

Growth inhibition and possible mechanism of oleamide against the toxin-producing cyanobacterium *Microcystis aeruginosa* NIES-843

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Abstract Oleamide, a fatty acid derivative, shows inhibitory effect against the bloom-forming cyanobacterium *Microcystis aeruginosa*. The EC₅₀ of oleamide on the growth of *M. aeruginosa* NIES-843 was 8.60 ± 1.20 mg/L. In order to elucidate the possible mechanism of toxicity of oleamide against *M. aeruginosa*, chlorophyll fluorescence transient, cellular ultrastructure, fatty acids composition and the transcription of the *mcyB* gene involved in microcystins synthesis were studied. The results of chlorophyll fluorescence transient showed that oleamide could destruct the electron accepting side of the photosystem II of *M. aeruginosa* NIES-843. Cellular ultrastructure examination indicated that the destruction of fatty acid constituents, the distortion of thylakoid membrane and the loss of integrity of cell membrane were associated with oleamide treatment and concentration. The damage of cellular membrane increased the release of microcystins from intact cells into the medium. Results presented in this study provide new information on the possible mechanisms involved and potential utilization of oleamide as an algicide in cyanobacterial bloom control.

Keywords Oleamide · *Microcystis aeruginosa* · Growth inhibition · Photosynthesis · Microcystins

Introduction

Eutrophication is accelerating by excessive nitrogen and phosphorus loading into water through sewage effluent and agricultural run-off on a global scale. Cyanobacteria frequently dominate in eutrophic waters and form water blooms; *Microcystis* is a typical genus of bloom-forming and microcystins-producing cyanobacterium. *Microcystis*-based water blooms pose high health risk to human due to release of hepatotoxic microcystins (Dittmann and Wiegand 2006).

Several approaches involved in chemical and physical interventions, e.g., CuSO₄ amendment, sonification, shading of sunlight, are proposed to control harmful algal blooms, but most of them are impracticable because of high cost, low efficiency and specificity, or subsequent secondary pollution (Shao et al. 2013a). Previous studies indicated that many biologically derived substances (BDSs), e.g., polyphenols (Nakai et al. 2000), ethyl 2-methylacetoacetate (Li and Hu 2005), α -ionone (Wu et al. 2011), alkaloids (Jančula et al. 2010), fatty acids (Nakai et al. 2005; Wu et al. 2006), showed inhibitory effects against the growth of bloom-forming cyanobacteria. As these substances originated from biological sources, most BDSs are easily biodegradable in aquatic environments and do not accumulate. Compared with non-degradable algicide CuSO₄, BDSs show more practicable and great potential in the harmful algae control. However, the public health risks of most BDSs are currently unknown. Though some BDSs may show high inhibitory effect against harmful cyanobacteria, they may also be toxic to humans. Take anthraquinone as an example, this substance

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shows strong inhibitory effect against cyanobacteria (Nanayakkara and Schrader 2008), while Müller et al. (1998) reported that some plant originated anthraquinone derivatives show genotoxicity. In this case, selection of a safe algicidal BDS is crucial in harmful algae control.

Among the reported BDSs, fatty acids and their derivatives are the relative safe ones for applications in harmful algae control. Fatty acids, e.g., oleic acid, linoleic acid, are the main constituents of plasma membranes and thylakoids, but they could destroy the integrity of plasma membrane of cyanobacteria and disturb the association of phycobilins with thylakoids (Wu et al. 2006). Though Wu et al. (2006) demonstrated that the plasma membranes and thylakoids are the targets of toxicity of fatty acids against cyanobacteria, it is not known how they affect the constituent of fatty acids in cyanobacterial cell. The effects of exogenous fatty acids on photosynthesis and microcystins synthesis and secretion remain unknown.

Kabara et al. (1972) reported that the antimicrobial effect of amine derivative of fatty acid is stronger than its corresponding free fatty acid. Recently, oleamide, an amine derivative of oleic acid, was reported to have inhibitory effects against some pathogenic bacteria (El-Moez et al. 2013). As a BDS, oleamide extensively exists in adlay, duckweed, and other plants (Wu et al. 2007; Lu et al. 2014; El-Moez et al. 2013). Previous work in our laboratory indicated that biological originated oleamide showed strong inhibitory effect on the growth of *M. aeruginosa* (non published data). Beside the characteristics of the ample raw materials for oleamide extraction, its chemical synthesis is also facile and inexpensive. These characteristics make oleamide a promising candidate as an environmental friendly algicide in cyanobacterial bloom control. However, the physiological responses and the related characteristics of microcystins of *M. aeruginosa* to oleamide exposure are till not available. In order to elucidate the effect of oleamide on the structure and the functions of plasma membranes and thylakoids of *M. aeruginosa* as well as the characteristics of synthesis and secretion of microcystins, the responses of chlorophyll fluorescence transient of photosynthesis II, fatty acids constituent, ultrastructural observation and, gene expression profiles involved in microcystins synthesis under oleamide treatment were investigated in this study.

