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# Properties of Dissolved Organic Carbon (DOC) released by Three Species of Blue-green Algae

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**남조류에 의해 배출된 용존유기탄소의 특성.** 최광순 · Akio Imai[1] · 김범철 · Kazuo Matsushige[1] (강원대학교 환경학과 [1]일본 국립환경연구소)

순수 배양한 남조류 3종(*Microcystis aeruginosa, Anabaena flos-aquae, Oscillatoria agardhii*)을 대상으로 식물플랑크톤에 의한 체외배출 용존유기탄소(extracelluar dissolved organic carbon; EOC)의 배출양상, 화학적성분, 자외선흡광 특성을 연구하였다. EOC의 화학적성분은 XAD-8, 양이온, 음이온수지를 이용하여 소수성 산(hydrophobic acids; AHSs), 소수성 중성(hydrophobic neutrals; HoNs), 친수성 산(hydrophilic acids; HiAs), 친수성 염기(hydrophilic bases; HiBs), 그리고 친수성 중성(hydrophilic neutrals; HiNs)으로 분류하였다. 3종의 남조류로부터 배출된 EOC의 양과 화학적조성은 종마다 상이하였고, 조류의 성장단계에 따라서도 다르게 나타났다. HiAs성분은 남조류가 배출한 EOC의 가장 많은 부분(25~92%)을 차지했고, 정체기로 갈수록 HiAs성분이 차지하는 비율이 감소하였다. 반면, HiBs와 HiNs 성분은 정체기로 진행될수록 비율이 증가하였다. 특히 HiNs성분은 성장초기에는 거의 배출되지 않다가 정체기에서는 전체 EOC의 상당한 부분을 차지하였다(*M. aeruginosa*: 44%, *A. flos-aquae*: 28%). AHSs성분은 *M. aeruginosa*에서 0.2~2.5%로 매우 작았지만, *A. flos-aquae* (8.7~16%)와 *O. agardhii* (7.5~16%)에서는 상당한 부분을 차지했다. 그러나 AHSs의 배출은 *M. aeruginosa*와 *O. agardhii*에서 성장이 진행될수록 증가한 반면, *A. flos-aquae*에서는 반대의 경향을 보였다. 남조류에 의해 배출된 EOC의 자외선흡광 특성도 종에 따라 상이한 결과를 보였다. 본 연구의 결과는 남조류의 종과 성장단계에 따라 배출되는 용존유기물의 특성이 다르다는 것을 시사한다.

**Key words : Extracelluar organic carbon, Fractionation, Blue-green algae, Growth phases**

## INTRODUCTION

Dissolved organic carbon (DOC) can function as an important carbon and energy source for heterotrophic bacteria and higher trophic levels in freshwater ecosystems (Wetzel *et al*., 1972; Amon and Benner, 1994; Carlson *et al*., 1994; Lampert and Sommer, 1997). There are two major sources of DOC in lake water: allochthonous origin from the catchment and autochthonous origin produced within lakes (Wetzel, 1983; Munster and Chrost, 1990; Meili, 1992). The relative contribution of these sources to the lake carbon budget depends on hydraulic retention time, development of the littoral zone, and trophic state. With progressing eutrophication autochthonous carbon becomes increasingly important (Wetzel, 1983).

One of the most important sources of autochthonous DOC in pelagic waters is extracellular

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organic carbon (EOC) released from phytoplankton. The EOC may occur as a result of active excretion of phtosynthetic product, leakage from senescent and death algal cells (Nalewajko, 1977; Sharp, 1977; Fogg, 1982; Chrost and Faust, 1983; Watanabe, 1984; Jensen *et al.*, 1985; Hama and Handa, 1987; Baines and Pace, 1991), and herbivorous grazing (Lampert, 1978; Park *et al.*, 1997).

There have been a number of reports concerning quantity, molecular size distribution, chemical composition and bacterial utilization of EOC in laboratory cultures as well as in natural phytoplankton in freshwater and marine environments (Fogg, 1971, 1982; Nalewajko, 1977; Mague *et al.*, 1980; Cole *et al.*, 1982; Chrost and Faust, 1980, 1983; Sφndergaard *et al.*, 1985; Riemann and Sφndergaard, 1986; Hama and Handa, 1987; Lignell, 1990; Siuda and Wcisko, 1990; Siuda *et al.*, 1991; Sundh, 1992; Sell, 1994; Nakano, 1996; Cho *et al.*, 1997). However, the release patterns and fractionation of EOC in different growth phases of algae are unknown, with the exceptions of the studies by Nalewajko and Lean (1972) and Lee and Lee (1996).

