Marine Pollution Bulletin 104 (2016) 113–120

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Insights into the binding interactions of autochthonous dissolved organic matter released from *Microcystis aeruginosa* with pyrene using spectroscopy

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ARTICLE INFO

Article history:
Received 3 October 2015
Received in revised form 26 January 2016
Accepted 29 January 2016
Available online 4 February 2016

Keywords:
Microcystis aeruginosa
Autochthonous
Dissolved organic matter (DOM)
Pyrene
Parallel factor (PARAFAC) analysis
Binding coefficient

ABSTRACT

The autochthonous dissolved organic matter (DOM) released by *Microcystis aeruginosa* (*M. aeruginosa*-DOM) during its growth period was characterized by spectroscopy. Furthermore, the relationships between the *M. aeruginosa*-DOM spectroscopic descriptors and the pyrene binding coefficient (K_{DOC}) values were explored. The results showed that the spectroscopic characteristics of the *M. aeruginosa*-DOM and the binding properties of pyrene were dynamically changed along with the algae growth. Pearson correlation analysis demonstrated that a higher pyrene K_{DOC} value was observed for the *M. aeruginosa*-DOM that has a higher humification index (HIX) value, a lower biological index (BIX) value and a lower absorption ratio (E2/E3). The presence of protein-like and long-wavelength-excited humic-like components may impose negative and positive effects on binding of pyrene by the *M. aeruginosa*-DOM, respectively. Principal component analysis (PCA) further supported that the binding affinity of pyrene may be primarily influenced by the humification degree of the *M. aeruginosa*-DOM.

1. Introduction

Dissolved organic matter (DOM) is a heterogeneous mixture of dissolved substances found ubiquitously in freshwater and marine environments. The molecules that compose aquatic DOM can be classified as allochthonous and autochthonous DOM (McIntyre and Gueguen, 2013). Allochthonous DOM generally refers to soils and catchment inputs brought by runoff, while autochthonous DOM is derived from algal production and microbial metabolism. Algogenic DOM, as one of the most important types of autochthonous DOM, arises from passive leakage by diffusion across the cell membrane and active exudation from algae (Thornton, 2014). Determining major controls on the biogeochemistry of DOM is thus essential not only for understanding the ecological functioning of aquatic ecosystems but also in predicting the bioavailability or toxicity of hazardous contaminants such as heavy metals and organic pollutants (Hur and Kim, 2009; Tonietto et al., 2014).

Polycyclic aromatic hydrocarbons (PAHs), a class of hydrophobic organic compounds (HOCs), widely distributed in freshwater and marine ecosystems. Some PAHs are known to be teratogenic, carcinogenic and mutagenic (Pérez-Fernández et al., 2015). The environmental behavior and bioavailability of PAHs being released into aquatic environments are greatly affected by DOM (Yu et al., 2011; Lu et al., 2013). The dissolved organic carbon (DOC) normalized binding coefficient (K_{DOC}) has been widely used to determine the intensity of interaction between PAHs and DOM (Chin et al., 1997; Mei et al., 2009). Many previous studies have revealed that the K_{DOC} value of a specific PAH largely depends on the chemical and structural nature of DOM, including polarity, molecular weight, aromaticity and aliphaticity (Mei et al., 2009).

Investigations on the correlations between physicochemical properties of DOM originating from various sources and binding affinity of PAHs may contribute to an estimation of the environmental behavior and ecological risk of PAHs (Hur and Kim, 2009; Hur et al., 2009). Although many studies have revealed relationship between characteristics of DOM and K_{DOC} values of PAHs, they have mainly focused on allochthonous DOM such as compost, soil, sediment and commercial humic substances (Plaza et al., 2009; Banach-Szott et al., 2014; Wang and Zhang, 2014). However, little is known about the extent of PAHs binding with autochthonous DOM released from algae during its growth period. As one of the most common species responsible for the nuisance blooms in freshwater resources, cyanobacterium *Microcystis aeruginosa* releases a large amount of autochthonous DOM (*M. aeruginosa*-DOM) from bloom formation to collapse. Recently, Leloup et al. indicated that the concentration and composition of the *M. aeruginosa*-DOM included dynamic variations at different culture phases (Leloup et al., 2013). These data imply that

