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Characteristics of algal organic matters (AOMs) generated by two different algae species

Se-Hyun Oh[1], Yunchul Cho*[2]

[1]R&D Team, K-AD, 2511 Sejong-ro, Sejong 30019, Republic of Korea
[2]Department of Civil and Environmental Engineering, Daejeon University,
62 Daehak-ro, Dong-gu, Daejeon 34520, Republic of Korea

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Abstract. Algal organic matters (AOMs) generated during algal blooms is known to lead to deterioration of water quality. In this study, the amounts of AOMs produced by two cyanobacteria such as Anabaena sp. and Oscillatoria sp. were measured during their growth, respectively. The results showed that AOM production continued from the exponential growth phase to the death phase. During the death phase, AOM production seemed to be sustained due to intracellular organic matter (IOM) released by dead cells. It was found that the specific UV absorbance (SUVA) values of extracellular organic matter (EOM) were relatively higher than those of IOMs in two algal species. Meanwhile, LC-OCD and XAD analyses were performed to understand the physicochemical characteristics of AOMs produced during the algal growth phases. The XAD analysis indicated that AOMs generated by both algal species had a high proportion of hydrophilic substances compared to hydrophobic and transphilic ones. The LC-OCD analysis showed that the order of high content of main components of AOMs was were high-molecular-weight biopolymers, humic-like substances, and building blocks.

Keywords: algal organic matter; cyanobacteria;, extracellular organic matter; hydrophobicity; LC-OCD

1. Introduction

Algal blooms result from a rapid increase of algae cells such as green algae and cyanobacteria in freshwater systems [18]. Changes in various environmental factors caused by global warming and anomalous weather events affect algal growth [13, 4]. In general, algae bloom causes deterioration of water quality as well as public health issue. Also, algae bloom result in the increase of turbidity and decrease of dissolved oxygen [17, 16]. Algal bloom can increase algal organic matter (AOM), which can serve as precursors of disinfection by products (DBPs)[6]. AOM is divided into extracellular organic matter (EOM) and intracellular organic matter (IOM). EOMs are metabolites produced by algal cells during the exponential and stationary growth phases, and IOMs are released from cell autolysis by aging or cell damage due to oxidation in water treatment process [26]. EOM is generally composed of polysaccharides, organic acids, proteins, amino acids, fats, organophosphates, and toxic substances [23, 7]. It has also been reported that EOM consists mostly of hydrophilic fractions [25].

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*Corresponding author, Professor, E-mail: ycho@dju.ac.kr

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In general, either EOM or IOM is not easily removed by chlorine, ozone and advanced oxidation treatment. Especially, ozone treatment has limitations in removing EOM, and cause damage to algal cells, resulting in the release of IOMs [15]. However, ozone oxidation has been reported to produce less DBPs than other oxidation processes [22].

Determination of physicochemical properties of AOMs is the prerequisite to effectively remove them in water treatment process. Various spectroscopy techniques of determine organic matter in aqueous phase such as fourier-transform infrared spectroscopy (FTIR) and fluorescence spectroscopy (FS) have been applied to understand characterization of AOM [21]. Recently, liquid chromatography-organic carbon detector (LC-OCD) and fluorescence excitation-emission matrix spectroscopy (FEEM) have been utilized for the characterization of natural organic matter (NOM) and AOM [2]. AOM can be determined by molecular weight using LC-OCD can be used to determine AOM based on molecular weight from high molecular weight compounds to low ones [9, 12, 11]. According to molecular weight AOM compounds can be categorized into several distinct fractions such as biopolymers (> 20,000 g/mol), humic substances (1000-20,000 g/mol), building blocks (humic substance fractions, including polycarboxylic acids) (350-500 g/mol), low molecular weight acids (< 350 g/mol), and etc.