The amount of EOC excreted varies with algal species and their physiological state. There is evidence that appreciable amounts of EOC accumulate in cultures during the stationary phase, but little during the exponential growth phase (Fogg, 1952; Allen, 1956; Lewin, 1956; Sharp, 1977; Lee and Lee, 1996). Other reports, however, indicate that high excretion rates also occur in the exponential phase (Watt and Fogg, 1966; Nalewajko and Marin, 1969).

The chemical composition of EOC also varies with growth phases of algae. Nalewajko and Lean (1972) reported that high molecular weight (HMW) compounds became predominant as cultures aged. Many studies have been done to fractionate EOC using gel- and ultra-filtration based upon the molecular weight distributions (Chrost and Faust, 1980; Chrost, 1981; Iturriaga, 1981; Sφndergaard and Schierup, 1982; Lancelot, 1984). EOC also can be fractionated by its hydrophobic-hydrophilic properties and functional groups. The hydrophobic-hydrophilic properties are related to the aromaticity of DOC, while the functional groups are related to the solubility of the organic molecules. Leenheer (1981) classified organic solutes in river waters quantitatively into hydrophobic-base, -acid, and -neutral fractions and hydrophilic-base, -acid, and -neutral fractions based upon their adsorption using nonionic (XAD-8) and ion-exchange resin adsorbents.

Because the aromaticity of DOC may result in an increase in ultraviolet (UV) absorbance, UV absorbance at 260 nm has been adopted as an index for the amount of refractory organic matter such as humus, lignin, and tannin-like compounds (Tambo and Kamei, 1978; Fukushima *et al.*, 1996). Therefore, fractionation of DOC using hydrophobic-hydrophilic properties and the ratio of UV absorbance to DOC can offer useful information on the characteristics of EOC released from algae.

The objectives of this study were as follows: 1) to examine the quantities and the release patterns of EOC in cultures of three cyanobacterial species at various growth phases; 2) to fractionate quantitatively EOC into five fractions; hydrophobic acids, hydrophobic neutrals, hydrophilic acids, hydrophilic bases and hydrophilic neutrals, using XAD-8, cation, and anion resins; and 3) to examine the optical properties of each EOC fraction.

## MATERIALS AND METHODS

### Cultures and Medium

Axenic cultures of *Microcystis aeruginosa* (NIES-44), *Anabaena flos-aquae* (NIES-44) and *Oscillatoria agardhii* (NIES-204) obtained from the Microbial Culture Collection at the Japanese National Institute for Environmental Studies (NIES) were used in this study. These algae are dominant species during summer and autumn in Lake Kasumigaura, Japan. The cultures were maintained on 1/5 CB medium (Lee and Lee, 1996) modified by the substitution of $K_2HPO_4$ as β-glycerophosphate and $NaHNO_3$ as Tris buffer. Since CB medium contains a high concentration of organic carbon, we modified the medium composition to diminish the organic carbon concentration. DOC concentrations within the medium after inoculation were below 1 mgC/l.

### Algal growth experiments

Cultures were grown axenically in 10-L polycarbonate bottles illuminated at 50 μE/$m^2$/sec under a light/dark cycle of 12 h : 12 h and 25°C temperature. Cultures were stirred by air bubbl-

es from a pump equipped with a 0.2-μm sterile filter. Phases of growth were divided into exponential and stationary. A period of linear increase in the logarithmic plot of the growth curve was considered to be the exponential growth phase, and the following stage in which biomass remained more or less stationary was considered to be the stationary phase. At each stage of growth, 1.2-L sub-samples were collected and the algae were separated by filtration using pre-combusted (450°C, 4 h) glass-fiber filters (Whatman GF/F). The residues on the filters were analyzed to measure particulate organic carbon (POC) and chlorophyll *a* concentration, and the filtrates were used to measure the concentration and characteristics of algal EOC.

### Fractionation of EOC excreted from algae

DOC excreted from algae was fractionated into hydrophobic acids (equivalent to aquatic humic substances, AHSs), hydrophobic neutrals (HoNs), hydrophilic acids (HiAs), hydrophilic bases (HiBs) and hydrophilic neutrals (HiNs) based on their adsorption onto a series of macroporous (or macroreticular) resin adsorbents. Nonionic Amberlite XAD-8 resin (20~60 mesh), strong cation exchange resin (Bio-Rad AG-MP-50, 50~100 mesh) and strong anion exchange resin (Bio-Rad AG-MP-1, 50~100 mesh) were used. The column capacity factor, k′, for separating hydrophobic acids through the XAD-8 resin column was 50. This separation condition was the same as that for isolating AHSs from natural waters using XAD-8 resin (Malcolm *et al*., 1989). The classification of dissolved organic compounds em-

