Characteristics and Reactivity of Algae-Produced Dissolved Organic Carbon

My-Linh Nguyen[1]; Paul Westerhoff, P.E.[2]; Lawrence Baker[3]; Qiang Hu[4]; Mario Esparza-Soto[5]; and Milton Sommerfeld[6]

Abstract: Algae (green, blue–green, and diatom) grown in inorganic media produced particulate and dissolved organic carbon (DOC). DOC produced by a green-alga contains 25% hydrophobic acids. DOC from all algae had specific ultraviolet absorbance values less than 2.0 m^{-1} $(mg/L)^{-1}$. Algae-produced DOC was biologically labile; greater than 60% degraded in bioreactors within 5 days. The biodegradable material likely included carbohydrates, amino acids, and amino sugars, which were present in hydrophobic acid isolates. Chlorination of algal DOC formed disinfection by-products; DOC from the green alga, *Scenedesmus quadricauda*, produced chloroform [0.53 micromole per mg carbon (μmol/mg C)], dichloroacetic acid (0.27 μmol/mg C), and trichloroacetic acid (0.14 μmol/mg C). This work complements other studies, which focused on algal total organic carbon (DOC and cellular material), and clearly demonstrates the importance of identifying algae-derived sources of DOC in water supplies and removing such DOC in water treatment plants prior to chlorination.

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Introduction

Algal growth often impairs surface water supplies used for drinking water by: (1) contributing to total organic carbon (TOC) and turbidity (e.g., algae cells), (2) producing taste and odor compounds, and (3) contributing precursors, which forms disinfection by-products (DBPs) upon chlorination, such as trihalomethanes (THMs) and haloacetic acids (HAAs). Autochthonous, algal-derived, dissolved organic carbon (DOC) can be a significant source of DBP precursors for drinking water treatment facilities. Although several studies have evaluated chlorination of TOC produced by algae, including particulate cell material, limited information exists on different types of algae, and on characteristics of DOC and amount and type of DBP production during chlorination.

Algal extracellular organic matter (EOM) and algal biomass (cells) have been shown to produce THMs (e.g., chloroform) following chlorine reaction. THM yields ranged from 0.23 to 3.2 μmol $CHCl_3$/mg TOC (μmol/mg C) (Wardlaw et al. 1991). Chloroform yields may vary depending on chlorine contact time, algal specie, and growth phase. Hoehn et al. (1980) studied THM formation of two green algae and two blue–green algae using chlorine contact times up to 24 h. The results suggested that chloroform yields for algal EOM (0.034–4.2 μmol/mg C) was as significant as those for algal biomass, with highest yields in the late exponential growth phase (Hoehn et al. 1980). Using a chlorine contact time of 24 h, THMs produced by intact algal cells and EOM from cultures of blue–green algae *Anabaena cylindrica* were approximately equal (Briley et al. 1980). With longer contact time (e.g., 7 days), data from Plummer and Edzwald (2001) showed that chlorination of algal cells (*Cyclotella* sp.) produced higher DBP level (>40 μg $CHCl_3$/L or 0.33 μM) than extracted EOM (<20 μg $CHCl_3$/L or 0.17 μM), although TOC data for the biomass were not available for yield comparison. Longer chlorine contact times may lead to cell lysis and release of intracellular DOC that contributed to higher DBP production. Results from the same study also showed that chloroform yields from EOM for the green alga *Scenedesmus quadricauda* (0.17 μmol/mg C) was lower than that for the diatom *Cyclotella* sp. (0.42 μmol/mg C). Because different methods were used to grow algae and extract EOM, it would be difficult to compare and differentiate DBP formation attributable to particulate cell materials versus DOC produced during growth for different studies and among different species.

During drinking water treatment, disinfectant addition usually occurs after particle removal processes. As such, chlorine reac-

[1]PhD, Project Manager, Awwa Research Foundation, 6666 W. Quincy Ave., Denver, CO 80235 (corresponding author). E-mail: mnguyen@awwarf.org

[2]PhD, Associate Professor, Dept. of Civil and Environmental Engineering, ECG 252, Arizona State Univ., Tempe, AZ 85287. E-mail: p.westerhoff@asu.edu

[3]PhD, Senior Fellow, Minnesota Water Resource Center, 173 McNeil Hall, 1985 Buford Ave.; St. Paul, MN 55108. E-mail: bakerenv@earthlink.net

[4]PhD, Assistant Professor, Dept. of Applied Biological Sciences, WAN 340A, Arizona State Univ. East, 7001 E. Williams Field Rd., Tempe, AZ 85212. E-mail: huqiang@asu.edu

[5]PhD, Professor-Researcher, Centro Interamericano de Recursos del Agua, Univ., Autónoma del Estado de México, Carretera Toluca-Atlacomulco km 14.5. E-mail: marioesparza@uaemex.mx

[6]PhD, Associate Dean, College of Liberal Arts and Sciences, SS 109, Arizona State Univ., Tempe, AZ 85287. E-mail: Milton.Sommerfeld@asu.edu

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tions with algal derived DOC become more important than reactions with intact algal cells. Further, the presence of algal EOM could impact water treatment processes. Paralkar and Edzwald (1996) showed that algal EOM increased coagulant demand. The large molecular weight polysaccharide fractions of EOM have been primarily implicated in this study. Widrig et al. (1996) found that coagulation poorly removed the organic nitrogen fragments (presumably EOM) monitored by pyrolysis gas chromatography mass spectrometry (PY-GC-MS). Understanding the nature of algal EOM will help elucidate the behavior of DOC during water treatment and may improve the effectiveness of DBP precursor removal.

The purpose of this paper is to compare the normalized production rates, structural characteristics, chlorine reactivity, and biological lability of DOC generated by three types of algae: green algae, blue–green algae, and diatoms. Algae cultures were grown in laboratory reactors with a bromide-free, inorganic growth media for periods of 3 to 7 days. DOC production rates were determined, and algal-produced DOC was analyzed to determine chemical characteristics and reactivity with chlorine to form DBPs. Larger-volume cultures were used to isolate and characterize fulvic acids (i.e., hydrophobic acid fraction). The structure and reactivity of the isolated fulvic acid from the algal culture was compared against fulvic acids previously isolated from different source waters.