The aim of this study was to comprehensively characterize AOMs produced by two different algal species (*Anabaena sp*., and *Oscillatoria sp*.). At the phylum level, both algal species are classified as *Cyanobacteria*. At the genus level, *Oscillatoria sp.* is non-heterocystous and exhibits oscillatory motility, whereas *Anabaena sp.* is heterocystous and shows a bead-like cellular arrangement. It has been reported that *Microcystis*, *Anabaena*, *Oscillatoria*, and *Aphanizomenon* species are dominant during algal bloom events in Korea [20].

In order to achieve the goal, the amounts of EOMs produced by two different algal species according to different growth phases were evaluated, and differences in physical and chemical properties between EOMs and IOMs were investigated using LC-OCD and organic matter fractionation analyses. Results obtained this study can be helpful in improving AOM control in drinking water treatment.

2. Mathematical modeling

In this study two different algae species were selected to understand the characteristics of AOMs. Both *Anabaena sp*. and *Oscillatoria sp*. were purchased from the Korean Collection for Type Cultures (KCTC), Korea. The algae cultivation was performed in a laboratory-scale culture system for 6 to 7 weeks (Table 1). BG-11 was used as a medium for algae cultivation (Table 2) [5]. Algae were collected from the algae cultivation system to construct the growth curve and analyze the characteristics of algae species. Algal cells were counted using a hemocytometer (Marienfeld Superior, Germany) and observed by optical microscopy (OptinityKCS3-63S, China). The hemocytometer plate is a specially designed slide with four grooves forming three platforms, which improve the counting of algae cells.

In order to prepare EOM solution, 50 mL of algae cultivation solution was centrifuged at 5000 rpm for 10 minutes using a centrifuge (Hanil Science Inc., Korea) and then the supernatant was filtered using a glass fiber membrane of 0.45 μm (Whatman GF/C Glass Microfiber Filter, UK). In case of IOM solution preparation, algae cultivations solution was centrifuged at 5000 rpm for 10 minutes. After the supernatant was removed, the algal precipitates were washed three times with deionized (DI) water and resuspended in DI water. The suspension was frozen at -24°C and melted

Table 1. Algal cultivation condition

Algal seed: Medium	1: 10
Lux	3000 ~ 5000
L: D	12 h: 12 h
Temperature (℃)	27
pH	7.5~8.5
Axenic/Xenic	Xenic

Table 2. BG-11 medium for algae cultivation

Compound	Amount (g/L)
$NaNO_3$	1.5
$CaCl_2.2H_2O$	0.036
Ferric ammonium citrate	0.012
$EDTA.Na_2.2H_2O$	0.001
K_2HPO_4	0.04
$MgSO_4.7H_2O$	0.075
Na_2CO_3	0.02
Trace metal solution[a]	1 ml/L

[a] H_3BO_3,2.86g/L;$MnCl_2.4H_2O$,1.81g/L;$ZnSO_4.7H_2O$,0.222g/L;$Na_2MoO_4.2H_2O$,0.39g/L;$CuSO_2H_2O$,0.079g/L;$Co(NO_3)_2.6H_2O$,0.049g/L.

Table 3. Operating conditions of LC-OCD

Item	Condition
Energy Consumption	ca. 1,5 KWh
Environment Temperature	ca. 20 ∼30℃ (25℃ recommended)
UV-Lamp Zero water Reactor	990 V/80 mA
UV-Lamp Thin Film Reactor	2000 V/40 mA
Carrier Gas	N_2 4.0 or 5.0, about 20 L/hr
Mobile Phase	Phosphate buffer 20 mmol, pH 6,58, about 2L/24hr
Acidification Solution	Phosphoric Acid pH 1,5, about 0.5L/24 hrs
Measuring Range TOC	<10ppb ∼ 5,000ppb
Measuring Range LC-OCD	>10ppb ∼ 5,000ppb
Detection Limit TOC	2∼10ppb
Detection Limit LC-OCD	5∼50ppb (per compound)
UV-Lamp Zero water Reactor	990 V/80 mA

at room temperature. After the freeze-thaw procedure was conducted three times, the suspension was treated with ultrasonic waves (Mujigae, Korea), and then filtered using 0.45m glass fiber membrane (Whatman GF/C Glass Microfiber Filter, UK).

