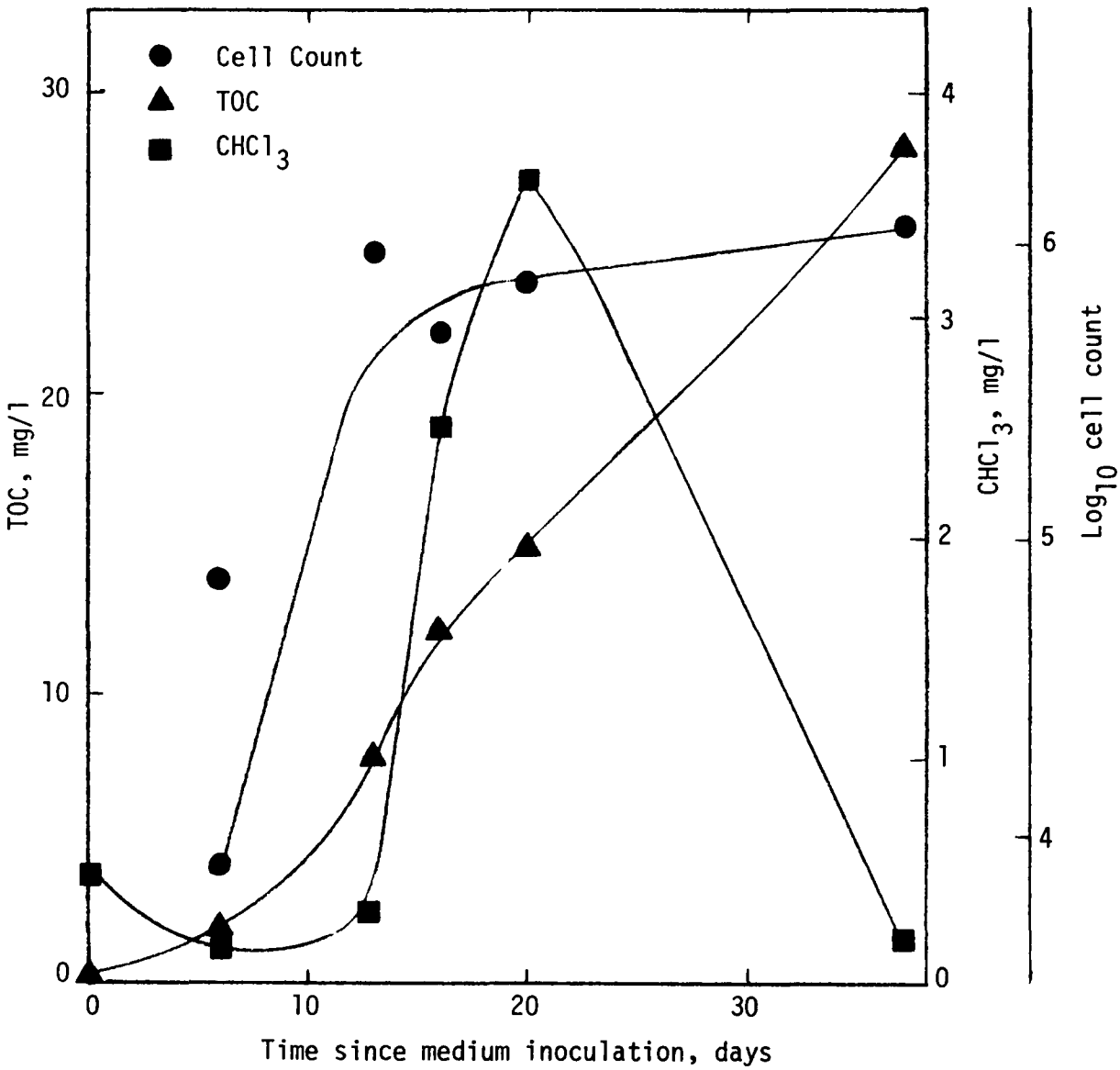


FIGURE 9. The relationship between growth of the blue-green alga, Oscillatoria tenuis "1566", the extracellular total organic carbon (TOC) present in the medium, and chloroform ($CHCl_3$) produced by chlorinating the medium filtrates with 20 mg/l chlorine.

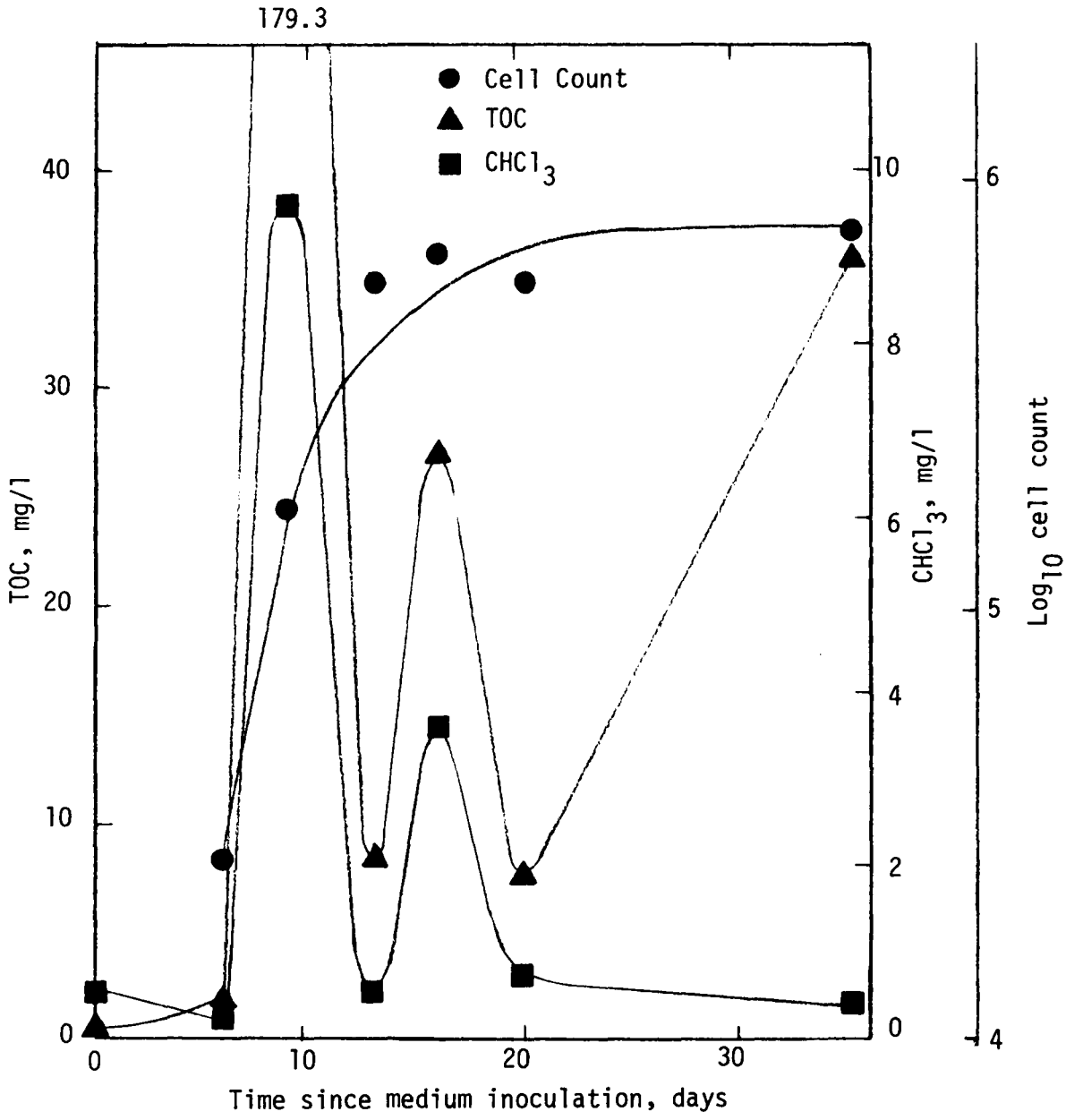


FIGURE 10. The relationship between growth of the blue-green alga, Oscillatoria tenuis "GLET", the extracellular total organic carbon (TOC) present in the medium, and chloroform ($CHCl_3$) produced by chlorinating the medium filtrates with 20 mg/l chlorine.

at nine days of growth. The THM values are the result of a single analysis, whereas the TOC values are averages of several replicates that were within ±2 percent of each other. The precision of the THM analysis has been shown to be approximately ±10 percent for the Bellar-Lichtenberg technique (100). No precision data for the LLE method are available, but the detection limit for $CHCl_3$ is 0.2 µg/l (101).

A general trend that can be seen in all species is increased $CHCl_3$ formation during the earlier phases of growth. The maximum $CHCl_3$ formation of S. quadricauda, A. flos-aquae, and O. tenuis "GLET" occurred during the exponential growth phase. Chloroform concentrations produced from filtrates of media containing C. pyrenoidosa (Figure 6) peaked twice, the maximum concentration appearing in the late exponential phase. (Because the precision of $CHCl_3$ analysis is approximately ±10 per cent, these peaks are regarded as distinctly different). The highest $CHCl_3$ concentration for O. tenuis "1566" appeared to occur in the early stationary phase. However, the cell densities for both O. tenuis "1566" and C. pyrenoidosa were still increasing slightly when the maximum $CHCl_3$ concentrations were reached.

It is obvious from these figures, that there were considerable fluctuations in the TOC content of the culture media throughout the growth cycles. With the exception of C. pyrenoidosa and O. tenuis "1566", early peaking of the TOC can be seen, followed by a significant decrease after 12 days of growth. This pattern of TOC development was not always evident, as can be seen in Appendix Figures A-9 -A-13, where supplementary TOC data are given. It should be noted that the blue-

green algae generally excreted more extracellular organic carbon than did the green algae.

One final observation regarding Figures 6-10 is that the uninoculated media contained low levels of TOC (approximately 0.40 mg/l) and yielded some $CHCl_3$ (approximately 500 µg/l) when chlorinated. These values were not subtracted from the analytical results obtained during the growth of the algae because the TOC concentrations fluctuated considerably and at some stages in the algal growth cycles, the $CHCl_3$ produced was lower than in that observed in the chlorinated blanks. The significance of this observation will be discussed later.

The carbon liberated by the algae was primarily non-volatile organic carbon. The volatile fraction was insignificant except in one instance involving O. tenuis "GLET" at 9 days of growth (Figure 10). On that date, the volatile fraction comprised 90 per cent of the TOC. This phenomenon was not observed again in a replicate culture (Appendix Figure A-13).

The changes in chloroform concentrations followed approximately the same patterns as changes in organic carbon in cultures of S. quadricauda, A. flos-aquae, and O. tenuis "GLET", especially during the early growth stages. In all species, the $CHCl_3$ concentrations were significantly less in the late stages of growth. However, the TOC was not always less. The blue-green algae media, when chlorinated, developed lower $CHCl_3$ concentrations when the cultures were from 30 to 37 days old than did the media in which green algae grew.

Figures 11-15 show the concentrations of TOC and the $CHCl_3$ formed

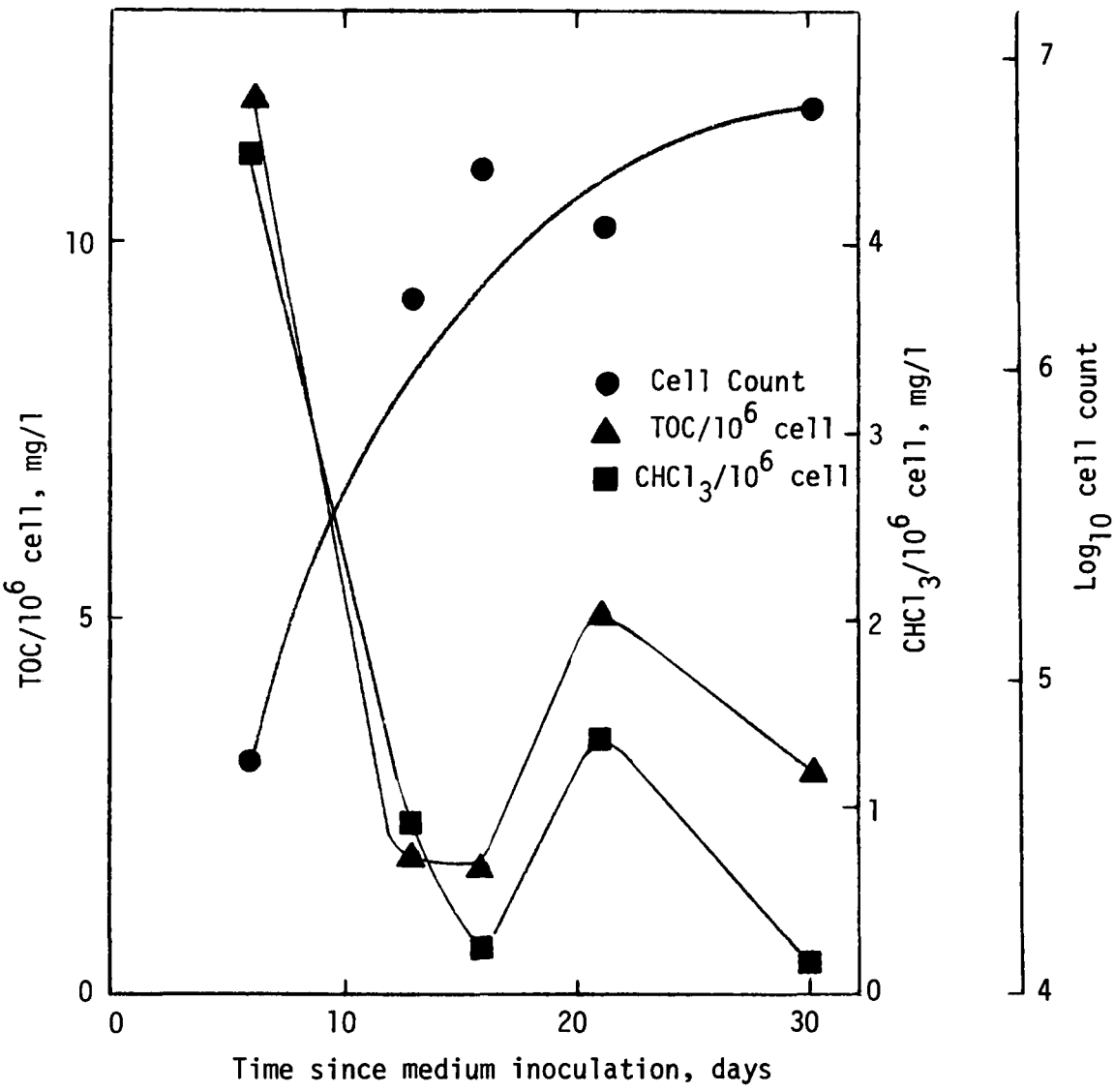


FIGURE 11. Variations in the production of algal extracellular product (as TOC) and chloroform ($CHCl_3$), on a per cell basis in chlorinated media filtrates shown in relation to algal growth for Chlorella pyrenoidosa.

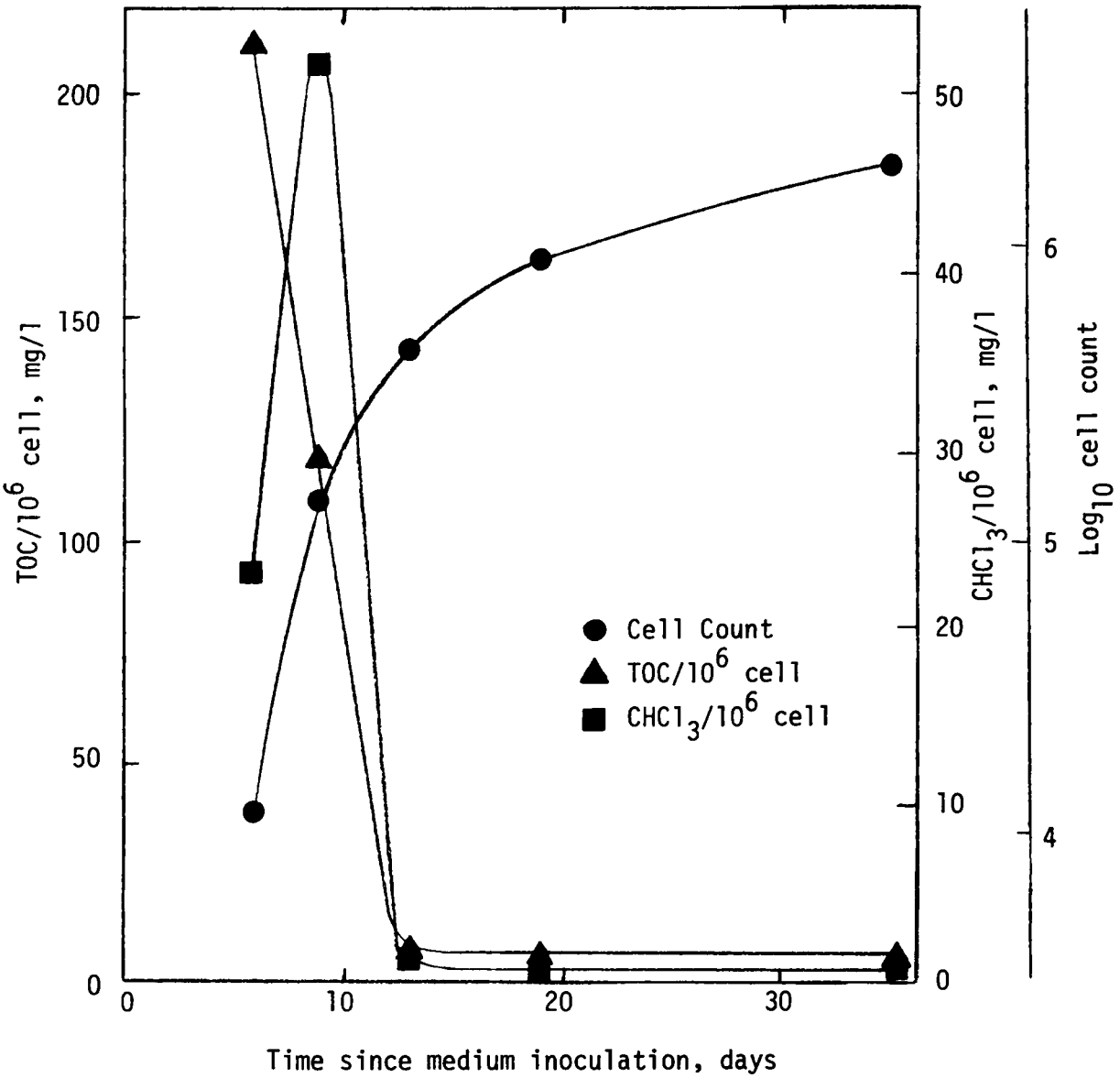


FIGURE 12. Variations in the production of algal extracellular product (as TOC) and chloroform ($CHCl_3$), on a per cell basis in chlorinated media filtrates shown in relation to algal growth for Scenedesmus quadricauda.