Materials and methods

Cyanobacterial strain, culture conditions and chemicals

Microcystis aeruginosa NIES-843 was originated from the National Institute of Environmental Science, Japan, and was kindly provided by Professor Renhui Li (Chinese

Academy of Sciences). *M. aeruginosa* NIES-843 was grown axenically in CT medium (Ichimura 1979) at 25 ± 1 °C under a photoperiod cycle of 12:12 light/dark. The light intensity was set as 30 $\mu\text{mol photons}/(\text{s}\cdot\text{m}^2)$. Oleamide (≥ 98 % purity) was purchased from Sigma-Aldrich (MO, USA).

Inhibitory effects of oleamide on the growth of *M. aeruginosa* NIES-843

Oleamide was dissolved in dimethyl sulfoxide (DMSO). One hundred microliters of oleamide solution were added into 250 mL Erlenmeyer flask containing 94.9 mL of autoclaved CT liquid medium, and then 5 mL of exponential-phase cultures of *M. aeruginosa* NIES-843 were transferred into it. The final volume was set as 100 mL. The oleamide concentration was set as 1, 2, 4, 8, and 16 mg/L, respectively. Oleamide was substituted with DMSO in control treatment. Our previous studies indicated that DMSO showed no observable effect on the growth and photosynthesis of *M. aeruginosa* NIES-843 at an amendment level of 0.1 % (v/v). The initial cell density was about 1×10^6 cells/mL. Each treatment was conducted with three replicas. All treatments were maintained under the cultural conditions as mentioned above.

Chlorophyll *a* determination

Chlorophyll *a* (Chl *a*) in the cells of *M. aeruginosa* NIES-843 was extracted using 90 % ethanol in darkness at 4 °C. The optical density was determined at 665 and 649 nm using a spectrophotometer. Chl *a* concentration was calculated using the following equation provided by Wang (2000).

$$C_a(\text{mg/L}) = 13.95A_{665} - 6.88A_{649}$$

Measurement of chlorophyll fluorescence transients

The chlorophyll fluorescence transients of each treatment were measured using a fluorometer (AquaPen-C AP-C 100, Photon Systems Instruments, The Czech Republic) after dark adaption for 15 min. The actinic light was set as 3000 $\mu\text{mol photons}/(\text{s}\cdot\text{m}^2)$. The parameters for specific energy fluxes (ABS/RC, TR₀/RC, ET₀/RC, DI₀/RC), energy flux ratios (ϕP_0 , ψ_0 , ϕE_0 , ϕD_0), relative value of $(1 - V_k/V_j)$, and performance index of photosystem II were derived or calculated from chlorophyll fluorescence transients according to the methods described by Christen et al. (2007) and Appenroth et al. (2001). The detailed information for these parameters is as follows: ABS/RC, the absorption of energy per reaction centre; TR₀/RC, trapping of energy per reaction centre at time zero; ET₀/RC, electron transport per reaction centre at time zero; DI₀/RC,

heat dissipation per reaction centre at time zero; φP_0 , maximum quantum yield of primary photochemistry; ψ_0 , probability that a trapped exciton moves an electron further than Q_A^- ; φE_0 , probability that an absorbed photon moves an electron further than Q_A^- ; φD_0 , maximum quantum yield of non photochemical de-excitation; PI_{ABS} , performance index of photosynthesis (Christen et al. 2007).

$$ABS/RC = (M_0/V_J)(1-F_0/F_M)$$

$$TR_0/RC = M_0/V_J$$

$$ET_0/RC = (TR_0/RC)\psi_0$$

$$DI_0/RC = (ABS/RC) - (TR_0/RC)$$

$$\varphi P_0 = 1 - (F_0/F_M)$$

$$\varphi D_0 = F_0/F_M$$

$$\psi_0 = 1 - V_J$$

$$\varphi E_0 = (1 - F_0/F_M)(1 - V_J)$$

$$PI_{ABS} = [RC/ABS][\varphi P_0/(1 - \varphi P_0)][\psi_0/(1 - \psi_0)]$$

$$(1 - V_K/V_J)_T = [(1 - V_K/V_J)_{\text{treatment}}]/[(1 - V_K/V_J)_{\text{Control}}]$$