Liquid chromatography-organic carbon detection (LC-OCD) measurements was performed to characterize AOMs. LC-OCD (DOC-LABOR, Germany) was used to measure EOMs produced by

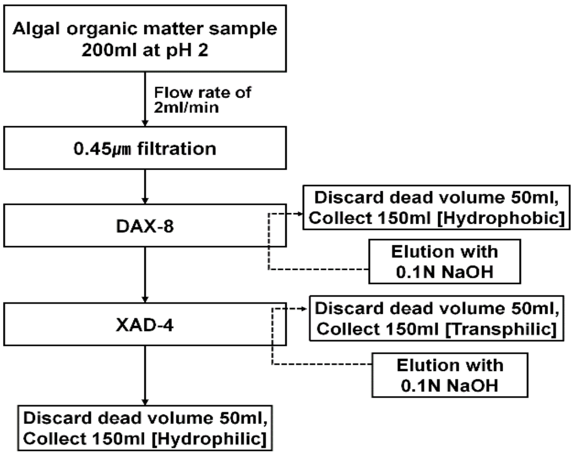

Figure 1. Schematic diagram of fractionation procedure

three different algae species. The relative reactions of organic carbon, ultraviolet and organic nitrogen at various residence times were measured by an on-line OCD and UV detector (Table 3). For understanding the characteristics of AOMs, various analytic methods for organics such as BOD, COD and TOC were applied. Total organic carbon (TOC) was determined using a TOC analyzer (Shimadzu, Japan). The aromaticity of AOMs were estimated through a specific UV absorbance (SUVA). Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) analyses were conducted according to the standard methods. In addition, the fractionation of AOMs was performed using the combined DAX-8 and XAD-4 resin procedure (Fig. 1) [10].

3. Results and discussion

3.1 Growth curves of two different algae species

The growth curves of *Oscillatoria sp*. and *Anabaena sp*. were shown in Fig. 2. The initial concentrations of algal cells were 2.3×105 cell/mL of *Oscillatoria sp*. and 3.3×104 cell/mL of *Anabaena sp*., respectively. The two different types of algae species were cultured in a batch system with an observation period of around 40 days.

The growth curves of the two algae species were similar to a typical microbial growth phase which showed the four distinct phases of growth. The growth curves of two different algal species had the adaptation phase, exponential phase, stationary phase, and death phase. In the stationary phase the maximum cell densities of *Oscillatoria sp*. and *Anabaena sp*. reached around 3.5×107 cell/mL and 8.3×105 cell/mL, respectively. Comparing the growth curves of the two different algae species, the growth cycle of *Anabaena sp*. seemed to be relatively shorter than *Oscillatoria sp*.

EOM concentrations of two algal species showed continuously increasing trends over a period of algal growth (Fig. 2). From the adaptation phase to the exponential phase, the concentration of EOM seemed to be increased rapidly. This trend is likely due to the increase of metabolites secreted by the rapid growth of algae cells. From the stationary phase to the death phase, the EOM