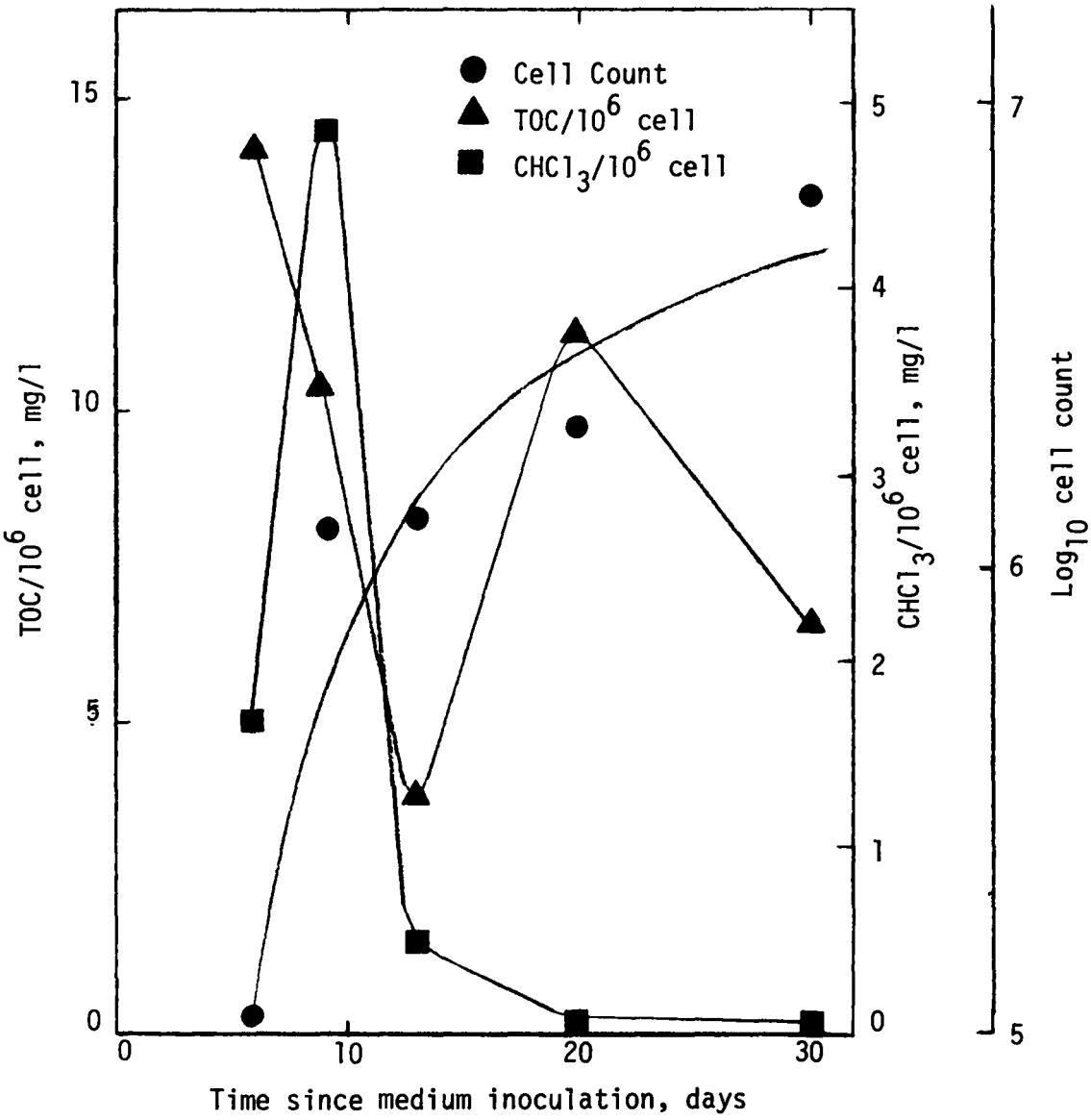


FIGURE 13. Variations in the production of algal extracellular product (as TOC) and chloroform ($CHCl_3$), on a per cell basis in chlorinated media filtrates shown in relation to algal growth for Anabaena flos-aquae.

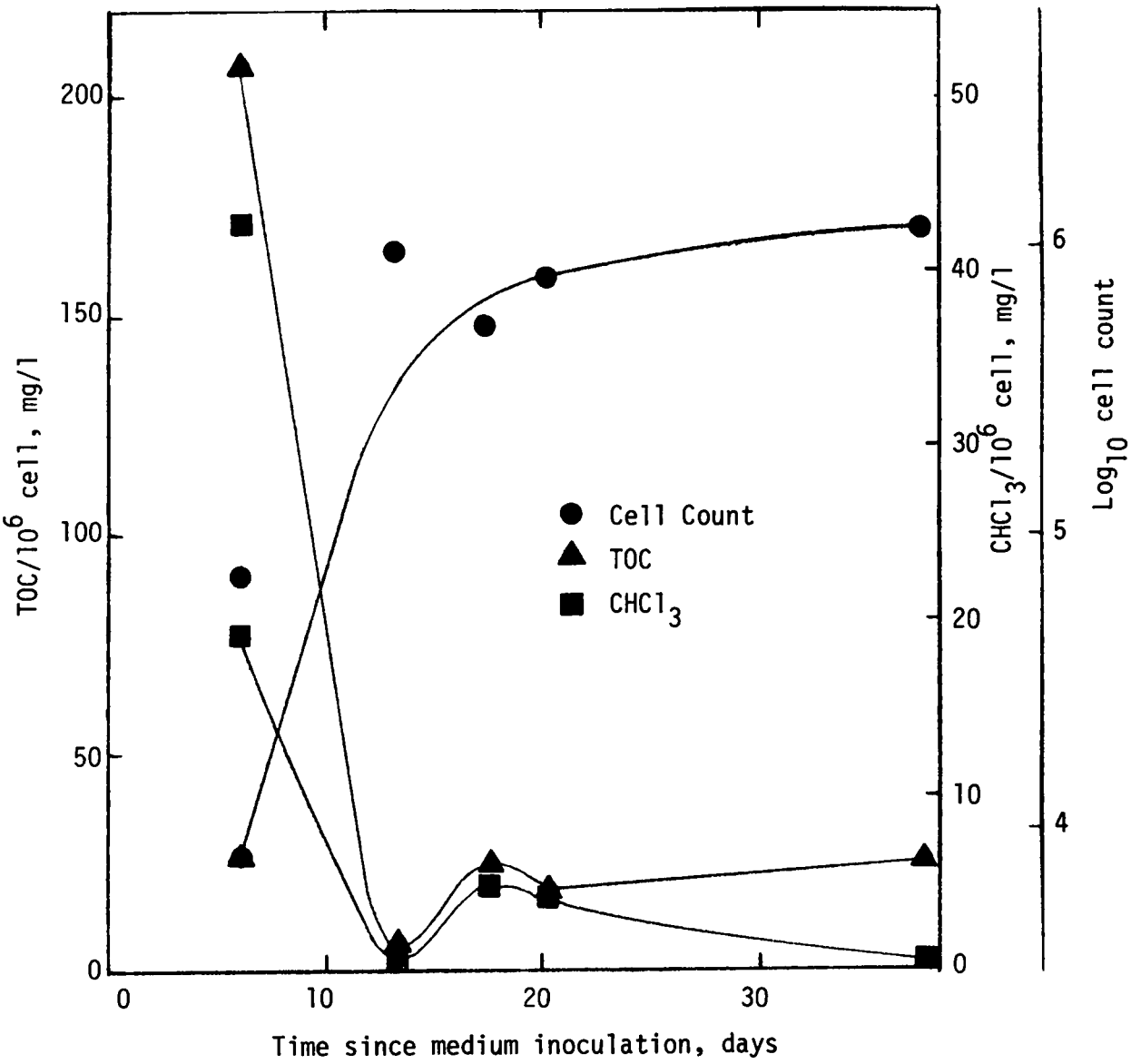


FIGURE 14. Variations in the production of algal extracellular product (as TOC) and chloroform ($CHCl_3$), on a per cell basis in chlorinated media filtrates shown in relation to algal growth for Oscillatoria tenuis "1566".

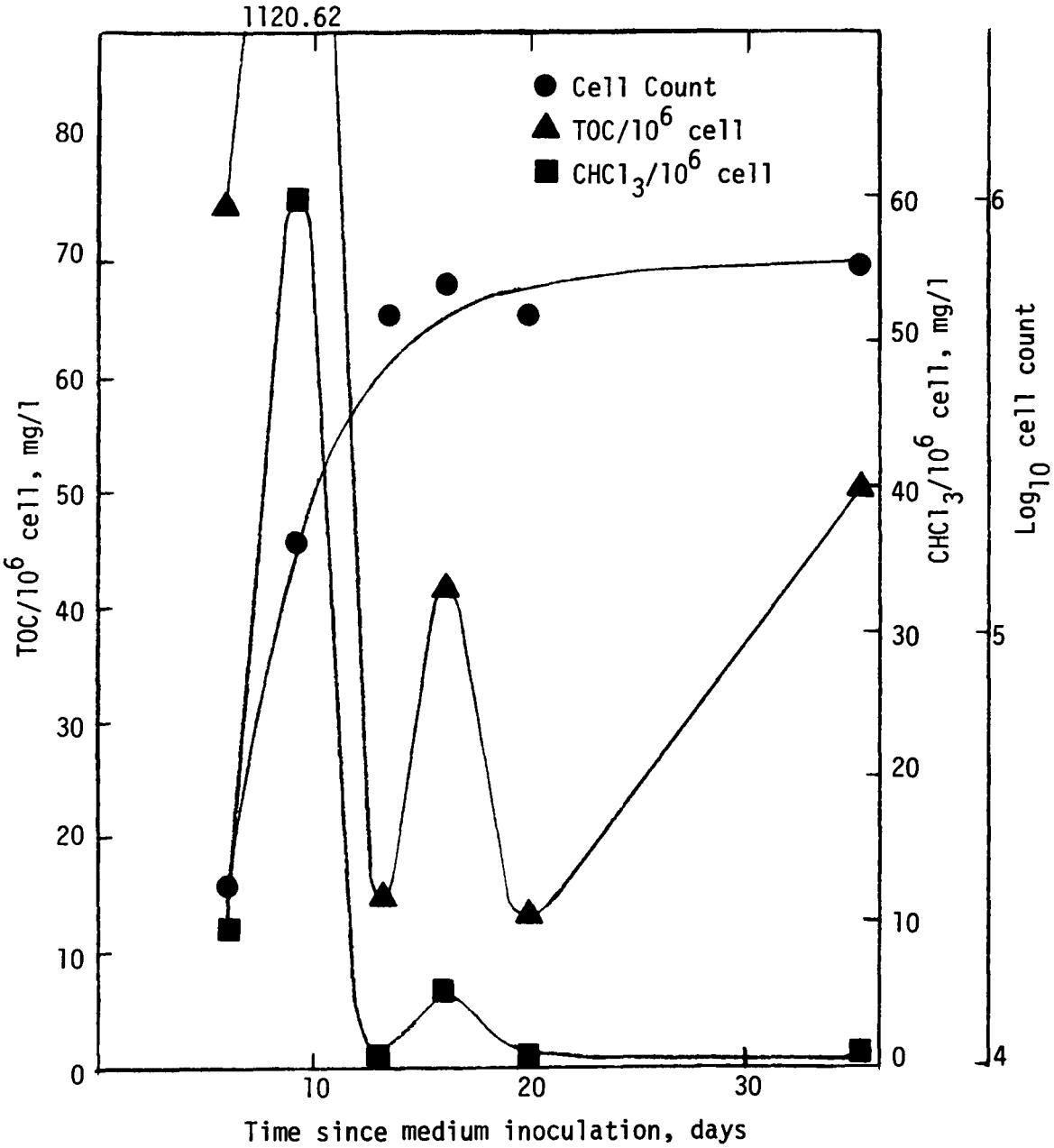


FIGURE 15. Variations in the production of algal extracellular product (as TOC) and chloroform ($CHCl_3$), on a per cell basis in chlorinated media filtrates shown in relation to algal growth for Oscillatoria tenuis "GLET".

per cell during growth. It is obvious that maximum carbon excretion and $CHCl_3$ formation occur during the log growth phase. Another feature that can be seen in species of the blue-green algae is a second increase in the TOC without a corresponding increase in $CHCl_3$ late in growth. This phenomenon was not as apparent in the green algae cultures. In them the pattern of $CHCl_3$ concentrations tends to resemble that of TOC concentrations throughout the growth cycle.

The concentrations of $CHCl_3$ produced per mg/l TOC are shown in Figures 16 and 17. A nonlinear relationship obviously existed in the blue-green cultures. An approximately linear relationship occurred with S. quadricauda but not with C. pyrenoidosa.

The percent yields of chloroform carbon from the TOC $\left(\frac{\text{mg } CHCl_3\text{-C} \times 100}{\text{mg TOC}}\right)$ were calculated and summarized in Figure 18. For C. pyrenoidosa, S. quadricauda, and A. flos-aquae, the maximum yields were observed early in the growth cycle. In cultures of O. tenuis strains, the yields tended to peak somewhat later. As can be seen, the $CHCl_3$ yields from the filterable carbon in blue-green algae cultures decreased to nearly zero during the late growth stages.

A very high $CHCl_3$ yield (15.7 per cent) was found in one sample of O. tenuis "1566" at six days of growth. However, a much lower yield was observed in a duplicate culture that was also six days old. The high yield was observed in a culture which grew quite rapidly in comparison to the replicate culture, and these data will be discussed in more detail later.

Samples sent to Texas for haloform analysis were analyzed for other

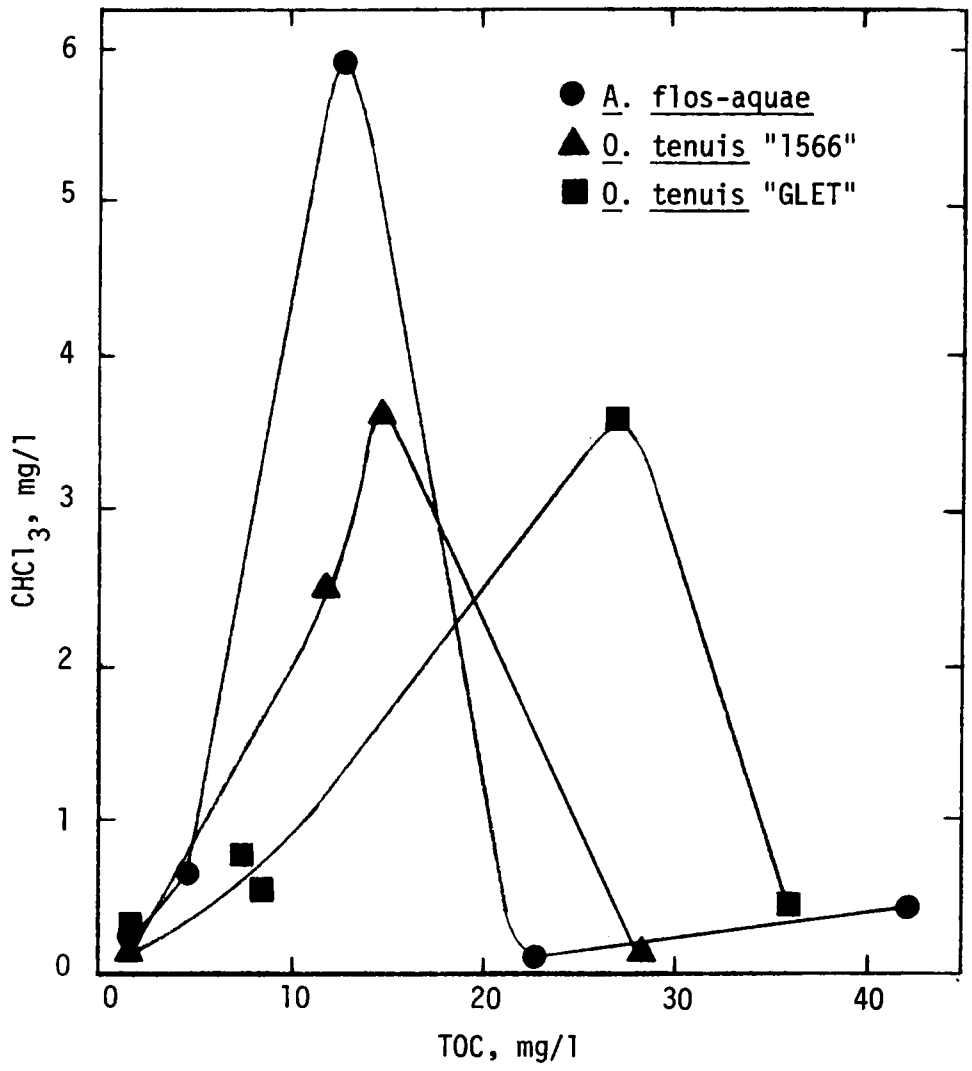


FIGURE 16. Variations in chloroform ($CHCl_3$) concentration with increasing organic carbon (TOC) concentration of the chlorinated filtrates of the blue-green algae: Anabaena flos-aquae, Oscillatoria tenuis "1566", and Oscillatoria tenuis "GLET".