Transmission electron microscopic observations

Microcystis aeruginosa NIES-843 was inoculated into CT medium containing 0, 2 and 16 mg/L of oleamide. Cells of *M. aeruginosa* NIES-843 were collected after 24 h of exposure by centrifuge at $10,000 \times g$ for 8 min, and then fixed and dehydrated according to the methods as described by Shao et al. (2013b). Thin sectioning and staining were performed according to the methods described by Ozaki et al. (2009). The stained cells were then examined using a transmission electron microscope (FEI Tecnai G² 20 TWIN, Eindhoven, The Netherlands).

Determination of fatty acids

The lipid in the cells of *M. aeruginosa* NIES-843 was saponified using 2 M KOH–methanol solution at 75 °C for 15 min. The saponified fatty acids were then further transmethylated using 2 M HCl–methanol solution at 75 °C for 15 min. Fatty acid methyl esters were analyzed using a gas chromatograph (GC-2014C, Shimadzu, Kyoto, Japan), equipped with a flame ionization detector (FID) and a capillary gas chromatography column (AT FFAP, 30 m 0.32 mm \times 0.33 μ m, Lanzhou Institute of Chemical Physics, CAS). The injector and FID detector temperature was set as 240 and 260 °C, respectively. The oven temperature was programmed as follows: initial temperature was hold at 150 °C for 1 min, and increased to 200 °C at a rate of 15 °C per min, and then further increased to 250 °C

with a gradient of 2 °C per min, and followed by holding at this temperature for 6 min. The pressure for nitrogen carrier gas (≥ 99.99 %) was set as 150 kPa.

Quantification of microcystins

The microcystins concentration in the medium was determined using enzyme-linked immunosorbent assay (ELISA) based on anti-microcystin-LR monoclonal antibodies. The microcystin ELISA kit was purchased from Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China), and the concentration of microcystins in the medium was determined according to manufacturer's instructions.

Transcription of the gene involved in the synthesis of microcystins

mcyB is a gene belonging to the gene cluster *mcyA-J* which involved in microcystins synthesis. The transcription of *mcyB* in *M. aeruginosa* NIES-843 was determined by qPCR after 24 h of exposure to oleamide. Total RNA isolation and reverse transcription (RT) were performed using the methods previously described by Shao et al. (2009). The qPCR was performed using a MyiQTM qPCR Detection System (Bio-Rad, Hercules, CA, USA). Forward primer (CCTACCGAGCGCTTGGG) and reverse primer (GAAAATCCCCTAAAGATTCCTGAGT) for *mcyB* were obtained from Kurmayer and Kutzenberger (2003). The qPCR thermal cycling scheme was set as: started at 95 °C for 3 min, and then followed by 40 cycles of 95 °C for 15 s, 59 °C for 30 s, 72 °C for 15 s. The index $2^{-\Delta\Delta Ct}$ was used to evaluate the transcription of *mcyB*, where $\Delta\Delta Ct$ was calculated as follows:

$$\Delta\Delta Ct = (Ct_{\text{targetgene}} - Ct_{16Srm})_{\text{stress}} - (Ct_{\text{targetgene}} - Ct_{16Srm})_{\text{control}}$$

Statistical analysis

Statistical analysis was conducted by one-way ANOVA using SPSS (version 13.0, SPSS Inc., Chicago, IL, USA). Difference was considered to be significant at $P < 0.05$ (LSD).

Results

Growth characteristics

The concentration of Chl *a* in the algal culture was used as an indicator for the growth of *M. aeruginosa* NIES-843. Compared with the control, oleamide showed no significant

effect on the growth of *M. aeruginosa* NIES-843 at a concentration of 0.5 mg/L on day 2 and 1.0 mg/L on day 6, respectively (Fig. 1). However, the growth of *M. aeruginosa* NIES-843 was significantly inhibited by oleamide at a concentration of 1.0 mg/L and higher on day 2. Regression analysis indicated that the EC_{50} (48 h) of oleamide on the growth of *M. aeruginosa* NIES-843 was 8.60 ± 1.20 mg/L.