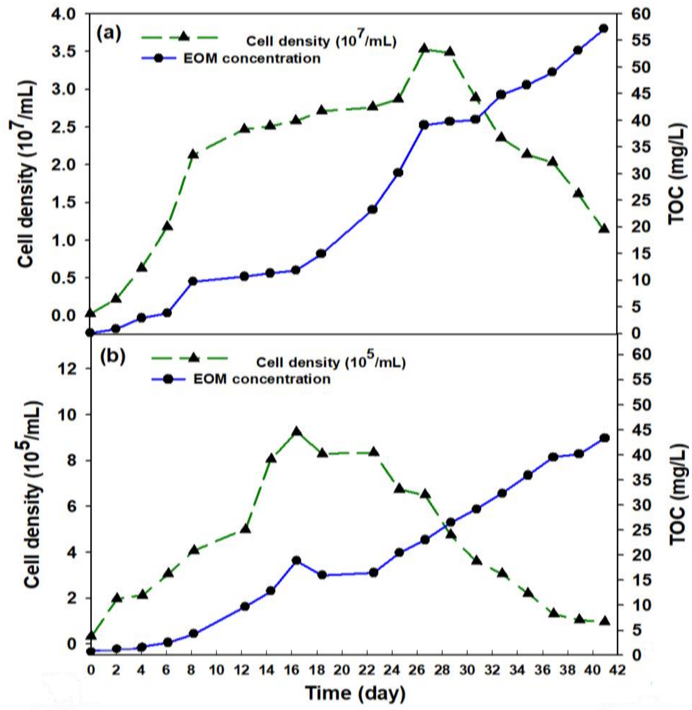

Figure 2. The growth curves of two different algae species (a: *Oscillatoria sp*., b: *Anabaena sp*.,)

concentrations gradually increased. The slow increase in organic matter concentration may be attributed to the release of IOM. Chen et al. [3] suggested that release of IOM increased because catabolism of algae exceeded the anabolism between the stationary phase to the death phase.

3.2 LC-OCD analysis of AOMs

The OCD chromatogram of *Oscillatoria*-AOM showed four main peaks which indicate biopolymers (> 20000 g/mol, 19-33 min), humics (1000-20000 g/mol, 33-46 min), building blocks (350-500 g/mol, 46-51 min) and low molecular weight (LMW) neutrals (<350 g/mol, weak, uncharged organic compounds, 64-80 min) in that order (Fig. 3a). In case of the organic composition of AOMs during the exponential growth phase, the proportions of biopolymers, humics, building blocks and LMW neutrals were 35%, 22%, 15% and 28% respectively (Fig. 3b). Interestingly, the proportion of biopolymers increased to 48% during the stationary growth phase. Compared to other substances, biopolymers are the most important organic components of AOMs. In the exponential growth phase, algae are known to release low molecular weight substances such as ethanoic acid and amino acids into the water, while the higher molecular substances such as polysaccharides are released into the water after entering the stationary phase [19].

On the other hand, the medium primarily consisted of building blocks and LMW neutrals. This implies that a significant portion of the building blocks for algal organisms could originate from the culture medium. Additionally, most of these low molecular weight organics (e.g., LMW acids and neutrals) may have originated from the culture media [21]. The BG-11 medium for the

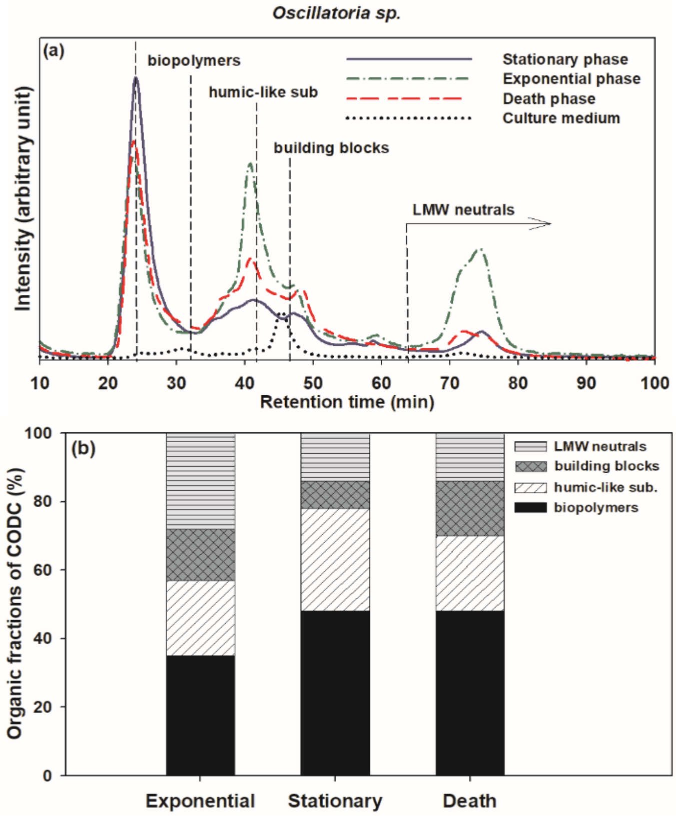

Figure 3. LC-OCD chromatograms (a) and fractionation (b) of AOMs from *Oscillatoria sp*. at different growth phases

Oscillatoria cultivation contained 1 mg/L of EDTA, which was used as a chelating agent to minimize the precipitation of metals in the medium. LC-OCD analysis of a culture medium solution showed a peak appearing at a similar elution time as the LMW neutrals peak (64-80 min) detected in the AOM sample.