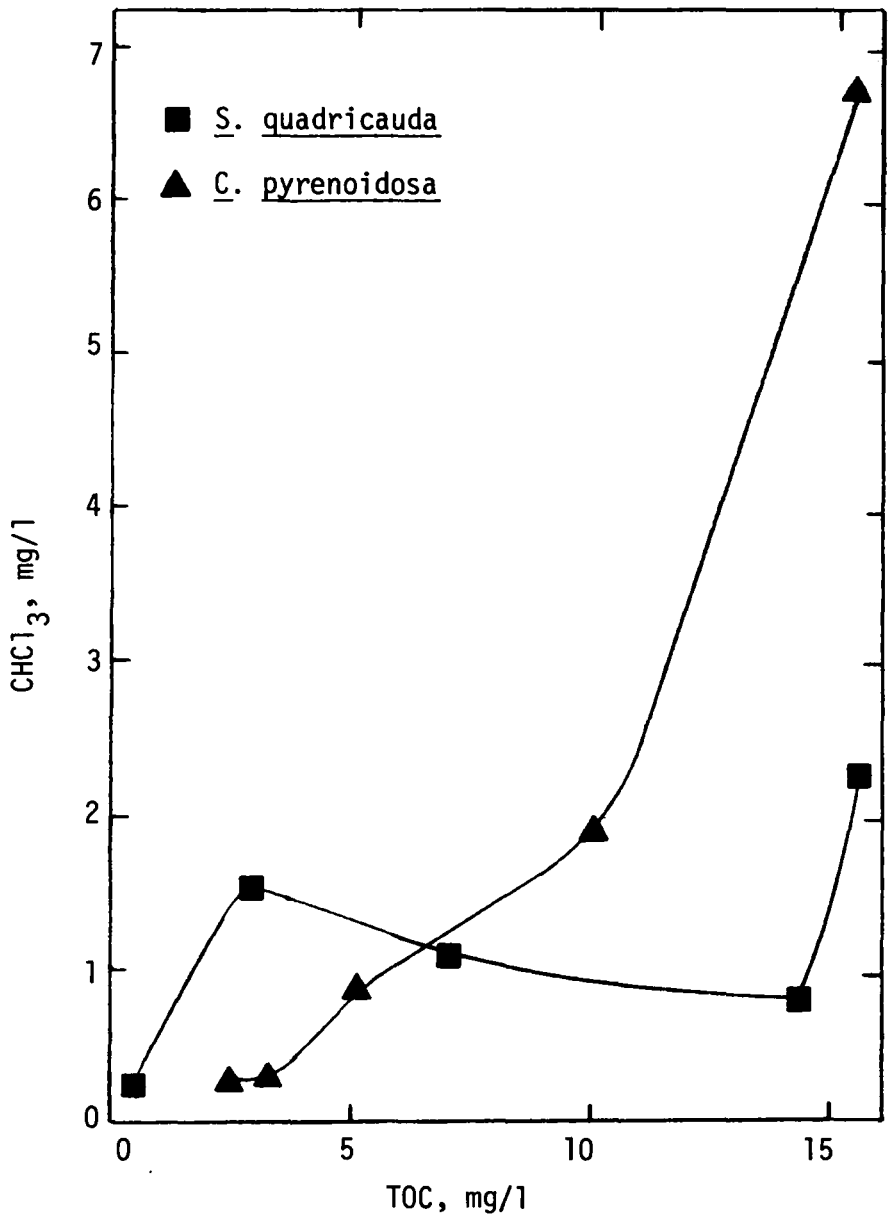


FIGURE 17. Variations in chloroform ($CHCl_3$) concentration with increasing organic carbon (TOC) concentration of the chlorinated filtrates of the green algae: Scenedesmus quadricauda and Chlorella pyrenoidosa.

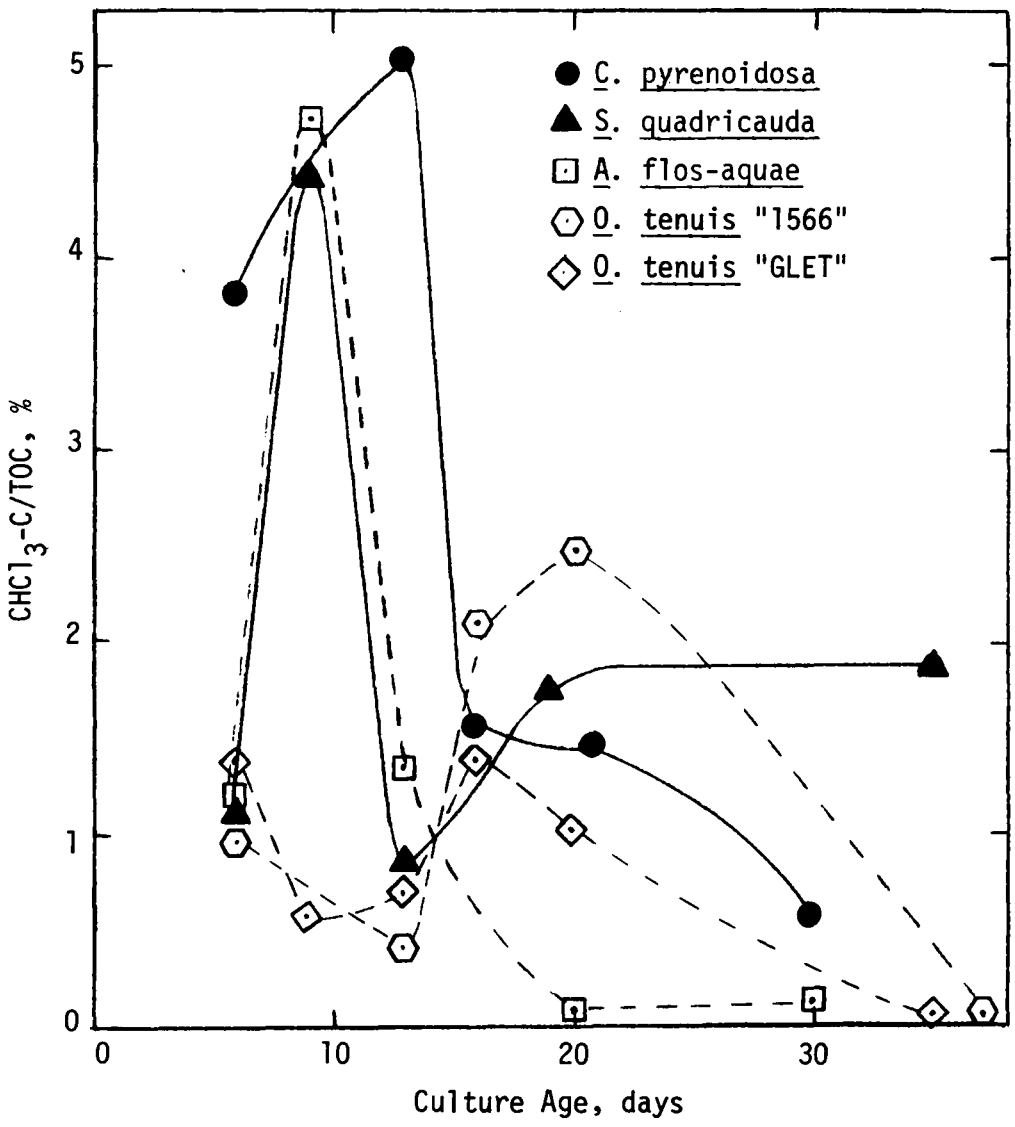


FIGURE 18. Variations in the chloroform-carbon ($CHCl_3$-C) yields [expressed as a percentage of the media filtrate total organic carbon (TOC)] with culture age for all the algae species.

halogenated organics in addition to $CHCl_3$. Low concentrations of CCl_4, $CHCl_2Br$, and $CHClBr_2$ were detected in some samples but in much lesser concentrations than $CHCl_3$. Bromoform was not detected in any of the samples.

The information used to construct Figures 4-16 can be found in Appendix Table A-6.

Model Compounds

Table 5 contains the data derived from experiments involving chlorination of solutions of pure compounds known to be algal extracellular products. Low yields of $CHCl_3$ were found to be produced by these compounds. In all cases, when chlorinated the solutions that were buffered to pH 9.3 produced more $CHCl_3$ than when they were buffered to pH 7.5. The per cent increase varied with the compound. For example, an increase of 43 per cent in $CHCl_3$ yield was found when alanine solutions were increased to pH 9.3, and a 323 per cent increase was demonstrated with mannose solutions.

Cell Mass Chlorination

Chlorination concentrations produced by the chlorination of algal biomass harvested at various times during their growth cycles are given in Table 6. There seemed to be a tendency for the yields ($CHCl_3$-C per unit TOC) to decrease with time of growth, but the trend was not as well defined as was evident with the yields from ECP. There was no discernible, direct relationship between the $CHCl_3$ and the TOC concentrations of these samples. It should be noted that the pH's of the cell suspensions generally were lower than those of filtrates.

TABLE 5. Haloforms Produced in Chlorinated Solutions of Organic Compounds Reported to be Extracellular Products of Algae

Compound	Chlorine dose, mg/l	pH	TOC mg/l	$CHCl_3$ µg/l	$CHCl_3$-C/ TOC, %	*Molar Yield of $CHCl_3$, %	CCl_4, µg/l	$CHCl_2Br$, µg/l
Mannose	20	7.5	11.14	24.5	0.022	0.132	-	0.17
	20	9.3	11.14	103.0	0.093	0.558	-	0.28
Mannitol	20	7.5	8.86	34.5	0.039	0.234	-	0.20
Xylose	20	7.5	10.50	43.0	0.041	0.205	-	0.24
Glucuronic Acid	20	7.5	10.65	33.0	0.031	0.186	-	0.15
Ascorbic Acid	20	7.5	11.01	13.7	0.012	0.072	-	0.14
Lactic Acid	20	7.5	16.29	16.8	0.010	0.040	-	0.11
Succinic Acid	20	7.5	12.95	25.2	0.020	0.080	-	0.13
	20	9.3	12.95	51.4	0.040	0.159	-	0.18
Fumaric Acid	20	7.5	10.41	17.2	0.017	0.068	-	0.10
α-ketoglutaric Acid	20	7.5	12.70	12.2	0.010	0.048	-	0.10
	20	9.3	12.70	32.8	0.026	0.130	-	0.15
Alanine	30	7.5	10.15	4.8	0.005	0.014	0.04	0.09
	30	9.3	10.15	6.8	0.007	0.020	-	0.14
Serine	30	7.5	11.13	4.7	0.004	0.013	0.09	0.27
Glutamic	20	7.5	8.89	10.7	0.012	0.060	-	0.08
Thiamine	30	7.5	10.03	40.8	0.041	0.492	-	0.12
Biotin	30	7.5	10.29	28.4	0.028	0.280	-	0.12
Gum Arabic	20	7.5	6.42	31.5	0.049	**	-	0.16
	20	9.3	10.19	139.0	0.137	**	0.06	0.59
Gum Karaya	20	7.5	4.40	40.9	0.093	**	-	0.13
Glycolic Acid	20	7.5	10.98	18.4	0.017	0.034	-	0.11
	20	9.3	10.98	69.0	0.063	0.126	-	0.19
Acid Phosphatase	30	7.5	12.72	422.0	0.333	**	0.03	0.10
Acetaldehyde	20	7.5	7.70	160.0	0.209	0.418	0.06	0.22

*Molar yield of $CHCl_3$, % = (moles $CHCl_3$/mole compound) x 100

**Cannot be calculated because molecular weights not known

TABLE 6. Characteristics of Cell Suspension and Haloform Yields After Chlorination with 50 mg/l Chlorine

Algae Species	Culture Age, days	pH	TOC, mg/l	$CHCl_3$, µg/l	$CHCl_3$-C /TOC, %	CCl_4, µg/l	$CHCl_2Br$, µg/l
C. pyrenoidosa	13	6.4	2.92	820	2.82	-	-
	16*	-	18.9	521	0.28	0.26	0.09
	20	7.9	15.2	860	0.57	-	-
	30	8.4	13.2	1500	1.41	-	-
S. quadricauda	9	-	20.7	1040	0.50	-	-
	13*	-	32.6	622	0.19	0.71	0.07
	19	7.0	52.4	910	0.17	-	-
	35	8.3	75.8	110	0.15	-	-
A. flos-aquae	9	6.7	12.2	890	0.73	-	-
	20	-	24.2	510	0.21	-	-
	30	-	30.5	1350	0.44	-	-
	35*	-	10.9	479	0.46	0.17	0.08
O. tenuis "1566"	6	-	2.1	820	3.89	-	-
	16*	-	11.4	470	0.41	0.14	0.09
	20	6.2	23.5	790	0.34	-	-
	37	-	20.0	1300	0.65	-	-
O. tenuis "GLET"	9	6.9	11.8	630	0.54	-	-
	16	-	18.9	770	0.41	-	-
	20*	-	10.6	553	0.52	0.41	0.08

*Haloform analyses were by the liquid-liquid extraction technique (101). All others were analyzed by the purge/trap technique (100).

Chlorination of Extracellular Carbon and Biomass of Aged Cultures

Tables 7 and 8 contain data derived from the experiments involving chlorination of aged cultures. The $CHCl_3$ yields from the ECP's of the aged cultures (Table 7) were significantly lower than those produced by the actively growing cultures (Appendix Table A-6). The yields observed from chlorination of the resuspended cells of the aged cultures (Table 8) were higher than those observed when filtrates were chlorinated, reflecting the tendency observed in the active cultures, i.e., a decrease in $CHCl_3$ yields from the chlorinated biomass with age of the culture.

Sediments

As seen in Table 9, low $CHCl_3$ yields were produced by chlorination of the organic carbon extracted from and contained within the sediment samples. Difference in the chloroform yields from sediment collected at the site of confluence or at the dam site were small, although the TOC concentrations were greater at the site of confluence.

Residual Chlorine

Chlorine residuals were not measured in the samples shipped away for haloform analysis; however, chlorine demand tests were conducted with filtrates of algal cultures after eight, twelve, and seventeen days of growth. Results of these experiments are given in Appendix Tables A-7 to A-9. A residual of at least 3.0 mg/l chlorine was maintained for one week in all media collected from 8-day old cultures (except for *O. tenuis* "GLET" which was not tested). As the culture aged, the chlorine

TABLE 7. Characteristics of Aged Algae-Culture* Filtrates and Haloform Yields after Chlorination with 20 mg/l Chlorine

Algae Species	Culture age, days	pH	TOC, mg/l	$CHCl_3$, µg/l	CCl_4, µg/l	$CHCl_2Br$ µg/l	$CHClBr_2$ µg/l	$CHCl_3$-C/ TOC, %
Chlorella pyrenoidosa	150	7.8	12.1	116.0	0.08	4.11	-	0.096
Scenedesmus quadricauda	150	8.0	5.5	68.5	0.04	0.08	0.38	0.126
Anabaena flos-aquae	154	8.1	25.1	17.6	0.05	0.34	0.37	0.007
Oscillatoria tenuis "1566"	150	7.8	16.9	238.0	0.06	0.06	0.064	0.141
Oscillatoria** tenuis "GLET"	124	8.0	95.8	285.0	0.09	0.15	-	0.030

*Cultures were maintained in continuous light for approximately 44 days then stored in the dark to promote death and decay

**was not moved to dark

TABLE 8. Characteristics of Aged Algae Cell Suspensions (in Distilled Water) and Haloform Yields* after Chlorination with 50 mg/l Chlorine

Algae Species	Culture Age, days	pH	TOC, mg/l	$CHCl_3$, µg/l	$CHCl_3$-C/ TOC, %	CCl_4, µg/l	$CHCl_2Br$ µg/l	$CHClBr_2$ µg/l
Chlorella pyrenoidosa	150	6.6	24.4	620	0.255	0.75	0.16	-
Scenedesmus quadricauda	150	6.3	38.8	838	0.214	0.76	0.09	-
Anabaena flos-aquae	154	6.8	32.8	602	0.184	0.53	0.11	-
Oscillatoria tenuis "1566"	150	6.7	44.3	525	0.119	0.40	0.26	-
Oscillatoria** tenuis "GLET"	124	6.5	76.1	828	0.109	0.39	0.49	-

*All haloform analyses were by the liquid-liquid extraction technique of Henderson et. al (101)

**Culture was not stored in the dark

TABLE 9. Haloform Yields upon Chlorination of Solids and Interstitial Waters from Sediments Collected at Two Sites in the Occoquan Reservoir

Site	Sediment	Dry Weight, g.	pH	TOC, mg/l	$CHCl_3$, µg/l	$CHCl_3$-C/ TOC, %	CCl_4, µg/l	$CHCl_2Br$, µg/l	$CHClBr_2$, µg/l
1	Dam Site: Interstitial Water	50.48	7.1	66.0	314	0.048	0.08	15.70	0.54
1	Dam Site: Washed Sediment	50.48	6.6	42.9	216	0.051	0.08	3.60	0.90
2	Confluence Site: Interstial Water	52.11	6.9	94.2	284	0.030	0.07	9.25	0.52
2	Confluence Site: Washed Sediment	52.11	6.3	31.9	303	0.095	0.14	4.95	-

demand was greater, but there was still a free-chlorine residual of at least 6.0 mg/l after sever hours contact in most culture filtrates. In some cases, however, the chlorine residual was less than 0.5 mg/l.

