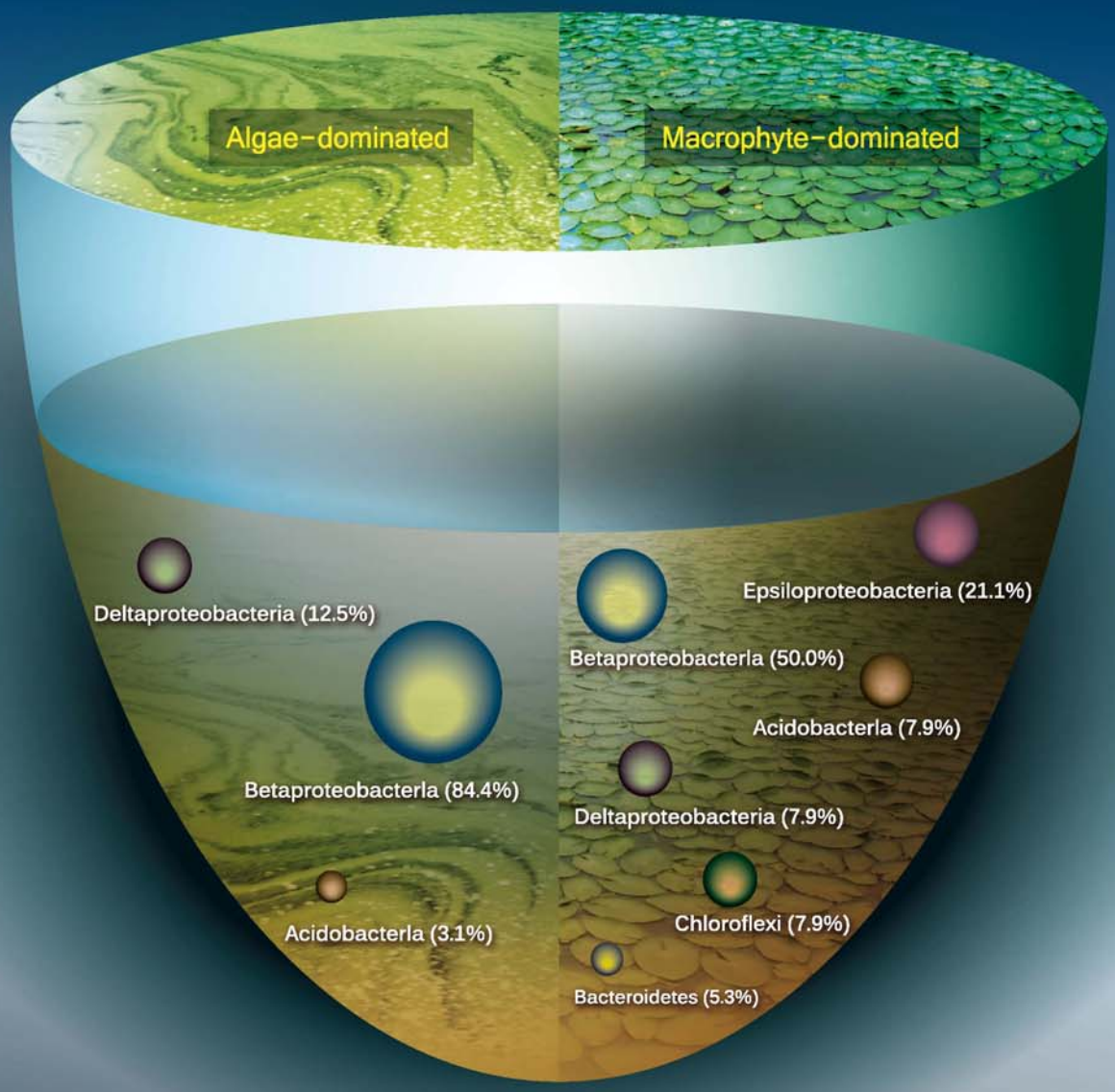
JES
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
June 1, 2013 Volume 25 Number 6
www.jesc.ac.cn



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Chinese Academy of Sciences

ISSN 1001–0742 **Journal of Environmental Sciences** Vol. 25 No. 6 2013

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Serial parameter: CN 11-2629/X*1989*m*207*en*P*25*2013-6

Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2013, 25(6) 1089–1097
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Organic matter produced by algae and cyanobacteria: Quantitative and qualitative characterization

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Received 30 October 2012; revised 16 January 2013; accepted 22 January 2013

Abstract

This work aims at characterizing organic matter produced by an alga *Euglena gracilis* and a cyanobacteria *Microcystis aeruginosa* and assessing the evolution of its characteristics during growth. A culture medium was optimized. The species growth phases were monitored using both visible spectrophotometry and flow cytometry cell counting. Organic matter fractionation according to hydrophobicity and specific UV absorbance (SUVA) index were used to specifically characterize the produced algal organic matter (AOM). The AOM characteristics were both growth phase and species dependent. However, a similar evolution was observed. The hydrophilic fraction (HPI) was the major fraction whatever the growth phases and was almost the only one produced during lag and exponential phases. It represented around 75% of AOM during exponential phase and then decreased when the stationary phase appeared. It represented 46% and 60% of the AOM during late decline phase for the cyanobacteria and the alga respectively. The hydrophobic (HPO) and transphilic (TPH) fractions started to appear from the beginning of the stationary phase with more hydrophobic compounds coming from intracellular organic material of dying cells. HPO and TPH percentages still increased during the decline phase probably because of two additional processes: photo-dissolution and leaching of particulate organic matter from cells fragments. A comparison of AOM during late decline phase and natural organic matter (NOM) from Glane River (France) underlined that AOM was more hydrophilic and presented a lower SUVA for each fractions than NOM. However, the difference between NOM and AOM hydrophobicity narrowed during decline phase.