For *Anabaena sp*., three major peaks were biopolymers (20-37 min), humic-like substances (37-45 min), and LMW neutrals (64-81 min) (Fig. 4a). The corresponding peak of the building blocks (45-50 min) exhibited relatively minor signals. Similar to *Oscillatoria sp*., building blocks mainly originate from the culture medium. Like *Oscillatoria*, biopolymers, humic-like substances, LMW neutrals, and building blocks exhibited varying proportions of organic matter for each growth period. The proportion of biopolymers in the stationary phase was higher (~56%) compared to the exponential growth phase (~41%) and death phase (~50%) (Fig. 4b). Additionally, the humic-like substances reached their highest percentage (22%) as a result of algal decomposition during the death phase.

3.3 Fractionation of AOMs

Fig. 5 presents the results of XAD fractionation on AOM (EOM and IOM) produced by *Oscillatoria sp*. and *Anabaena sp*. in the exponential and stationary phases. In two algal species,

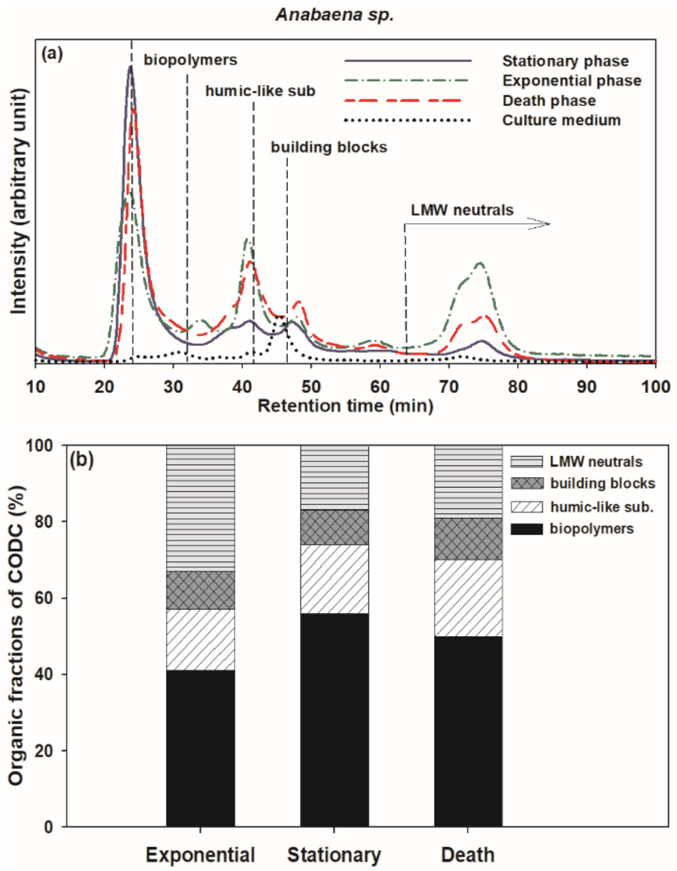

Figure 4. LC-OCD chromatograms (a) and fractionation (b) of AOMs from Anabaena sp. at different growth phases

