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Effect of propionamide on the growth of *Microcystis flos-aquae* colonies and the underlying physiological mechanisms

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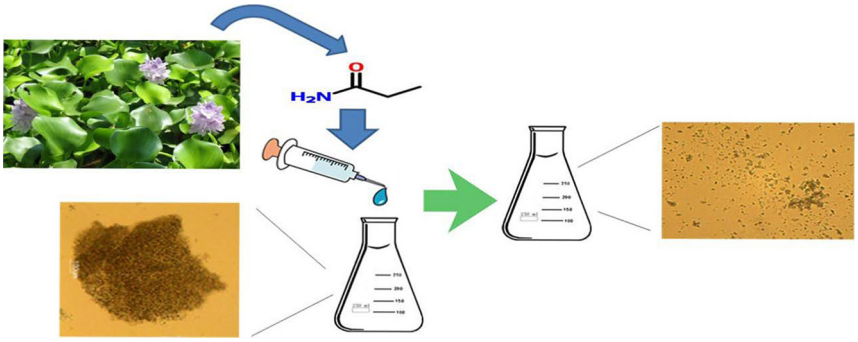
HIGHLIGHTS

- This study first reports propionamide (PA) allelopathy affecting *M. flos-aquae* colony growth/form.
- PA (2 mg L^{-1}) strongly inhibited *M. flos-aquae* colony growth and induced colony disintegration.
- PA (2 mg L^{-1}) reduced EPS synthesis and photosynthetic efficiency and affected MC synthesis.
- PA (2 mg L^{-1}) had no potential risk to the ecological safety of aquatic environments.

GRAPHICAL ABSTRACT



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ABSTRACT

Reducing the formation and growth of *Microcystis* colonies is an important prerequisite for the effective prevention and treatment of cyanobacterial blooms. *Microcystis flos-aquae* colonies was selected to investigate the potential of propionamide for use in controlling cyanobacterial blooms. Propionamide, one of the major allelochemicals in the root exudates of *E. crassipes*, was tested using different concentrations (0, 0.2, 1, and 2 mg L^{-1}) and dosing methods (one-time addition, semi-continuous addition, and continuous addition) to assess its effect on the growth of *M. flos-aquae* colonies. The results showed that in the presence of different concentrations of propionamide, the growth of *M. flos-aquae* colonies followed a logistic growth model, with a higher degree of fit at lower propionamide concentrations. With the semi-continuous addition of 2 mg L^{-1} propionamide, the growth of *M. flos-aquae* colonies was markedly inhibited; the relative inhibition ratio of algal cells reached >90% at day 7 of co-culture, and the colonial form gradually disintegrated, transforming mainly into unicellular and bicellular forms and small colonies (average diameter < 50 μm). Following the semi-continuous addition of 2 mg L^{-1} propionamide, the exopolysaccharide content, the chlorophyll-*a* concentration, and the maximum photochemical efficiency of photosystem II (Fv/Fm) trended downward in *M. flos-aquae* colonies, whereas the relative expression of the microcystin (MC) biosynthetic genes, *mcy*A and *mcy*H, was upregulated overall. Importantly, the synthesis of intracellular microcystin-LR (MC-LR) was decreased after an initial increase, and the extracellular MC-LR concentration did not differ significantly from that in the control group ($p > 0.05$). Moreover, an acute toxicity test showed that 2 mg L^{-1} propionamide was generally non-toxic to *Daphnia magna*. In conclusion, appropriate use of propionamide could effectively control the expansion of *M. flos-aquae* colonies without potential risks to the ecological safety of aquatic environments; therefore, propionamide can actually be used to regulate cyanobacterial blooms in natural waters.

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1. Introduction

With increased water eutrophication and an enhanced greenhouse effect, blue-green algal (cyanobacteria) blooms have continued to expand, seriously affecting the landscape and the ecological functions of water (Paerl and Huisman, 2008). As one of the important large freshwater lakes in China, Taihu Lake has been plagued by cyanobacterial blooms in recent years. Studies have shown that during the period from May to June in each year, *Microcystis flos-aquae* is the main species of algae bloom (Chen et al., 2003). Under natural conditions, *M. flos-aquae* mainly occurs in the colonial form and causing serious microcystin (MC) contamination (Yamamoto and Nakahara, 2009; Codd et al., 2005). The formation and growth of *M. flos-aquae* colonies, as well as the persistent maintenance of the colonial form, are critical conditions for cyanobacterial blooms (Yamamoto et al., 2011; Zhu et al., 2014). Therefore, how to effectively inhibit the expansion of *M. flos-aquae* colonies has emerged as a hot topic and frontier area in aquatic environmental research.

Secondary metabolites (i.e., allelochemicals) produced during the growth of aquatic plants have received increased research efforts and attention from the standpoint of their use in algal inhibition by allelopathy. This interest is attributable to several advantages of semiochemicals, including their easy degradation in the environment and high ecological security (Hilt and Gross, 2008). Jin et al. (2003) suggested that up to 90% inhibition of *Chlorella vulgaris* and *Scenedesmus obliquus* can be produced by propionamide and pelargonic acid which are the main effective antialgal allelochemicals from root exudates of *Eichhornia crassipes*. Liu et al. (2011) observed that propionamide significantly inhibited the growth of unicellular *Microcystis aeruginosa*. Moreover, propionamide has high water solubility. In acute toxicity studies, the lowest published lethal concentration for propionamide inhaled by rats is 8000 ppm (Budavari, 1989), and that intravenously injected into rabbits is 230 mg kg^{-1} (Maurras, 1911), suggesting that this compound is only weakly toxic. Thus, propionamide is suitable for use in the control of algal blooms in natural waters.