Key words: algae; cyanobacteria; organic matter; XAD fractionation; SUVA; flow cytometry
DOI: 10.1016/S1001-0742(12)60208-3

Introduction

The natural organic matter (NOM) in natural waters can be from both autochthonous and allochthonous natural origins and anthropogenic activities. The production by living organisms (mainly phytoplankton and bacteria) are autochthonous sources of NOM whereas allochthonous sources of NOM include soils and catchment inputs brought by runoff. NOM structure and characteristics are thus complex (Gondar et al., 2008).

To improve knowledge on organic matter (OM) properties, several laboratory techniques are commonly used: 3D fluorescence spectroscopy, UV-Visible spectroscopy, mass spectrometry, FT-IR or GC-MS pyrolysis (Maurice et al., 2002; Parlanti et al., 2002; Leenheer and Croué, 2003; Navalon et al., 2010). However, the structure of NOM is so complex that a full characterization would be very expensive and difficult. A way to characterize NOM consists in separating organic molecules in several fractions having the same properties. Organic molecules can be separated according to their hydrophobic character (Thurman, 1985; Leenheer and Croué, 2003). The protocol uses XAD resins in series to separate organic compounds in hydrophobic (HPO), transphilic (TPH) and hydrophilic (HPI) fractions. Each fraction is characteristic of different environmental processes and the repartition is dependent of the origin of compounds. In natural waters, the state of humification can thus vary considerably. Wang et al. (2009) showed that hydrophobic materials with a high molecular weight were mainly from allochthonous origin. The autochthonous compounds presented a more

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hydrophilic character with lower molecular weight in comparison with the allochthonous compounds. Thus, as the autochthonous contribution is more important in lakes than in rivers, the HPI fraction percentage is more important in these latter ones than in river waters (Imai et al., 2001).

Eutrophication phenomenon has been recently of major concern especially because of its impact on water resources management and drinking water production. Indeed, the seasonal proliferations of phytoplankton (algae and cyanobacteria) result in disruptions of ecosystem functioning with modifications of food webs (Oliver and Ganf, 2000), in organic material release responsible for color, taste and odor (Dokulil and Teubner, 2000) and in production of cyanobacterial hazardous toxin. Important quantities of organic matter are released during proliferations but are not totally consumed by heterotrophic bacteria. The enrichment in organic matter of water resources during eutrophication is thus of major concern as these organic compounds accumulate.

The algal organic matter (AOM) released is composed of extracellular organic matter (EOM) produced by living cells and intracellular organic matter (IOM) from cell autolysis which is generated during the population growth and decline (Nguyen et al., 2005). The AOM is mainly composed of polysaccharides, proteins (30% to 55% of dry algal biomass (González López et al., 2010)), peptides, amino acids and other organic acids like fatty acids (Cardozo et al., 2007), giving to AOM a highly hydrophilic character. Several authors already studied the AOM properties using XAD fractionation but these studies were restricted to the exponential and stationary phases. The AOM composition is linked to the considered species, its growth phase, the age of the culture and the environmental conditions (Pivokonsky et al., 2006). The concentrations in HPO and TPH in AOM vary largely and depend on the culture conditions. However, the HPI fraction is always the predominant fraction and 57% to 71% of AOM is hydrophilic (Her et al., 2004; Nguyen et al., 2005; Henderson et al., 2008; Zhang et al., 2011; Li et al., 2012).

As a part of NOM, the AOM inputs may impact the NOM characteristics because the AOM properties differ from the one of NOM (Henderson et al., 2008). In natural water not affected by eutrophication, the NOM is generally composed of 10% to 20% of hydrophilic compounds whereas the AOM is much more hydrophilic. Recurrent inputs of AOM may thus lead to the increase of the hydrophilic fraction of NOM. It is thus necessary to assess how water resources can be enriched in organic matter during and after bloom phenomenon to better understand the changes in NOM properties and adapt water treatment processes.

The objective of this work was first to study under laboratory cultivation, the characteristics of the AOM produced by one alga, *Euglena gracilis*, and one cyanobacteria, *Microcystis aeruginosa*, species commonly found in water resources, from bloom formation to collapse, and second to evaluate the AOM evolution in the long term. This work required the optimization of a growth medium, the species cultivation and the monitoring of AOM characteristics during life cycle. The AOM characteristics were investigated by organic matter fractionation according to hydrophobicity and specific UV absorbance (SUVA) index, an indicator of the aromaticity of the organic molecules. This work then focused on the evolution of AOM characteristics over time and in particular during decline phase with a comparison between NOM and AOM properties after the cells death.

1 Materials and methods

Two species: one alga, *E. gracilis* and one cyanobacteria, *M. aeruginosa*, were cultivated in laboratory conditions to assess the organic matter produced by phytoplanktonic bloom.

All analyses were performed in duplicate from samples grown on separate occasions.

1.1 Culture conditions

Non axenic strains of *E. gracilis* and *M. aeruginosa* were obtained from the Ecosystems and Toxic Interactions Unit, "Regulations, Development, and Molecular Diversity" Department in the National Museum of Natural History (Paris, France).

The culture medium used in this study was adapted following the work by Hadjoudja et al. (2009) to meet the specific requirements of this work and the biological needs of the studied species. As dissolved organic carbon (DOC) is the parameter used to quantify organic matter production from phytoplankton, the culture medium must thus contain as little as possible organic carbon in order to avoid any disturbance in DOC measurements and thus to decrease the uncertainty on the DOC production. Indeed, as few DOC production by phytoplankton is expected (in the order of some tens mg C/L), organic pH buffers like MOPS (3-(N-morpholino) propane sulfonic acid) for example cannot be used because of the huge quantity of DOC brought by organic molecules (in the order of g C/L). A phosphate buffer was thus preferred to maintain pH at 7.4 at the beginning of the experiments. In the case of *E. gracilis*, additions of NaOH 0.1 mol/L were necessary to regulate pH in the range of the one determined for natural waters. No regulations were necessary for *M. aeruginosa*.