Methods

Phase I

This study utilized three algal species: A diatom isolated from Phoenix metropolitan surface water supply, *Chaetoceros muelleri,* and two strains from the University of Texas (Austin, Tex.) Culture Collection of Algae, *Oscillatoria prolifera* (a blue–green alga, UTEX No. 1270) and *Scenedesmus quadricauda* (a green alga, UTEX No. 76). Algal species were cultured in a laboratory apparatus [Fig. 1] that contained six glass culture tubes (4.5 cm inner diameter × 60 cm: 950 mL liquid volume) suspended in a constant temperature bath (26±1 °C). Fluorescence lamps provided illumination of 95±5 μmol m^{-2} s^{-1}. Filtered, sterilized [GelmanVersapor (Pall Gelman Sciences Inc., New York) 0.45 μm membrane] and humidified air was bubbled through the tubes to provide CO_2 and mixing.

All three algal species were grown in an inorganic growth medium (pH 8.0) with TOC < 0.2 mg/L (Hoehn et al. 1980). Stock nutrient, glassware, and equipment were sterilized by autoclaving at 121°C and 15 psi for 30 min. Algal growth was monitored daily by optical density at 730 nm (OD_{730}) (Shimadzu UV/Vis 1601 Spectrophotometer). Chlorophyll *a* was measured according to *Standard Method* 10200H (APHA/AWWA/WEF 1995) using the same spectrophotometer. Linear correlations were developed between OD_{730} and dry algal biomass, and between OD_{730} and chlorophyll *a* for each species (Table 1). Glassware for DOC analysis was acid-washed and ashed at 550°C. DOC was defined as filtrate passing an ashed (550°C) Whatman GF/F filter (~0.7 μm). Triplicate samples (60 mL each) were collected and filtered (550°C ashed GF/F) and stored in the dark at 4°C for subsequent DOC, ultraviolet absorbance (or UV absorbance) (UVA), and disinfection by-product (i.e., THMs and HAAs) formation potential analyses.

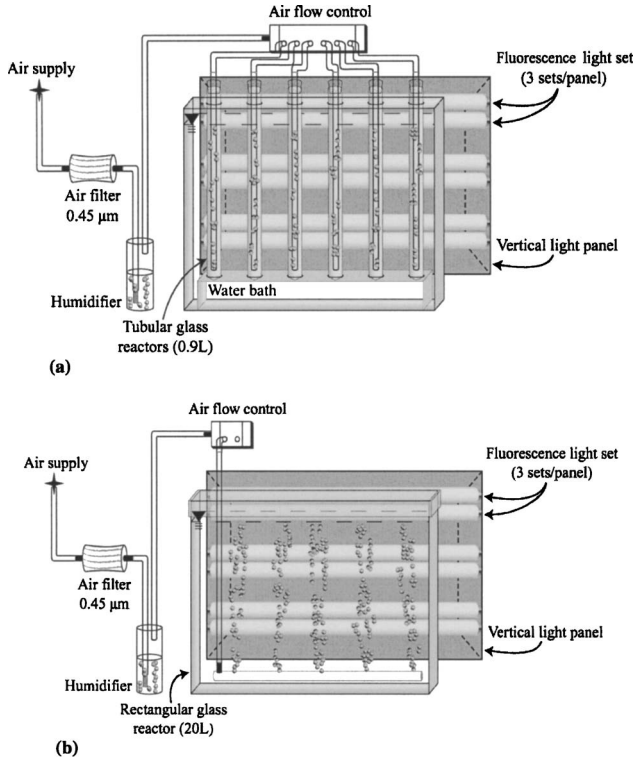

Fig. 1. Apparatus for algae experiments: (a) Phases I–small batch reactor tubes (4.5 cm ID × 60 cm L per tube); and (b) Phases II–large volume ractor(67 cm L × 10 cm W × 45 L per tube).

Phase II

A large quantity of algae-metabolite DOC was produced for structural characterization. The green alga (*Scenedesmus*) was grown in two 20 L glass tanks using the same growth medium as in Phase I. The narrow width of the reactor (10 cm) assured light penetration throughout the experiment [Fig. 1(a)]. To increase volume, nutrient medium was added at intervals of several days until day 17, when nutrient addition was discontinued and measurement began. OD_{730} was measured to monitor growth. Samples were taken at various stages of growth (days 17, 19, 22, 27, and 37) to measure DOC concentration, THM and HAA formation potential, and biodegradable DOC ($BDOC_5$). On day 37 (stationary stage, OD_{730}=1.1 cm^{-1}), the culture in the two tanks (~40 L) was harvested. After the culture settled for 2 h, the supernatant was filtered using Balston Whatman Glass Fiber filters, DH/AH, which are capable of removing >99.9% of 1 μm par-

Table 1. Linear Relationships (Slopes) between the Optical Density at 730 nm and Other Biomass Indicators

Algae species	Dry mass: optical density ratio [(mg/L)/cm^{-1}]	Chlorophyll *a*: optical density ratio [(μg/L)/cm^{-1}]	Dry mass: chlorophyll *a* ratio (mg/μg)
Scenedesmus quadricauda	647 (r^2=1.00)	154 (r^2=0.95)	4.10 (r^2=0.95)
Oscillatoria prolifera	840 (r^2=0.97)	250 (r^2=0.98)	3.14 (r^2=0.97)
Chaetoceros mulleri	960 (r^2=0.99)	5,190 (r^2=0.99)	0.199 (r^2=0.99)

Table 2. Summary of the Characteristics and Reactivity of Bulk Algal Dissolved Organic Carbon (DOC)

	Phase I			Phase II
Parameters	Scenedesmus quadricauda	Oscillatoria prolifera	Chaetoceros mulleri	Scenedesmus quadricauda
SUVA [m^{-1}/(mg/L)]	1.5±0.5	0.8±0.1	1.0±0.4	1.1±0.3
THM:DOC (μg/mg)	48±12	30±4.3	30±6.6	64±14
HAA:DOC (μg/mg)	NA	NA	NA	60±7.7
$DOC:OD_{730}$ [(mg/L)/cm^{-1}]	3.3±3.7	16±6.6	18±10	2.9±0.7
$THM:OD_{730}$ [(μg/L)/cm^{-1}]	150±120	510±230	510±340	180±31
$HAA:OD_{730}$ [(μg/L)/cm^{-1}]	NA	NA	NA	180±60
$BDOC_5$ (%)	NA	NA	NA	62±6.0

Note: SUVA=specific ultraviolet absorbance; THM=trihalomethane; HAA=haloacetic acids; OD_{730}=optical density at 730 nm; $BDOC_5$=biodegradable DOC; and NA=not applicable.

ticulate cellular material. The acidified (pH 2.00±0.05) filtrate was isolated by adsorption chromatography using macroporous resins (XAD-8) (Aiken et al. 1992). The hydrophobic acid fraction was obtained from elution of the XAD-8 resins with 0.1 N NaOH, and the eluent was desalted (to remove Na^+) by passing it through H^+-saturated cation exchange resins. The resulting concentrate was lyophilized for further analyses.

