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Photoalteration in Biodegradability and Chemical Compositions of Algae-derived Dissolved Organic Matter

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자외선에 의한 조류기원 용존유기물의 생분해도 및 화학조성변환. 최광순* · Akio Imai · Kazuo Matsushige · Takashi Nagai · 김용환 · 김범철[1] (일본 국립환경연구소, [1]강원대학교 환경과학과)

자외선에 의한 조류기원 용존유기물의 특성변화를 조사하기 위하여 배양한 남조류 두종 (*Microcystis aeruginosa, Oscillatoria agardhii*)의 체외배출용존유기물에 자외선 처리전과 처리후의 생분해도와 유기물의 화학적조성을 비교하였다. 자외선처리는 pyrex용기에 시료를 넣고 인공자외선램프(UVA: 40 W/m^2, UVB: 2 W/m^2)로 24시간 조사하였다. 유기물의 화학적조성은 XAD-8, 양이온, 음이온수지를 이용하여 소수성 산(hydrophobic acids; HoA), 소수성 중성(hydrophobic neutrals; HoN), 친수성 산(hydrophilic acids; HiA), 친수성 염기(hydrophilic bases; HiB), 그리고 친수성 중성(hydrophilic neutrals; HiN)의 5개 분획으로 분류하였다. 자외선처리동안 유기물의 농도변화는 거의 없었던 반면 생분해도는 자외선처리 전에 비해 현저히 감소하였다(*M. aeruginosa*: 17%, *O. agardhii*: 28% 감소). 또한 자외선 처리 전과 후의 유기물의 화학적조성도 상당한 차이를 보였다. 자외선처리 후 HiB분획(단백질, 아미노산류)은 감소한 반면 HiA분획(카복실산류)은 증가하였다. 유기산분석에서도 3종류의 카복실산이 자외선처리 후 증가하는 것으로 나타났다. 일반적으로 수체의 유기물은 자외선에 의해 난분해성 유기물이 분해되거나 잘게 쪼개져 이분해성 유기물로 전환되는 것으로 알려졌다. 그러나 본 연구에서는 조류기원의 이분해성 유기물의 경우는 자외선에 의해 완전한 광분해는 보이지 않았지만 난분해성 유기물로 전환되었고 화학적조성도 바뀌었다. 이는 유기물의 기원과 종류에 따라 자외선에 대한 영향이 다르다는 것을 시사한다.

Key words : algal DOM, UV effects, photoalteration, biodegradability, chemical composition

INTRODUCTION

Dissolved organic matters (DOM), one of the major pools of organic carbon in aquatic ecosystems, can be an important source of carbon and energy for both heterotrophic microorganisms and higher trophic levels (Amon and Benner, 1994; Lampert and Sommer, 1997; Wetzel, 2001). However, only a minor portion of DOM is involved in a fast carbon cycle, while the remainder is resistant to microbial degradation (Søndergaard and Borch, 1992; Søndergaard *et al.*, 1995; Wetzel, 2001). Much attention has been paid to the role of ultraviolet (UV) radiation on the biological cycling of DOM in aquatic systems. The UV light can alter or cleave the DOM into smaller and more labile organic molecules

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(Miller and Zepp, 1995; Wetzel *et al*., 1995; Amon and Benner, 1996; Moran and Zepp, 1997; Wetzel, 2000). As a result, UV radiation can increase the pool of labile DOM in aquatic systems and thereby enhance bacterial production.

However, these processes may be limited to the allochthonous DOM, which is mainly comprised of aromatic, recalcitrant and high molecular weight compounds. Recent studies noted that the autochthonous DOM (originating primarily from phytoplankton), which is composed of relatively labile compounds, is resistant to photodegradation (Thomas and Lara, 1995; Tranvik and Kokalj, 1998; Pausz and Herndl, 1999). Furthermore, labile DOM such as proteinaceous substrates and phytoplankton exudates can be transformed into recalcitrant forms after UV radiation exposure (Naganuma *et al*., 1996; Tranvik and Kokalj, 1998; Pausz and Herndl, 1999; Obernosterer *et al*., 2001). These results suggest that the autochthonous DOM seems to be resistant to photodegradation, but obviously altered in its characteristic. With progressing eutrophication autochthonous DOM becomes increasingly important. If lake waters displaying this feature are also exposed by UV radiation, this may affect the carbon cycling and pools of the aquatic systems. Nevertheless, there is little information on the photoalteration of the autochthonous DOM. Especially there have been very few studies on photochemical change in chemical composition of autochthonous DOM.