Residual chlorine was also determined in the suspensions of cells harvested after sixteen days of growth (Appendix Table A-10). A dose of 41.8 mg/l chlorine provided a residual greater than 1.0 mg/l after 48 hours of contact in suspensions of C. pyrenoidosa, A. flos-aquae, and O. tenuis "1566". The cell suspension of S. quadricauda did not show any residual after 24 hours; however, since residual determinations were made prior to that time, it is not possible to state how long a residual actually did persist.

Changes in pH During Growth

Results of pH variations of the cultures during growth are recorded in Appendix Table A-11. The range in Ph was from 8.4 to 10.5. It appears that the pH increases during early growth, then decreases slightly. The pH's of samples at the time they were analyzed for haloforms are recorded with the other data pertaining to these samples.

Bacterial Analysis

All species of algae utilized in this study were found to be contaminated with bacteria. The cultures of A. flos-aquae, C. pyrenoidosa, and S. quadricauda appeared to be contaminated with one bacterial type, and both strains of O. tenuis appeared to contain two types. Although the cultures were contaminated, the bacterial populations were not dense enough to cause visible turbidity in the algal culture medium. Identification of the bacteria was not performed, and no attempt was made to

quantify the populations. It should be mentioned that the study, as it was originally designed, did not include any provisions for routine bacterial enumerations in the algae cultures during their life cycle. It should be stated that the bacteria that were observed were isolated from cultures that were approximately four weeks old, and there is no way to actually know at what algae culture age the bacterial populations began to increase.

V. DISCUSSION

During the discussion, it should be kept in mind that this investigation was conducted under laboratory conditions which are quite different from the conditions found in the natural environment. Nevertheless, the results can be used as a rational basis for postulating the impact that algae growth in drinking water sources may have in intensifying THM problems in finished water.

Changes in Total Organic Carbon Concentration of the Filtrates as the Algae Culture Ages

It is apparent from an examination of Figures 6-15, that the greatest liberation of organic material by algal cells occurs during the exponential phase of growth, a fact that is consistent with reports in the literature (42, 43, 47). The fluctuations seen in TOC concentrations during the growth cycle could be due either to utilization of the extracellular carbon by the algae themselves, by the bacteria, or by both. It is possible that the inorganic carbon concentration in the culture medium became limited, as the cultures were not continuously aerated, and the algae may have subsisted partially by heterotrophic utilization of organic carbon.

As was pointed out earlier, the changes in TOC concentrations did not always follow the same pattern as the algae populations increased. Time of sampling may have had an effect, as excretion and utilization rates may have varied from day to day. The TOC data shown in Appendix Figures A-9-A-13 were obtained from analysis of samples taken from one

culture flask during the growth of a single culture of algae, whereas that shown in Figures 6-10 in the Results section of this thesis were obtained from analysis of individual cultures that were sacrificed at varying intervals after inoculation. The latter method of obtaining data during an algae growth cycle most likely would account for the observed differences between the absolute values of TOC seen in a continuous culture from day-to-day and the individual flasks. Obviously, the cultures grown in separate flasks could not be maintained in completely synchronous growth.

During the late growth stages of S. quadricauda, A. flos-aquae, and O. tenuis "GLET" (Figures 7, 8, and 10) it was observed that the TOC concentrations increased a second time. This pattern may have been caused by products of algal metabolism, an increase in bacterial products, or both.

Changes in Chloroform Concentrations in the Chlorinated Medium During the Algal Growth Cycle

As was seen in Figures 6-10 there were considerable variations in the $CHCl_3$ concentrations with time in the chlorinated media of all the algal species examined in this study. There are several possible explanations for these observed fluctuations. First, the concentration of the extracellular carbon may have been a factor. During the early growth stages high $CHCl_3$ concentrations generally occurred at a time when the apparent organic carbon excretion was greatest. However, during later growth this relationship of $CHCl_3$ concentrations and TOC concentrations is not as evident, especially in the blue-green algae,

which may indicate a changing type of precursor carbon produced by the algae as the culture aged. The hypothesis that there was a change in precursor type is substantiated by the patterns of $CHCl_3$ yields per unit precursor carbon upon chlorination of the ECP (Figure 18). These variations give some indication of the nature of the carbon excreted by the algae during the growth cycle. The decrease in the $CHCl_3$ yields during the later growth stages indicates that the ECP produced by the algae was comprised of carbon compounds that were less reactive in the haloform reaction than the compounds liberated early in the growth cycle. Algal cultures have been found to excrete low-molecular weight compounds during their early growth stages and higher molecular weight compounds during later growth (43, 57). The substances released during the later stages of the life cycle may not yield high concentrations of haloforms when chlorinated. Further evidence of a changing precursor is seen in the non-linear relationship of $CHCl_3$ concentrations per unit carbon precursor (Figures 16 and 17). Rook (16) found a linear increase in the $CHCl_3$ formation upon chlorination of increasing concentrations of a single compound (humic acids) which would not be seen if the precursor type changed.

Another possible explanation for the decline in the $CHCl_3$ concentrations in the chlorinated medium is that the products of bacterial growth have begun to increase in concentration as the algal culture aged and that these products did not serve as high haloform-yielding precursors. As mentioned previously, there is no direct evidence that the bacterial populations in fact were increasing but this possibility cannot be ignored.

Another factor that may have affected the observed variations in the $CHCl_3$ concentrations was the varying pH of the algae cultures (Appendix Table A-11). The pH generally was higher during early growth when maximum $CHCl_3$ formation occurred. The haloform reaction is known to be pH dependent so pH fluctuations in the cultures may have influenced the yields of $CHCl_3$ that were observed.

It is also possible that the chlorine may have been exhausted before the haloform reaction was complete in some of the samples. There was a period of several weeks (two weeks for the samples sent to Texas, up to eight weeks for the samples sent to California) from the time the samples were chlorinated until the haloform analysis were performed. The chlorine demand of some samples may have been greater than others because of the changing nature of the ECP with culture age. The data did show, however, that a chlorine residual persisted in all samples for at least seven hours after dosing (Appendix Tables A-7 to A-9). It has been shown that the haloform reaction proceeds most rapidly during the first twelve hours in other systems (16, 17), and in the vast majority of tests involving ECP, the free residuals did not fall below 1.0 mg/l chlorine. Therefore, it is likely that the haloform reaction was virtually complete even though the chlorine may have been eventually exhausted in some of the samples after seven hours contact.

It is likely that the variations in TOC concentrations, the nature of the ECP, and the media pH all influenced the $CHCl_3$ concentrations that were observed in the chlorinated media. Of these, the changing nature of precursor carbon and, to a lesser extent, the concentration of the extracellular carbon probably exerted the greatest influence.

Differences in Chloroform Formation of Chlorinated Filtrates of the Blue-Green Algae and Green Algae

During the early growth stages the blue-green algae generally showed a higher concentration of extracellular carbon and higher $CHCl_3$ formation upon chlorination than did the green algae. However, the $CHCl_3$ yields per unit extracellular carbon were higher for the green algae than for the blue-green algae, with the possible exception of A. flos-aquae, indicating that the green algae tend to excrete higher-$CHCl_3$-yielding compounds. The $CHCl_3$ concentrations produced in the chlorinated medium for the blue-green algae were lower in the late growth stages than were produced in the green algae cultures. The blue-green algae are known to excrete large amounts of nitrogenous substances (75, 76) and late growth often is characterized by an increase in the production of mucilagenous material; these substances may not yield high concentrations of haloforms when chlorinated. It appears that high $CHCl_3$ formation from the chlorinated filtrates of the blue-green algae may be more closely related to greater carbon excretion. However, the general trend for both the blue-green and green algae is increased formation of $CHCl_3$ upon chlorination of the ECP during the exponential phase of growth.

Chloroform Yields from Algal Extracellular Carbon

Table 10 contains a statistical summary of the data concerning the $CHCl_3$ yields observed after chlorination of the algal ECP, and Table 11 compares the data with other values reported in the literature when different precursors were involved. It is apparent that many of the

TABLE 10. Chloroform Yields (Carbon Basis) from the Carbon Present in Algal Extracellular Metabolites.

Algae Species	Range ($CHCl_3$-$C/_{TOC}$, %)	Mean	Standard Deviation
C. pyrenoidosa	0.59-4.96	2.46	1.83
S. quadricauda	0.85-4.37	1.98	1.40
A. flos-aquae	0.04-4.67	1.46	1.88
O. tenuis "1566"	0.07-2.44	1.90	1.04
O. tenuis "GLET"	0.13-1.34	0.84	0.48

TABLE 11. A Comparison of the Chloroform Yields (Carbon Basis) from Algal Extracellular Metabolites and from Studies with Humic Substances Reported in the Literature.

Range ($CHCl_3$-$C/_{TOC}$, %)	Investigators: Precursor Studied (Ref).
0.04-4.96	This work: ECP of Algae (all species included)
0.3-0.9	Rook: Fulvic Acid Extract (16)
0.7 (pH 6.7), 1.4 (pH 9.2)	Stevens et. al: Aldrich Humic Acid (25)
0.5-1.6	Babcock and Singer: Humic Acid Extract (20)

$CHCl_3$ yields calculated from the data during this study fall within the range of those reported for humic and fulvic acids. However, it also appears that the ECP produced by four of the five cultures of algae at certain stages in their life cycle yielded higher levels of $CHCl_3$ than have been reported for the other types of precursors. The $CHCl_3$ yields from the algal ECP, at some times during growth, exceeded the yields reported for the humic and fulvic acids by as much as four times, a fact that indicates the algal ECP may be at least equally as important as the humic substances under many conditions and possibly more important under others. More discussion of this follows later.

An unusual observation made during the analysis of the nine-day old culture of O. tenuis "GLET" was that approximately 90 per cent of the TOC in the medium was volatile. In other cultures, the volatile component was insignificant. However, the $CHCl_3$ yield on that day was not significantly higher than on other days, suggesting that perhaps the volatile compounds produced by algae are not significant contributors in the haloform reaction.

Another unusual observation was that the $CHCl_3$ yield from the six-day old culture of O. tenuis "1566" was approximately 16 per cent. This value was not included in the statisitical analysis of the yields (Table 11) because it obviously was an anomaly and would have biased the analysis. This high yield on that particular day may indicate that cultures which grow quickly excrete highly reactive compounds early in their growth cycle.

Studies with Model Compounds

The model compounds utilized in this investigation (Table 5) did not produce $CHCl_3$ concentrations equivalent to those produced from the algal ECP. The percent yields (molar basis) from these compounds were lower by a factor of 100 than those reported by Morris and Baum (15) who found as much as 100 per cent yields from some compounds. Rook (105) also found significantly higher yields from certain model substances which may contain structures similar to important functional groups in the fulvic acids. The compounds reported by Morris and Baum and by Rook generally were more complex and contained more haloform reactive functional groups than did the ones selected for this experiment.

As was expected, the yields of $CHCl_3$ from the model compounds were greater at the higher pH's. This could be due to enhanced enolization or to the fact that the final hydrolysis of the trihalogenated hydrocarbon occurs more readily at an elevated pH. Both of these theories are supported in the literature (15, 16). It is also possible that the ionic species (OCl^-) of the hypochlorous acid, which predominates at pH 9.3 is more reactive with the organic enol than the nonionic species (HOCl). It is possible that the yields would be increased at even higher pH values, especially with compounds such as mannose that have high pKa values. (The pK_1 values for most sugars generally range from 11.5-12.5). However, the pH's selected for the experiments in this study were reasonable for surface waters where algae may abound, but higher pH's may be important in water treatment plants that practice lime-soda softening and the effect of pH on THM-yields may become more

important under these circumstances. Acetaldehyde, which is known to be reactive in the haloform reaction, was not as reactive as was expected. Chlorination at a higher pH (greater than 9.3) may have resulted in a greater yield.

Chloroform Formation by Algal Biomass

Algal biomass was chlorianted so that it could be determined whether the biomass or the ECP were more significant as $CHCl_3$ precursors. This phase of the investigation was regarded to be an important component of the total study because algae growing in a water supply can either enter the treatment plant and be chlorinated or die and fall to the sediments, thus becoming associated with the sediment organic material.

Analysis of Table 6 reveals that the active algal cells, generally produced less $CHCl_3$ when chlorinated than did the ECP, but the yields were still within the range reported for the humic and fulvic acids. There are several possible explanations for the lower yields. First, the types of organic materials contained within the cell may be less reactive with chlorine than the ECP liberated during growth. Second, the pH of the harvested cell suspensions were lower than that of the filtrates. Third, the chlorine may have been exhausted before the reaction was complete, as the chlorine demands of the cells was greater than for the metabolites. This is unlikely, however, as the cell suspensions of the algae, with the exception of S. quadricauda, maintained a free chlorine residual of greater than 1.0 mg/l after 48 hours contact (Appendix Table A-10).

The general trend of decreasing yields with culture age indicates

that the type of carbon associated with the cells changes with time. Possibly a greater incorporation of protein and carbohydrate material occurs as the culture ages. For example, the older blue-green algae produce more mucilagenous material which would be associated with the cells as well as with the ECP. Morris and Baum (15) reported a $CHCl_3$ yield per unit TOC (obtained from the percent molar yield) from purified chlorophyll of approximately 0.28 per cent (Table 3). The $CHCl_3$ yields produced upon chlorination of the algal biomass in this study were consistent with this report.

Chloroform Formation Potential of Aged Algal Cultures and Sediments

There were low $CHCl_3$ yields from the biomass and ECP of aged cultures, a phenomenon consistent with the data presented previously for yields from the growing cells and ECP of growing cells. It appears that little of the carbon associated with or released by the aged cultures is reactive in the haloform reaction.

Yields of $CHCl_3$ from two carbon fractions of the sediments were investigated: 1) the soluble fraction, present in the interstitial water within the sediments which consists of carbon released by dead algal cells and the products of bacterial degradation of a variety of organic substances and 2) the insoluble (particulate) fraction consisting primarily of dead algal cells and other particulate organic debris. These studies indicated that the carbon associated with the sediments (Table 9) is not significant as presursor material. The $CHCl_3$ yields upon chlorination of the sediments were similar to those from chlorination of the aged algal cells and, to a lesser extent, of the

actively growing cells. It should be noted, that there was little difference in the yields obtained from sediments collected at the site of confluence of Bull Run and Occoquan Creek where relatively fresh organic material from runoff and sewage treatment plants is deposited and the dam site, approximately 11.5 miles away, where the organic material in the sediments most likely is more stabilized by bacterial decomposition than deposited at the confluence.

Implications in Water Treatment

It should be pointed out that the algal populations grown in culture were more dense than would be expected under normal circumstances in lakes and streams. However, in a bloom situation, the cell count in natural waters often reaches 10^6 cells per ml. In addition, algal cells generally liberate more organic material *in situ* than in culture (44, 58, 67).

From the data collected in this investigation, it is obvious that the algal ECP is more significant than the biomass in THM formation. The substances liberated by algae are soluble therefore, may not be readily removed during water treatment. It is possible, though, that these substances could be removed by coagulation, flocculation, and sedimentation during routine water treatment as they probably adsorb readily to particulates. However, if heavy prechlorination is practiced, these extracellular organics could be a significant source of precursors for THM production. They may be especially pronounced if blooms occur near the raw water intake.

A factor that would tend to minimize the problem is that the com-

pounds excreted by algae are readily biodegradable and do not persist in the water for long periods of time. It may be assumed that, under normal conditions, these products do not pose any problem.

It is also obvious from this study that the compounds liberated by young, actively growing algal cells are the most significant as precursor carbon. Consequently, it seems important that the bloom be controlled in the early stages to minimize the input of high-yield organic material. To accomplish such control, the algae population in the water would have to be monitored routinely and at the first indication of a developing bloom, remedial action (such as the application of copper sulfate) would have to be taken.