Analyses

DOC was analyzed with a Shimadzu TOC 5050A carbon analyzer, and UV absorbance of DOC at 254 nm (UVA_{254}) was measured with a Shimadzu UV/Vis 1601 Spectrophotometer. Specific absorbance (SUVA) was calculated as UVA_{254} divided by DOC.

Fluorescence

Fluorescence measurements were conducted with a Perkin Elmer LS 50B fluorescence instrument and reported in arbitrary units (AU). Samples were prepared for fluorescence analysis by dilution with 0.1 N KCl to adjust DOC concentrations to ~1 mg/L (to allow direct comparisons of fluorescence intensities) and acidification to pH 3. Excitation-emission matrices (EEM) were collected according to the method presented in Westerhoff et al. (2001) and Chen et al. (2003).

Structural and Elemental Analyses

XAD-8 isolates were subject to solid-state ^{13}C nuclear magnetic resonance (NMR) and elemental (CHN) analyses (Perkin-Elmer 2400 Series II CHNS/O Analyzer). Suwannee River fulvic acid (SRFA), the standard aquatic fulvic acid of the International Humic Substances Society, was analyzed for comparison. Combustion at 750°C for 2 h was performed to determine the ash content of the DOC isolates (Reddy et al. 1989), and all elemental data are reported on an ash-free basis. Solid-state ^{13}C-NMR analysis of DOC isolates was conducted according to Drewes et al. (1999) using a Varian Inova plus 400 NMR spectrometer at a frequency of 100.59 MHz (on a 4 mm Jakobson Mas probe). Hexamethylbenzene (17.3 ppm) was used as a reference standard.

Disinfection By-Product Methods

Samples for THM and HAA formation potentials were buffered at pH 7.0, then chlorinated with NaOCl at a chlorine:DOC ratio of 5:1 (mg Cl_2:mg C) and incubated in the dark at 20°C for 7 days. The dosage and residual of chlorine (as Cl_2) were measured spectrophotometrically according to the DPD *Standard Method* 4500-CI (APHA/AWWA/WEF 1995). Residual chlorine was quenched with sodium thiosulfate ($Na_2S_2O_3$) for THM analyses and with ammonium chloride (NH_4Cl) for HAA analyses. THM samples were analyzed according to U.S. EPA Method 551.2 using a HP 5890 Series II Gas Chromatograph. HAA samples were analyzed (U.S. EPA Method 502.2) at the Water Service Laboratory in the City of Phoenix Water Services Department.

Biodegradable Dissolved Organic Carbon Method

Biodegradable DOC was quantified according to the simplified BDOC method proposed by Allgeiers et al. (1996). In each 250 mL ashed (550°C) amber bottle, 100 mL of sample was added to 20 mL of biologically active sand. The bottles (reactors) were kept in the dark for 5 days at room temperature (25–27°C) with constant shaking to improve oxygen and DOC mass transfer. DOC samples were collected and filtered (GF/F) immediately after inoculation (DOC_0) and at the end of the five-day incubation period (DOC_5). The difference between DOC_0 and DOC_5 is the amount of biodegradable DOC ($BDOC_5$).

Results

Summary of the production, characteristics and reactivity of bulk and resin isolated algal-DOC from Phase I and Phase II experiments are provided in Tables 2 and 3. The following sections present separately the results for the two experimental phases.

Phase I Algae Experiments

Algae Growth Kinetics

Correlations between OD_{730}, chlorophyll *a*, and dry biomass weight are summarized in Table 1. Algae growth exhibited lag, log, and stationary growth phases [Fig. 2(a)]. Based on OD_{730}, logarithmic-phase specific growth rates were 0.016, 0.026, and 0.048 h^{-1} for the green, blue–green, and diatom species, respectively. During the logarithmic growth phase, the green alga produced more biomass per unit chlorophyll *a* (4.1 mg/μg) than the blue–green alga (3.1) or diatom (0.2). The diatom produced the most dry-biomass weight and chlorophyll *a* per unit OD_{730}, and the lowest dry-biomass weight per unit chlorophyll *a*.

Organic Matter Production

POC and DOC were produced in the 0.9 L reactors. The kinetics of DOC production during the growth experiments is presented in Fig. 2(b). The diatom and blue–green alga produced in excess of 20 mg/L of DOC, whereas the green alga produced between 10 and 12 mg/L. DOC production paralleled biomass production

Table 3. Characteristics of Algal Dissolved Organic Carbon (DOC) and Suwannee River Fulvic Acid by Fluorescence Spectrometry

Sample	Age (day)	DOC (mg/L)	Ultraviolet absorbance (m^{-1})	Specific ultraviolet absorbance [m^{-1}/(mg/L)]	Fluorescence index ratio
SRFA	NA	1.0	4.1	4.1	1.4
Algal-FDHA	37	1.0	2.3	2.3	1.8
Scenedesmus quadricauda	2.75	1.5	2.1	1.4	1.6
	4	4.0	4.4	1.1	1.6
	6	12	9.4	0.8	1.7
Chaetoceros mulleri	0.75	1.4	1.5	1.0	2.0
	1.25	1.7	1.9	1.0	1.8
	2	16	10	0.6	1.9
	3	19	9.6	0.5	1.7
	4	20	9.5	0.6	1.7
Oscillatoria prolifera	0	1.1	1.1	0.9	2.4
	1	3.5	2.4	0.7	2.9
	2	11	7.8	0.7	3.0
	5	25	16	0.7	2.8

Note: Bulk algal DOC concentrations were diluted to 1 mg/L for fluorescence measurements.