The purpose of this study was to examine the photoalteration of algal DOM produced from two blue-green axenic cultures by comparing their biodegradability and DOM fraction distributions before and after UV exposure. Bacterial degradation test was used as a measure of biodegradability of algal DOM. The algal DOM was fractionated into five classes using three kinds of resin adsorbents. Some organic acids newly produced during UV exposure also were analyzed with capillary electrophoresis system.

MATERIALS AND METHODS

Preparation of algal DOM

To obtain the algal-derived DOM, two axenic cultures of *Microcystis aeruginosa* and *Oscillatoria agardhii* that were isolated from Lake Kasumigaura (Japan) were grown axenically in 10 *l* polycarbonate bottles at 25°C and about 50 $\mu E/m^2/sec$ under a light/dark cycle of 12 h : 12 h on CB medium. The cultures were stirred by air bubbles provided from a pump equipped with a 0.2 μm sterilizing filter. Since the standard CB medium contains a high concentration of organic carbon, we modified the medium composition by substituting K_2HPO_4 for β-glycerophosphate and $NaHNO_3$ for Tris buffer. DOC concentrations in the medium after inoculation were below 0.5 mgC/l. When the cultures reached their stationary phase, they were filtered through pre-combusted (450°C for 4 h) Whatman GF/F glass-fiber filters. The filtrates were used as sources of the algae-derived DOM.

UV irradiation experiments

For the UV radiation treatment, one liter of DOM sample was put in 1.3 *l* pyrex bottle of a photo-reaction apparatus (USHIO, Japan) and the radiation experiments were conducted for 2, 4, 6, 8, 10, 12 and 24 h. UV light source was provided by UM-452 lamp which emitted the light of nearly all UV wavelength ranges (220 to 400 nm). Since pyrex bottles used in this experiment selectively cut off UV light shorter than 280 nm (the transmission of the pyrex was zero at 280 nm, and was 70% at 320 nm), short UV radiation (UV-C) was not included. UV radiation was measured with a radiometer (MI-340 UV meter, Eikoseiki, Japan), equipped with a UV-A sensor (316～400 nm) and a UV-B sensor (280～315 nm).

Biodegradability experiments

Biodegradability of algal DOM before and after UV exposure was quantified through a series of microbial degradation experiments. A portion (200 ml) of the algal DOM samples before and after UV exposure was poured into pre-combusted 300-ml glass bottles (550°C for 4 h), and then 1 ml of bacteria concentrate were added to give an initial bacterial abundance of around 10^5 cells/ml. Water for the bacteria inoculum was collected from Lake Kasumigaura. The bottles were then incubated in darkness at room temperature (ca 20°C) for 20 days. Ten milliliters of sub-samples for DOC determination were collected from the bottles after 0, 1, 2, 3, 4, 5, 7, 10, 15 and 20 days. The biodegradability experiments were performed in triplicate.

Table 1. Classification of organic compounds for dissolved organic matter in natural waters.

Fraction	Solute compound classes
hydrophobic acids (HoA)	humic substances (humic and fulvic acids)
hydrophobic neutrals (HoN)	hydrocarbons, carbonyl compounds
hydrophilic acids (HiA)	carboxylic acids (fatty and hydroxyl acids), sugar acids
hydrophilic bases (HiB)	protein, amino acids, aminosugars
hydrophilic neutrals (HiN)	oligosaccharides, polysaccharides

DOM fractionation

The DOM samples before and after UV treatment were fractionated into five fractions: hydrophobic acids (HoA), hydrophobic neutrals (HoN), hydrophilic acids (HiA), hydrophilic bases (HiB), and hydrophilic neutrals (HiN), based on their adsorption on a series of macroporous 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 for fractionation. The fractionation procedure was according to Imai *et al.* (2002). Appropriate classification of organic compounds according to the DOM fraction is listed in Table 1 (Leenheer, 1981; Thurman, 1985).

Chemical analyses

Some carboxylic acids that were found to be major products formed during UV exposure were analyzed on a capillary electrophoresis (CE) system (Quanta 4,000, Waters). A 70 cm fused silica capillary (75 μm inner diameter), and a 100 mM sodium boric acid buffer containing 0.5 mM of an electro-osmotic flow modifier (OFM-BT, Waters) was used for the analyses. A separation voltage of 15 kV was applied and the analytes were detected by indirect UV detection at 185 nm. Standard curves (10~1,000 μg/l) were made for the three detected carboxylic acids (oxalic, formic, and acetic acids).