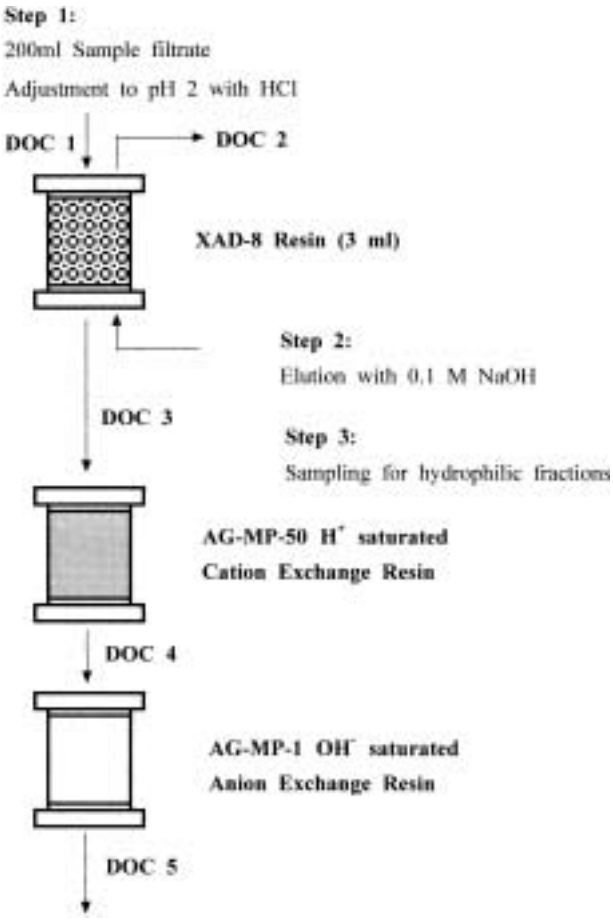


**Fig. 1.** A flow scheme of the DOC fractionation procedure.

**Table 1.** Fractionation of dissolved organic materials employed in this study and the representative materials of each component

| Fraction (Abbr.) | Solute compounds |
|---|---|
| hydrophobic acids (AHSs) | humic substances (humic acids, fulvic acids) |
| hydrophobic neutrals (HoNs) | carbonyl compounds |
| hydrophilic acids (HiNs) | sugar acids, fatty acids, hydroxyl acids |
| hydrophilic bases (HiBs) | protein, aminosugars |
| hydrophilic neutrals (HiNs) | carbohydrates (oligosaccharides, polysaccharides) |

ployed in this study is summarized in Table 1 (Leenheer, 1981).

The XAD-8 resin was cleaned and conditioned according to Thurman and Malcolm (1981). Three milliliters (wet volume) of the XAD-8 resin were packed into a glass column and rinsed 3 times, alternating from 0.1 M NaOH to 0.1 M HCl just before application of the sample. A blank sample was collected in the final rinse with 0.1 M HCl (B1). Both AG-MP-50 (hydrogen-form) and AG-MP-1 (chloride-form) resins were Soxhlet-extracted with methanol for 24 h. AG-MP-1 was then converted into free-base form with 1 M NaOH and rinsed with Milli-Q water (Milli-Q SP.TOC, Millipore). Glass columns containing 6 ml (wet volume) of the cation resin and 12 ml (wet volume) of the anion resin were connected in series and conditioned by pumping about 1-L Milli-Q water. Blank samples (B2 and B3) were

collected from each column after the conditioning.

A flow scheme of the DOC fractionation procedure is shown in Fig. 1 (Imai *et al*., 1998). The steps of the DOC fractionation procedure were as follows: 1: acidify the filtrate (DOC1) to pH 2.0 with 6 M HCl; pass 200 ml of the filtrate through the XAD-8 column by peristaltic pump at a flow rate of about 1 ml/min; and rinse the column with 1~2 bed volumes of 0.1 M HCl; 2: elute the column in the reverse direction with more than 3 bed volumes of 0.1 M NaOH at a flow rate not exceeding 0.5 ml/min (DOC2), and measure the elutant volume; 3: pump the effluent from the XAD-8 column (DOC3) through the series of cation-anion resin columns at a flow rate of about 1 ml/min and after pumping 1~2 bed volumes of the sample, collect elutant samples (DOC5 and DOC4) from the anion resin column and then from the cation resin column.