Chlorophyll fluorescence transients

Ten important parameters involved in specific energy fluxes (ABS/RC, TR₀/RC, ET₀/RC, DI₀/RC) and energy flux ratios (ϕP_0 , ψ_0 , ϕE_0 , ϕD_0), relative value of $(1 - V_k/V_j)$, and performance index (PI_{abs}) of photosystem II were derived from chlorophyll fluorescence transients (Fig. 2). On day 2, the variation of these ten parameters exhibited identical trend in the treatments of 0.5, 1, 2, 4, 8 mg/L, but these parameters showed a contrary pattern in the treatment of 16 mg/L. The parameters PI_{abs}, ϕP_0 and ϕE_0 were higher than those of the control in the treatments of 0.5, 1, 2, 4, 8 mg/L on day 2, but they were lower than the control in the treatment of 16 mg/L; the parameters ABS/RC, DI₀/RC and ϕD_0 were lower than the control in the treatments of 0.5, 1, 2, 4, 8 mg/L, but they were higher than the control in the treatment of 16 mg/L; the parameters ET₀/RC and ψ_0 showed no significant difference with those of the control in the treatments of 0.5, 1, 2 mg/L, but they were lower than the control in the treatments of 4, 8 and 16 mg/L. The variation of these ten parameters on day 6 was different from that on day 2. These ten parameters exhibited a similar variation trend in all oleamide

treatments on day 6. The parameters, PI_{abs}, ϕP_0 , ϕE_0 , ψ_0 , ET₀/RC, were higher than those of the control in all oleamide treatments on day 6, while DI₀/RC and ϕD_0 were lower than those of the control.

Fatty acids composition

Figure 3 shows the effect of oleamide stress on the fatty acid constituent in *M. aeruginosa* NIES-843 on day 2. According to the results of GC determination, seven fatty acids, specifically C16:0, C18:0, C18:1, C18:2, C18:3 α , C18:3 γ , and C18:4, were the main fatty acids in the cells of *M. aeruginosa* NIES-843. Among these seven fatty acids, C16:0 was the primary fatty acid in the control treatment, and the relative ratio of C16:0, C18:0, C18:1, C18:2, C18:3 α , C18:3 γ and C18:4 in the control treatment was 49.72, 10.24, 8.93, 12.43, 8.23, 6.10, and 4.34 %. However, the relative content of C18:1 increased drastically with the increase of oleamide concentration in the medium, and it accounted for 84.97 % of these seven fatty acids in the treatment of 16 g/L, whereas, the relative content of all other six fatty acids decreased along with the increase of oleamide concentration. The fatty acid constituent on day 6 exhibited the same pattern as that on day 2 (data not presented).

Ultrastructure of *M. aeruginosa* NIES-843

Compared with the control, oleamide treatment did not induce obvious ultrastructural change on the cell wall and carboxysome (Fig. 4). However, the thylakoid membrane in the cell of *M. aeruginosa* NIES-843 was distorted by oleamide at a concentration of 2 mg/L, and both the

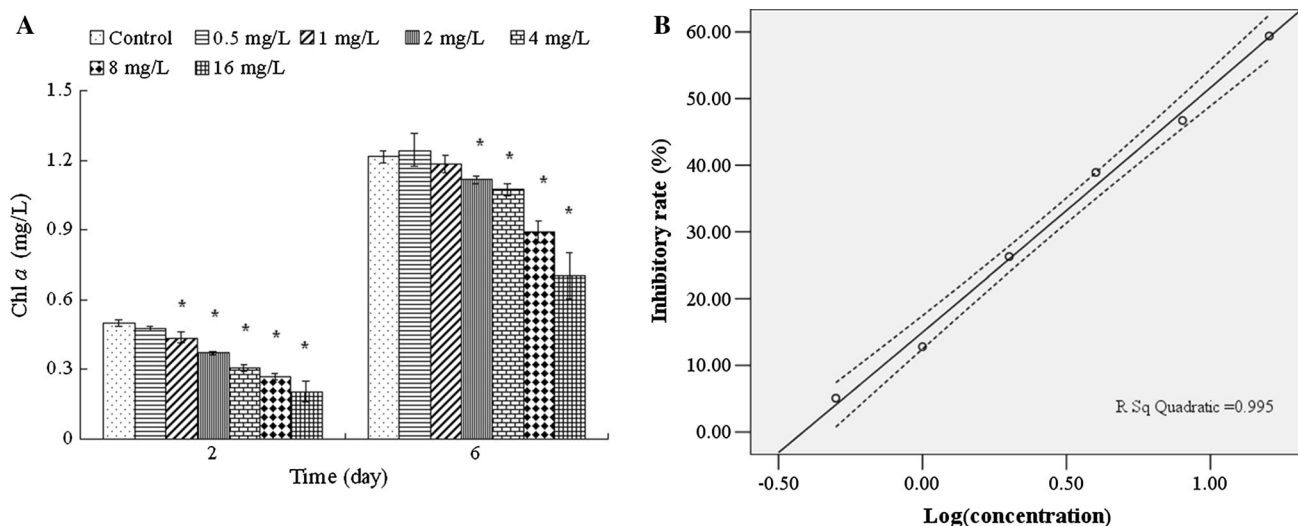


Fig. 1 Effects of oleamide on the growth of *M. aeruginosa* NIES-843 during incubation. **a** Changes of Chl *a* concentration over time under the condition of oleamide treatments; **b** Regression analysis between