DOC was measured as non-purgeable DOC with a Shimadzu TOC-5,000 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% of coefficient of variance (CV). Potassium hydrogen phthalate (Kanto Chemical Co., Tokyo) was used as standard.

RESULTS AND DISCUSSION

It is well recognized that UV radiation can alter the DOM pool in natural waters by complete degradation into CO_2, and by cleaving into more smaller and labile molecule enhancing the bacterial utilization. The photochemical removal of DOC into CO_2 in many natural waters shows a wide range of 0 to 60%, depending on the DOM sources, light sources, and time of light exposure (Wiegner and Seitzinger, 2001). In this study, no significant changes of dissolved organic carbon (DOC) were observed in algal DOM during UV radiation exposure, showing a constant levels of 12.34±0.08 mgC/l in *M. aeruginosa* and 8.68± 0.05 mgC/l in *O. agardhii* (Fig. 1). This implies that complete degradation of algal DOM to CO_2 did not occur during UV exposure. The UV treatment (for 24 h under 42 W/m^2) used in the present study corresponds to the level shown the photochemical effect in other natural waters. Hence, no change of algal DOC in this study is not due to the light treatment, but probably the DOM sources having resistant to UV radiation. Similar results were reported in other studies with phytoplankton exudates (Thomas and Lara, 1995; Tranvik and Kokalj, 1998).

On the other hand, there was a great difference

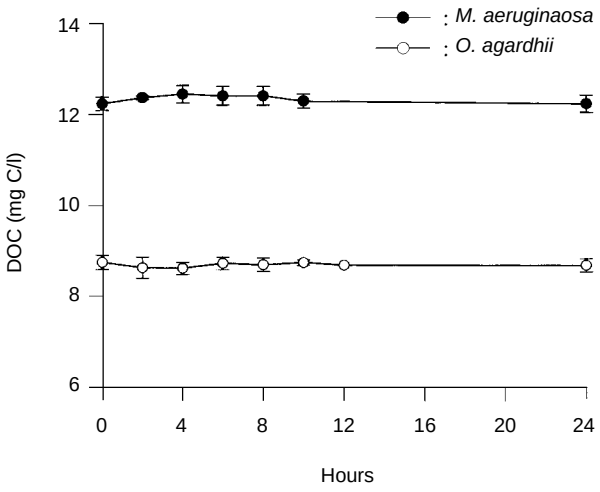

Fig. 1. Changes in concentrations of algal derived DOC with UV exposure times.

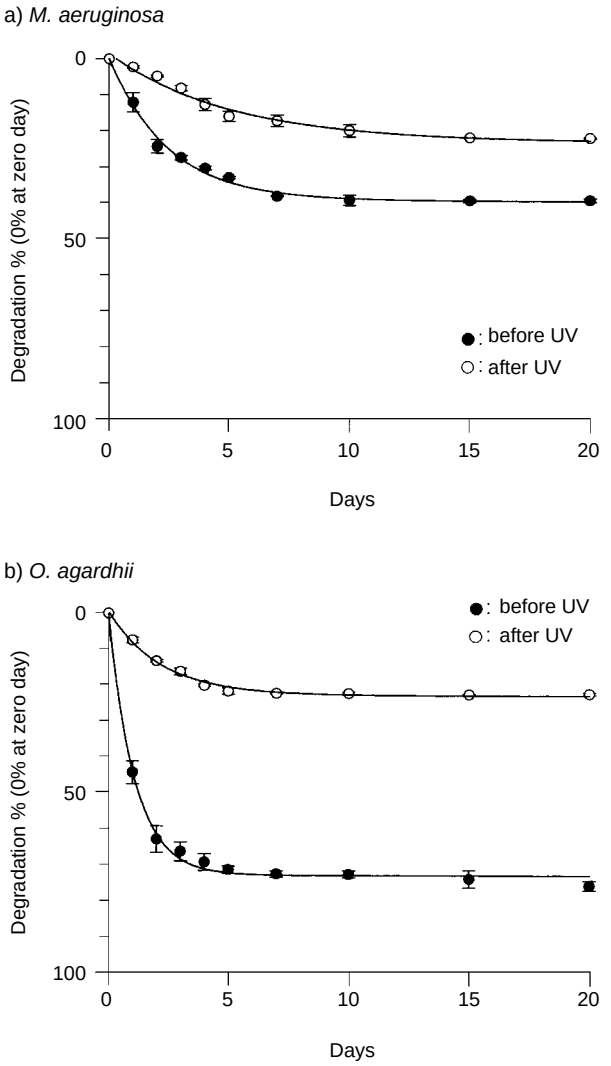

Fig. 2. Degradation curves of algal DOM before and after UV exposure. Bars represent standard deviation.