The cultures were grown in 1 L erlenmayer flasks filled up with 500 mL of synthetic sterilized medium (121°C for 15 min) whose composition is described in **Table 1**. This study required inoculation of 20 erlenmayer flasks for each species. The 20 flasks were inoculated at the same time and two of them were sacrificed for each measurement point. The initial DOC content reached 1.8 mg C/L. The two species were grown in a versatile Envi-

Table 1 Composition of the culture medium*

Product	Concentration (mol/L)	Theoretical input of DOC	Provider
Macronutrients			
K_2HPO_4	5.00×10^{-4}		VWR Prolabo
KH_2PO_4	5.00×10^{-4}		VWR Prolabo
NH_4Cl (*E. gracilis*)	1.73×10^{-3}		Fluka
or $NaNO_3$ (*M. aeruginosa*)	1.73×10^{-3}		Fluka
$MgSO_4 \cdot 7H_2O$	1.03×10^{-4}		Fluka
$CaCl_2 \cdot 2H_2O$	2.16×10^{-4}		Chem-Lab
Na_2CO_3	1.89×10^{-4}		VWR Prolabo
Micronutrients			
H_3BO_3	4.63×10^{-5}		Acros Organics
$MnCl_2 \cdot 4H_2O$	9.10×10^{-6}		Sigma
$Na_2MoO_4 \cdot 2H_2O$	1.60×10^{-6}		Sigma
$CoSO_4 \cdot 7H_2O$	2.00×10^{-7}		Acros Organics
$ZnSO_4 \cdot 7H_2O$	8.00×10^{-7}		VWR Prolabo
$CuSO_4 \cdot 5H_2O$	3.00×10^{-7}		VWR Prolabo
$EDTANa_2$	1.17×10^{-5}	1.4 mg C/L	Chem-Lab
$Fe(NO_3)_3 \cdot 9H_2O$	1.16×10^{-5}		Sigma-Aldrich
Vitamin B1	3.00×10^{-6}	0.4 mg C/L	Sigma-Aldrich
Vitamin B12	7.40×10^{-10}	6×10^{-4}mg C/L	Biochemika
Total		1.8 mg C/L	

* Data are adapted from Hadjoudja et al. (2009).

ronmental Test Chamber MLR-351 (SANYO Electric Co., Japan) equipped with fluorescent lamps FL405S-W/37 (Mitsubishi/Osram, Japan) under 2000 lux illumination (30 μmol photon/(m^2·sec)) with a 15 hr/9 hr light/dark cycle at (23 ± 1)°C. Cultures were daily mixed by hand.

1.2 Growth monitoring

The algal and cyanobacterial growths were daily monitored until the beginning of the stationary phase by measuring absorbance at 685 and 750 nm, respectively, by using a Pharmaspec 1700 spectrophotometer (Shimadzu, Japan) with 1 cm-long quartz cells. These wavelengths corresponded to maximum cultures absorption when drawing spectrum. The cell counting and the viability determination were performed by flow cytometry on a weekly basis during the first 6 weeks of culture because of the fast evolution of the system. Two erlenmeyer flasks were sacrificed for each analysis. After the late stationary phase, the analyses frequency was reduced to a monthly basis. A FACSCalibur (Becton Dickinson, USA) flow cytometer equipped with an argon laser (488 nm excitation) and a red-emitting diode (635 nm excitation) was used to count cells. The flow cytometer recorded orange fluorescence from phycoerythrin (564–606 nm, FL2) and red fluorescence from chlorophyll *a* (> 650 nm, FL3). The calibration was daily checked by using 6 μm fluorescent beads (Kit CaliBRITE containing FITC, PE and PerCP beads). Measurements were realized in duplicates.

1.3 Algal organic matter characterization

1.3.1 Sample preparation

The culture medium was systematically centrifuged at 6000 ×*g* during 20 min at 4°C and then filtered on 0.45 μm nitrate cellulose membrane in order to separate dissolved and particulate organic matter such as algae cells and detritus.

1.3.2 Dissolved organic carbon analyses

DOC analyses were performed by a TOC-L analyzer (Shimadzu, Japan, precision ± 50 μg C/L) according to the Non-Purgeable Organic Carbon measurement procedure. First, samples were acidified with HCl 1 mol/L and sparged with high purity air to eliminate inorganic carbon and volatile organic compounds. Non volatile organic carbon was then combusted in a 720°C oven and the subsequently produced CO_2 was quantified by IR spectroscopy.

1.3.3 XAD fractionation

The qualitative and quantitative enrichment of the system by OM was assessed by organic matter fractionation. The used protocol was adapted and optimized from Aiken et al. (1992) and Malcolm and Mac Carty (1992).

The humic acids (HA) were precipitated by acidification at pH 2 with HCl 37% and separated by filtration on 0.45 μm membranes. Samples 306 mL were then passed through DAX-8 and XAD-4 resins in series. The hydrophobic compounds (HPO) were adsorbed on DAX-8 resin, the transphilic compounds (TPH) on XAD-4 resin and the hydrophilic compounds (HPI) were not adsorbed on any resin. In the literature two values of partition coefficient k' are commonly used: 50 and 100. Labanowski and Feuillade (2011) studied the impact of the k' coefficient value on the adsorption of hydrophilic compounds. They advised using low k' and low DOM concentration. As AOM is known to be mainly hydrophilic, a k' value of 50 was chosen for this study. The filtration flow and the resins volumes were respectively fixed at 50 mL/hr and 5 mL. The concentrations of each fraction were determined by DOC measurements. HA were not considered in the repartition because of their poor percentage in DOC (< 3% for both species). Only three fractions will be thus presented.