oleamide concentration and inhibitory rate on 48 h. Average values \pm standard deviation ($n = 3$). Asterisk means lower than the control ($P < 0.05$)

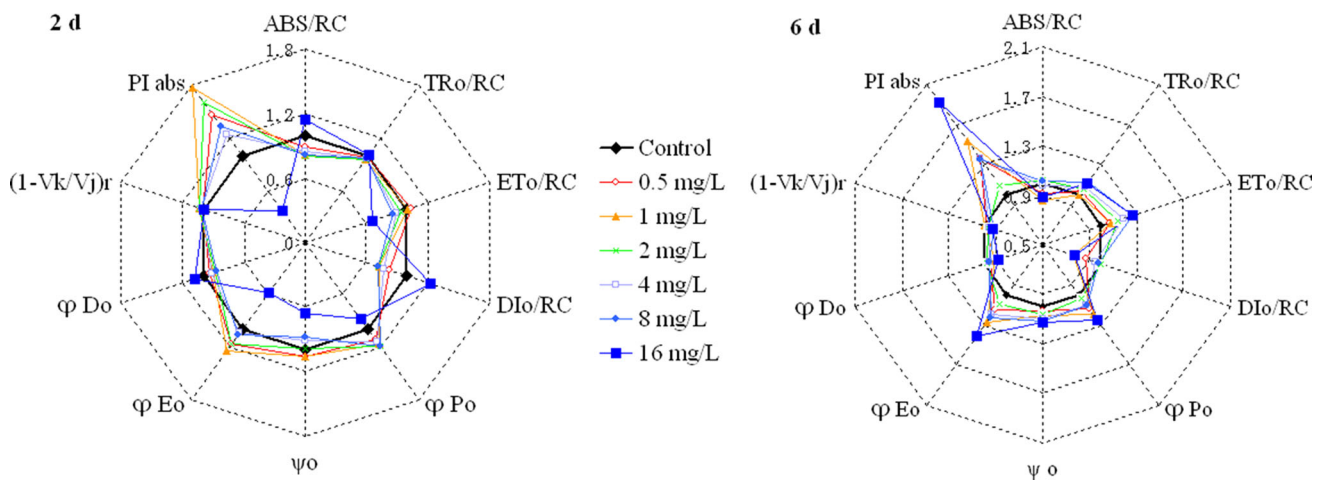


Fig. 2 Radar plot of the parameters originated from chlorophyll fluorescence transients. *Left* on day 2; *right* on day 6

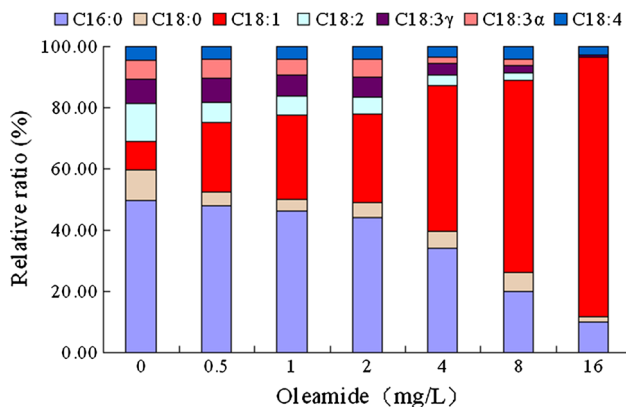


Fig. 3 Effects of oleamide on the relative ratio of seven main fatty acids in the cells of *M. aeruginosa* NIES-843

thylakoid membrane and the cell membrane were all damaged by oleamide at a concentration of 16 mg/L. The content of polyphosphate granules decreased under oleamide treatment.

Microcystins concentration in the medium

The microcystin concentration in the medium was not influenced by oleamide at concentration ≤ 4 mg/L on day 2 (Fig. 5), whereas it was significantly higher than the control at 8 and 16 mg/L. On day 6, the microcystin concentration in the medium was lower than that of the control in the treatments of 1, 2, and 4 mg/L, but it showed no significant difference with that of the control at oleamide concentrations of 0.5, 8, and 16 mg/L.