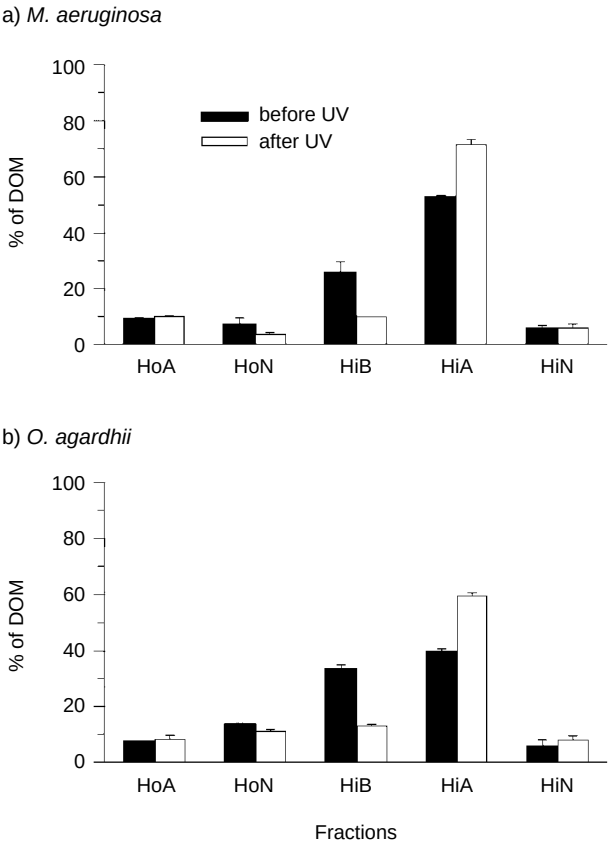

Fig. 3. Proportions (%) of DOM fractions for before and after UV treated samples. Bars represent standard deviation.

in the biodegradability between before and after UV exposure both in two algal DOM sources. Microbial degradations were reduced in the UV exposed algal DOM by 17% in *M. aeruginosa* and 53% in *O. agardhii*, respectively (Fig. 2). Decomposition rates also were two times lower in UV exposed algal DOM (0.20/day in *M. aeruginosa* and 0.45/day in *O. agardhii*, respectively) than in raw algal DOM (0.40/day in *M. aeruginosa* and 0.91/day in *O. agardhii*, respectively). The decreased bacterial activity on UV exposed algal DOM has also been reported in other studies (Tranvik and Kokalj, 1998; Pausz and Herndl, 1999). They found that microbial activity on the UV exposed algal DOM was inhibited by 15 to 20%, while the loss of DOC was less than 1% during the UV exposure. These results indicate that algal DOM can be altered qualitatively without complete degradation by UV radiation. Thus, we further tried to fractionate the algal DOM before and after UV exposure to understand the change of their chemical compositions caused by UV radiation.

The hydrophilic bases (HiB) and acids (HiA) were dominant fractions of the algal DOM (more than 70% both in *M. aeruginosa* and *O. agardhii*), although the proportion of each fraction differed with the sources of algal DOM (see black bars in Fig. 3). Hydrophobic fractions (HoA and HoN) contributed only 16% in *M. aeruginosa* and 20% in *O. agardhii*, respectively. After UV radiation exposure, the proportions of the HiB (protein-like DOM) and HiA (carboxylic acids-like DOM) fractions were considerably changed compared with other fractions (see white bars in Fig.