1.3.4 SUVA analyses

The UV absorbance at 254 nm was measured by using a PharmaSpec 1700 spectrophotometer (Shimadzu, Japan, precision ± 0.005 cm^{-1}) with 1 cm-long quartz cells. The Specific UV absorbance (SUVA index) which is defined as the ratio of the UV absorbance to the DOC values allows estimating the aromaticity of each fraction and correlates with the hydrophobicity of organic molecules (Weishaar et al., 2003; Croué, 2004). The SUVA index was calculated for each organic matter fraction produced by alga and cyanobacteria during stationary and decline phases.

2 Results and discussion

2.1 Determination of growth phases and algal organic matter production

Figure 1 shows the evolution of absorbance, cell counting and DOC respectively produced by *E. gracilis* and *M. aeruginosa*. The absorbance measures and the cell counting data were well correlated and allowed to define growth phases.

For both species, four growth phases of different duration were observed: a short lag phase at the beginning of the experiment with an adaptation to the culture medium, an exponential phase during which population increased quickly, a stationary phase characterized by a growth slowdown because of a lack of nutrients and at the end of the experiment, a decline phase with cells death. The durations of the lag and exponential phases were quite similar for both species: about 5 days long for the lag phase, and about 2 weeks for the exponential phase which began after day 6 of culture and finished after day 21. However, the growth phase was only achieved after day 28 of cultivation for both species and after the end of the exponential phase the behavior of the two species differed. The stationary phase was about 34 days long (from day 22 to day 56 of culture) for *E. gracilis* and population reached a maximum of 420,000 cells/mL after 28 days. For *M. aeruginosa*, the growth was slowed during one week from day 22 to day 28 culturing day and population was greatly reduced after only 35 days of cultivation. *M. aeruginosa* maximum population was about 11,800,000 cells/mL on day 28. For both species, the late stationary phase was marked by a change in culture color from green to brown and yellow, which underlined the deterioration of cells health.

The production rate in DOC (mg C/(L·day)) depended on the growth phase (**Fig. 1a**, **b**). Whatever the specie, it progressively increased during exponential phase with the increasing cells number. During this phase, DOC was mainly produced by cells metabolism. The maximum production rate in DOC was reached during stationary phase (0.53 mg C/(L·day) for *E. gracilis* and 0.48 mg C/(L·day) for *M. aeruginosa*) because of the production of DOC by cells autolysis and living cells metabolism. During the decline phase, the production rate in DOC tended to decrease probably because of the decrease of DOC production by cells metabolism following cells death.

The *E. gracilis* production of DOC was higher than the one of *M. aeruginosa* for each growth phase despite the maximum population was lower. As a consequence, at the end of the experiment, the DOC concentrations were higher for the alga (49.4 mg C/L) than for the cyanobacteria (21.6 mg C/L). The DOC concentration still increased even a long time after the cells death.

Figure 2 presents the cytograms obtained for *E. gracilis* and *M. aeruginosa* after 28, 42 and 77 days of culture, i.e., during stationary and decline phases. The position of the populations' dots plot was dependent of the content in chlorophyll *a* (FL3) and phycoerythrin (FL2) of the cells. Mendoza-Cózatl and Moreno-Sánchez (2005) and Reynolds (1984) estimated the content in chlorophyll *a* of *E. gracilis* and *M. aeruginosa* of 4.38 pg Chl-*a*/cell and 0.36 pg Chl-*a*/cell. The alga was thus richer in chlorophyll-*a* than the cyanobacteria, explaining why FL3 autofluorescence for *E. gracilis* was more intense than for *M. aeruginosa*.

A shift in autofluorescence dots plots for populations of *E. gracilis* and *M. aeruginosa* from stationary phase to decline phase was observed in these cytograms. This shift was in agreement with the results of cell counting.

2.2 Evolution of algal organic matter characteristics

2.2.1 Quantitative study

Figure 3 shows the relationship between growth phases and absolute dissolved organic matter concentrations in the culture medium separated into four fractions according to their hydrophobic character (concentrations are expressed in mg C/L). HA were not considered because of their low contribution to DOC (< 3%).

