Ecotoxicology and Environmental Safety 157 (2018) 134–142

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

PAHs would alter cyanobacterial blooms by affecting the microcystin production and physiological characteristics of *Microcystis aeruginosa*

ARTICLE INFO

Keywords:
Microcystis aeruginosa
Phycobiliprotein
Microcystin
Polycyclic aromatic hydrocarbons
Antioxidant system

ABSTRACT

The wide presence of polycyclic aromatic hydrocarbons (PAHs) in lakes necessitates a better understanding of cyanobacteria metabolites under the contamination of PAHs. The *M. aeruginosa* strain PCC7806 was selected to investigate the effects of naphthalene and pyrene on the physiological and biochemical reactions of cyanobacteria, including antioxidant defense system (superoxide dismutase, catalase), intracellular microcystin (MC) content, phycobiliprotein (phycocyanin, allophycocyanin) contents, and specific growth rate. Naphthalene and pyrene altered the growth of the *M. aeruginosa* strain, reduced the contents of phycocyanin and allophycocyanin, and stimulated the activities of antioxidant enzymes without lipid peroxidation. Remarkably, the intracellular MC content was significantly increased by 68.1% upon exposure of *M. aeruginosa* to 0.45 mg L^{-1} naphthalene, and increased by 51.5% and 77.9% upon exposure of *M. aeruginosa* to 0.45 mg L^{-1} pyrene and 1.35 mg L^{-1} pyrene, respectively ($P < 0.05$). Moreover, significant correlations were observed between these physiological reactions, referring that a series of physiological and biochemical reactions in *M. aeruginosa* worked together against the PAH contamination. Considering that MCs are the most studied cyanobacterial toxins, our results clarified that the promoting MC production by PAH contamination cannot be neglected when making related risk assessments of eutrophic waters.

1. Introduction

Toxic cyanobacterial bloom, which not only causes taste and odor problems but also produces toxins, has become a widespread and critical problem in aquatic environments all over the world (Ding et al., 1999). Among the dominant cyanobacteria, *Microcystis* is the most widespread cyanobacterial genus in eutrophic lakes and is known to produce cyanobacterial hepatotoxins termed microcystins (MCs) (Carmichael, 1992). MC contamination in eutrophic lakes can be accumulated in fish, bivalves and other invertebrates and then cause harmful effects to human health by transference through the food web. Previous research has suggested that MCs can cause liver damage by inhibiting essential enzymatic functions in hepatic tissue (Yoshizawa et al., 1990).

MC concentrations in water bodies depend not only on the abundance of toxin-producing *Microcystis* strains but also on the MC production by toxigenic strains (Yang et al., 2015). The population of cyanobacteria (Pomati et al., 2004; Parsons et al., 2015) and MC production (Wang et al., 2007; Al-Ammara et al., 2015) can be affected by some environmental stresses, including pollutants. Al-Ammara et al. (2015) noted that some environmental factors could alter MC concentrations by affecting the abundance of toxin-producing strains in a cyanobacterial population and/or their toxin production (Al-Ammara et al., 2015). As a ubiquitous toxic freshwater cyanobacterium isolated from a lake in China, the *M. aeruginosa* strain PCC7806 has usually been used to investigate the ecophysiology of *M. aeruginosa* (Downing et al., 2005; Frangeul et al., 2008).

Significant levels of PAHs, which are common constituents of combustion residues, have been detected in lakes in China (Lu et al., 2010). Even at low dosages, the exposure of *M. aeruginosa* to PAHs would have effects on its growth (Stoichev et al., 2011). In the surface layers of Taihu lake, corresponding to sedimentation from 1980 to 1990, the surface sediments of ΣPAH_s concentrations were 1180 and 530 μg kg^{-1} in Meiliang Bay and Xukou Bay, respectively (Liu et al., 2009). Meanwhile, PAHs in sediments have a profound deleterious effect on benthic communities (Bennett et al., 2000). Although growth inhibition (in term of specific growth rates) of *M. aeruginosa* by several contaminations has been widely recognized as direct evidence of contamination (Cerezo et al., 2015), reduction of cell growth was only observed at specific levels of PAHs. PAHs could promote the growth of *M. aeruginosa* at specific levels (Zhu et al., 2012), suggesting that colonies of *M. aeruginosa* have a high tolerance to PAH contamination. Thus, increasing PAH contamination in an aquatic system might alter the occurrence of cyanobacterial blooms and MC contamination in lakes, which could not be neglected as making risk assessments of eutrophic waters.

Due to the poor understanding of the effects of PAHs on *M. aeruginosa*, related risk assessments for PAHs and cyanobacterial blooms are significantly complicated. To better elucidate the bloom sustainment of colonial *M. aeruginosa* in eutrophic reservoirs and lakes, the physiological and biochemical responses of *M. aeruginosa* to PAH contamination should be clarified. The present study investigated the effects of PAHs on a series of physiological and biochemical processes of the *M. aeruginosa* strain PCC7806 to determine the main factors of PAHs affecting cyanobacterial blooms.