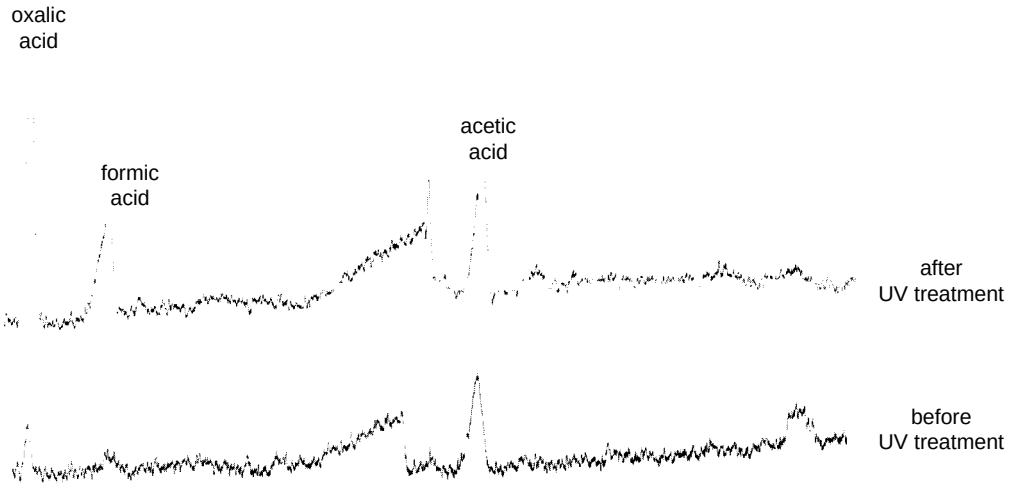

Fig. 4. Electropherograms for before and after UV treated samples (*M. aeruginosa*) indicating UV−induced increase of oxalic, formic and acetic acids.

3). In both algal DOM sources the fractions of the protein−like DOM decreased considerably (16.8% and 20.0% of DOM, respectively) after UV exposure. In contrast, the carboxylic acid−like DOM increased as much as the decrease of the protein−like DOM after UV exposure. Differences in the chemical compositions of algal DOM between before and after UV exposure also provided evidence that algal DOM changed qualitatively by UV radiation.

Contradictory results were reported by Thomas and Lara (1995), who found no changes in chemical compositions as well as concentration of algal DOM after UV exposure. The differences between their and our results may be due to the algal DOM used in two experiments. We used freshly produced algal DOM, while the DOM used in their experiments had been aged in the presence of bacteria for 8 months. During the long incubation, bacteria would utilize initially labile constituents that also would be changeable by UV radiation. Thus, initially labile DOM might be not involved in their experiments in spite of the fact that they are important fraction of algal DOM.

To clarify the increase of the carboxylic acids−like fraction after UV exposure, we measured several carboxylic acids (oxalic, formic, and acetic acids) with capillary ion electrophoresis (CE) system. The three carboxylic acids increased after UV exposure in both algal DOM sources, although the extent of increase for each organic acid differed with the sources of algal DOM (Table 2). Especially, a substantial increase of oxalic acid (410 μg/l) and formic acid (530 μg/l) after the UV exposure was observed in DOM from *M. aeruginosa* (Fig. 4, Table 2). In general, carboxylic acids are easily decomposable materials for bacteria (Allard *et al*., 1994; Bertilsson and Tranvik, 1998; Wetzel, 2000). Hence, the increased HiA fraction (probably produced as photo−product of HiB fraction) may not be linked to the recalcitrance of algal DOM by UV exposure. Further research is needed to clarify the mechanism of the photoalteration of algae−derived DOM in aquatic ecosystems.

Table 2. Results of the CIE analysis indicating an increase of carboxylic acids after UV exposure. The values are averages of duplicates.(Unit: μg/l)

Carboxylic acids	*M. aeruginosa*		*O. agardhii*	
	before	after	before	after
Oxalic acid	50	460	70	180
Formic acid	30	560	550	560
Acetic acid	150	200	30	150

In the present study, we presented that algal DOM can be photochemically altered in its chemical composition and biodegradability. The photoalteration of algal DOM is likely to have an influence on the carbon cycle and pool in aquatic systems, especially algal DOM is important carbon source, e.g. by making algal DOM unavail-

able for the production of bacteria.