VI. SUMMARY AND CONCLUSIONS

The objectives of this study were to determine if algal ECP and biomass are significant THM precursors and to demonstrate any differences in the THM formation potential during the different stages of the algal growth cycle. Four species of algae (two green and two blue-green) were grown in an inorganic medium in continuous light and constant temperature. At various times during the growth cycle, samples were sacrificed and the cells and medium were separated. Both were chlorinated and analyzed for THM's and TOC.

Solutions of single compounds, reported in the literature as being constituents of algal ECP also were chlorinated after pH adjustments to 7.5 and 9.3 and analyzed for THM production and TOC. Additional studies included investigations to determine the THM formation potential of the soluble inorganic carbon and biomass contained within aged algae cultures and of the TOC associated with sediments collected from two reservoir sites.

The significant conclusions derived from this study are:

1. Algal ECP is generally more significant than the biomass as THM precursor.
2. The greatest formation of THM's and greatest excretion of high-yield compounds (measured as TOC) usually occurs during the exponential phase of growth and tends to decrease as the culture ages.
3. The yields of $CHCl_3$ upon chlorination of algal ECP and biomass are within the range of, and often exceed, those reported for

the humic substances. Therefore these substances may be considered at least as important as THM precursors as the humic substances. Under some conditions, such as when an algal bloom develops near the intake of a water treatment facility, may be more important than the humic substances as THM precursors.

4. Chlorination of single compounds at pH 9.3 results in significantly higher $CHCl_3$ yields that chlorination at pH 7.5.
5. It is difficult to make any definite conclusions about the differences in the THM formation potential of the green algae and blue-green algae as each species behaved differently. As can be seen in the two strains of O. tenuis, even algae of the same species behave differently. However, the following generalizations can be made: (a) It appears that the blue-green algae generally liberate more organic carbon and produce higher absolute concentrations of $CHCl_3$ upon chlorination during early growth than do the green algae. (b) The green algae excrete higher $CHCl_3$-yielding compounds than the blue-green algae, with the exception of A. flos-aquae. (c) The species of the blue-green algae tend to produce less $CHCl_3$ upon chlorination of the medium late in the growth cycle than do species of the green algae.

VII. RECOMMENDATIONS

In future studies, the following changes in experimental procedures are recommended:

1. Algae should be cultured in one large container so that TOC and THM data would truly be representative of changes that occur as the population densities increase. This procedure would eliminate spurious data induced by the differing rates of algae growth in small, individual cultures that are sacrificed at different times during the growth cycles.
2. Chlorine residuals after 24 hours contact and pH should be taken on all samples to be analyzed for haloforms to determine the significance of these parameters on the haloform formation.
3. Bacterial growth (by quantification) should be monitored during the algal growth cycles.
4. Replicate experiments of each algal species would make comparisons of the variations in TOC and haloform formation upon chlorination during algal growth more reliable.

The following additions to the experiment are suggested to gain a better understanding of the changes in haloform concentrations as the algal culture ages:

1. Characterization of the molecular type of compounds found in the growth medium during the different states of algal growth should be accomplished.

2. Repetition of the experiments with other species of the blue-green algae and green algae and other types of algae important in drinking water supplies should be accomplished to determine the relative significance of each algal type as THM precursors.
3. Repetition of the experiments with axenic cultures of algae and with mixed cultures of algae and bacteria to determine any differences in haloform yields upon chlorination should be accomplished.

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APPENDIX

TABLE A-1. Procedures for Preparing Low-Organic Water

1.	Reflux-redistilled water: Reflux - Add 0.4 ml H_2SO_4 and 0.7 ml permanganate to 300 ml glass-distilled water, boil 3-5 min., connect to reflux apparatus and reflux 1/2-1 hr. Distill - Collect 600 ml reflux water, neutralize with NaOH and transfer to a distilling flask. Boil 3-5 min., connect to distilling apparatus.
2.	Glass-distilled water, distilled and stored in an all glass system.
3.	Glass-distilled water, chlorinate with 10 mg/l chlorine and purged with N_2 gas for 30 min.
4.	Glass-distilled water, boiled 15 min.
5.	Glass-distilled water, treated under ultraviolet (U.V.) light for 30 min.
6.	Glass-distilled water, treated under U.V. light 18 hrs.
7.	Glass-distilled water, purged with ozone for 5 min.
8.	Glass-distilled water, purged with ozone for 30 min.

TABLE A-2. Results of THM Analysis of Chlorinated Water Prepared by Different Methods. All Samples Chlorinated with 10 mg/l Chlorine.

Method	$CHCl_3$, µg/l	CCl_4, µg/l	$CHBr_3$, µg/l	$CHClBr_2$, µg/l
Reflux-Redistill	11, 12			
Glass distilled	31, 8, 15, 15	0, 5, 0	0, 8, 0	0
Glass distilled-N_2purge	71			
Glass distilled-boiled	17			
Glass distilled U.V. (30 min.)	16			
Glass distilled U.V. (18 hr.)	1.9, 2.0			
Glass distilled ozonate, 5 min., tygon tubing	54, 68	0, 1	3, 11	2, 0
Glass distilled ozonate, 30 min., tygon tubing	33, 42	0, 1	27, 25	0
Glass distilled ozonate, 5 min., glass tubing	127, 249, 188	0	2, 4	0
Glass distilled ozonate, 30 min., glass tubing	168, 227, 11	0	2, 0	0

TABLE A-3. Characteristics and Haloform Analysis of Algal Culture Media Prepared with and without EDTA.

	Cl_2 dose, mg/l	pH	TOC, mg/l	$CHCl_3$, µg/l
Media with EDTA	20	9.1	2.00	8200
	20	9.7	2.00	12200
	10	8.5	2.00	7300
	10	8.7	2.00	6400
	50	-	-	12700
Media without EDTA	20	9.2	0.40	376, 520
	10	8.9	0.40	360, 360

TABLE A-4. Total Organic Carbon Data of Stock Solutions for Preparing the Algal Culture Medium. Each Stock Solution was Diluted: 1 ml stock per ℓ glass-distilled water.

Stock Solution	TOC, mg/l
$Ca(NO_3)_2$	0.044
$NaNO_3$	0.128
K_2HPO_4	0.041
$MgCl_2 \cdot 6H_2O$	0.135
$MgSO_4$	0.009
$NaHCO_3$	0.206
Micronutrient Solution	0.098
H_3BO_3	0.050
$FeSO_4$	0.076

TABLE A-5. Results of Haloform Analysis of Filtrates of Algal Cultures Grown in Culture Medium Containing EDTA. Samples Dosed with 20 mg/l Chlorine.

Algal Species	Culture Age, days	TOC, mg/l	$CHCl_3$, mg/l	Log_{10} Cell Number
Chlorella pyrenoidosa	3	15.0	10.1	5.11
	7	11.0	10.2	6.50
	13	17.4	4.2	6.90
	29	21.4	1.9	7.12
Scenedesmus quadricauda	5	14.7	13.1	5.18
	8	16.3	4.3	5.56
	13	14.2	8.6	5.78
	21	97.0	9.7	6.33
Oscillatoria tenuis "1566"	8	16.9	10.5	5.14
	18	18.3	6.6	5.62
	21	28.5	7.9	6.11
Anabaena flos-aquae	4	13.5	10.4	5.54
	10	24.0	9.4	6.28
	20	37.1	1.8	6.90

TABLE A-6. Characteristics of Algal Culture Filtrates and Haloform Yields after Chlorination with 20 mg/l Chlorine

Algae Species	Culture Age, days	pH	Cell Count	TOC, mg/l	$CHCl_3$, µg/l	CCl_4 µg/l	$CHCl_2Br$ µg/l	$CHCl_3$-C TOC, %	mg TOC 10^6 cells	mg $CHCl_3$ 10^6 cells
Chlorella pyrenoidosa	6*	-	5.5×10^4	0.7	245	-	1.08	3.77	11.87	4.45
	13	9.13	1.66×10^6	3.0	1500			4.96	1.83	0.90
	16*	9.68	3.16×10^6	7.2	1100	0.08	2.17	1.534	1.62	0.25
	20	-	4.45×10^6	15.7	2290			1.465	4.97	0.73
	30	9.10	4.87×10^4	14.4	840			0.586	2.96	0.17
Scenedesmus quadricauda	6*	-	1.2×10^5	2.5	276	-	1.19	1.094	211.08	23.00
	9	9.3	1.3×10^5	15.4	6700			4.369	118.46	51.54
	13*	-	4.45×10^5	3.5	294	-	1.92	0.847	7.83	0.66
	19	-	8.6×10^5	5.2	880			1.713	5.93	1.01
	35	8.4	1.86×10^5	10.2	1900			1.876	5.47	1.02
Anabaena flos-aquae	6*	-	1.1×10^6	1.6	182	-	0.07	1.175	14.09	1.65
	9	10.1	1.22×10^6	12.7	5900			4.665	10.41	4.84
	13*	-	1.28×10^6	4.9	633	-	0.71	1.307	3.80	0.50
	20	9.4	6.3×10^6	22.6	100			0.044	11.3	0.05
	30	9.8	2.0×10^3	42.1	460			0.101	6.68	0.07
Oscillatoria tenuis "1566"	6*	-	8.0×10^4	1.7	152	-	0.87	0.925	206.25	19.00
	6	7.9	7.3×10^5	2.0	3100			15.66	27.23	42.47
	13*	-	9.64×10^5	7.6	303	0.03	0.60	0.400	7.88	0.31
	16*	-	5.0×10^5	12.0	2500	-	0.53	2.092	24.0	5.0
	20	8.7	7.5×10^5	14.8	3600			2.443	19.73	4.8
	37	7.8	1.13×10^6	28.3	190			0.067	25.04	0.17
Oscillatoria tenuis "GLET"	6*	-	2.5×10^4	1.9	246	0.04	1.71	1.328	74.4	9.84
	9**	9.4	1.6×10^5	179.3	9600			0.538	1120.63	60.00
	13*	-	5.5×10^5	8.2	551	0.14	3.81	0.672	14.96	1.00
	16	-	6.49×10^5	27.0	3600			1.339	41.60	5.55
	20*	8.6	5.5×10^5	7.5	754	0.06	0.71	1.008	13.66	1.37
	35	8.4	7.14×10^5	35.9	471			0.131	50.28	0.66

*Haloform analyses were by the liquid-liquid extraction procedure (101). All other haloform analyses by the Bellar-Lichtenberg procedure (100).
**Volatile organic carbon = 108.0 mg/l.

TABLE A-7. Free Chlorine Residual of Algal Culture Filtrates at Eight Days of Growth

Algae Species	Contact Time	Chlorine Dose, mg/l			
		4	8	12	16
Chlorella pyrenoidosa	2 hr.	0.4	5.0	8.5	10.8
	1 wk.	1.5	3.4	4.5	3.0
Scenedesmus quadricauda	2 hr.	1.7	5.0	8.7	11.4
	1 wk.	0	1.3	5.2	6.6
Anabaena flos-aquae	2 hr.	2.7	5.0	8.5	11.2
	1 wk.	0	1.8	5.1	6.7
Oscillatoria tenuis "1566"	2 hr.	2.2	5.5	8.7	12.0
	1 wk.	0	3.5	6.5	7.7
Oscillatoria tenuis "GLET"	2 hr.	2.0	5.2	8.8	11.4
	1 wk.	0	3.7	6.0	5.8

TABLE A-8. Chlorine Residual of Algal Culture Filtrates at 12 days of Growth. Residual readings Taken after 7 hrs. Contact.

Algae Species	pH	Chlorine Residual, mg/l	Chlorine Dose, mg/l									
			5	10	15	20	25	30	35	40	45	50
Chlorella pyrenoidosa	10.2	Free	0.8	2.5	6.5	-	14.5	-	18.5	-	30.0	-
		Total	3.5	4.5	10.0	-	17.4	-	21.6	-	35.0	-
Scenedesmus quadricauda	10.4	Free	1.7	5.0	8.9	-	18.6	-	30.5	-	36.8	-
		Total	3.0	6.5	12.3	-	20.8	-	34.5	-	41.5	-
Anabaena flos-aquae	10.5	Free	1.4	1.0	0.7	0.3	0.5	0.5	6.0	-	8.0	9.3
		Total	3.0	1.5	1.1	1.5	2.0	5.5	11.0	-	13.6	15.0
Oscillatoria tenuis "1566"	10.2	Free	1.0	0.7	0.7	0.7	0.5	1.5	1.5	4.5	10.5	14.5
		Total	1.5	1.5	1.2	1.0	0.9	2.0	2.5	6.2	12.5	17.5
Oscillatoria tenuis "GLET"	10.0	Free	0.7	2.2	6.3	-	15.0	-	23.5	-	32.5	-
		Total	1.2	3.6	8.8	-	18.5	-	27.5	-	37.0	-

TABLE A-9. Chlorine Residual of Algal Culture Filtrates at 17 days of Growth. Residual readings Taken after 7 hrs. Contact.

Algae Species	pH	Chlorine Residual, mg/l	Chlorine Dose, mg/l									
			5	10	15	20	25	30	35	40	45	50
Chlorella pyrenoidosa	9.5	Free	1.1	2.7	7.6	9.0	-	16.7	-	-	-	40.0
		Total	1.8	4.4	10.2	12.2	-	20.0	-	-	-	47.5
Scenedesmus quadricauda	10.0	Free	1.8	7.2	13.6	15.8	-	25.0	-	-	-	42.8
		Total	2.1	8.8	16.0	18.5	-	28.0	-	-	-	47.7
Anabaena flos-aquae	10.3	Free	0.3	0.5	0.7	0.3	0.7	1.2	-	2.0	-	10.0
		Total	1.0	1.0	1.7	2.5	2.3	3.2	-	4.5	-	12.5
Oscillatoria tenuis "1566"	8.7	Free	0.2	0.5	1.4	0.5	-	0.6	-	-	0.9	1.0
		Total	1.0	1.1	2.3	1.0	-	1.1	-	-	1.4	1.5
Oscillatoria tenuis "GLET"	9.2	Free	1.7	3.0	3.0	6.6	-	15.3	-	-	-	34.5
		Total	3.0	3.9	4.7	9.0	-	17.8	-	-	-	40.0

TABLE A-10. Free Chlorine Residuals of Algae Cell Suspensions (in Distilled Water) at 16 Days of Growth.

Algae Species	Contact Time, hours	Chlorine Dose, mg/l		
		8.36	20.9	41.8
Chlorella pyrenoidosa	24	0	0.4	6.2
	48	0	0	1.9
Scenedesmus quadricauda	24	0	0	0
	48	0	0	0
Anabaena flos-aquae	24	0	0.8	4.7
	48	0	0	1.2
Oscillatoria tenuis "1566"	24	0	2.4	5.3
	48	0	0.3	1.4

TABLE A-11. Variations in pH with the Algal Culture Age.

Algae Species	Culture Age, days				
	1	4	8	12	19
Chlorella pyrenoidosa	8.6	8.7	9.6	10.2	9.5
Scenedesmus quadricauda	8.5	8.5	9.9	10.4	10.0
Anabaena flos-aquae	8.6	9.15	10.3	10.5	10.3
Oscillatoria tenuis "1566"	8.75	8.7	10.5	10.2	8.7
Oscillatoria tenuis "GLET"	8.4	8.7	9.6	10.0	9.2

TABLE A-12. Raw Total Organic Carbon Data of Algal Cell Suspensions (in Distilled Water).