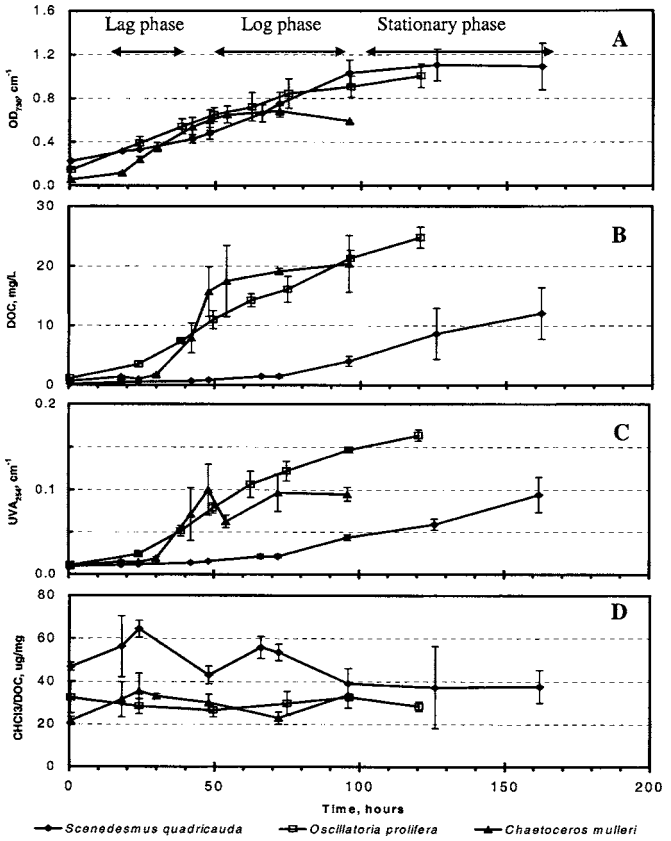

Fig. 2. Phase I—(a) growth, (b) dissolved organic carbon production, (c) ultraviolet absorbance values, and (d) disinfection by-products reactivity for different algal species. Error bars represent the standard deviation of triplicate samples.

[Figs. 2(a and b)]. Logarithmic-phase specific DOC production rates were 0.03 and 0.09 h^{-1} for the green alga and diatom, respectively. Following a lag-growth phase, a linear DOC production rate of 0.24 mg/L DOC per hour (r^2=0.99, n=6) was observed for the blue–green alga. Shortly after reaching the stationary growth phase the cultures became yellow-brownish, and cells flocculated into clumps. Filtration of samples during this period resulted in the formation of a viscous layer on the filter, possibly due to production of extracellular polymeric materials by the alga. DOC yields per unit biomass differed for each alga. The highest rate of normalized DOC production ($\Delta DOC/\Delta OD/\Delta t$, mg L^{-1} cm^{-1} h^{-1}) occurred during the early stationary growth phase. The green alga had the lowest normalized production rate during this growth phase, at 2.1 mg L^{-1} cm^{-1} h^{-1}, compared to the blue–green alga (3.8) and diatom (21). Overall, the average DOC production rates for the three species were 0.55, 2.2, and 5.7 mg L^{-1} cm^{-1} h^{-1}, respectively. The blue–green alga had the highest DOC production per unit chlorophyll *a* per hour [9.0 μgC(μgChl-*a*)$^{-1}$ h^{-1}], followed by the green alga (3.6) and diatom (1.1).

UV absorbance at 254 nm (UV_{254}) by organic molecules results primarily from aromatic compounds. The inorganic nutrient media included nitrate (15 mg/L N), which also absorbs at UV_{254} (0.6 m^{-1} in the filtered media for the green alga). Nitrate concentrations were not monitored over time in the experiments but would be expected to rapidly decrease (Hu et al. 2000). Fig. 2(c) shows gradual increases of UVA_{254} values over time, indicating the production of aromatic compounds.

Fluorescence data (filtered samples), at multiple excitation (λ_{ex}) and emission (λ_{em}) wavelengths (i.e., EEM), were obtained at an equivalent DOC of 1 mg/L for select samples during each growth experiment. The location of peak fluorescence intensity shifted toward lower wavelengths during the lag phase but remained relatively constant during the log phase. For example, the peak fluorescence at λ_{em}=445 nm shifted from $\lambda_{ex}\sim$250 nm to λ_{ex}=215 nm for all three cultures. After a few days of growth, the blue–green alga exhibited higher peak fluorescent intensities and a different EEM location (103 AU; λ_{ex}=215 nm, λ_{em}=442 nm) than either the green alga (49 AU; λ_{ex}=220 nm, λ_{em}=339 nm) or diatom (44 AU; λ_{ex}=220 nm, λ_{em}=333 nm). A slight increase in peak fluorescence intensity (AU) occurred between the lag and log growth phases.

An EEM of the EOM obtained on day 6 from the Phase I green alga culture is presented in Fig. 3(a). Three fluorescence regions of higher intensity were observed (indicated by arrows in Fig. 3(a)). The higher fluorescence region at λ_{em}=310 nm may result from protein-like EOM, which is associated with aromatic amino acids (e.g., tyrosine and tryptophan) and other nitrogenous materials (Coble 1996; Ismaili et al. 1998; Determann et al. 1994, 1998; McKnight et al. 2001; Westerhoff et al. 2001). The two peaks at longer emission wavelengths (between 400 and 450 nm) may be associated with more fulvic-like material, even though the alga was cultured in the absence of organic matter in the growth media.

DBP Formation

The main THM produced in the DBP formation experiments was chloroform (~99% of total THM) [Fig. 2(d)]. Bromide was purposefully omitted to minimize brominated THM formation and the resulting complexity of data interpretation. Absolute THM concentrations increased with time, but DOC reactivity in forming chloroform (μg $CHCl_3$/mg C) did not vary significantly as a function of growth phase. DOC produced by the green alga was more reactive in forming chloroform (48±12 μg/mg C;