Transcription of *mcyB*

The transcriptional profiles of *mcyB* was not modified by oleamide at concentrations of 0.5, 1, 2 and 4 mg/L on day

2, but it was depressed at 8 and 16 mg/L (Fig. 6). However, the transcription of *mcyB* in all oleamide treatments showed no significant difference with that of the control on day 6.

Discussion

Oleamide is a fatty acid derivative existed in many plants, e.g., adlay, duckweed, onion, garlic, wheat germ and *Nigella sativa* (Wu et al. 2007; Lu et al. 2014; El-Moez et al. 2013). Extracts dominated by oleamide from wheat germ and other plants showed strong inhibitory effects against selective pathogenic bacteria (El-Moez et al. 2013). The results presented here extended the antibacterial spectrum of oleamide from pathogenic bacteria to cyanobacteria. Our results indicated that the EC_{50} of oleamide on the growth of *M. aeruginosa* NIES-843 was 8.60 ± 1.20 mg/L. Compared with other reported biological originated algicidal substances, the inhibitory effect of oleamide against *M. aeruginosa* is weaker than polyphenols, e.g., gallic acid (EC_{50} : 1.0 mg/L), pyrogallol acid (EC_{50} : 0.65 mg/L), (+)-catechin (EC_{50} : 5.5 mg/L), ellagic acid (EC_{50} : 5.1 mg/L) (Nakai et al. 2000), but it is stronger than that of other BDSs, e.g., β -ionone (EC_{50} : 21.2 mg/L) (Shao et al. 2011), 4-hydroxybenzoic (EC_{50} : 36.3 mg/L) (Wang et al. 2008).

Chlorophyll fluorescence transients can reflect the structure and efficiency of photosystem II (Strasser et al. 1995). The relative value of $[1 - (V_K/V_J)]$ can reflect the state of oxygen evolution complex (Appenroth et al. 2001). Results in Fig. 2 showed that the relative value of $[1 - (V_K/V_J)]$ in the treatments of oleamide showed no significant difference with that of the control, suggesting that oxygen evolution complex of photosystem was not the

Fig. 4 Effects of oleamide on the ultrastructure of *M. aeruginosa* NIES-843. CW cell wall, CM cell membrane, TM thylakoid membrane, DTM distorted thylakoid membrane, CB Carboxysome, PG hole previously occupied by polyphosphate granules