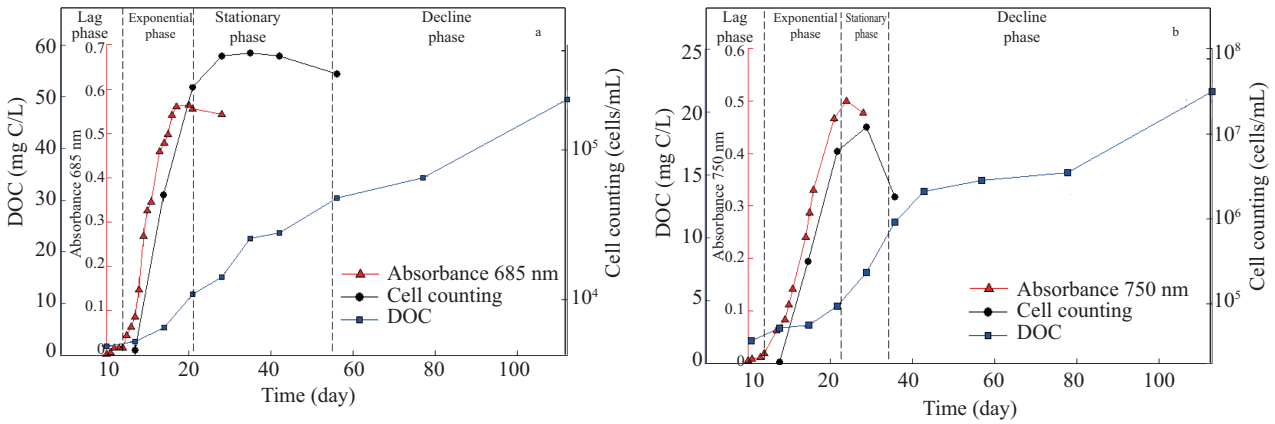

Fig. 1 Growth phases determined by absorbance 685 nm for *E. gracilis* and 750 nm for *M. aeruginosa* measuring and cell counting vs. evolution of DOC released by *E. gracilis* (a) and *M. aeruginosa* (b).

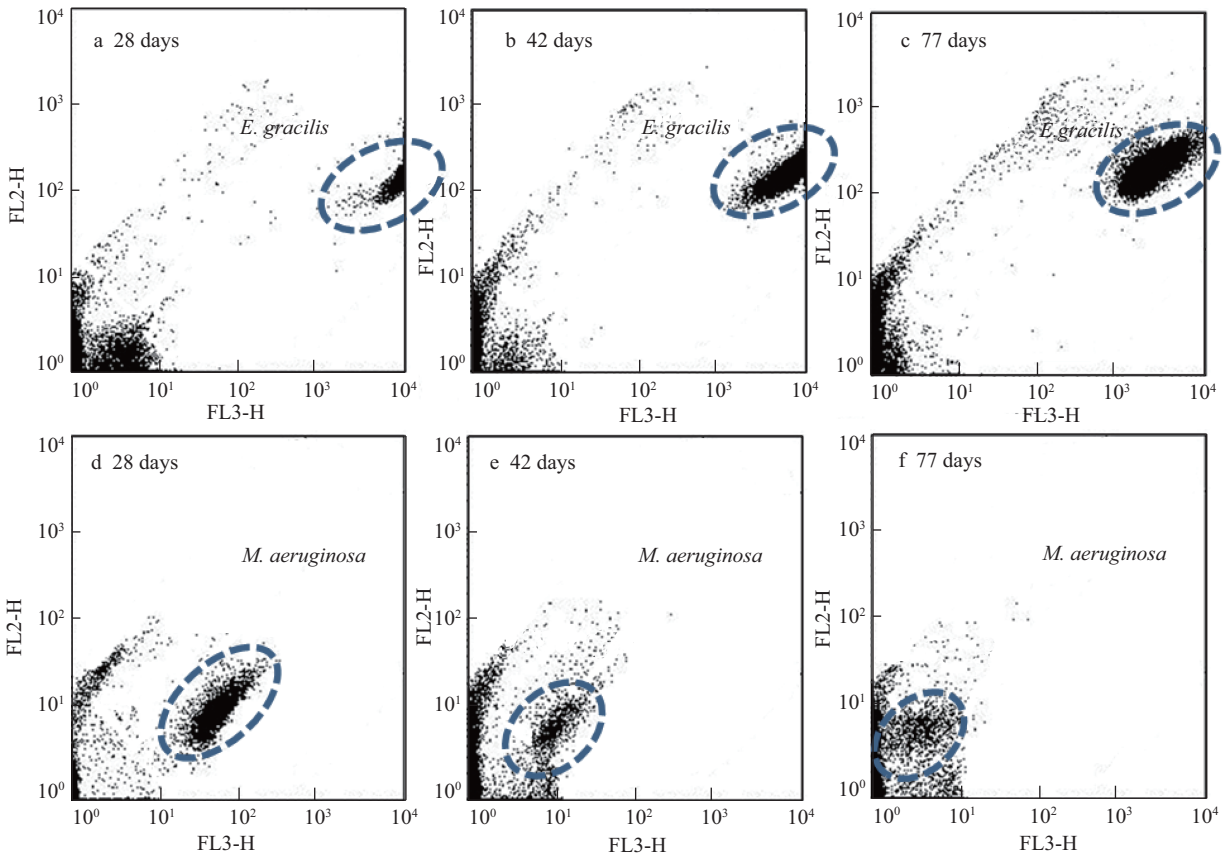

Fig. 2 Cytograms showing the autofluorescence decrease of chlorophyll-*a* (FL3) and phycoerythrin (FL2) for *E. gracilis* (a) (population dots plot is partially out of the window), (b) and (c) and *M. aeruginosa* (d, e and f) after respectively 28, 42 and 77 days of cultivation.

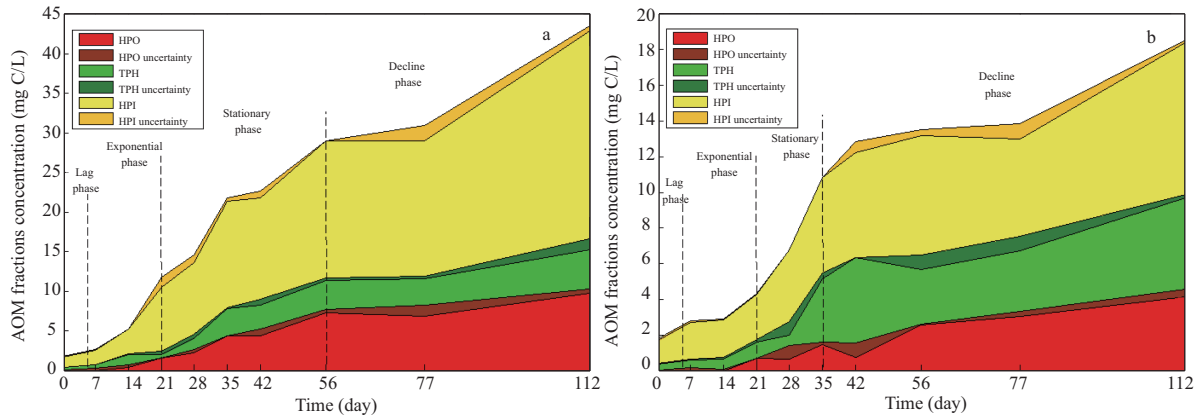

Fig. 3 Evolution of concentrations of HPO, TPH and HPI produced by *E. gracilis* (a) and *M. aeruginosa* (b) from bloom formation to collapse.