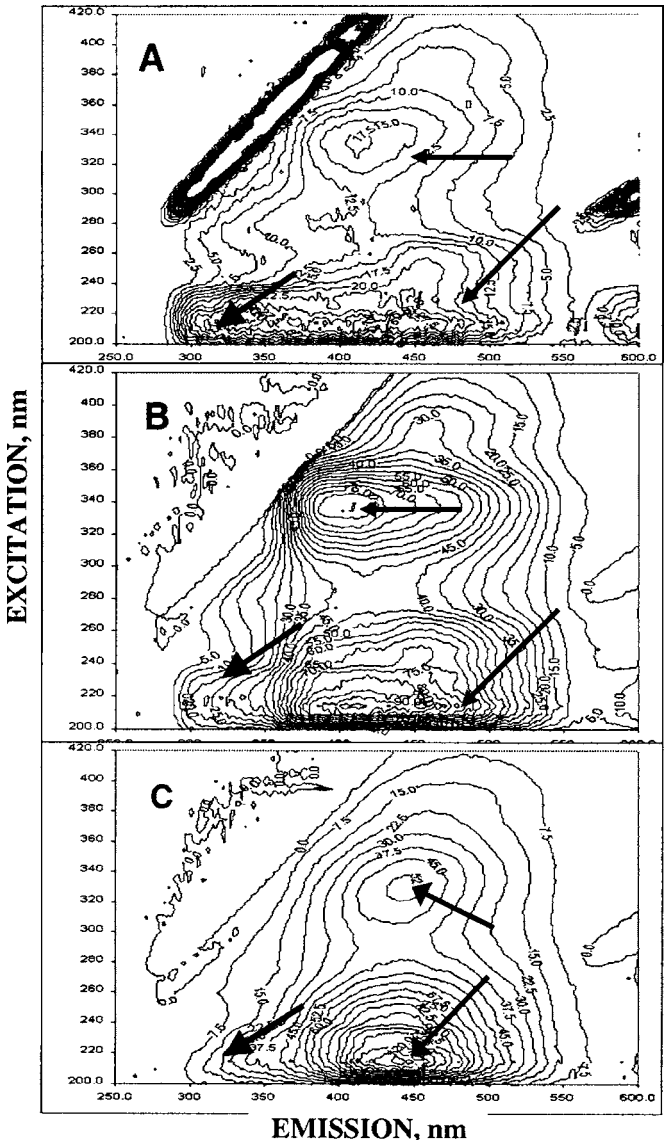

Fig. 3. Excitation-emission matrix (EEM) spectra [dissolved organic carbon (DOC)=1 mg/L, pH 3]: (a) bulk DOC produced from *S. quadricauda*, (b) Phase II green algae culture, and (c) Suwanee River fulvic acid. Samples were diluted to 1 mg/L dissolved organic carbon for direct comparisons of intensities.

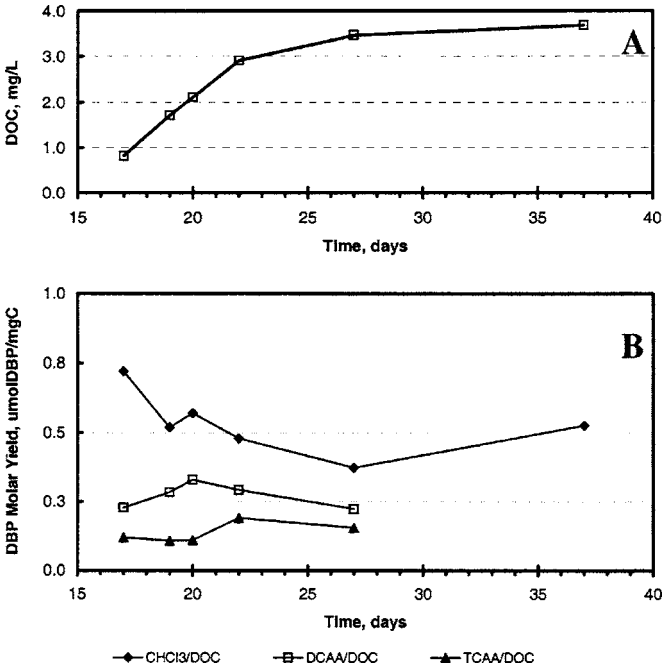

Fig. 4. Reactivity of algae-produced dissolved organic carbon (DOC), Phase II—*Scenedesmus*, to forming disinfection by-products (DBP): (a) DOC concentration and (b) DBP molar yield over time

0.40±0.10 μmol/mg C) than DOC produced by the blue–green (30±4 μg/mg C; 0.25±0.03 μmol/mg C) or the diatom (29±6 μg/mg C; 0.24±0.05 μmol/mg C).

Phase II Algae Experiments

Scenedesmus, the green alga, had lower specific growth rates (0.002–0.003 h^{-1}) in the 20 L reactors than in the 0.9 L reactors (0.016 h^{-1}). OD_{730} increased from ~0.55 cm^{-1} on day 17 to 1.1 cm^{-1} at day 37.

Organic Matter Production

DOC concentrations increased from less than 1 mg/L on day 17 to 3.8 mg/L by day 37 [Fig. 4(a)]. DOC production rate during the exponential growth phase was 0.18 mg L^{-1} cm^{-1} h^{-1}. Although UVA_{254} values also increased over time from 1.1 m^{-1} (day 17) to 5.7 m^{-1} (day 37), the increase was incongruous with DOC concentrations. No observed increase in UVA value (2.9 m^{-1}) occurred between days 22 and 27. The average SUVA value was 1.1 m^{-1} $(mg/L)^{-1}$. The maximum SUVA value [1.6 m^{-1} $(mg/L)^{-1}$] was measured at day 37.

$BDOC_5$ values during the large-volume experiment ranged from 0.5 mg/L during early log growth to 2.4 mg/L during the stationary phase. As a percentage of the DOC present, $BDOC_5$ fractions ranged between 54±6% on day 17 and 71±5% on day 37. The average of all $BDOC_5$ data between days 17 and 37 was (62±6.0%; n=5). Kinetic BDOC experiments were conducted over 7 days. After 7 days, the BDOC was 2.6 mg/L (77% of the total DOC). Approximately 40% of the DOC degraded within 24 h, and 60% degraded within 3 days. Therefore, both easily degradable and more slowly biodegradable DOC was present in the algae culture. More DOC than UVA material was removed biologically, and SUVA doubled from 1.3 (day 0) to 2.7 m^{-1} $(mg/L)^{-1}$ during the 5 day DBOC test.

Disinfection By-Product Formation

The average chloroform reactivity for DOC produced by *Scenedesmus* was slightly higher in the 20 L reactors (63±14 μg/mgC) than in the 0.9 L reactors (48±12 μg/mgC). The molar yield of DBPs normalized to DOC over time during the Phase II experiments is presented in Fig. 4(b). Chloroform had the highest molar yield (~0.53 μmol/mgC), followed by dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA). DCAA and TCAA were plotted separately since different DOC precursor material has been implicated in their production (Reckhow et al. 1990). Less than 6% of the total haloacetic acid, composed of nine different compounds, was present as monochloroacetic acid (MCAA). Algae-produced DOC was more reactive to forming DCAA (0.27 μmol/mgC) than TCAA (0.14 μmol/mgC), yielding an average TCAA:DCAA ratio of approximately 0.52 μmol/μmol (ranging from 0.33 to 0.69). The total HAA