DOC fractionation was analyzed in duplicate. After fractionation, DOC and UV absorbance were measured for the samples of DOCs 1-5 and the blank. We neglected the hydrophobic base (HoB) fraction since it is known to be very small in quantity (Imai *et al*., 1998). Each DOC fraction was calculated as follows:

$$\text{AHSs} = \text{DOC2} \times (\text{elutant volume})/(\text{sample volume}) \quad (1)$$

$$\text{HoNs} = \text{DOC1} - \text{B1} - \text{AHSs} - \text{DOC3} \quad (2)$$

$$\text{HiBs} = (\text{DOC3} - \text{B1}) - (\text{DOC4} - \text{B2}) \quad (3)$$

$$\text{HiAs} = (\text{DOC4} - \text{B2}) - (\text{DOC5} - \text{B3}) \quad (4)$$

$$\text{HiNs} = \text{DOC5} - \text{B3} \quad (5)$$

The average values of B1, B2, and B3 were $0.04 \pm 0.10$ mgC/l (n = 29), $0.001 \pm 0.000$ mgC/l (n = 18) and 0.001 (0.000 mgC/l (n = 18), respectively. These DOC concentrations were less than the blank values reported by Leenheer (1981). Background contamination was thus successfully held down. The blank DOC from the XAD-8 column during 0.1 M NaOH elution was neglected since its concentration was found to be very low in comparison with the DOC concentration of DOC2. Milli-Q water was adjusted to pH 2 with HCl and passed through the XAD-8 resin column and then through the cation-anion resin columns. The DOC concentration of B2 was lower than that of B1. Consequently, the DOC blank contribution from the XAD-8 column to DOCs 4 and 5 was negligible.

### Chemical analyses

DOC measurements were conducted as non-purgeable dissolved organic carbon with a Shimadzu TOC-5000 total organic carbon analyzer equipped with Pt catalyst on quartz wool. At least triplicate measurements were made for each sample and analytical precision was within 1% coefficient of variation (CV). Potassium hydrogen phthalate (Kanto Chemical Co., Tokyo) was used as the standard. UV absorbance was measured with a Shimadzu UV-2500 UV/VIS spectrometer at 260 nm using a 1-cm path length quartz cell. All samples were adjusted to pH 2 with HCl before measurement. Milli-Q water was used as the blank. UV absorbance at 260 nm was selected because (1) the maximum UV absorbance of phenolic, benzene carboxylic and polycyclic aromatic compounds (p-p* transition) that are considered significant components of AHSs occur at wavelengths of 200~290 nm; and (2) the presence of nitrate and borate does not affect the measurement of UV absorbance at 260 nm. UV absorbance at 260 nm has been reported to be a sensitive index for refractory DOC in water (Tambo and Kamei, 1989).

The quantity of DOC released by the algae was calculated as the increase of DOC in the medium during incubation. POC was determined by CHN coder (Yanaco MT-5, Shimadzu). Chlorophyll *a* was determined by extraction with 100% methanol in a refrigerator overnight (Marker *et al*., 1980).

## RESULTS

### Growth of algae

POC concentrations in all cultures increased exponentially from the beginning of cultivation without a lag phase (Fig. 2). The growth patterns were similar for *M. aeruginosa* and *A. flos-aquae*; the exponential phase was continued from day 1 to day 8, and then the stationary phase began. The maximum POC in the two cultures reached 74 and 125 mgC/l during the stationary phase, respectively. The POC concentration in *O. agardhii* reached a maximum of 17 mgC/l more rapidly, on day 8, and then decreased.

In all cultures, chlorophyll *a* concentration followed the growth pattern of POC, but differed among the species and age of culture (Fig. 2); chlorophyll *a* content during the exponential phase