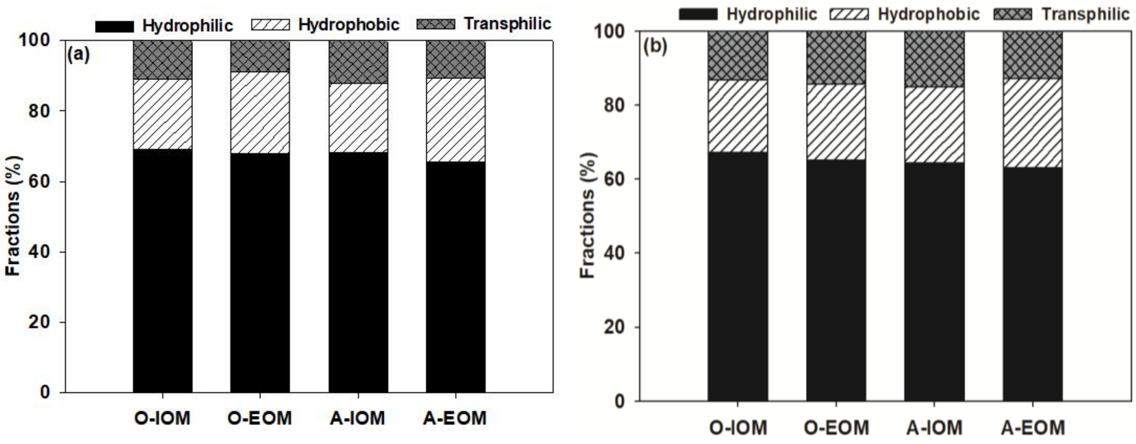

Figure 5. Comparison of the percentages of HPO, TPH and HPI of AOM from exponential phase (a) and stationary phase (b) (O-EOM and O-IOM: EOM and IOM produced by *Oscillatoria sp*., A-EOM and A-IOM : EOM and IOM produced by *Anabaena sp*.)

Table 4. Characteristics of AOMs produced by two algae species at the different growth phases

Growth phase	Algae species	AOM	TOC (mg/L)	SUVA
Exponential	*Oscillatoria sp.*	EOM	33.14	1.10
		IOM	8.157	0.86
	Anabaena sp.	EOM	31.86	1.16
		IOM	8.833	0.89
Stationary	*Oscillatoria sp.*	EOM	62.00	1.20
		IOM	6.70	0.84
	Anabaena sp.	EOM	51.30	1.03
		IOM	7.90	0.98
Death	*Oscillatoria sp.*	EOM	64.48	1.10
		IOM	1.86	0.81
	Anabaena sp.	EOM	55.86	1.08
		IOM	2.07	0.87

the main component of AOM was hydrophilic organic matter, which are consistent with those of other literature [17, 24].

AOM is mainly composed of polysaccharides, proteins, peptides, amino acids, and other organic acids such as fatty acids, resulting in the high hydrophilicity of AOM [1, 14]. Compared to EOM, IOM had a higher proportion of hydrophilic organic matter and a lower proportion of hydrophobic organic matter. The percentages of the hydrophilic portion for the EOM of both algal species in the exponential phase were 68% for *Oscillatoria sp*. and 66% for *Anabaena sp*., respectively. In case of the stationary growth phase, percentages of the hydrophilic portion for *Oscillatoria sp*. and *Anabaena sp*. were 65% and 63%, respectively. These results are in good agreement with the literature [8, 17, 24, 7]. On the other hand, characteristics of AOMs of two different algal species at the different growth phases were summarized (Table 4). SUVA values of EOM of two species were higher than those of IOM at each growth phase. The results revealed that EOM contained more hydrophobic organic matters than IOM. Also, these results were similar to those from the fractionation of AOMs.

4. Conclusions

The growth curves of two different algal species appeared to follow typical microbial growth patterns. All the algal species showed their AOM concentrations continuously increased over the entire algal growth period. During the death phase the slow increase in organic matter concentration is likely attributed to the release of IOM. On the other hand, LCD-OCD analysis showed biopolymers are main components for two algal species. However, the proportion of components of AOMs showed slightly different trends for two algal species. This suggests that the composition of organic matter varies with different algal growth phases. In case of XAD resin fractionation, two algal species predominantly produced hydrophilic components. This result is likely due to AOMs which are primarily composed of polysaccharides, proteins, peptides, and other organic acids. The results obtained from this study can serve as fundamental data for the effective control and treatment of algal-derived organic matter in drinking water treatment process.

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