The initial DOC concentration in the culture medium was 1.74 mg C/L, which is in agreement with the theoretical DOC content in the culture medium (**Table 1**). DOC was mainly composed of HPI at a concentration of 1.40 mg C/L, mostly induced by EDTA (1.17×10^{-5} mol/L) and 0.34 mg C/L of TPH due to vitamins B1 (3×10^{-6} mol/L) and B12 (7.4×10^{-10} mol/L). These values were not subtracted from DOC measurements to calculate AOM fraction concentrations because the fate of these organic compounds is not known. Indeed, biological transformation and consumption may affect their behavior towards adsorption on XAD resins or reduce their contribution to DOC over time.

Figure 3a shows that during the lag phase, only little DOC was produced by *E. gracilis*, correlated with few changes in cells number. During this phase, DOC was only composed of HPI (increased by 0.58 mg C/L).

Then during the exponential phase, DOC increased significantly and AOM produced was mainly composed of HPI (increased by 7.41 mg C/L) whereas HPO and TPH did not evolve substantially (for each, increase < 1.4 mg C/L).

During the stationary phase, HPI still increased (by 7.94 mg C/L) in parallel to a high increase in HPO (by 6.19 mg C/L) and, to a lesser extent, in TPH concentrations (by 3.09 mg C/L).

During the decline phase, HPI was still actively produced (increased by 9.53 mg C/L) while HPO and TPH kept increasing (by 2.57 and 2.43 mg C/L respectively). Nevertheless the increase was less intensive than during stationary phase even if the duration of the decline phase was almost twice of that of the stationary phase.

The evolution of the organic fractions concentrations was similar for *M. aeruginosa* and *E. gracilis* (**Fig. 3b**). Indeed, the HPI fraction of *M. aeruginosa* was the only one significantly produced during the lag and the exponential phases (increased by 1.18 mg C/L). The HPO and TPH fractions did not evolved significantly during the exponential phase. During the stationary phase, the TPH concentration increased by 2.84 mg C/L while the HPO fraction only increased by 0.88 mg C/L. The HPI fraction still increased during this growth phase (by 2.78 mg C/L).

During the decline phase, the HPI fraction was still actively produced (increased by 3.33 mg C/L) and TPH fraction increased to a lesser extent than during the stationary phase (increased by 1.44 mg C/L). However, HPO concentration increased in a larger extent than during the stationary phase (increased by 2.95 mg C/L).

The repartition of the HPO, TPH and HPI fractions presented a similar evolution during the growth phases for *E. gracilis* and *M. aeruginosa* (**Fig. 3a**, **b**). The HPI compounds were produced in large quantities at each growth phase from bloom formation to collapse. Only hydrophilic compounds were produced during the lag and the exponential phases. This was mainly due to cell metabolism as mortality was low.

The HPO and TPH compounds were not produced in substantial quantities during the lag and the exponential phases but their concentrations started increasing from stationary phase during which they were most intensively released and they kept rising regularly during the decline phase. The HPO and TPH compounds seemed to correlate with the increase in mortality and the release of intracellular organic material. Moreover, the HPI initially produced may be transformed into TPH and HPO compounds according to the theories of polyphenols (Stevenson, 1982) and supramolecules (Piccolo et al., 2000). Such a phenomenon is observed in natural environments.

2.2.2 Qualitative study

Table 2 shows the evolution of the repartition of the organic matter fractions of the AOM produced by *E. gracilis* and *M. aeruginosa* during the exponential and the stationary phases.

These values are in accordance with the high percentage in hydrophilic fraction (between 57% to 71%) obtained by the previous publications (Her et al., 2004; Nguyen et al., 2005; Henderson et al., 2008; Zhang et al., 2011; Li et al., 2012) for the algae and the cyanobacteria. However, the difference in percentages between the different studies may be explained by different culture conditions and the media used.

Table 2 OM fractions (±3%) produced by *E. gracilis* and *M. aeruginosa* during the exponential and the stationary phases

Growth phase	Fractions by *E. gracilis* (%)			Fractions by *M. aeruginosa* (%)		
	HPO	TPH	HPI	HPO	TPH	HPI
Exponential	15	10	75	2	23	75
Stationary	18	13	69	20	19	61

Little changes in AOM characteristics were observed between the exponential and the stationary phases according to the authors previously cited and **Table 2**. These values are the only ones available in literature. They were thus not sufficient to conclude on both the evolution of the AOM characteristics at each growth phase and the fate of these organic compounds a long time after stationary phase. Indeed, the DOC concentrations still increased after the cells death showing that there were still DOC inputs (**Fig. 1a**, **b**). However, the contribution of each organic fraction to the total DOC may be different from the stationary phase. Therefore, the characteristics of the AOM were likely to further evolve during the decline phase.

The increase of AOM hydrophobicity during the stationary and the decline phases (**Fig. 3**) seemed to result from the release of intracellular organic material of high hydrophobic character. Indeed, for both species, the contribution of humic substances (HPO and TPH) to the DOC substantially increased from the beginning of the stationary phase and progressively during the decline phase.