Algae Species	Culture Age, days	TOC, mg/l
Chlorella pyrenoidosa	13	2.890, 2.950
	20	18.7, 19.1
	16	15.0, 15.5
	30	11.9, 15.0, 12.6
Scenedesmus quadricauda	9	14.5, 19.8, 23.4, 30.8, 15.1
	13	31.7, 33.1, 36.8, 31.6, 33.6
	19	52.2, 52.6
	35	80.3, 73.7, 73.4
Anabaena flos-aquae	9	12.2, 12.2
	20	24.0, 24.3
	30	34.0, 30.9, 27.2
	13	17.9, 16.7, 17.5, 17.1
Oscillatoria tenuis "1566"	6	2.540, 1.880, 1.935
	16	11.7, 11.2
	20	24.2, 19.9, 26.4
	37	23.3, 17.4, 19.4
Oscillatoria tenuis "GLET"	9	12.8, 8.5, 16.1, 19.8
	16	18.4, 20.4, 18.0
	20	8.1, 10.3, 16.0, 8.0
	30	25.1, 16.3, 12.6, 19.4, 17.0

TABLE A-13. Raw Total Organic Carbon Data of Algae Culture Filtrates

Algae Species	Culture Age, days	TOC, mg/l
Chlorella pyrenoidosa	6	0.595, 0.710
	13	2.940, 3.130
	16	7.050, 7.355
	20	15.8, 15.5, 15.8
	30	13.1, 17.1, 12.9
Scenedesmus quadricauda	6	2.835, 2.220
	9	15.3, 15.9, 15.0
	13	3.545, 3.425
	19	5.270, 5.050
	35	9.250, 10.185, 10.59, 10.66
Anabaena flos-aquae	6	1.635, 1.475
	9	13.8, 10.0, 14.3
	13	5.305, 4.420, 4.470
	20	29.1, 18.4, 20.3
	30	38.8, 44.5, 43.1
Oscillatoria tenuis "1566"	6	1.665, 1.640
	13	7.6
	6	2.060, 1.505, 2.380, 2.045
	16	12.2, 11.0, 12.5
	20	14.9, 14.8
	37	27.1, 30.9, 26.9
Oscillatoria tenuis "GLET"	6	1.845, 1.875
	9	179.5, 179.0
	13	8.220, 7.300, 9.165
	16	27.2, 24.8
	20	7.015, 8.015
	35	28.3, 33.6, 35.7

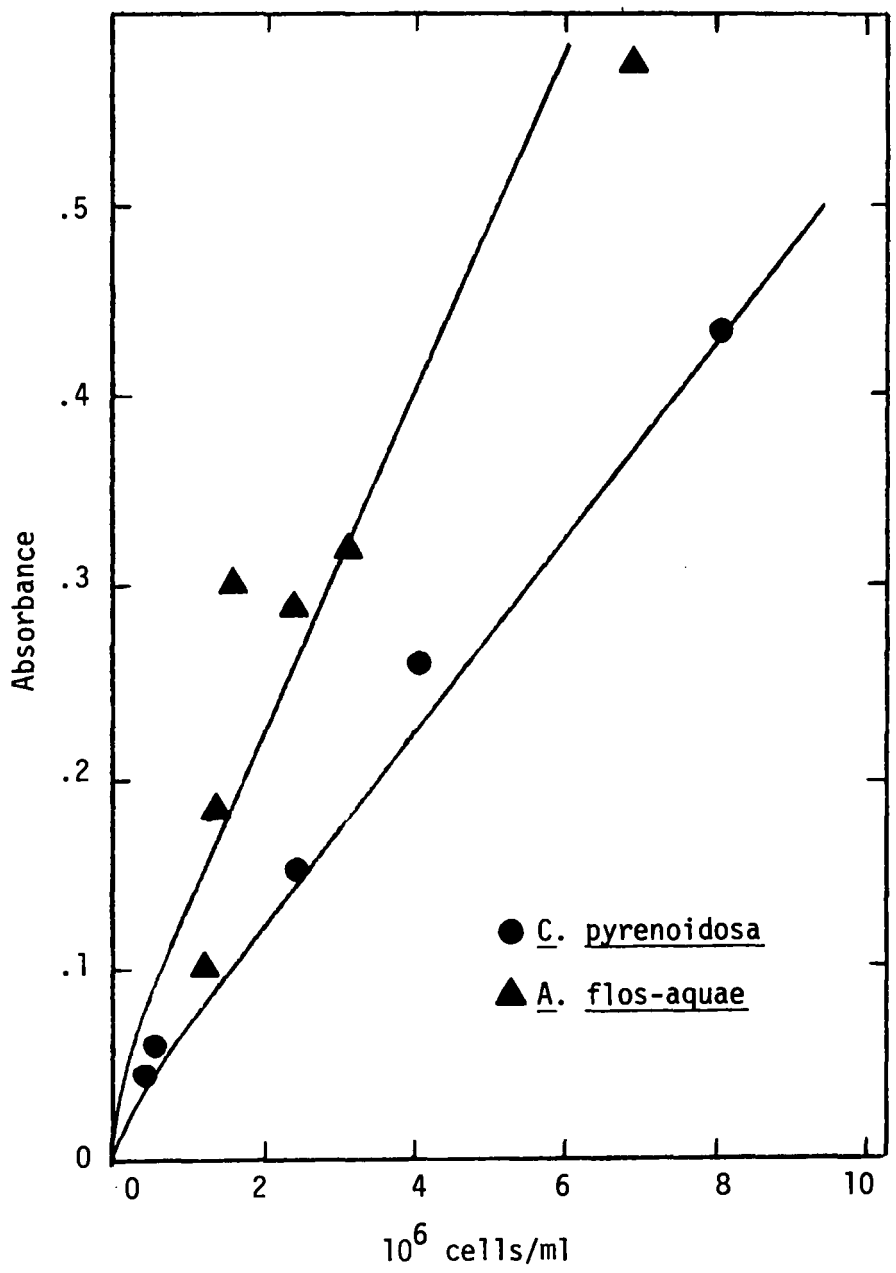

FIGURE A-1. Variations in population density of Chlorella pyrenoidosa and Anabaena flos-aquae as a function of absorbance. Absorbance readings taken at 680 nm. Least squares fit - due to clustering, all data not included.

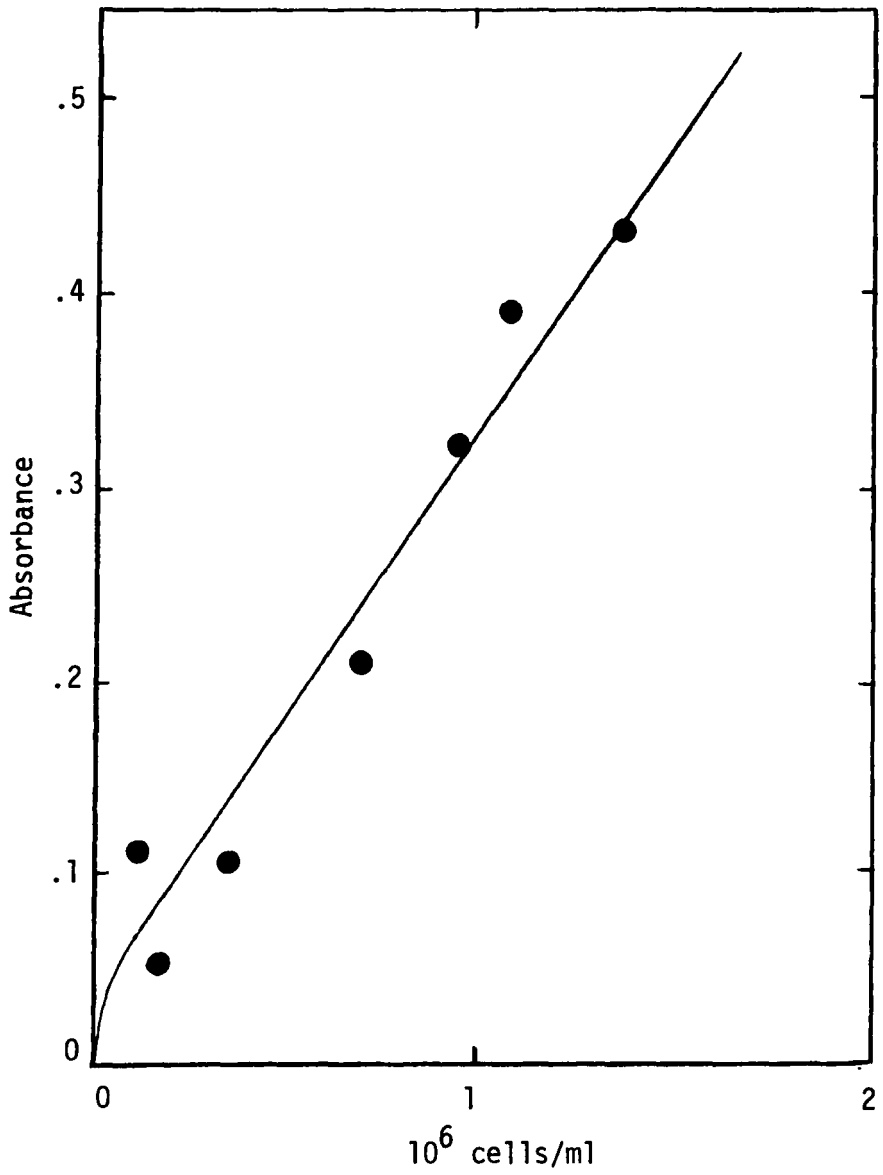

FIGURE A-2. Variations in population density of Scenedesmus quadricauda as a function of absorbance. Absorbance readings taken at 680 nm. Least squares fit - due to clustering, all data not included.

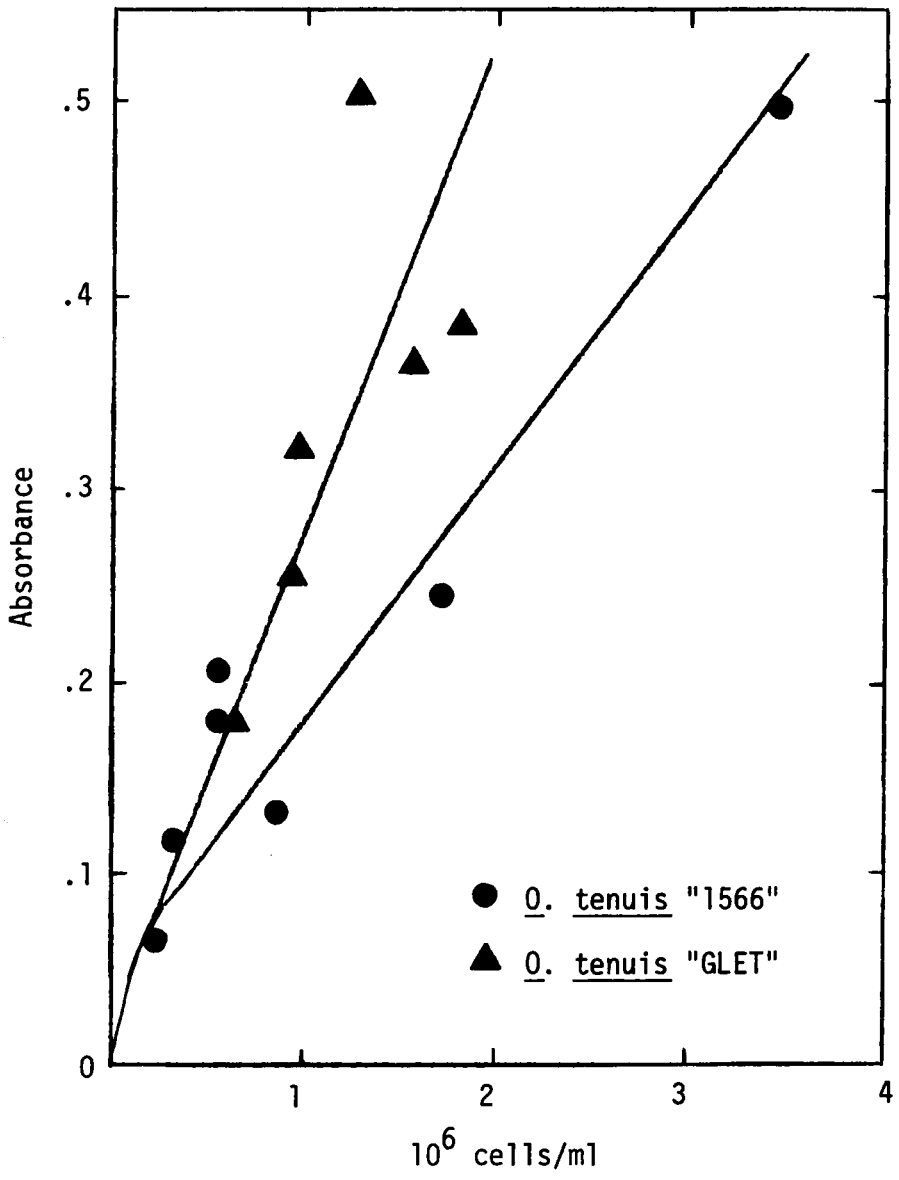

FIGURE A-3. Variations in population density of Oscillatoria tenuis "1566" and Oscillatoria tenuis "GLET" as a function of absorbance. Absorbance readings taken at 680 nm. Least squares fit - due to clustering, all data not included.

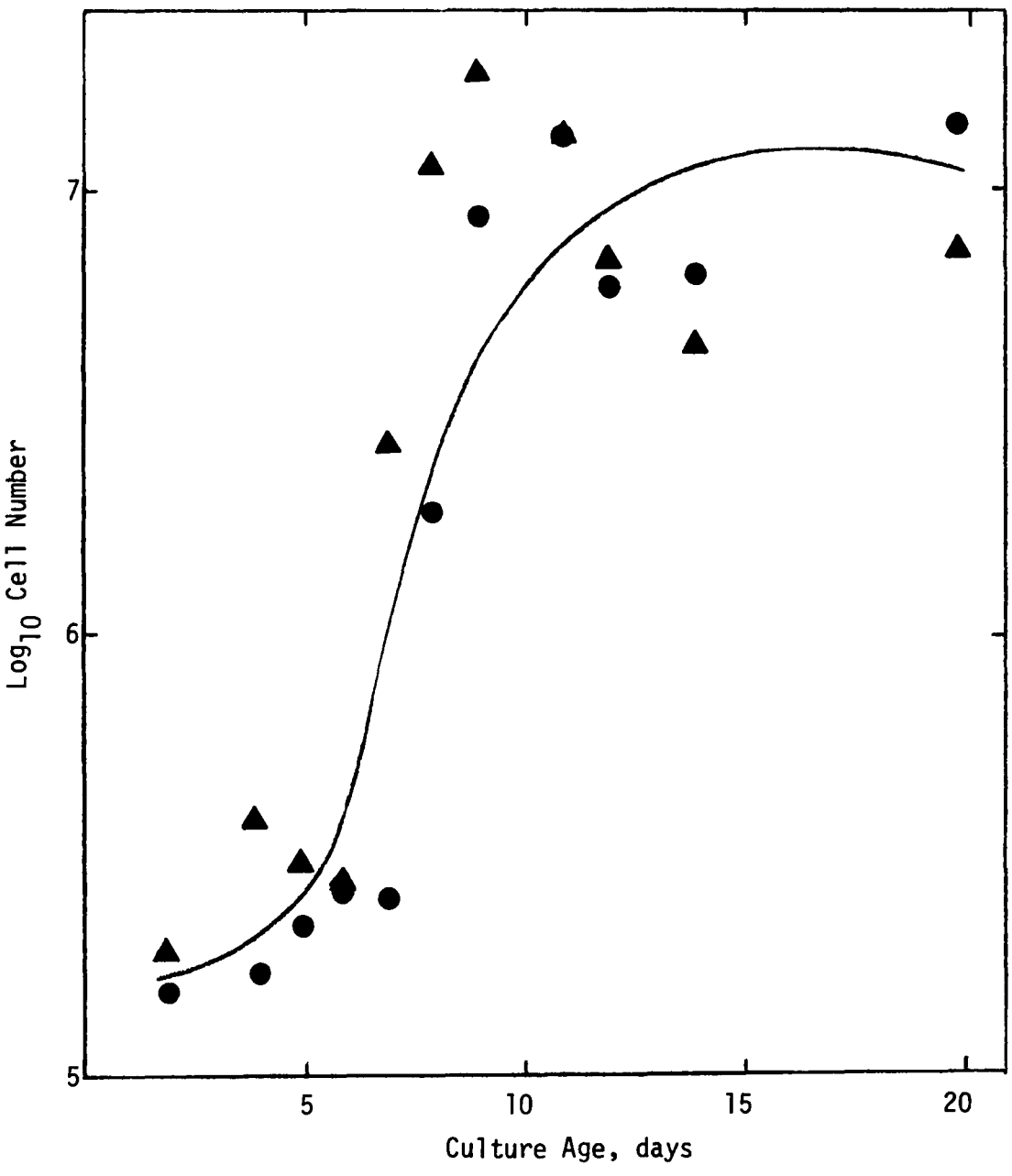

FIGURE A-4. Growth curve for Chlorella pyrenoidosa. Symbols represent two separate cultures.

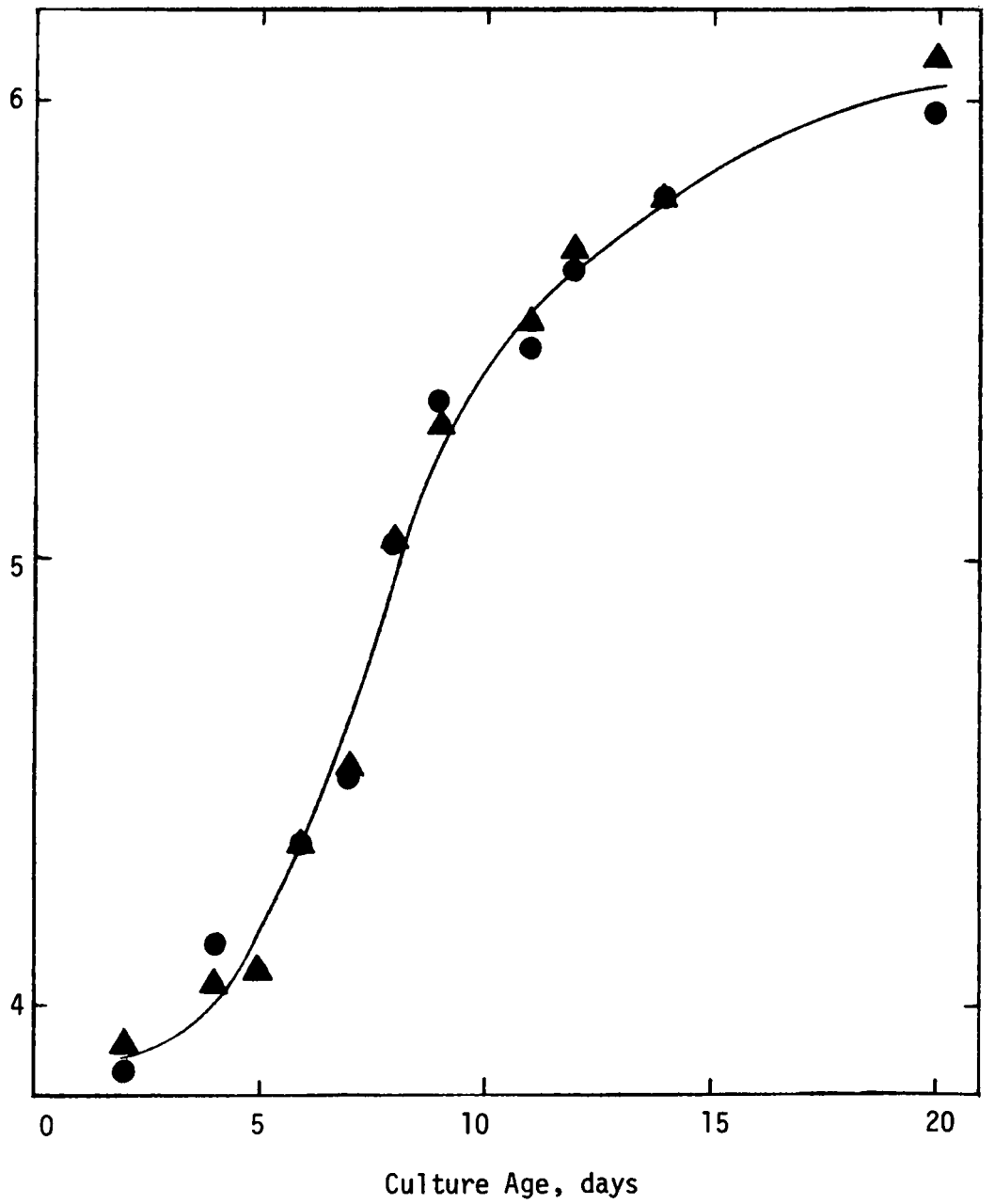

FIGURE A-5. Growth curve for Scenedesmus quadricauda. Symbols represent two separate cultures.

