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Effects of different algaecides on the photosynthetic capacity, cell integrity and microcystin-LR release of *Microcystis aeruginosa*

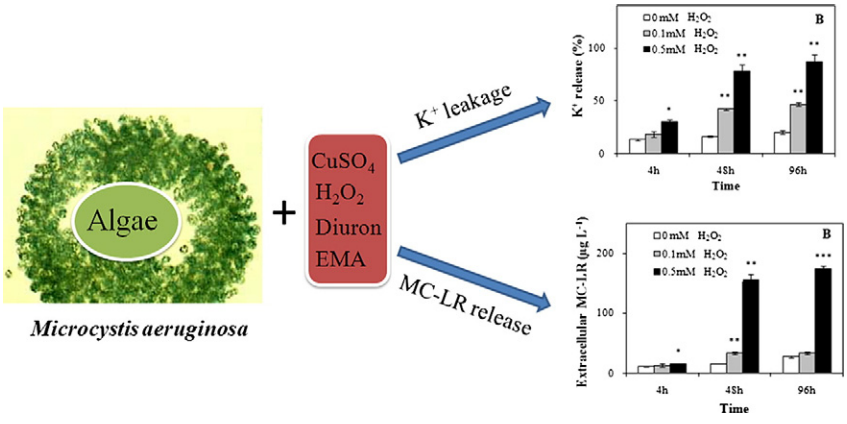
Shiqing Zhou [a], Yisheng Shao [a,b,*], Naiyun Gao [a], Yang Deng [c], Junlian Qiao [a], Huase Ou [a], Jing Deng [a]

[a] State Key Laboratory of Pollution Control and Resource Reuse, Tongji University, Shanghai 200092, China
[b] China Academy of Urban Planning & Design, Beijing 100037, China
[c] Department of Earth and Environmental Studies, Montclair State University, Montclair NJ 07043, United States

HIGHLIGHTS

- PAM was used to investigate the effects of algaecides on *Microcystis aeruginosa*.
- We estimate the release of potassium (K^+) from cell membrane for cell lysis.
- The risk of microcystin-LR release was evaluated after algaecides exposure.
- The order of MC-LR release potential was copper sulfate>hydrogen peroxide> diuron>ethyl 2-methylacetoacetate.

GRAPHICAL ABSTRACT



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ABSTRACT

Bench scale tests were conducted to study the effects of four common algaecides, including copper sulfate, hydrogen peroxide, diuron and ethyl 2-methylacetoacetate (EMA) on the photosynthetic capacity, cell integrity and microcystin-LR (MC-LR) release of *Microcystis aeruginosa*. The release of potassium (K^+) from cell membrane during algaecide exposure was also analyzed. The three typical photosynthetic parameters, including the effective quantum yield (Φ_e), photosynthetic efficiency (α) and maximal electron transport rate ($rETR_{max}$), were measured by a pulse amplitude modulated (PAM) fluorometry. Results showed that the photosynthetic capacity was all inhibited by the four algaecides, to different degrees, by limiting the energy capture in photosynthesis, and blocking the electron transfer chain in primary reaction. For example, at high diuron concentration (7.5 mg L^{-1}), Φ_e, α and $rETR_{max}$ decreased from 0.46 to 0.19 ($p < 0.01$), from 0.20 to 0.01 ($p < 0.01$) μmol electrons m^{-2} s^{-1}/μmol photons m^{-2} s^{-1}, and from 160.7 to 0.1 ($p < 0.001$) μmol m^{-2} s^{-1} compared with the control group after 96 h of exposure, respectively. Furthermore, the increase of algaecide dose could lead to the cell lysis, as well as release of intracellular MC-LR that enhanced the accumulation of extracellular MC-LR. The order of MC-LR release potential for the four algaecides was $CuSO_4$ > H_2O_2 > diuron > EMA.