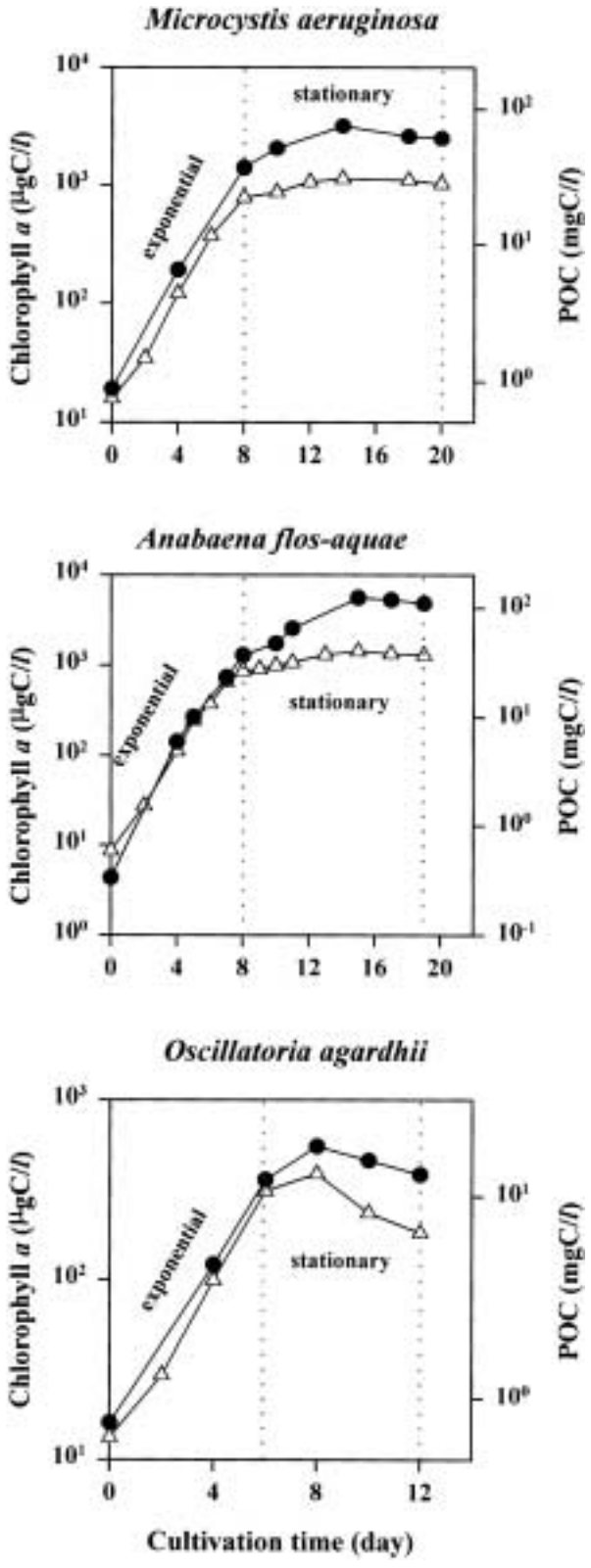


**Fig. 2.** Time course of chlorophyll *a* (open triangles) and POC concentrations (closed circles) in the three axenic cultures.

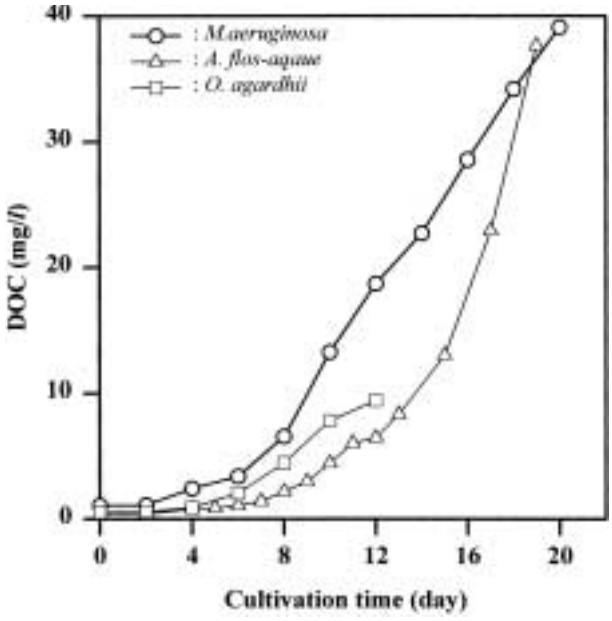


**Fig. 3.** Time course of DOC concentration in the three axenic cultures.

of growth tended to be higher than those in other phases.

### DOC release from algae

The quantities and release patterns of DOC also differed among algal species and incubation time (Fig. 3). DOC concentrations in culture media of *M. aeruginosa*, *A. flos-aquae* and *O. agardhii* increased continuously from 1.0, 0.4 and 0.6 mgC/l at the beginning to 39.1, 37.7 and 9.5 mgC/l at the end of the experiment, respectively. In *M. aeruginosa*, DOC concentration increased slowly during the beginning of the exponential phase, but a distinct increase of DOC was observed after day 8. In *A. flos-aquae*, although the biomass increased 100-fold during the exponential growth phase (Fig. 2), DOC concentrations remained at a low level (Fig. 3). However, the DOC increased exponentially as the culture entered the stationary phase. The quantity of EOC from *O. agardhii* was only one fourth that of the other species, although the release pattern of EOC was similar to that of the other species.

The ratios of DOC/POC (specific DOC excretion) were considerably higher in the stationary than in the exponential phase (Fig. 4). Relatively low values were observed in *A. flos-aquae*. The ratios during the exponential phase decreased or changed less with incubation time, whereas the