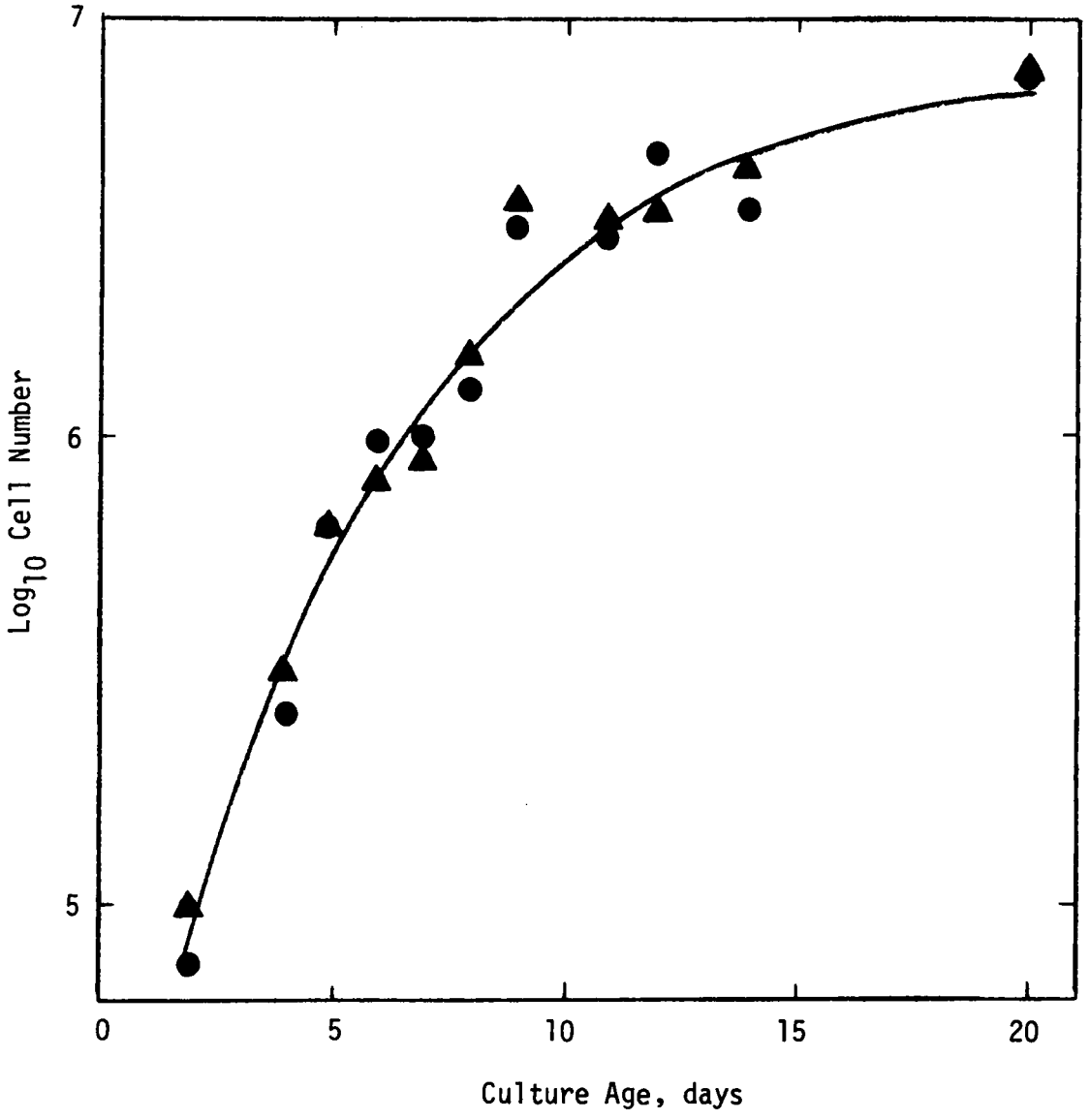

FIGURE A-6. Growth curve for Anabaena flos-aquae. Symbols represent two separate cultures.

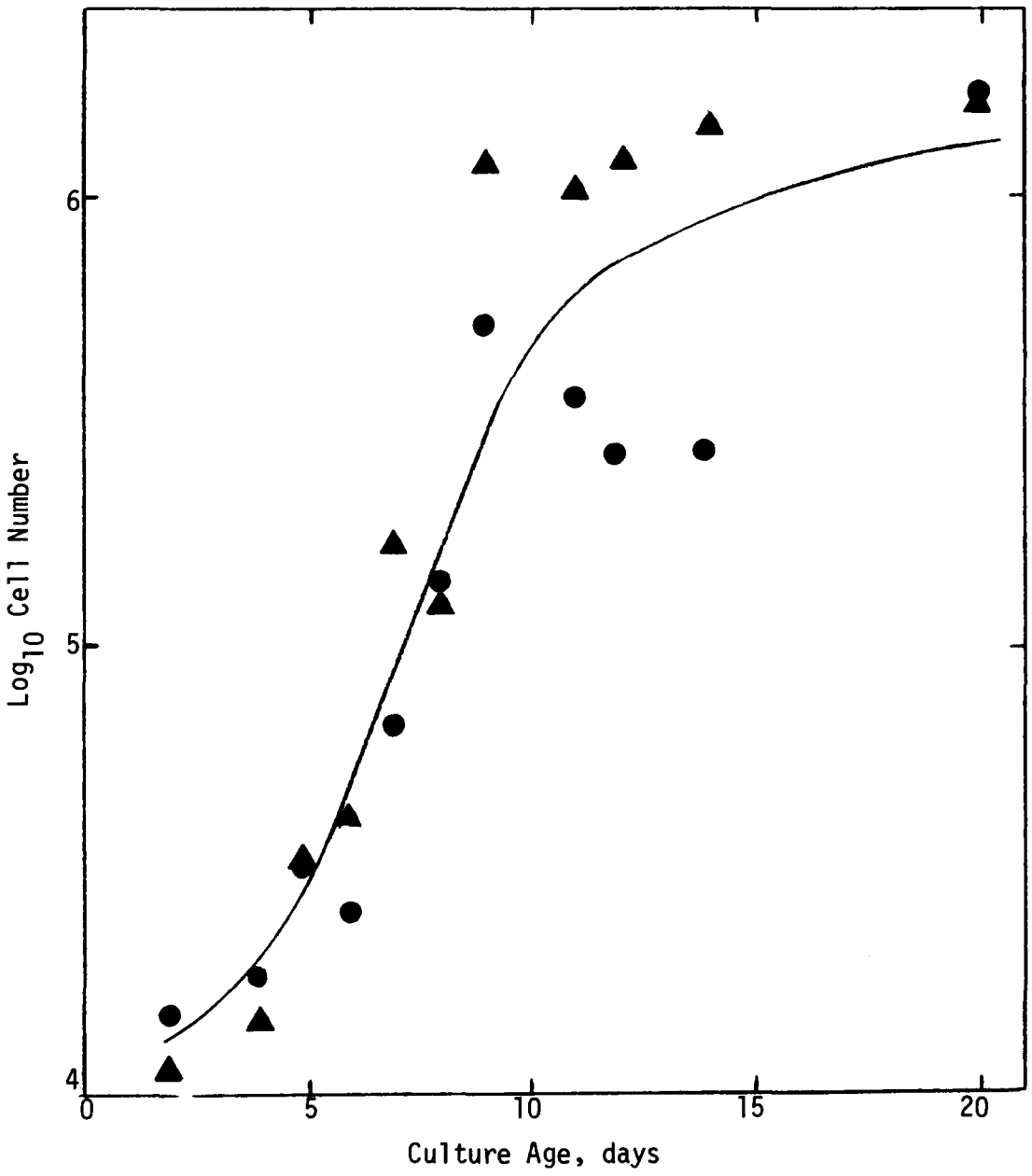

FIGURE A-7. Growth curve for Oscillatoria tenuis "1566". Symbols represent two separate cultures.

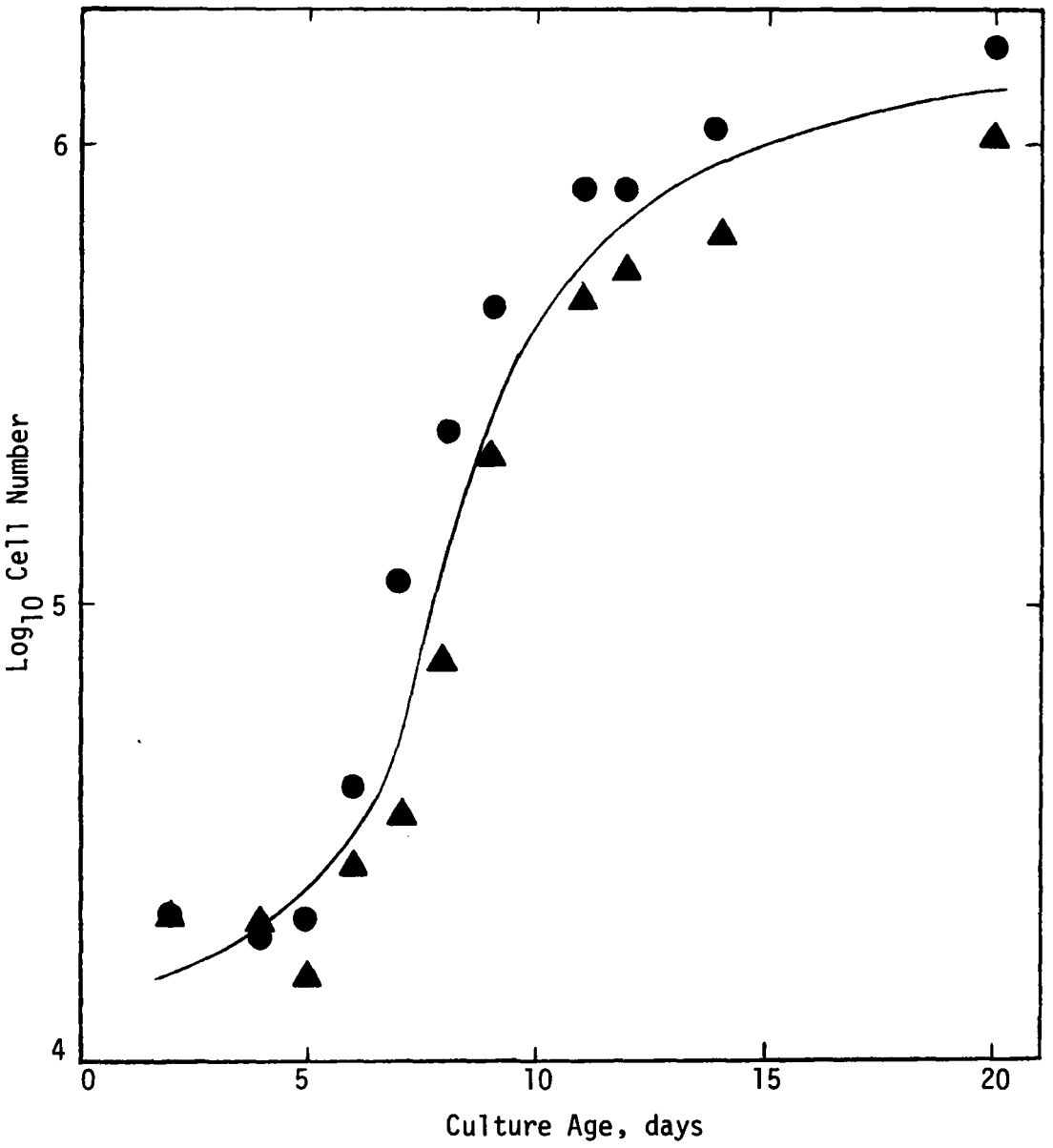

FIGURE A-8. Growth curve for Oscillatoria tenuis "GLET". Symbols represent two separate cultures.

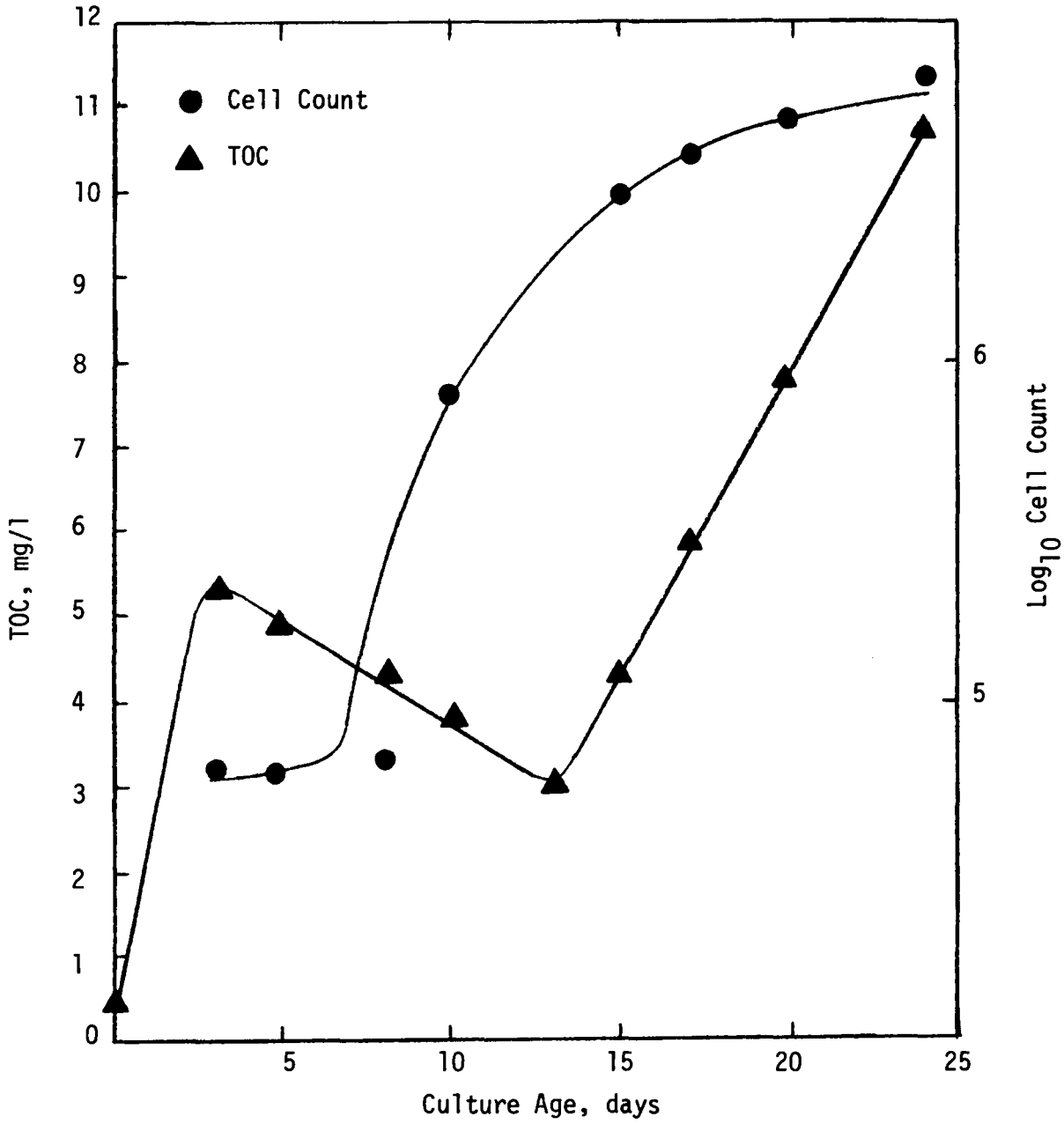

FIGURE A-9. Variations in extracellular organic carbon (as TOC) and cell count as a function of the culture age for Chlorella pyrenoidosa.