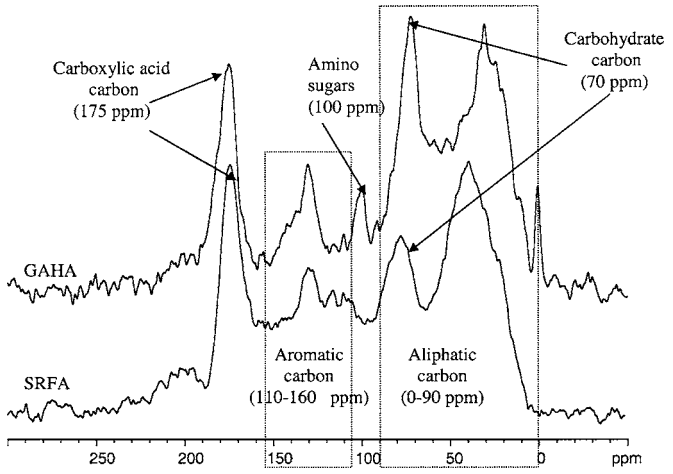

Fig. 5. Solid state ^{13}C nuclear magnetic resonance spectra for the Suwannee River fulvic acid (SRFA) and Phase II green algae culture, the hydrophobic acid fraction of dissolved organic carbon produced from a green alga

molar yield, normalized to DOC (0.44 μmol/mgC), was slightly lower than the molar yield of THMs (chloroform).

Isolated Algal Organic Matter Characterization

EOM was isolated from the Phase II green algae culture on day 37. Approximately 25% of the DOC was isolated as hydrophobic acids (XAD-8 isolate eluted with NaOH). In addition, approximately 10% of the initial DOC was not eluted from XAD-8 resins with NaOH; this fraction would be classified as hydrophobic neutral material. The freeze-dried hydrophobic acid isolate from the Phase II green algae culture (algal-FDHA) was further characterized. Algal-FDHA had a nitrogen content of 5% on an ash-free basis; the C:N ratio was 8.5 on a weight basis.

Spectroscopic analysis of algal-FDHA indicated the presence of a heterogeneous mixture of aliphatic and aromatic material. SUVA for algal-FDHA was 2.3 m^{-1} $(mg/L)^{-1}$, suggesting the presence of aromatic carbon. The EEM for algal-FDHA is presented in Fig. 3(b), and in Fig. 3(c) SRFA is also shown for comparison. Two regions of high fluorescence intensities were observed for algal-FDHA at $\lambda_{em} \sim 410$ nm, and it exhibited higher DOC normalized fluorescence intensity than the nonfractioned (i.e., bulk DOC) sample prior to XAD-8 fractionation. The solid-state ^{13}C-NMR spectrum for algal-FDHA is presented in Fig. 5 along with the spectrum for SRFA obtained on the same instrument for comparison. Algal-FDHA contained both aliphatic (0–110 ppm) and aromatic (110–160 ppm) carbon. It also exhibited sharp peaks near 70, 100, and 130 ppm, shift regions associated with carbohydrates, amino sugars, and protonated and alkyl-substituted aromatic carbon, respectively.

Chlorination of algal-FDHA and SRFA produced THMs. Algal-FDHA produced only chloroform (0.57 μmol/mg C), and the chloroform yield was greater than that resulting from chlorination of the bulk non-fractionated DOC (0.40 μmol/mg C). The chloroform yield from algal-FDHA was ~30% less than that obtained for SRFA.

Discussion

Algal Organic Matter Production

Production of biomass (dry weight basis) relative to chlorophyll *a* production was significantly lower for the diatom compared to the green or blue–green algae. The three different algal cultures also had different yields of DOC per chlorophyll *a* or cell abundance (i.e., OD_{730}). The green algae had the lowest normalized (to OD_{730}) DOC and chlorophyll *a* yields. Despite comparable yields of DOC, the diatom produced approximately 20 times more chlorophyll *a* than the blue–green algae. Neither the production of chlorophyll *a* nor the growth rate of algae appeared to be correlated with DOC production in the three algal cultures studied. Therefore, extrapolation of these findings in single algae cultures to natural systems with mixed algae cultures would suggest that neither cell counts nor chlorophyll *a* production should be correlated with DOC production.

The release of algal EOM occurs via two processes: (1) Diffusion driven by equilibrium between intra- and extracellular concentration (Type I) and (2) irreversible degradation of the surface of the cell (Type II) (Watt 1966). Type I products are low molecular weight intermediate products of metabolism (glycolic and amino acids) that are lost by diffusion through cell membrane due to high concentration within the cells. Releases of Type I EOM are relatively dominant during the exponential growth phase. In general, healthy algae (late exponential stage) produced more EOM per unit biomass than the algae with declining population (measured by chlorophyll *a*) (Watt 1966). Laboratory results has shown that dilution of algal population caused increase in Type I EOM releases per unit carbon fixation (or biomass); higher dilution (>90%) causes more significant increases in EOM release (Fogg 1966; Watt 1966). Type II products are higher MW materials (polysaccharides) that represent the leaching of the surface of senescent cells (Watt 1969, 1966).

Using the correlations between optical densities (OD_{730}) and chlorophyll *a* values that were established in Table 1, the ranges of chlorophyll *a* were: 35 to 169 μg/L for the green algae, 36 to 252 μg/L for the blue–green algae, and 290–3,500 μg/L for the diatoms. Algal densities used in this study were significantly higher than generally found in natural systems (<20 μg/L of chlorophyll *a*). Therefore, the release of Type I EOM per unit biomass in the more diluted natural system could be higher than those measured in lab cultures during the exponential phase. On the other hand, Type II releases from senescent cells in lab cultures would be significantly higher than in natural systems due higher cell biomass. In natural systems (e.g., lakes) the kinetics of release of EOM would have characteristics of Type I. Fogg et al. (1965) estimated EOM release in natural system (using radiocarbon method) in the range between 7 and 50% of total carbon fixed in the photic zone of water column.

Algal Organic Matter Characteristics

Both growth and DOC production rates for the green algal culture were approximately an order of magnitude lower in Phase II (20 L reactor) compared to Phase I (0.9 L reactor) experiments. Lower growth rates in the larger reactors may have been due to less light penetration into the reactor (i.e., longer pathlength through the water column). However, SUVA values [~1 $m^{-1}(mg/L)^{-1}$] and fluorescence intensities for organic mater from Phase I and Phase II experiments were similar.