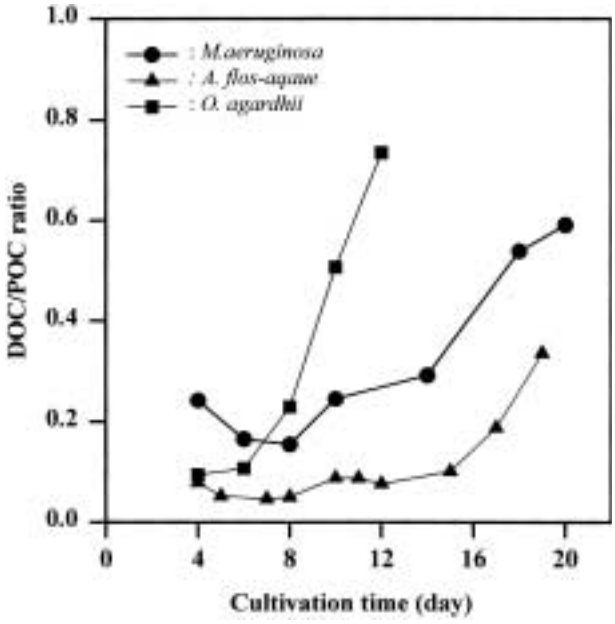


**Fig. 4.** Time course of the DOC/ POC ratio in the three axenic cultures.

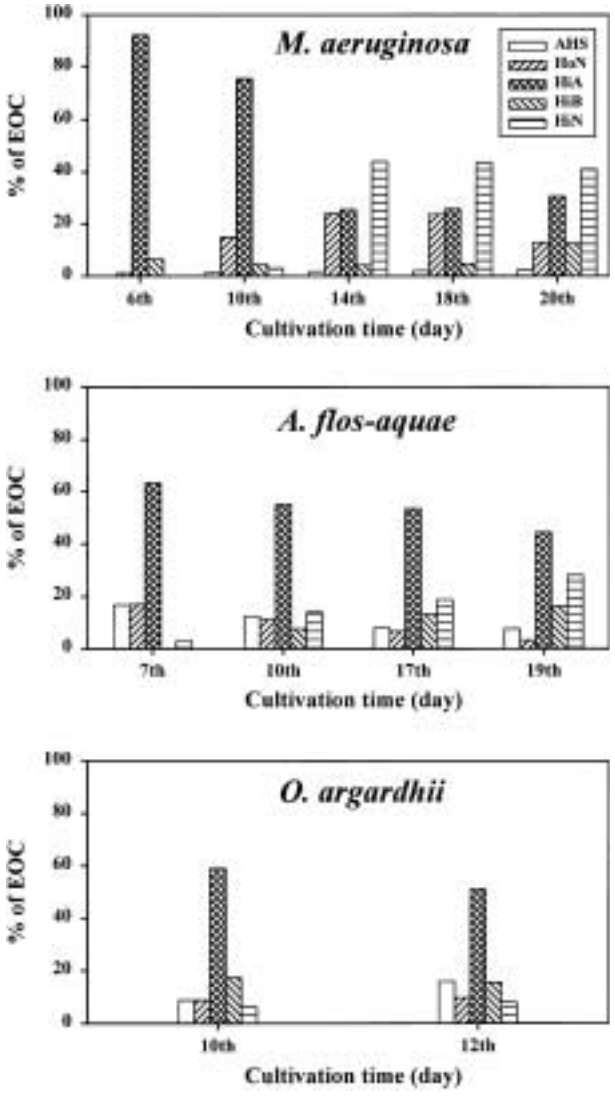


**Fig. 5.** Fractions of EOC released by the three axenic cultures.

ratios after exponential phase increased considerably. Although the quantity of EOC released by *O. agardhii* was considerably smaller than that by the other species (Fig. 3), the DOC/POC ratios in this species were larger (Fig. 4).

In all cultures, the percentage of EOC (PEOC) in total organic carbon (DOC+POC) consistently increased with incubation time. *M. aeruginosa* excreted relatively more DOC than did the other species, especially during the exponential phase (15.7~31.6% in *M. aeruginosa*, 5.3~16.7% in *A. flos-aquae*, and 9.1~31.5% in *O. agardhii*).

## Fractionation of EOC

As was the case for quantity of EOC, the chemical composition of EOC from the algae varied among species and the growth phase (Fig. 5). The HiA fraction was predominant during the exponential phase in *M. aeruginosa* and during all phases in *A. flos-aquae*. The HiA fraction in *O. agardhii* also was dominant (Fig. 5).

The proportion of HiAs to EOC decreased with age of culture (from 92% to 25% in *M. aeruginosa* and from 63% to 44% in *A. flos-aquae*). In contrast, the proportions of HiBs and HiNs increased as cultures aged. In particular, the HiN fraction increased from 0% to 44% of EOC in *M. aeruginosa* and from 3.0% to 28% in *A. flos-aquae*, respectively. The proportion of AHSs ranged from 0.2% to 2.5% in *M. aeruginosa*, 7.5% to 16% in *A. flos-aquae* and 8.7% to 16% in *O. agardhii*, respectively. The proportions of AHSs increased with culture age in *M. aeruginosa* and *O. agardhii*, but the reverse trend was shown by *A. flos-aquae*. The proportion of the HoN fraction ranged from 15% to 24% in *M. aeruginosa*, 3.7% to 17% in *A. flos-aquae*, and 8.7% to 9.6% in *O. agardhii*. The highest proportion of HoN fraction occurred during the stationary phase in *M. aeruginosa*, while *A. flos-aquae* this proportion decreased with age of culture as observed for the AHS fraction (Fig. 5).