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http://dx.doi.org/10.1016/j.marpolbul.2016.01.053
0025-326X/

studying the binding properties of PAHs with the *M. aeruginosa*-DOM during the growth period and its potential impact on the interaction with PAHs would help us to better understand the fate and bioavailability of PAHs in aquatic environments, especially during algal blooms.

UV–visible absorption spectroscopy and fluorescence spectroscopy have the potential to be useful techniques for exploring the characteristics of chromophoric DOM (CDOM) and fluorescence DOM (FDOM) in DOM from different origins (Coble, 1996; Yamashita et al., 2008; Zhang et al., 2013). Fluorescent measurements are rapid, sensitive, non-destructive and can be performed in real time and *in situ* to probe the physicochemical composition of DOM with special instruments. In particular, fluorescence excitation–emission matrix (EEM) spectroscopy can acquire the entire fluorescence characteristics of DOM, and thus, it has become one of the most popular techniques to investigate the information on the structure and the composition of DOM (Coble et al., 1998; Yamashita et al., 2008). Different fluorophores (protein-like and humic-like) in DOM can be distinguished based on the traditional peak-picking technique (Coble, 2007). However, peak-picking is time consuming and may not be reliable enough to identify the fluorescence components. In comparison, parallel factor (PARAFAC) analysis can break down complex EEM spectra of DOM into independent fluorophores to provide more information on DOM characterization. EEMs–PARAFAC analysis has been used to characterize DOM from various sources, such as algal-derived DOM (Herzi et al., 2013b) and other environment samples of DOM, such as soil, sludge, and compost (He et al., 2013; Gabor et al., 2015).

Recently, a number of spectroscopic descriptors have been suggested to predict binding affinity of PAHs with allochthonous DOM (Hur et al., 2009; Hur et al., 2014). However, little is known about the correlations between spectroscopic properties of autochthonous DOM from algae during the entire growth period and the binding affinity of PAHs. Thus, the main objective of this study was to assess the dynamics of interactions between PAH and the *M. aeruginosa*-DOM at various phases. Furthermore, the relationships between the spectroscopic descriptors and the K_{DOC} values were investigated for understanding the critical factors, controlling the capacity of the *M. aeruginosa*-DOM for binding PAH. Pyrene was chosen as a model PAH compound due to it being one of the most abundant PAHs in environmental media (Rudnick and Chen, 1998; Yang et al., 2014). Moreover, many studies on interactions between PAH and allochthonous DOM have been performed using pyrene as a model substance (Chefetz et al., 2000; Hur and Kim, 2009).

2. Materials and methods

2.1. M. aeruginosa culture

The cyanobacterial strain *M. aeruginosa* FACHB-315 was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China) and was cultured using standard BG11 medium (Rippka et al., 1979). The cultures were inoculated in 250-mL Erlenmeyer flasks containing 100 mL fresh BG11 medium with an initial density of 6.10×10^5 cells mL^{-1} under a diurnal cycle of 14 h light and 10 h dark with light irradiance of 2400 ± 200 lx at 22 ± 1 °C. All experimental utensils and media were autoclaved to minimize bacteria activity before assembly and operation. Moreover, the algae was cultured in a clean room. Triplicate Erlenmeyer flasks were sacrificed for sample points. All cultures were shaken three times daily to avoid agglomeration, and their position was exchanged randomly to avoid error produced by light.