The increase during the stationary phase and the early decline phase was probably due to cell lysis and the release of IOM which may be composed of organic material of higher hydrophobic degree than the EOM produced during the exponential phase (Fang et al., 2010).

The increase in humic substances concentrations after cells death may be due to two other main processes: photo-dissolution and leaching of cells fragments. Indeed several authors showed that the light exposure of particulate organic material (POM) resulted in a significant dissolved organic matter (DOM) generation through photo-dissolution processes (Kieber et al., 2006; Mayer et al., 2006; Pisani et al., 2011). Indeed, the photo-induced reactions can affect POM structure as well as DOM because both can absorb light at similar wavelengths (Kieber et al., 2006). These processes led to break down larger molecules into smaller photo-products by light absorption and therefore the exposure of organic particles to light can result in the transfer of particulate carbon to the dissolved phase (Pisani et al., 2011). Mayer et al. (2009) demonstrated that simulated solar irradiation of algal membrane at various decay stages led to the conversion of 10% to 30% of particulate organic matter

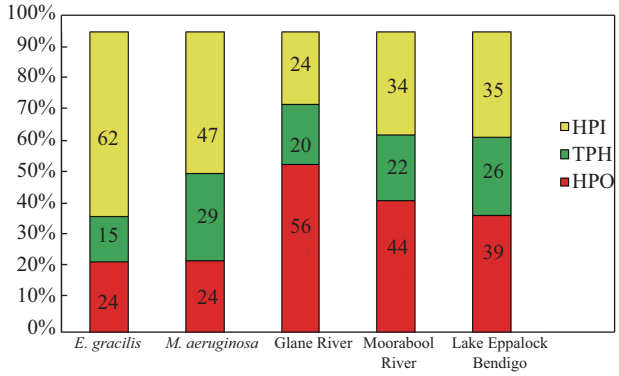

Fig. 4 Comparison of the percentages of HPO, TPH and HPI of dissolved organic matter from different origins. Distributions are given after 112 days of cultivation for *E. gracilis* and *M. aeruginosa*. Results of Glane River are described in Labanowski and Feuillade (2009) and those of Moorabool River and Lake Eppalock Bendigo are from Gray et al. (2007).

into DOM. Under dark conditions, the leaching of POM was also an important source of DOM but to a lesser extent than photo-dissolution according to Pisani et al. (2011).

2.3 Comparison of the characteristics of AOM and NOM

2.3.1 Organic matter fractionation

The percentages of HPO, TPH and HPI in laboratory cultures of *E. gracilis* and *M. aeruginosa* at the end of the experiment (112 days of cultivation corresponding to the advanced decline phase) were compared with the ones of natural waters from River Glane, France (Labanowski and Feuillade, 2009), Moorabool River, Australia and Lake Eppalock Bendigo, Australia (Gray et al., 2007) (**Fig. 4**). A long time after bloom formation, the organic matter produced by algae and cyanobacteria had similar HPO percentages (24%) and was mainly composed of hydrophilic compounds. The HPI fraction thus represented about half of the DOM generated by bloom for *M. aeruginosa* and even more (60%) for *E. gracilis*. The two species differed by their TPH and HPI content. The DOM produced by *E. gracilis* was more hydrophilic (62%) than the one coming from *M. aeruginosa* (47%), the TPH fraction of which percentage (29%) being twice as great as the algal one.

In accordance with the studies of Thurman and Malcolm (1981) and Martin-Mousset et al. (1997) in which humic substances represented about 40% to 60% of DOM from natural waters, HPO was the major fraction of Glane River (almost 50% of DOM), Moorabool River and Eppalock Lake (40%) waters. The Glane River was not affected by eutrophication phenomena and thus the hydrophilic compounds represented only 24% of DOM. This low HPI content underlined the low contribution of autochthonous sources. However, Moorabool River and Eppalock Lake were affected by recurrent bloom phenomena, which can explain why they had a higher HPI content than Glane River.

The characteristics of AOM and NOM were different because the AOM was much more hydrophilic than NOM, which was composed of more hydrophobic compounds coming from allochthonous sources. However, the AOM became more hydrophobic with the age of the culture and the difference of the characteristics between NOM and AOM narrowed during the decline phase. Recent results have shown that old cultures of *E. gracilis* (one year old) contain 48% of HPI, 20% of TPH and 32% of HPO, i.e., almost ten percent of HPI less than 112 days old cultures. So AOM seems to become more and more hydrophobic but this process is very slow and after one year, AOM is still mainly hydrophilic. As in the long term scale AOM remains mainly hydrophilic, the hydrophilic contribution to NOM in eutrophic waters is expected to increase because of annually inputs of fresh AOM.

2.3.2 SUVA index

The SUVA index determined for both species and for each organic fraction at stationary (28 days) and late decline phases (after 112 days of cultivation) are reported in **Table 3**, as well as SUVA index of the River Glane, France (Labanowski and Feuillade, 2009) for comparison. It was used to determine the aromaticity of the produced algal organic matter and to compare the aromatic character of fractions from different origins.

The global SUVA index of the AOM produced by both species was lower than the one of River Glane whatever the growth phase (**Table 3**). This result was in accordance with the high hydrophilic character of the AOM described in **Fig. 4** when compared to natural waters. Weishaar et al. (2003) showed that high SUVA index correlated with high aromaticity of organic compounds and with high hydrophobic character of organic molecules.