Table 4. Comparison of Chemical Properties of Dissolved Organic Carbon Isolates (XAD-8) from Algae and other Aquatic DOC Sources

Source	Isolate	Sampling location	Specific ultraviolet absorbance at 254 nm	^{13}C nuclear magnetic resonance (%C)[a]				
			$[m^{-1}\ (mg/L)^{-1}]$	Al (0–90 ppm)	Ar (110–160 ppm)	Ar-OH (140–160 ppm)[b]	COOH (160–190 ppm)	CN (wt/wt)
Scenedesmus quadricauda	FDHA[c]	Lab cultures	2.3	64	12	3.3	15	8.5
Autochthonous	FXL[d]	Lake Fryxell, Antarctica[d]	1.9	60	13	3.8	20	18
Autochthonous	HRE[d]	Lake Hoare, Antarctica	NA	66	12	NA	20	19
Autochthonous	PNL	Pony Lake, Antarctica	NA	65	17	NA	17	11
Allochthonous	SRFA[c]	Suwannee River, Ga.	4.2	50	21	7.1	16	82
Allochthonous	CCK[d]	Coal Creek, Colo.	4.3	38	27	9.3	20	56
Allochthonous	OGR[d]	Ogeechee River, Ga.	3.7	47	25	8.1	18	58
Allochthonous	BKL[d]	Black Lake, N. C.	4.1	NA	17	NA	NA	47

Note: NA=not applicable.

[a]%C ^{13}C nuclear magnetic resonance data does not total 100% as %C ketone and anomeric carbon are not shown.

[b]% Ar–OH carbon is a subset of % Ar carbon assumed to contain phenolic substances.

[c]Solid state ^{13}C nuclear magnetic resonance analysis (Algal-FDHA: this study).

[d]Liquid state ^{13}C nuclear magnetic resonance analysis (Westerhoff et al. 1999; McKnight et al. 1994; Reckhow et al. 1990).

Algal-FDHA only accounted for 25% of the DOC. Algal-FDHA isolated after Phase II culturing had a higher SUVA $[2.3\ m^{-1}\ (mg/L)^{-1}]$ and higher DOC normalized fluorescence than the bulk DOC. SUVA and fluorescence data are indicators of aromatic carbon. Therefore, aromatic carbon produced by the green algal culture was preferentially isolated by XAD resin (Thurman 1985).

The C:N ratio (8.5) for algal-FDHA is low relative to C:N ratios (>45) from other hydrophobic acid fractions previously isolated from lakes and rivers in temperate climates with allochthonous sources of organic matter (Table 4). However, the C:N ratio for algal-FDHA is only slightly lower than the C:N ratio (11–19) for three lake systems in Antarctica where DOC production is hypothesized to be dominated by algal processes (i.e., autochthonous). A nitrogen enriched isolate (one with low C:N, such as algal-FDHA) is therefore a likely indicator for algal-derived organic matter.

Nitrogenous components in algal-derived organic matter primarily include hydrolysable amino acids, but terpenoids (MIB, Geosmin) or cyanotoxins (microcystins) may also be present (Westerhoff and Mash 2002). Some organic nitrogen can produce nitrogenous DBPs or serve as precursors for THMs and HAAs (Hureiki et al. 1994; Ueno et al. 1996; Reckhow et al. 2001). Based on a synopsis of extracellular products from algae, nitrogenous substances are often liberated from algae (Fogg 1966). blue–green algae (nitrogen fixers) have received considerable attention since they can excrete up to 45% of their total fixed nitrogen as org-N. Solid-state ^{13}C-NMR for algal-FDHA indicated a dominant peak at 100 ppm that probably represents the anomeric carbon unit of amino sugar components. Although usually fractionated as colloidal or hydrophobic neutrals, amino sugars (e.g., acetamides) originating from microorganism cell walls are also nitrogen enriched and could contribute to the low C:N ratio (Leenheer et al. 2001; Rostad et al. 1997). Algae and bacteria cell wall integrity emerges from cross-linked peptide chains of N-acetylglucosamine and N-acetylmuramic acid, for example, resulting in the presence of colloidal aminosugars.

Solid-state ^{13}C-NMR revealed the presence of amino sugars, carbohydrates, and other organic matter fractions (i.e., nonhydrophobic acid fractions) in algal-FDHA as well. BDOC experiments indicated that a larger percentage (>60%) of the DOC from Phase II experiments was biodegraded. The amino sugars, amino acids, and carbohydrates were probably a major fraction of the biodegraded material. Rostad et al. (1997) hypothesized that colloidal natural organic matter (NOM), predominantly amino sugars, was readily biodegradable, and as the size of NOM decreased the colloids transformed into nitrogen-rich humic materials.

Based upon solid-state ^{13}C-NMR, algal-FDHA contained mostly protonated and alkyl-substituted aromatic carbon (110–140 ppm), whereas SRFA contained mostly phenolic aromatic carbon (140–160 ppm). Overall, algal-FDHA had lower aromatic carbon content than SRFA. SUVA value, an indicator of aromatic carbon content, was also lower for algal-FDHA compared to SRFA. The non-fractionated EOM sample had relatively low SUVA values $[<2.0\ m^{-1}\ (mg/L)^{-1}]$ and were lower than algal-FDHA $[2.3\ m^{-1}\ (mg/L)^{-1}]$. This suggests that the non-fractionated EOM contained less aromatic carbon than the algal-FDHA isolate. Aromatic carbon also fluoresces, and on a DOC normalized basis algal-FDHA had a higher fluorescence response than nonfractioned EOM [Figs. 3(a and b)]. EEMs for algal-FDHA and SRFA also revealed differences. The fluorescence EEM peaks for algal-FDHA were shifted toward slightly shorter wavelengths than those for SRFA [Fig. 3(c)], which may be related to the different types of aromatic carbon detected by solid-state ^{13}C-NMR. A fluorescence index (FI) was calculated for all samples as the ratio of fluorescence intensity at shorter (450 nm) to longer (500 nm) emission wavelengths (with $\lambda_{ex}=370$ nm) (Table 3). McKnight et al. (2001) showed that FI values of DOC from autochthonous sources (~1.9) are higher than DOC from allochthonous sources (~1.4). Additional work indicated that the relationship between FI ratios was related to organic nitrogen content and suggested that algae contributed to the shift in FI values. Thus, FI values could be used as indicators of algal-derived organic matter (Wolfe et al. 2002). Our work supports the previous field observations. FI ratios (1.6–3.0) for DOC produced in algae cultures in this study were similar to ratios for fulvic acids isolated from algae-dominated waters (1.7–2.0). Nitrogenous aromatic amino acids (e.g., tryptophan or tyrosine) and/or protonated and alkyl-substituted aromatic material may result in fluorescence at shorter wavelengths than phenol-substituted aro-

matic carbon, thus resulting in larger FI values. Overall, solid-state ^{13}C-NMR, SUVA, and fluorescence (EEM or FI) indicate the formation of aromatic carbon by algae.