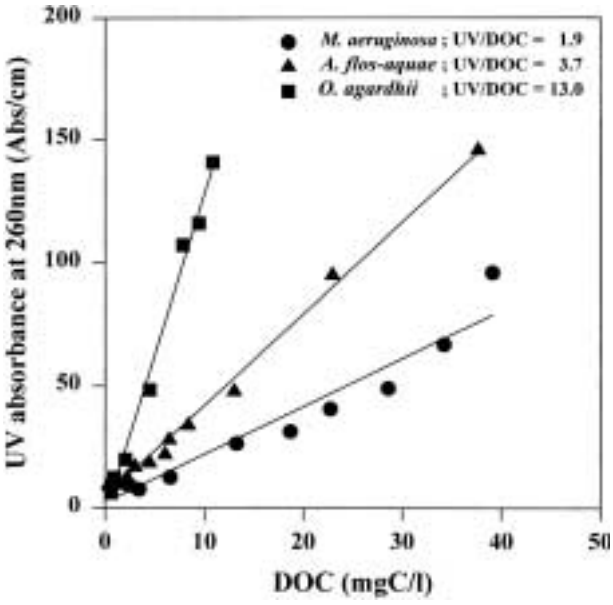


**Fig. 6.** Relationship between UV absorbance and DOC in the three axenic cultures.

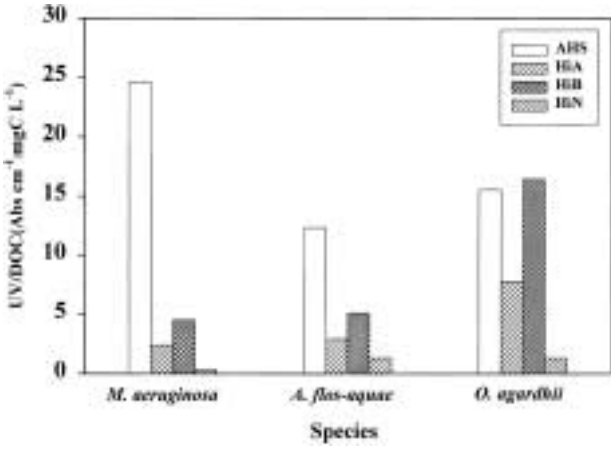


**Fig. 7.** UV absorbance : DOC ratios of the three axenic cultures.

### UV absorbance vs. DOC ratios

Positive linear relationships were observed between UV absorbance (at 260 nm) and DOC concentration in all cultures (Fig. 6). The slope, i.e. the ratio of UV absorbance to DOC, was much higher in *O. agardhii* (13.0 Abs · $cm^{-1}$/mgC · $L^{-1}$) than in *M. aeruginosa* (1.9 Abs · $cm^{-1}$/mgC · $L^{-1}$) and *A. flos-aquae* (3.7 Abs · $cm^{-1}$/mgC · $L^{-1}$).

The ratios for *M. aeruginosa* and *A. flos-aquae* fractions were AHSs > HiBs > HiAs > HiNs in magnitude, different from the order in *O. agardhii* (HiBs > AHSs > HiAs > HiNs) (Fig. 7). Although the AHS fraction in *M. aeruginosa* was minor component (< 2.4%), the UV absorbance: DOC ratio was very high (24.6 Abs · $cm^{-1}$/mgC · $L^{-1}$) In contrast, the ratio in *O. agardhii* (15.5 Abs · $cm^{-1}$/mgC · $L^{-1}$) was less than that for the the HiB fraction (16.4 Abs · $cm^{-1}$/mgC · $L^{-1}$).

## DISCUSSION

The present study indicates that the release pattern, chemical composition and optical property of DOC excreted by three blue-green algal species differed with species and growth phase. The PEOC reached 31.6% in *M. aeruginosa*, 16.7% in *A. flos-aquae*, and 31.5% in *O. agardhii* at the end of growth. Nalewajko and Lean (1972) also found that the lowest value of PEOC was observed in *A. flos-aquae* (12.7%) among the four cultures of different species. DOC excretion from *A. flos-aquae* and *O. agardhii* was relatively low during the exponential growth phase. This result was demonstrated by the previous reports that the excretion of EOC by algal culture was negligible during the exponential phase (Fogg, 1952; Allen, 1956; Lewin, 1956, Sharp, 1977; Lee and Lee, 1996). In contrast, DOC excretion from *M. aeruginosa* was large during the exponential as well as during the stationary growth phase. However, the DOC/POC ratios were several times lower in the exponential than in the stationary phase (Fig. 4), indicating that DOC excretion was negligible during the exponential phase. Most DOC may released during the stationary phases.