The SUVA indexes of AOM fractions from *E. gracilis* were higher than the ones coming from *M. aeruginosa* whatever the growth stage, except for the HPI fraction. The HPO and TPH fractions from the alga were thus composed

Table 3 SUVA index of OM fractions from different origins

Origin		Global SUVA index (L/(cm·g C))	Fractions SUVA index		
			HPO	TPH	HPI
E. gracilis	Stationary phase	13.9 ± 0.1	19.5 ± 1.6	17.1 ± 1.4	8.4 ± 0.6
	Decline phase	11.2 ± 0.1	26.4 ± 3.9	13.5 ± 1.4	6.9 ± 0.1
M. aeruginosa	Stationary phase	10.7 ± 1.3	12.0 ± 0.4	7.3 ± 0.6	11.9 ± 0.5
	Decline phase	10.4 ± 0.8	19.0 ± 2.7	4.0 ± 1.0	11.6 ± 0.4
Glane River		18.3 ± 0.2	21.4 ± 0.2	16.2 ± 0.2	12.7 ± 0.2

of organic molecules of higher aromatic and hydrophobic character than the ones coming from the cyanobacteria, except for the HPI fraction.

However, the SUVA index of the HPI fraction from *E. gracilis* and the SUVA index of the TPH fraction from *M. aeruginosa* were very low when compared to other HPI and TPH fractions of different origins. Indeed, the SUVA index values of the HPI fraction of *M. aeruginosa* and Glane River were very close and higher when compared to *E. gracilis*. In the same way, the SUVA index values of the TPH fraction from *E. gracilis* and natural waters were similar and higher than the one of *M. aeruginosa*. These two fractions were thus very atypical and their properties differed from the one of natural waters.

Moreover, both TPH fractions produced during the stationary and the decline phases from *M. aeruginosa* exhibited unusual characteristics because of their lower SUVA index when compared to the HPI fraction.

The SUVA index of the organic fractions from *E. gracilis* and *M. aeruginosa* had the same evolution between the stationary and the decline phases. The SUVA of the HPO fractions substantially increased while those of the TPH fractions decreased. The SUVA of the HPI fractions only slightly decreased as the global SUVA index of the two species. The TPH and mainly the HPI fractions highly influenced the AOM characteristics.

Li et al. (2012) studied the extracellular and the intracellular organic matter characteristics of *M. aeruginosa* during the exponential phase. They showed that the SUVA of the HPO fraction was higher for intracellular organic material (9.8 L/(cm·g C)) than for extracellular organic material (5.1 L/(cm·g C)). The SUVA of the HPO fraction increased between these two phases certainly because the intracellular organic material was released during the stationary and the decline phases. They also showed that the SUVA of the TPH fraction of the intracellular organic compounds (8.6 L/(cm·g C)) was lower than for EOM (21.3 L/(cm·g C)) which is in accordance with the decreasing values of SUVA observed in this study. Then, the SUVA of the HPI fraction of IOM was slightly higher (9.6 L/(cm·g C)) than that of EOM (6.8 L/(cm·g C)), explaining why the SUVA did not evolve significantly between the stationary and the decline phases.

3 Conclusions

The AOM produced by *E. gracilis* and *M. aeruginosa* was quantified and characterized from bloom formation to collapse by fractionation according to the hydrophobic character. The AOM production and the evolution of its characteristics depended on both the specie and the growth phases.

The organic matter generated by phytoplanktonic bloom increased quantitatively and its characteristics kept evolving a long time after bloom collapsed. A stabilization was only reached during the late decline phase.

The DOC production by phytoplankton seemed to result from various processes depending on the growth phases. During the lag and the exponential phases, the DOC was essentially produced by cell metabolism as HPI because cell death was low. During the stationary phase, the biological activity still produced DOC as HPI but the cell decay resulted in intracellular compounds release as TPH, HPO and HPI with an increase of the HPO and the TPH contribution to AOM. During the decline phase, the metabolism contribution to DOC was low because of the decrease in cells number and then the release of intracellular compounds was important. Moreover, two other processes contributed to DOC, photo-dissolution and detritus leaching from cells fragments.

The properties of AOM are very different when compared to the one of NOM. The AOM was mainly hydrophilic although it became more hydrophobic in the long term. Then, during the decline phase, a fewer difference in the hydrophobic character of AOM and NOM was observed. Due to its high hydrophilic character, the eutrophic origin of organic matter can be thus identified by fractionation according to hydrophobicity. SUVA index of AOM was low when compared to the one of NOM and it did not evolve significantly between the stationary and decline phases.

Algal and cyanobacterial blooms are recurrent, periodical and intensive phenomena responsible for high organic matter inputs in water resources. Because of the specificities of AOM and the high quantity of organic matter produced, these blooms may thus disturb the dynamics of water resources. It may increase the hydrophilic content and decrease the SUVA index of NOM depending on the season. These impacts may also be observed and intensified in the long term because the organic matter tends to accumulate and it could limit the possible uses of the water resources.

Acknowledgments

The authors thank the FEDER (European Found for Regional Development) and the Regional Council of Limousin for their financial support. The authors are grateful to Bruno Védrine and Marilyne Vergnoux from Mêtis Biotechnologies for their help with flow cytometry and valuable discussions. Philippe Chazal is also thanked for its contribution to the improvement of the English language.

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Journal of Environmental Sciences (Established in 1989)

Vol. 25 No. 6 2013

Supervised by	Chinese Academy of Sciences	**Published by**	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental		Elsevier Limited, The Netherlands
	Sciences, Chinese Academy of Sciences	**Distributed by**	
Edited by	Editorial Office of Journal of	Domestic	Science Press, 16 Donghuangchenggen
	Environmental Sciences		North Street, Beijing 100717, China
	P. O. Box 2871, Beijing 100085, China		Local Post Offices through China
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Editor-in-chief	Hongxiao Tang	**Printed by**	Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742