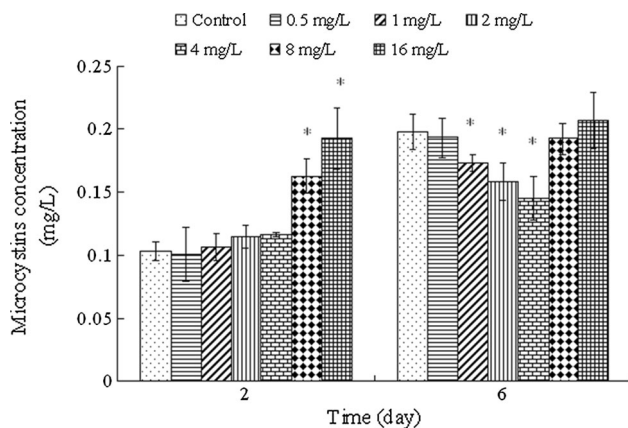
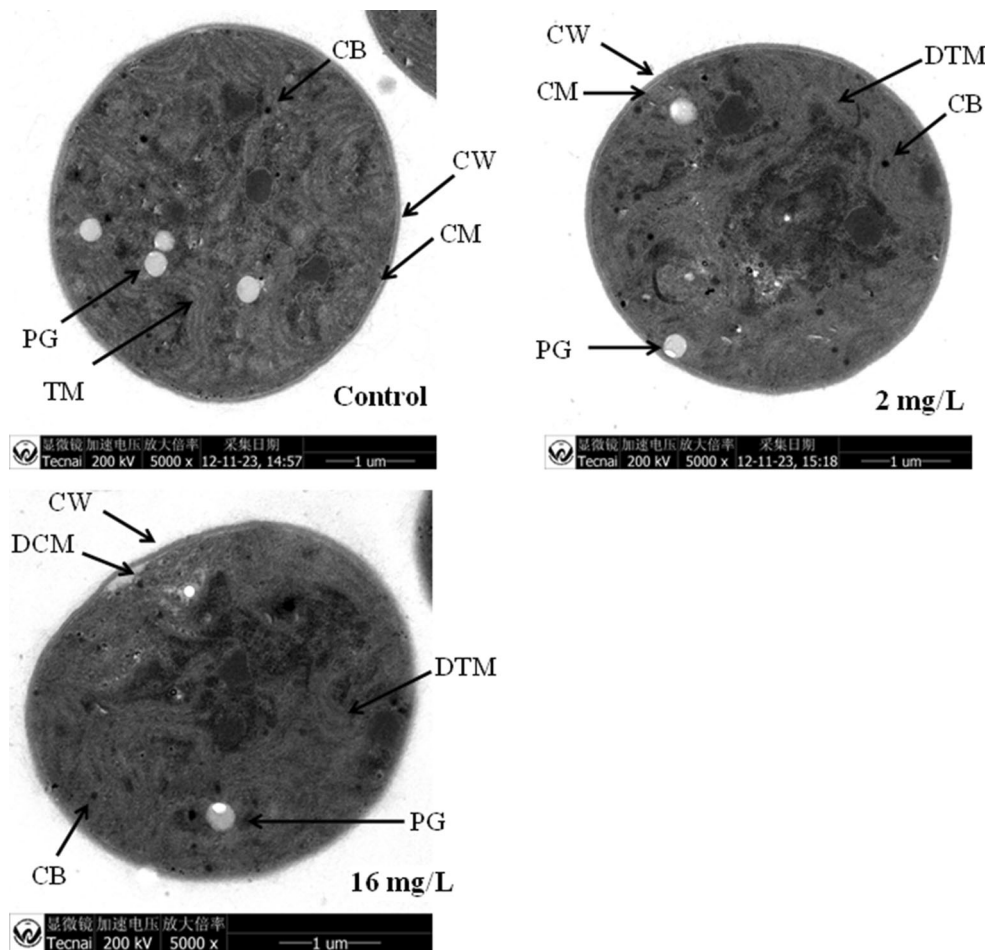


Fig. 5 Effects of oleamide on the microcystin concentration in the medium. Average values \pm standard deviation ($n = 3$). Asterisk means higher or lower than the control ($P < 0.05$)

target of oleamide against *M. aeruginosa* NIES-843. According to the model of energy fluxes of photosynthetic apparatus proposed by Strasser and Strasser (1995), the parameter ϕP_0 and ψ_0 represents the state of electron donating side and accepting side of the photosystem II,

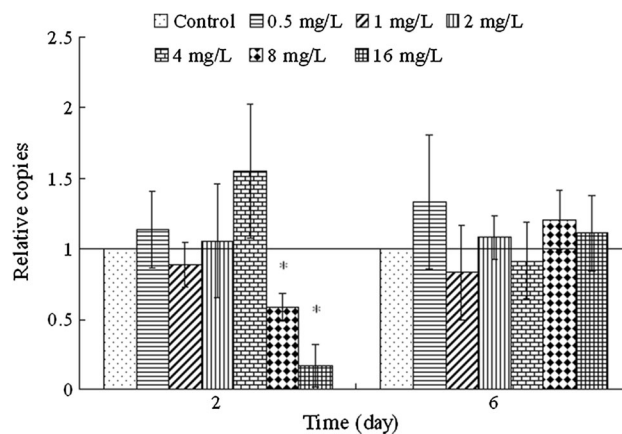


Fig. 6 Effects of oleamide on the transcription of *mcyB*. Average values \pm standard deviation ($n = 3$). Asterisk means lower than the control ($P < 0.05$)

respectively. Compared with the control, the parameter ψ_0 significantly decreased at an oleamide concentration of 4 mg/L and higher on day 2, whereas the value of ϕP_0 was significant lower than the control only at 16 mg/L at the same time. These results suggested that electron accepting

side of photosystem II was more susceptible to damage by oleamide. Previous study indicated that *Saccharomyces cerevisiae* needed more energy to cope with the stress from exogenous fatty acids (Kasemets et al. 2006). We speculate that, similar to *S. cerevisiae*, higher energy in the cell of *M. aeruginosa* NIES-843 was needed to cope with the stress from oleamide, and this may be the reason for the increase of ϕP_0 , ψ_0 , and ϕE_0 under the treatment of oleamide at low concentrations in this study. The variation of the parameters, such as ϕP_0 , ψ_0 , ϕE_0 , and PI_{abs} in the treatment of 16 mg/L exhibited contrary trend on day 2 versus day 6. We deduced that the damage of photosystem caused by oleamide was the reason for the decrease of ϕP_0 , ψ_0 , ϕE_0 and PI_{abs} on day 2 at an oleamide concentration of 16 mg/L, while the recovery and high bio-energy demand to cope with oleamide stress may be the reason for the increase of these parameters on day 6.