2. Material and methods

2.1. Experimental design

The *M. aeruginosa* strain PCC7806 used in this study was provided by the Institute of Hydrobiology, Chinese Academy of Sciences.

https://doi.org/10.1016/j.ecoenv.2018.03.052
Received 27 December 2017; Received in revised form 13 March 2018; Accepted 23 March 2018

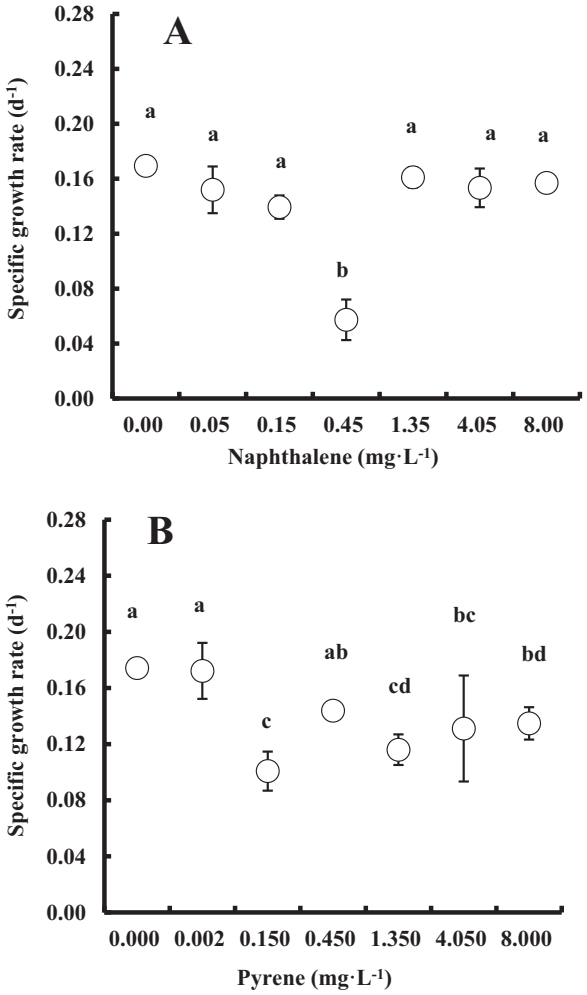

Fig. 1. The growth of the *M. aeruginosa* strain PCC7806 exposed to naphthalene (A) and pyrene (B). Data are means ± SD of the three replicates. Different lowercase letters indicate significant differences between the treatments ($P < 0.05$).

Naphthalene and pyrene were selected as representative PAHs. The exposure media were prepared by adding different levels of naphthalene (0 mg L^{-1}, 0.05 mg L^{-1}, 0.15 mg L^{-1}, 0.45 mg L^{-1}, 1.35 mg L^{-1}, 4.05 mg L^{-1}, respectively) or pyrene (0 mg L^{-1}, 0.002 mg L^{-1}, 0.15 mg L^{-1}, 0.45 mg L^{-1}, 1.35 mg L^{-1}, 4.05 mg L^{-1}, respectively) in 500-mL flasks containing 300 mL of blue-green medium (BG11). The lowest exposure levels of PAHs were designed based on the average PAH levels in lakes in China (Liu et al., 2009), and the NOEC values of PAHs were measured in terms of their population growth inhibition of *Scenedesmus subspicatus*, a green alga (Djomo et al., 2004). The stock solution of PAHs was prepared in methanol and used in all the exposure experiments. The methanol level in the blue-green medium (BG11) did not exceed 0.01%, at which level methanol has no effects on the growth of *M. aeruginosa*. The same methanol levels were added to the control. Each treatment was performed in triplicate. The exponentially growing cells were used as inocula for the start of the experiments (the initial cell density was approximately 10^4 cells·mL^{-1}). For testing the acute toxicity of the PAHs, after 120 h of incubation, specific volumes of algal suspensions were retrieved for further measurement (Chen et al., 2016a, 2016b).

All cultures were incubated at 25 ± 1 °C under cool white fluorescent light at an intensity of 1600 lx with a light: dark period of 12 h:12 h. The BG11 medium contained 0.075 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.036 g L^{-1} $CaCl_2{\cdot}2H_2O$, 0.006 g L^{-1} citric acid, 0.006 g L^{-1} ferric citrate, 0.001 g L^{-1} EDTA sodium salt, 0.02 g L^{-1} Na_2CO_3, and 1 mL of trace elements mix (containing in g·L^{-1}: H_3BO_3, 2.86; $MnCl_2{\cdot}2H_2O$, 1.81; $ZnSO_4{\cdot}7H_2O$, 0.222; $Na_2MoO_4{\cdot}2H_2O$, 0.39; $CuSO_4{\cdot}5H_2O$, 0.079; and $Co(NO_3)_2{\cdot}6H_2O$, 0.0494) (Stanier et al., 1971).

2.2. Cell density analyses

The biomass of *M. aeruginosa* was determined by measuring their optical density (OD) at 450 nm with a spectrophotometer (Downing et al., 2005; Wang et al., 2017), as the Eq. (1):

$$N = 70.575 \quad OD_{450} + 6.323, \tag{1}$$

where N represents the cell numbers (cells mL^{-1}), and OD_{450} are the optical density (OD) at 450 nm with a spectrophotometer.

For testing the growth rate of *M. aeruginosa*, specific volumes of algal suspensions were retrieved for measuring the density of cells at 0, 24, 48, 72, 96, 120, 144 h after cultivation. The specific growth rate of the *M. aeruginosa* strain is identified as the correlation slope of cell densities and the cultivation time, and was computed by the regression analysis as Eq. (2) (Al-Ammara et al., 2015) for the period between samplings therefore representing the mean μ for that period.

$$\mu = \frac{\ln N_n - \ln N_0}{t_n - t_0}, \tag{2}$$

where μ represents the algal specific growth rate, d^{-1}, and N_0 and N_n are the cell numbers at time t_0 (beginning of the test) and time t_n, respectively (cells mL^{-1}).