Li et al. (2015) showed that unicellular algae and algal cell colonies exhibit different tolerances to allelochemicals. However, many laboratory works concerning *E. crassipes* allelopathy has focused on the unicellular algae rather than on the algal cell colonies. Moreover, the allelopathic effect of aquatic plants on algae growth inhibition is influenced by multiple factors (Mulderij et al., 2003; Qian et al., 2009; Bährs and Steinberg, 2012), including the algal species and the allelochemical type, as well as the allelochemical dose and dosing method. In this paper, propionamide was tested using different doses and dosing methods to assess its effect on the growth of *M. flos-aquae* colonies and to determine the optimal allelopathic conditions. Under the optimal conditions, the changes in *M. flos-aquae* colony morphology and other major physiological response parameters were observed to clarify the mechanism by which propionamide affects the growth of *M. flos-aquae* colonies. Moreover, a test of acute toxicity to *Daphnia magna* was conducted to evaluate the ecological safety of propionamide for non-target aquatic organisms and provide theoretical guidance.

2. Materials and methods

2.1. Allelochemical and experimental algal species

The experiments were conducted using an analytical reagent "propionamide" purchased from Sigma-Aldrich Co. *M. flos-aquae* colonies (FACHB 1344) were obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences. The algal culture conditions referred to Gan et al. (2012). Upon reaching the exponential growth phase, the *M. flos-aquae* colonies was aseptically transferred into fresh culture medium for experimentation.

2.2. Experimental designs

2.2.1. Selection of an optimal propionamide dose for M. flos-aquae colonies inhibition

Regarding the national standard "Chemicals-Alga growth inhibition test" of China (General Administration of Quality Supervision, Inspection and Quarantine of PRC, Standardization Administration of PRC, 2008), propionamide was added to the algal culture, and the final concentration was controlled at 0, 0.2, 1, and 2 mg L^{-1}, with three replicates per concentration. The culture conditions were the same as those described in Section 2.1. The cultures were shaken manually twice daily, and their positions were exchanged to ensure uniform illumination for each treatment. This culture procedure was followed in all the subsequent experiments. The experiment lasted 11 days. The algal density was measured daily for each treatment group (Li and Hu, 2005), and the relative inhibitory rates of propionamide at various concentrations were calculated per Section 2.4.1. Based on these results, the $EC_{50,\ 3d}$ values of propionamide on *M. flos-aquae* colonies were obtained through a fitting calculation using the probability unit approach (Hayes, 1989). The colony diameter was measured at 0, 3, 6, and 10 days of the experiment. The results were analyzed to determine the optimal propionamide dose for the inhibition of *M. flos-aquae* colonies.

2.2.2. Selection of an optimal propionamide dosing method for M. flos-aquae colonies inhibition

Using the results presented in Section 2.2.1, propionamide was added to sterile BG11 medium at a concentration that provided the optimal dose. The resulting allelochemical solution was added to the algal culture by three dosing methods: one-time addition (OTA), semi-continuous addition (SCA), and continuous addition (CA). For each treatment group, a control group was established following the same dosing method, except that the allelopathic solution was replaced with sterile BG11 medium. Three replicates were established for each group. The experimental period and the measurement times of the algal density and colony diameter were the same as those described in Section 2.2.1. The results were analyzed to determine the optimal propionamide dosing method for the inhibition of *M. flos-aquae* colonies.

The different propionamide dosing method treatments were as follows. For the OTA group, 150 mL of allelopathic solution and 10 mL of algal culture were added to a 250 mL Erlenmeyer flask without any supplement until the end of the experiment. For the SCA group, 100 mL of allelopathic solution and 10 mL of algal culture were added initially, followed by supplementation with 10 mL of allelopathic solution every other day from day 2 until day 10. For the CA group, 50 mL of allelopathic solution and 10 mL of algal culture were added initially, followed by a supplement of 10 mL of algal culture every day from day 1 until the end of the experiment (day 10).

2.2.3. Study on the underlying physiological mechanisms for the influence of propionamide on M. flos-aquae colonies

Based on the results of Sections 2.2.1 and 2.2.2, the allelopathic conditions required for the strongest growth inhibition of *M. flos-aquae* colonies were identified, including the optimal propionamide dose and dosing method. Next, these conditions were jointly applied to the *M. flos-aquae* colonies. The morphology of the colonies was then observed, and other major physiological response parameters of algal were measured, including the extracellular polysaccharide (EPS) content, chlorophyll-*a* (Chl-*a*) concentration, maximum photochemical efficiency of photosystem II (PSII) or Fv/Fm, MC biosynthesis gene (*mcy*) expression, and extracellular/intracellular microcystin-LR (MC-LR) contents. The changes in these parameters with the addition of propionamide were examined to clarify the mechanism underlying the propionamide effect on *M. flos-aquae* colonies under optimal conditions. The experiment lasted 11 days, with three replicates for each