Algal Organic Matter Reactivity with Chlorine

DOC from the green alga was slightly more reactive in forming THMs and HAAs than that from the blue–green algae or diatom. The magnitude of chloroform yield in the current study is consistent with previously reported data for chlorination of organic materials (EOM) for the green algae; (0.18–0.34 μmol/mg C), diatom (0.42 μmol/mg C) and blue–green algae (0.50 μmol/mg C) (Plummer and Edzwald 2001).

The total HAA molar yield, normalized to DOC, was approximately equal to the molar yield of THMs (chloroform). Algae-produced DOC was more reactive to forming DCAA than TCAA, and the average TCAA:DCAA ratio of approximately 0.52 μmol/μmol was comparable to the ratio of 0.6 μmol/μmol observed in Plummer and Edzwald (2001) from the chlorination of the diatom (*Cyclotella*). In contrast, Reckhow et al. (1990) reported a TCAA:DCAA ratio of ~2.0 μmol/μmol for 10 aquatic humic and fulvic acids with allochthonous origins.

Data from Phase I indicated a linear correlation between chloroform concentration and UVA_{254} with a slope of 45–49 μg$CHCl_3$/L (~0.38 μM) per unit absorbance (m^{-1}) for the green algae (r^2=0.98), blue–green algae (r^2=0.97), and the diatom (r^2=0.81). During Phase II experiments, chloroform production (0.39 μmol/mg C) was linearly correlated with UVA_{254} (33 μg L^{-1} m^{-1}, r^2=0.92). Further, total HAA formation (60 μg/mg C) was linearly correlated with UVA_{254} (89 μg L^{-1} m^{-1}, r^2=0.99). These findings strongly suggest that aromatic carbon is a principal precursor for THM and HAA formation, even for DOC of algal origin. Other nonaromatic precursor materials (e.g., most amino acids, ketones) may be important, but the presence of these compounds would not be indicated by UVA_{254}.

Although the UVA_{254} correlation with DBP yields is significant, an alternative hypothesis is that N-enriched aromatic proteins (e.g., tryptophan and tyrosine) and/or other amino acids are important DBP precursors. In chlorination experiments with amino acids, Hureiki et al. (1994) observed the following order of $CHCl_3$ production (higher to lower reactivity): tryptophan > tyrosine ≫ histidine > aspartic acid > threonine > lysine > alanine > serine. Croue et al. (1999) hypothesized that nitrogenous units of NOM could be an important class of di-HAA precursor sites, perhaps due to high chlorine demands. Further, high chlorine demands by nitrogenous material may lower the available chlorine (i.e., Cl_2/DOC ratio) during the reaction, resulting in preferential formation of DCAA over more halogenated products (e.g., TCAA) (Croue et al. 1999). Croue et al. (1999) found that tri-HAA was greater than di-HAA in several NOM fractions, but not in the hydrophilic base fraction, which contained elevated nitrogen levels. Therefore, the low C:N ratio of algae-produced DOC could mean that nitrogenous components of algal-derived DOC are important DBP precursors.

Conclusions

This study examined the nature and reactivity of DOC produced by several types of laboratory-grown algae and demonstrated the potential significance of algae as a source of DOC and DBP precursors. It differs from previous studies that characterize both POC and DOC in solution together. The following summarizes the key findings of this study.

- Algae produce DOC that is reactive to formation of chlorination DBPs. DOC produced by the green alga was more reactive in forming chloroform (0.40 μmol/mg C) than the blue–green alga (0.25 μmol/mg C) or diatom (0.24 μmol/mg C). Algae-produced DOC was more reactive to forming DCAA (0.27 μmol/mg C) than TCAA (0.14 μmol/mg C), corresponding to an average TCAA:DCAA ratio of approximately 0.5 μmol/μmol.
- SUVA values for the algae-produced DOC were low [<2 m^{-1} $(mg/L)^{-1}$], as was SUVA for the XAD-isolated algal-FDHA [2.3 m^{-1} $(mg/L)^{-1}$]. By comparison, SUVA values for XAD isolates from allochthonous sources are generally >3.7 m^{-1} $(mg/L)^{-1}$.
- The FI for alga-derived DOC in this work ranged from 1.6 to 3.0, and is consistent with conclusions from field data that suggest FI values >1.6 may be attributable to algal processes.
- Algal-FDHA had a low C:N ratio (8.5 wt/wt), indicating an enrichment of organic nitrogen compounds (probably amino sugars and amino acids). Solid state ^{13}C-NMR indicated the presence of nonphenolic aromaticity (protonated, alkyl, or nitrogen substituted) in the algal-FDHA, which differed from the range and peak of phenolic carbon observed in the SRFA spectrum. Algal-FDHA also contained carbohydrates and amino sugars.
- Algae-produced DOC was easily biodegraded, with $BDOC_5$ ranging from 50 to 70% of DOC. High biodegradability is conferred by the presence of labile carbohydrates and proteins.

The common characteristics of algae-derived DOC appear to be organic nitrogen enriched; lower in hydrophobic acid content, aromatic carbon content, phenol substitution, and SUVA values in comparison to DOC derived from terrestrial sources. Further, the characteristics of fulvic acids isolated from algae-produced DOC are similar to those of fulvic acids isolated from Antarctic lakes whose DOC largely comes from autochthonous sources. DOC obtained from algae may serve as an "end-member" in a continuum of DOC sources, with plant- or soil-derived DOC (or groundwater DOC) on the other extreme of the continuum (Mash et al. 2004).

The results in this study demonstrate the importance of algal DOC as a source of DBP precursors for THMs and HAAs, including a tendency to produce DCAA rather than TCAA. Although algal DOC and algal-FDHA had lower THM reactivity compared to SRFA, algae-produced DOC could nevertheless be an important THM precursor in reservoirs with substantial autochthonous production. In addition, algae-produced DOC and algal hydrophobic acids differ from other terrestrial-dominated humic and fulvic acids in terms of DBP reactivity and carbon structure (e.g., functional groups and nitrogen content). Understanding these characteristics will be useful in the development of more rapid techniques to differentiate DOC sources, leading to better management and treatment of source water.

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