ABSTRACT

The effect of ultraviolet (UV) radiation on the characteristics of algae-derived dissolved organic matter (DOM) was examined by comparing the biodegradability and DOM fraction distribution of algal DOM before and after UV exposure. Algal DOM from two axenic cultures of *Microcystis aeruginosa* and *Oscillatoria agardhii* were irradiated for 24 h at a UV intensity of 42 W/m^2. A complete degradation of algal DOM during the UV exposure did not occur, remaining at constant concentrations of dissolved organic carbon (DOC). After UV exposure, however, microbial degradations were reduced by 17% in *M. aeruginosa* and 53% in *O. agardhii*, respectively, and decomposition rates also were two times lower in UV exposed algal DOM. In addition, the chemical compositions of algal DOM altered substantially after UV radiation exposure. The proportions of hydrophilic bases (HiB; protein-like DOM) decreased considerably in both algal DOM sources after UV exposure (16.8% and 20.0% of DOM, respectively), whereas those of hydrophilic acids (HiA; carboxylic acids-like DOM) increased as much as the decrease of the HiB fraction. Capillary ion electrophoresis (CE) analysis showed that several carboxylic acids increased significantly after UV exposure, further confirming an increase in HiA fractions. The results of this study clearly indicate that algal DOM can be changed in its chemical composition as well as biodegradability without complete degradation by UV radiation.

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REFERENCES

Allard, B., H. Borén, C. Pettersson and G. Zhang. 1994. Degradation of humic substances by UV radiation. *Environ Int.* **20**: 97-101.

Amon, R. M. W. and R. Benner. 1994. Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. *Nature*, **369**: 549-552.

Amon, R.M.W. and R. Benner. 1996. Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen on the Amazon River system. *Geochim Cosmochim Acta*. **60**: 1783-1792.

Bertilsson, S. and L.J. Tranvik. 1998. Photochemically produced carboxylic acids as substrates for freshwater bacterioplankton. *Limnol Oceanogr.* **43**: 885-895.

Imai, A., T. Fukushima, K. Matsushige, Y.H. Kim and K. Choi. 2002. Characterization of dissolved organic matter in effluents from wastewater treatment plants. *Wat Res.* **36**: 859-870.

Lampert, W. and U. Sommer. 1997. *Limnoecology*. 96-97. Oxford University, New York.

Leenheer, J.A. 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. *Environ Sci Technol.* **15**: 578-587.

Miller, W.L. and R.G. Zepp. 1995. Photochemical production of dissolved inorganic carbon from terrestrial organic matter: significance to the oceanic organic carbon cycle. Geophys Res Lett. **22**: 417-420.

Moran, M.A. and R.G. Zepp. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnol Oceanogr.* **42**: 1307-1316.

Naganuma, T., S. Konish, T. Inoue, T. Nakane and S. Sukizaki. 1996. Photodegradation or photoalteration? Microbial assay of the effect of UV-B on dissolved organic matter. *Mar Ecol Prog Ser.* **135**: 309-310.

Obernosterer, I., R. Sempéré and G.J. Herndl. 2001. Ultraviolet radiation induces reversal of the bioavailability of DOM to marine bacterioplankton. *Aquat Microb Ecol.* **24**: 61-68.

Pausz, C. and G.J. Herndl. 1999. Role of ultraviolet radiation on phytoplankton extracelluar release and its subsequent utilization by marine bacterioplankton. *Aquat Microb Ecol.* **18**: 85-93.

Søndergaard, M., B. Hansen and S. Markager. 1995. Dynamics of dissolved organic carbon lability in a eutrophic lake. *Limnol Oceanogr.* **40**: 46-54.

Søndergaard, M. and N.H. Borch. 1992. Decomposition of dissolved organic carbon (DOC) in lakes. *Arch Hydrobiol Beih Ergebn Limnol.* **37**: 9-20.

Thomas, D.N. and R.J. Lara. 1995. Photodegradation of algal derived dissolved organic carbon. *Mar*

Ecol Prog Ser. **116**: 309–310.
Tranvik, L. and S. Kokalj. 1998. Decreased biodegradability of algal DOC due to interactive effects on UV radiation and humic matter. *Aquat Microb Ecol*. **14**: 301–307.
Thurman, E.M. 1985. Organic geochemistry of natural waters. Martinus Nijhoff, Boston.
Wetzel, R.G. 2000. Natural photodegradation by UV-B of dissolved organic matter of different decomposing plant sources to readily degradable fatty acids. *Verh Internat Verein Limnol*. **27**: 2036–2043.
Wetzel, R.G. 2001. Limnology. 3th ed. Academic Press, New York.
Wetzel, R.G., P.G. Hatcher and T.S. Bianchi. 1995. Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. *Limnol Oceanogr*. **40**: 1369–1380.
Wiegner, T.N. and S.P. Seitzinger. 2001. Photochemical and microbial degradation of external dissolved organic matter inputs to rivers. *Aquat Microb Ecol*. **24**: 27–40.

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