2.2. M. aeruginosa growth assay and DOM extraction

M. aeruginosa growth was monitored by measuring optical density (OD) at 680 nm with a UV–visible spectrophotometer (Carry-50, Varian, USA) at intervals of three days during the entire experimental period (Jang et al., 2004). The *M. aeruginosa*-DOM was obtained by centrifuging the cell suspension at 6000 rpm for 10 min, and subsequently, the supernatant was filtered through pre-combusted (450 °C, 4 h) GF/F filters (Herzi et al., 2013a). All the *M. aeruginosa*-DOM samples were normalized to a pH of 7 using HCl (0.5 mol L^{-1}) and NaOH (0.5 mol L^{-1}) prior to measurements of spectroscopic properties and binding experiments.

2.3. DOC concentration analysis

DOC concentrations of the *M. aeruginosa*-DOM were measured using a total organic carbon analyzer (TOC-L CPH, Shimadzu, Japan) with an automatic sample injector. The relative precision of DOC analysis was less than 2% (n = 3).

2.4. UV–visible absorption analysis

The UV–visible absorption spectra were recorded over the range of 250 to 700 nm using a UV–visible spectrophotometer, and Milli-Q water was used as a reference blank. The absorption coefficients $a(\lambda)$ (m^{-1}) were calculated based on optical path length and absorbance at wavelengths (λ = 250–700 nm) (Green and Blough, 1994). CDOM concentration is expressed using the absorption coefficient (m^{-1}) at a wavelength of 355 nm. The molecular weight of DOM was evaluated from the ratio of the absorptions at 250 and 365 nm [E2/E3]. Generally, higher E2/E3 ratio reflects lower average molecular weight and aromaticity (Peuravuori and Pihlaja, 1997). The ratio of absorptions at 465 and 665 nm [E4/E6] is related to the degree of condensation of the aromatic carbon network, molecular weight and polarity (Chen et al., 1977; Kang and Xing, 2008).

2.5. Fluorescence measurement and PARAFAC analysis

Fluorescence EEM spectra were obtained using a fluorescence spectrophotometer (Cary Eclipse, Varian, USA) with the emission wavelength (λ_{em}) ranging from 300 to 550 nm at 2 nm increments by varying the excitation wavelength (λ_{ex}) from 250 to 450 nm at 5 nm increments and with a scan speed of 1200 nm min^{-1}. The slits for excitation and emission were set as 10 nm and 5 nm, respectively. The absorbance data of all samples were recorded to eliminate the inner-filter effect using a previous method (Ohno, 2002). The majority of water Raman scatter peaks were removed by subtracting the EEM spectrum of Milli-Q water from the sample EEM spectra. Fluorescence intensities were Raman normalized using the area under the water Raman peak at λ_{ex} 350 nm and reported in Raman units (R.U.) (Lawaetz and Stedmon, 2009).

PARAFAC modeling was performed using the DOMFluor toolbox (http://www.models.life.ku.dk/) for MATLAB 7.0 according to the procedure recommended in Stedmon and Bro (Stedmon and Bro, 2008).

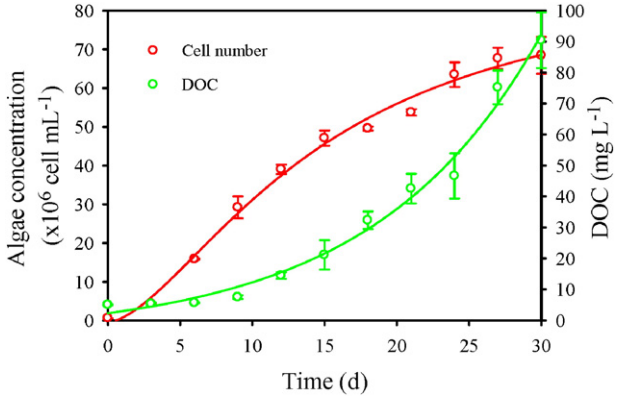

Fig. 1. Growth variation of *M. aeruginosa* and evolution of DOC released by *M. aeruginosa*.