Previous studies indicated that the disturbance of fatty acid constituents in plasma membrane could interfere with the functions of the membrane, and lead to loss of plasma lemma integrity (Stevens and Hofemyer 1993). As indicated from Fig. 3, the relative ratio of the main fatty acids changed drastically under oleamide stress. The disturbance of fatty acid composition induced by oleamide may be the reason for the damage of thylakoid membrane and the cellular membrane in the cell of *M. aeruginosa* NIES-843. Results of ultrastructural observation by TEM indicated that both the thylakoid membrane and the cellular membrane were all damaged by oleamide at 16 mg/L. These results suggest that the mechanism of toxicity of oleamide against *M. aeruginosa* NIES-843 may also be involved in the damage of membrane.

The synthesis and degradation of oleamide in the mammals and plants received intensive study, and some biochemical pathways for the synthesis and degradation are available. However, in prokaryotes, the information for the synthesis and degradation of oleamide is still very limited. Oleamide is derived from oleic acid. The degradation of oleamide in the plants and animals is the initial hydrolysis to oleic acid and ammonia by fatty acid amide hydrolase (Farrell et al. 2012; Kim et al. 2010). According to the result of fatty acid determination, we deduce that *M. aeruginosa* NIES-843 could hydrolyze oleamide into oleic acid and ammonia since only the content of oleic acid in the cell increased sharply along with the increase of oleamide concentration in the medium, whereas, the other six fatty acids all continue to decrease under oleamide stress.

The transcriptional pattern of *mcyB*, in response to oleamide treatment, was not in line with the variation of microcystins in the medium in this study. The transcription of *mcyB* was depressed at 8 and 16 mg/L, whereas the microcystins in the medium increased under these two

oleamide concentrations. Microcystins are mainly accumulated within the cells (Juttner and Luthi 2008). The microcystins in the medium were the parts released from inside of cells. Results of ultrastructural observation indicated that the cell membrane was damaged by oleamide at 16 mg/L. Thus, we postulate that the damage of cellular membrane caused the increase of microcystins release, which led to higher concentration of microcystins in the medium under the treatments of 8 and 16 mg/L. As indicated in Fig. 1, the growth of *M. aeruginosa* NIES-843 was inhibited by oleamide at a concentration of 1–16 and 2–16 mg/L on day 2 and 6, respectively, but the transcription of *mcyB* was not influenced by oleamide at a concentration of 0.5–4 and 0.5–16 mg/L on day 2 and 6, respectively. The microcystin concentration in the culture medium in the treatment of 1, 2 and 4 mg/L of oleamide were lower than that in the control. We deduce that the higher cell density in the control corresponds to higher microcystins concentration in the medium.

Beside existed in many plants, oleamide is also existed in many mammals. The function of oleamide in mammals received extensive study, and it has been elucidated to have many physiological functions in mammals, e.g., sleep-induction (Cravatt et al. 1995), modulation of gap junction communication in glial cells (Boger et al. 1998), and reducing anxiety (Akanmu et al. 2007). However, the information about the physiological function of oleamide in plants is limited. Previous studies suggested that the function of oleamide in plants may involve in seedling development, hormone signaling and plant–microbe interactions (Teaster et al. 2007; Tripathy et al. 1999; Kim et al. 2010). Results presented in this study provide some new information about possible inhibitory mechanism of oleamide against cyanobacteria. Though no adverse effect on mammals was reported and given the characteristics of simple chemical synthesis, biodegradable and relative stronger inhibitory effect against *Microcystis*, the possibility of using oleamide as an algicide needs further study on the ecological and public health risks to avoid devastating events irreversibly before large scale of applications can be made.

Conclusion

The EC_{50} of oleamide on the growth of *M. aeruginosa* NIES-843 was 8.60 ± 1.20 mg/L. Oleamide could induce the disturbance of fatty acids constituent in the cell of *M. aeruginosa* NIES-843, which led to the distortion of thylakoid membrane, the loss of integrity of plasma membrane and the release of microcystins. The electron accepting side of the photosystem II is a target of toxicity by oleamide against *M. aeruginosa* NIES-843.

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Conflict of interest Authors have no conflict of interest on the research results reported here. This article does not contain any studies with human participants or animals performed by any of the authors.

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