* Corresponding author at: State Key Laboratory of Pollution Control and Resource Reuse, Tongji University, Shanghai 200092, China. Tel.: +86 21 65982691.
E-mail address: yishengshao@163.com (Y. Shao).

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1. Introduction

Cyanobacterial (blue-green algae) blooms have increasingly occurred worldwide in various water sources and become a global problem. Certain dominant cyanobacterial species in the algae blooming (e.g. *Microcystis aeruginosa*) can produce intracellular metabolites, such as microcystins (MCs), taste and odor-causing substances, and other algae organic matters (AOMs), into water during the cell growth and lysis. The microcystins are highly hepatotoxic and can cause serious liver disease (Carmichael, 2001). The undesirable taste and odor-causing substances significantly degrade the quality of raw water, and are poorly removed by traditional water treatment technologies (Zhang et al., 2010). Moreover, AOMs have been demonstrated to serve as precursors of numerous disinfection by-products (DBPs) during chlorination (Fang et al., 2010; Lee et al., 2007). Therefore, the effective removal or inhibition of cyanobacterial blooming is critically important to prevent or minimize these cyanobacteria-derived water quality issues.

Currently, many methods have been proposed and studied to inhibit the cyanobacterial blooming. Restriction of nutrient including nitrogen (N) and phosphorus (P) in water sources is an essential and long-term method. However, this pathway is significantly unavailable for most of the areas across the world due to economical limitations. Therefore, short-term chemical remediation measures, such as addition of metals (e.g. copper sulfate, $CuSO_4$), oxidants (e.g. hydrogen peroxide, H_2O_2), herbicides (e.g. diuron) and natural compound-derived chemicals (e.g. ethyl 2-methylacetoacetate (EMA)), have received great attention to reduce the proliferation of cyanobacteria, particularly in the summer. At the same time, the chemical reagents should be used as the last chance due to their negative ecological consequences including the release of toxins, persistence and bioaccumulation (Jančula and Maršálek, 2011). Some efforts have been made since to evaluate chemical reagents for bloom control. Ma et al. (2012) investigated the release and degradation behaviors of toxins from *M. aeruginosa* after its exposure to chlorine. It was reported that chlorination damaged cell membranes and contributed to the release of intracellular substances such as toxins, K^+, and chlorophyll-*a*. In another study, Qian et al. (2010) observed that copper sulfate, hydrogen peroxide and N-phenyl-2-naphthylamine had different impacts on inhibiting photosynthesis-related and microcystin-related gene transcription of *M. aeruginosa*, which blocked the electron transport chain, enhanced reactive oxygen species accumulation and overwhelmed the antioxidant system. Among various chemical reagents, $CuSO_4$ may be the most well-known algaecide due to its toxicity against algae and very low cost. H_2O_2 is an efficient oxidizing agent against cyanobacterium without any toxic effect on fish at a dose of <19.7 mg L^{-1} (Gaikowski et al., 1999). Diuron (3-[3, 4-dichlorophenyl]-1, 1-dimethylurea) is algaecidal because it can inhibit algal photosynthesis by preventing the oxygen production and blocking the electron transfer at the level of photosystem II of photosynthetic micro-organisms and plants (Giacomazzi and Cochet, 2004). EMA derived from reed is an allelochemical with a demonstrated inhibitory activity on bloom-forming cyanobacterium (Li and Hu, 2005).