The major component of EOC in all species was the HiA fraction. Although the specific EOC chemicals fractionated by the three resin adsorbents were not identified in this study, the classification of organic solutes by Leenheer (1981) suggested that the HiA fraction consists of sugar acids, fatty acids, and hydroxyl acids (Table 1). Previous studies provided the evidence that the major components of DOC released from phytoplankton during active photosynthesis are amino acids, glycolate and sugars (Hellebust, 1965; Tanaka *et al*., 1974; Mague *et al*., 1980; Watanabe, 1980; Hino, 1988). Hama and Handa (1987) reported that when *Microcystis aeruginosa* was dominant in Lake Suwa, Japan, the EOC was comprised of low molecular weight (LMW, < 500 dalton) compounds, which contained amino acids,

oligopeptides and other organic acids. This means that LMW compounds are quantitatively important products released from blue-green algae during active photosynthesis.

The chemical composition of EOC also varied with physiological state of the algae. The proportion of HiA fraction decreased with culture age, whereas the proportions of HiBs and HiNs increased. In particular, the HiN fraction (oligo- and polysaccharides) during the stationary phase accounted more than 40% of extracellular products of *M. aeruginosa* (Fig. 5). Other reports indicate that HMW compounds become predominant as cultures age (Nalewajko and Lean, 1972; Sϕndergaard and Schierup, 1982). The HMW substances of EOC from phytoplankton in lakes were in most cases dominated by polysaccharides (Hama and Handa, 1987; Sundh, 1992), implying that the release of HiB and HiN fractions was due to lysis of senescent and decaying algal cells. Although the fractions in *A. flos-aquae* and *O. agardhii* also increased as cultures aged, the HiAs were still major components of EOC. AHSs formed a minor component (0.2～2.5%) of EOC in *M. aeruginosa*, but were more substantial in *A. flos-aquae* (7.5～16%) and *O. agardhii* (8.7～16%). The proportion of the AHS fraction also differed with algal species and culture age.

Differences in the ratios of UV absorbance to DOC also provide evidence that the composition of EOC differed among algal species. UV absorbance at 260 nm has been used as an index of the quantity of humic substances, which are mainly aromatic compounds (Schnitzer, 1977; Tambo and Kamei, 1978; Fukushima *et al*., 1996). The ratios of UV absorbance: DOC in the AHS fraction were considerably higher than those observed in hydrophilic components (Fig. 7). As a result, the low ratios in *M. aeruginosa* was due to the low proportion of AHSs compared with other species (Fig. 5). In *O. agardhii*, however, the ratio for the HiB fraction (proteins and amino sugars) was larger than that for the AHS fraction. Zumstein and Buffle (1989) reported that polysaccharides and proteins showed low UV absorbance. It is unclear why the UV absorption of the HiB fraction was higher than that of the AHS fraction in *O. agardhii*.

## ABSTRACT

The amount, chemical composition and optical property of extracellular dissolved organic carbon (EOC) by phytoplankton were examined using axenic cultures of *Microcystis aeruginosa*, *Anabaena flos-aquae*, and *Oscillatoria agardhii*. The extracellular organic matter was categorized into five fractions (hydrophobic acids; AHSs, hydrophobic neutrals; HoNs, hydrophilic acids; HiAs, hydrophilic bases; HiBs, and hydrophilic neutrals; HiNs) using three adsorbent resins (XAD-8, cation, and anion). The release pattern and chemical composition of EOC varied with algal species and their growth phases. Percentage of extracellular release increased with age in all cultures. HiAs were the dominant component of EOC in all cultures, whereas the proportion of HiAs decreased with age in all cultures. In contrast, the proportions of HiBs and HiNs increased as cultures aged. In particular, the HiN fraction increased from 0% to 44% of EOC in *M. aeruginosa* and from 3.0% to 28% in *A. flos-aquae*, respectively. The proportion of AHSs was higher in the cultures of *A. flos-aquae* (7.5～16%) and *O. agardhii* (8.7～16%) than *M. aeruginosa* (0.2～2.5%). The proportions of AHSs increased with culture age in *M. aeruginosa* and *O. agardhii*, but decreased in *A. flos-aquae*. The specific UV absorbance also varied among species; 1.9 Abs · $cm^{-1}$/mgC · $L^{-1}$ for *M. aeruginosa*, 3.7 Abs · $cm^{-1}$/mgC · $L^{-1}$ for *A. flos-aquae*, and 13.0 Abs · $cm^{-1}$/mgC · $L^{-1}$ for *O. agardhii*. The results of this study indicates that DOC excreted by three blue-green algae differed with species and the growth phase.

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