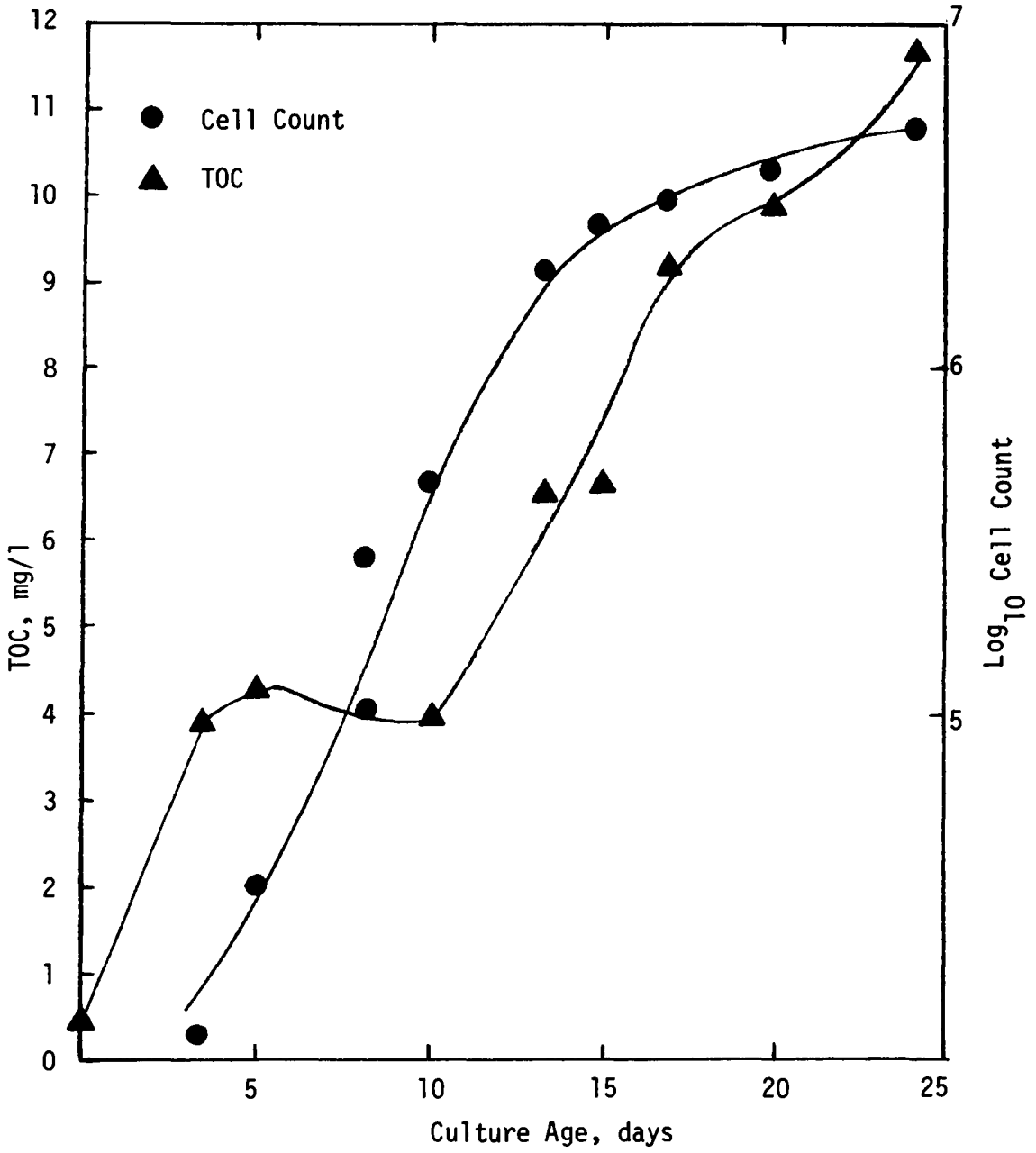

FIGURE A-10. Variations in extracellular organic carbon (as (TOC) and cell count as a function of the culture age for Scenedesmus quadricauda.

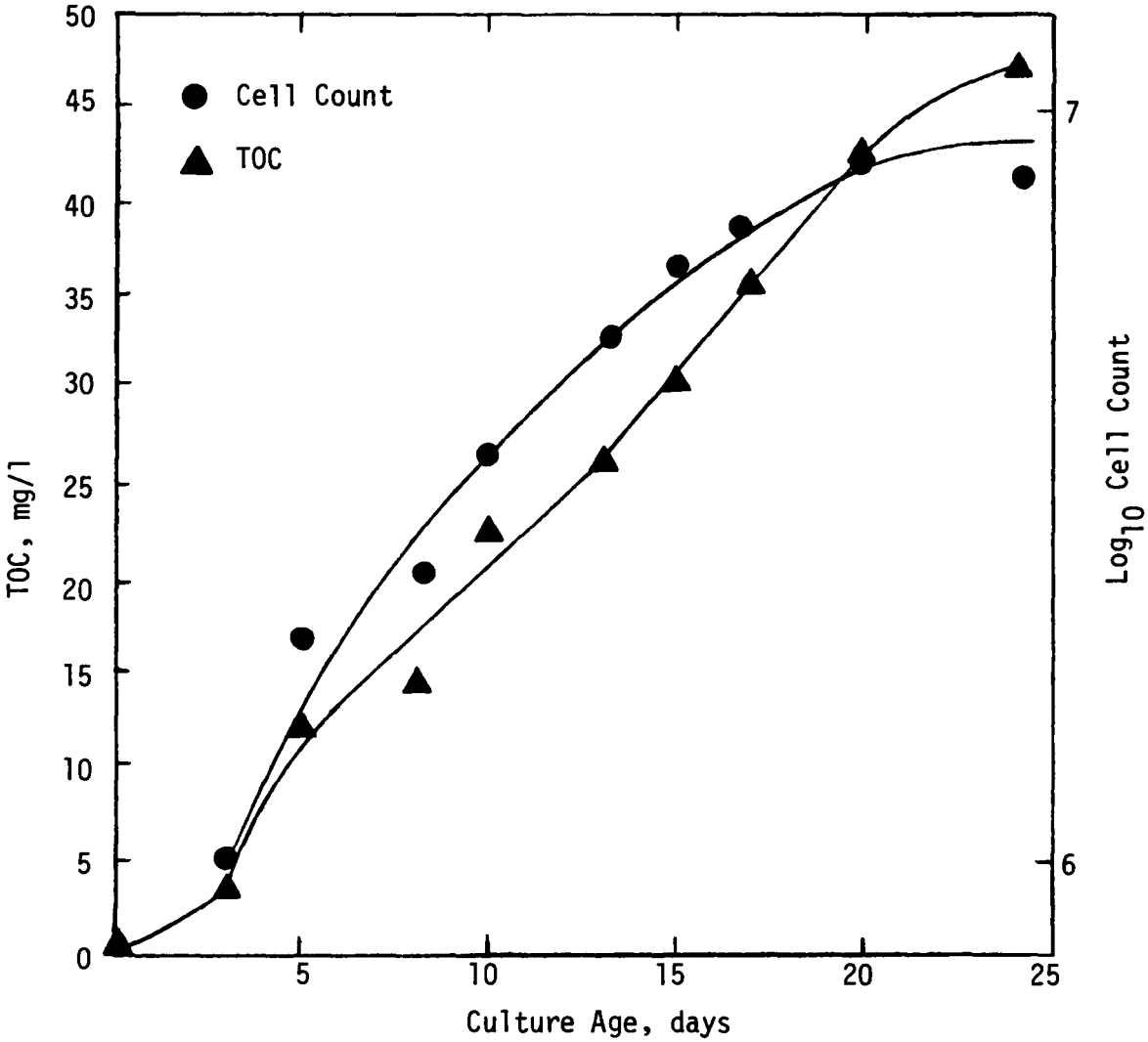

FIGURE A-11. Variations in extracellular organic carbon (as TOC) and cell count as a function of culture age for Anabaena flos-aquae.

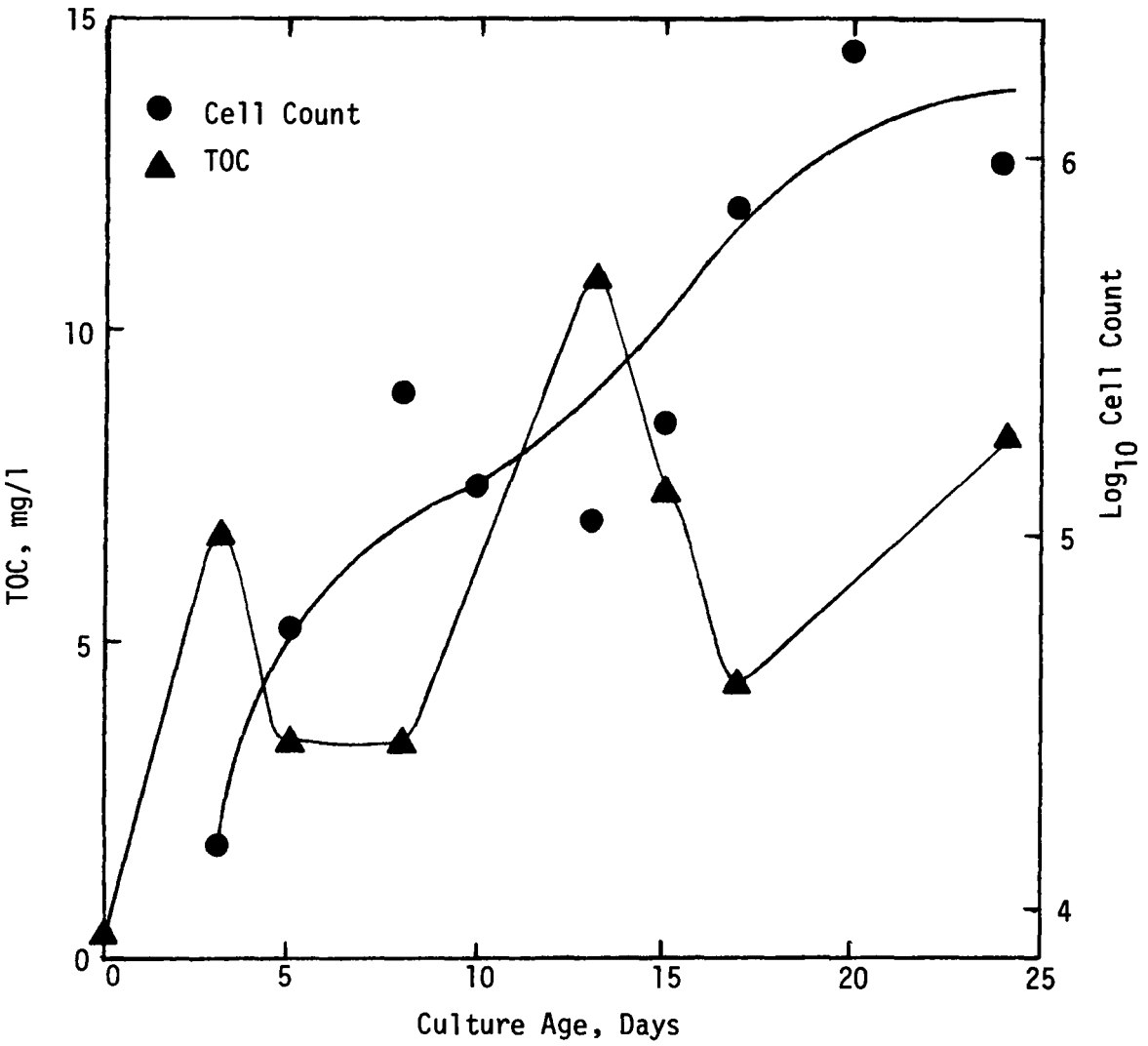

FIGURE A-12. Variations in extracellular organic carbon (as TOC) as the culture ages for Oscillatoria tenuis "1566".

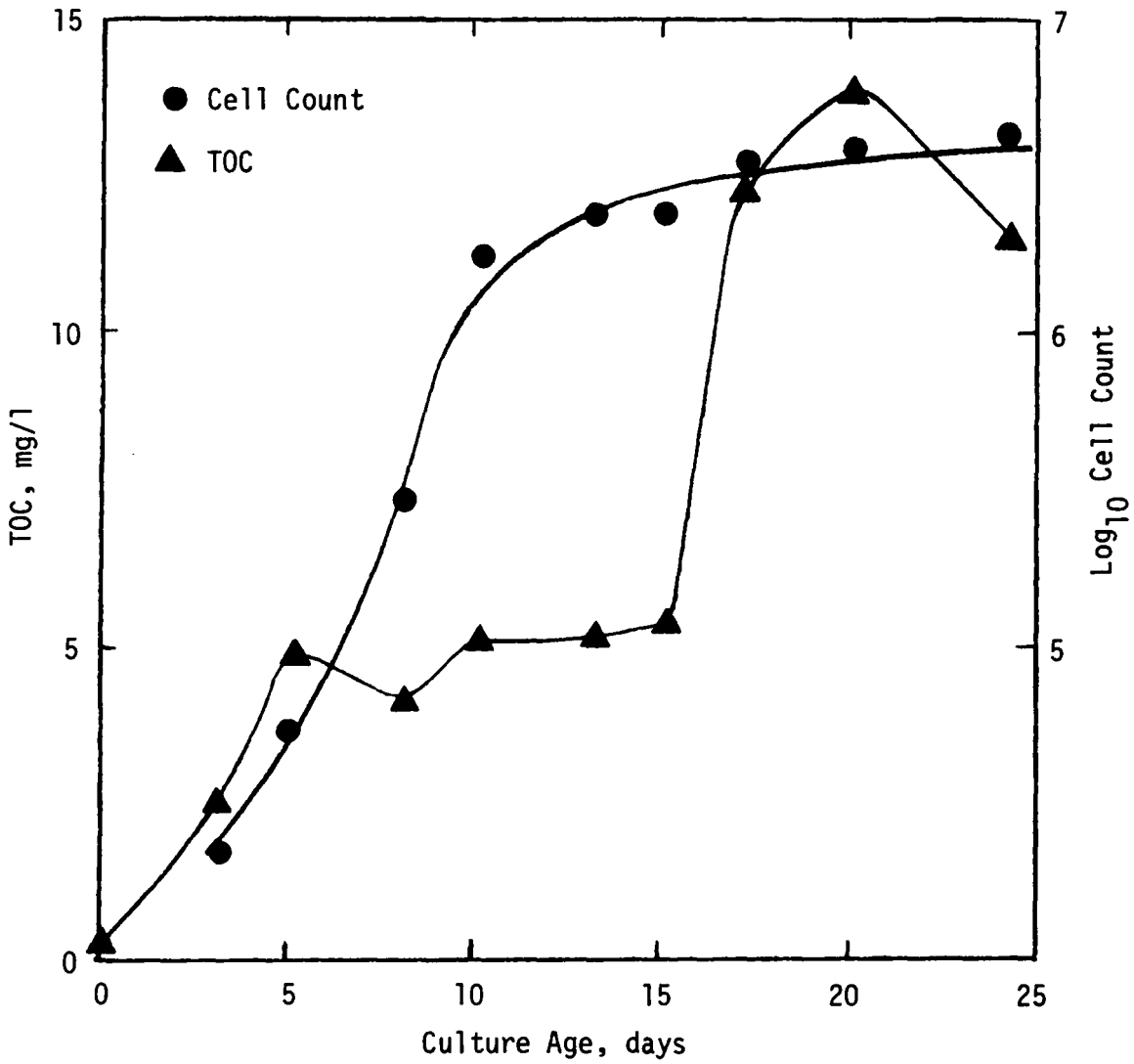

FIGURE A-13. Variations in extracellular organic carbon (as TOC) and cell count as a function of culture age for Oscillatoria tenuis "GLET".

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TRIHALOMETHANE FORMATION POTENTIAL OF ALGAL EXTRACELLULAR PRODUCTS AND BIOMASS

by

Barbara Craddock Thompson

(ABSTRACT)

A study was conducted to determine the importance of algae as sources of orgnanic precursors in the haloform reaction. Two species each of green and blue-green algae were grown in laboratory conditions and harvested periodically during their growth cycle. The principal part of the study was directed toward determining the patterns of extracellular products excretion into the medium and of the chloroform produced after chlorination. Similar studies of cell suspensions were conducted. The study also included an analysis of the chloroform yields from chlorinated reservoir sediments and pure compounds known to be algal extracellular metabolites.

The results indicated that the extracellular products of growing algal cells have the greatest potential as haloform precursors and that the greatest excretion of high chloroform-yielding compounds generally occurred during exponential growth. The blue-green algae were found to liberate more organic carbon than the green algae and produced higher chloroform concentrations upon chlorination. However, the green algae generally showed excretion of higher chloroform-yield compounds.

The chloroform yields per unit organic carbon for the algal extracellular products often exceeded those reported for the humic substances and, therefore, could be considered of equal or greater importance as

haloform precursors under certain conditions. Yields from solutions of pure compounds and from the sediments were significantly lower than those from the extracellular products and biomass of growing algal cells. Increasing the pH of the solutions of pure compounds resulted in higher chloroform yields, a result that has been demonstrated by others.