As an autotroph species, cyanobacteria rely heavily upon on the photosynthetic energy conversion using oxygenic photosynthetic systems, similar to plants. The photosynthetic apparatus, including photosystem II (PS II), photosystem I (PS I) reaction centers and phycobilisomes (PBS), plays a key role in photosynthesis of cyanobacteria (Ou et al., 2012a). Recently, the pulse amplitude modulated (PAM) fluorometry, based on the measurement of chlorophyll-*a* fluorescence, provides a powerful method to assess the properties of photosynthetic apparatus, particularly the activity of PS II (Ralph and Gademann, 2005). Through monitoring the changes of fluorescence parameters, the living state of cyanobacteria can be directly and easily quantified. There are three most useful parameters related to photosynthesis on PS II. The dimensionless effective quantum yield (Φ_e) is the proportion of energy used for photosynthesis to the total energy absorbed by chlorophyll associated with PS II (Genty et al., 1989). Φ_e can reflect the rate of linear electron transport, thereby indicating the overall photosynthesis rate (Maxwell and Johnson, 2000). The second photosynthetic parameter α represents the efficiency of light capture under light limited conditions and the last parameter $rETR_{max}$ is the maximal relative electron transport rate in PS II (Ihnken et al., 2010). Our previous efforts demonstrated that all the three parameters could well explain the immediate and long-term impacts of UV-C irradiation or potassium permanganate on the photosynthetic capacity of *M. aeruginosa* (Ou et al., 2012a,b).

However, the information regarding the impacts of the four most common algaecides (i.e. copper sulfate, hydrogen peroxide, diuron and EMA) on the photosynthesis capacity and integrity of *M. aeruginosa*, as well as the release of microcystin-LR (MC-LR), is rather limited. The objective of this study was to study the photosynthetic effects of the four algaecides on *M. aeruginosa*, and the release of MC-LR during the cells lysis.

2. Materials and method

2.1. Materials and reagents

A toxic strain of *M. aeruginosa* (FACHB-912) was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China) and cultivated using BG11 medium in 1 L conical flasks. The reactors were placed in an incubator at a controlled temperature of 25 °C. To simulate the nature light, the reactor was also illuminated by ~30–40 μmol photos m^{-2} s^{-1} tubular fluorescent lamps with a 12-h diurnal cycle every day. All the reagents and solvents were at least of analytical grade except as noted. Copper sulfate ($CuSO_4$), hydrogen peroxide (H_2O_2), and diuron were supplied from Sinopharm Chemical Reagent Co. Ltd., China. EMA was purchased from Sigma-Aldrich, USA.

2.2. Experimental procedures

The *M. aeruginosa* cultivations were harvested during their exponential periods with OD_{680} at ~0.2 (number 4.0×10^6 cells mL^{-1}). The cell number was determined by an YS100 microscope (Nikon, Japan) using the hemacytometer counting method and by measuring its absorbance at 680 nm (OD_{680}) with a UV–Visible spectrophotometer (UV 4802, Unico, USA). The regression equation between OD_{680} (Y) and the number of cells (X, $\times 10^6$ cell mL^{-1}) was established as $Y = 0.055\ X - 0.005$ ($R^2 = 0.99$). Different concentrations of algaecide stock solutions were prepared by dissolving appropriate amounts of the four algaecides in the culture medium, respectively. Thereafter, certain amounts of the stock solutions were spiked into the 200 mL cultivations to achieve the desired algaecide concentrations. During the contact with algaecides, the cultures were shaken for several minutes in an orbital shaker to ensure a complete solution mixing state.

After 4 h contact time, 10 mL sample was harvested for immediate PAM and optical density (OD_{680}) analyses. Another 10 mL sample was filtered with a 0.7 μm GF/F glass-fiber filter (Whatman, UK) for K^+ analysis. Furthermore, 20 mL sample was collected for the intracellular and extracellular MC-LR analysis. The residual solutions were cultivated under the identical conditions mentioned in Section 2.1 after all designated doses of the selected algaecides. The same analyses were repeated every other day. At a minimum, all the tests were conducted in triplicates.

2.3. Analytical methods

2.3.1. Photosynthetic capacity

In the tests to measure the in vivo data with the PHYTO-PAM phytoplankton analyzer (Walz, Germany), at each designate sampling time, 5 mL sample was immediately analyzed subsequent to harvesting from the culture. Without dark adaption, the effective quantum yield (Φ_e) can be calculated as:

$$\Phi_e = \Delta F / F'_m = \left(F'_m - F_s\right)/F'_m \quad (1)$$