Physiological Responses of *Microcystis aeruginosa* PCC7806 to Nonanoic Acid Stress

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ABSTRACT: A recent study has shown that nonanoic acid (NA) is one of the strongest allelochemicals to a cyanobacterium *Microcystis aeruginosa*, but the physiological responses of *M. aeruginosa* to NA stress remain unknown. In this study, physiological characters such as the growth rate, photosynthetic processes, phosphorus and nitrogen uptake kinetics, and the contents of intracellular microcystin of *M. aeruginosa* PCC7806 were studied under the NA stress. The results showed that the growth rates of *M. aeruginosa* PCC 7806 were significantly inhibited in all NA stress treatments during first 3 days after exposure, and the growth rate was recovered after 5-day exposure. After 2-day exposure, the contents of both phycocyanin and allophycocyanin per cell decreased at NA concentration of 4 mg L−1, and oxygen evolution was inhibited even at the concentration of 0.5 mg L−1, but carotenoid content per cell was slightly boosted in NA stress. Physiological recovery of *M. aeruginosa* PCC7806 was observed after 7-day exposure to NA. It was shown that NA stress had no effect on uptake of nitrogen, but could stimulate the uptake of phosphorus. The contents of intracellular microcystin have not been affected in all NA treatments in contrast with the control.

Keywords: nonanoic acid (NA); *Microcystis aeruginosa*; stress; physiological responses

INTRODUCTION

Water blooms formed by cyanobacteria have been posing a worldwide environmental issue in recent decades. *Microcystis aeruginosa* is one of the most important organisms responsible for water blooms, and its polypeptide metabolites—microcystins could significantly inhibit the eukaryotic protein phosphatases PP1 and PP2, indicating microcystins’ hepatotoxicity associated with liver cancer and tumors in humans and wildlife (Carmichael, 1995). Controlling the growth of harmful *M. aeruginosa* is crucial in the process against eutrophication and water blooms. Various methods have been proposed to control cyanobacterial blooms, but most of them were impracticable because of high cost or subsequent secondary pollution (Anderson, 1997). Some aquatic plants were known to inhibit the growth of water bloom-forming cyanobacteria by the excretion of inhibitory substances (Gross, 2003; Mulderij et al., 2007), and this phenomenon is termed as allelopathy originated from the report of Molisch (1937), who described direct or indirect effects of one plant on another by producing chemical compounds released into the environment. The allelopathy of aquatic macrophytes to microalgae is very complex or even
controversy. It is also difficult to distinguish allelopathy from resource competition, such as the competition for nutrients or light (Reigosa et al., 1999). For example, Brammer (1979) and Brammer and Wetzel (1984) stated that nutrient limitation rather than allelopathy attributed for the absence of phytoplankton in the presence of aquatic macrophyte *Stratiotes aloides*, while Mulderij et al. (2006) demonstrated that it was allelopathy rather than nutrients limitation responsible for lower phytoplankton biomass in the vicinity of *Stratiotes aloides*. Intensive studies about the allelopathic effects of extracts and exudate of macrophytes have been performed in recent years, and many chemicals have been screened out and proven to have algicidal activity, such as eugeniin (Saito et al., 1989), gallic acids, pyrogallic acid, (+)-catechin, ellagic acid (Nakai et al., 2000), nonanoic acid (NA) (Nakai et al., 2005), ethyl-2-methylacetocitosa was 0.5 mg L\(^{-1}\) (Nakai et al., 2005). Interestingly, nylon, 2, 3, 4 mg L\(^{-1}\), respectively. DMSO was substituted by methanol (0.01% v/v) in nitrogen uptake experiment, because DMSO interfere the detection of NO\(_3\) when using the spectrophotometric method. Prior to the present experiments, we already observed that 0.2% (v/v) DMSO and 0.015% (v/v) methanol had no obvious effect on the growth and photosynthetic processes of *M. aeruginosa* PCC 7806. Each treatment was replicated three times, and the initial cellular concentration was 6.1 \times 10\(^2\) cell \(\mu\)L\(^{-1}\). All treatments were cultured in the same conditions mentioned above. The growth of *M. aeruginosa* was counted at interval of 24 h for 7 days using a hemacytometer. The growth rate was calculated as in Eq. 1.

\[
\text{Growth rate} = (\ln N_t - \ln N_i)/ (t_2 - t_1) \tag{1}
\]

### Determination of Photosynthetic Pigments

Cultures were sampled on 2nd and 7th day respectively to measure contents of photosynthetic pigments. Chlorophyll \(a\) (Chl \(a\)) and carotenoids were extracted with 80% acetone, and the whole procedure was carried out in darkness at 4\(\C\). Chl \(a\) and carotenoid contents were determined with spectrophotometer at 663, 645, 450 nm. Chl \(a\) and carotenoid contents were calculated according to Richards and Thompson (1952). For determinations of phycocyanin (PC) and allophycocyanin (APC), cell suspension was centrifuged at 8000 \(\times\) g for 10 min, and the supernatant was discarded and the pellet was resuspended in phosphoric acid buffer solution (PBS) (0.05 M, pH 6.8). Resuspended cells were broken by sonication, and the solution was centrifuged for 10 min at 7500 \(\times\) g, and absorbencies of supernatant were determined at 565, 620, and 650 nm (Abelson and Simon, 1988).

### Measurement of Oxygen Evolution

Oxygen evolution was measured with a Clark-type oxygen electrode (Hansotech) on 2nd and 7th day after inoculation. Oxygen evolution was determined at 25\(\C\) under a light intensity of 500 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) PAR.

### MATERIALS AND METHODS

#### Strain and Culture Conditions

*Microcystis aeruginosa* PCC 7806 was originally obtained from the Pasteur Culture Collection of Cyanobacteria in France and kindly provided by the Freshwater Algae Culture Collection of the Chinese Academy of Sciences. This strain produces several bioactive compounds, mainly the microcystins MCYST-LR, and additionally (D-Asp3) MCYST-LR and cyanopeptolin depsipeptides (Bister et al., 2004). It was grown in BG\(_{11}\) liquid medium (pH 9) (Rippka et al., 1979), under a 12:12 LD cycle with a intensity of 80 \(\mu\)mol photons s\(^{-1}\) m\(^{-2}\) provided by cool white fluorescent tubes at 25\(\C\) ± 1\(\C\). NA (purity ≥ 97.5%) was purchased from Sinopharm Group Chemical Reagent Ltd. (Shanghai, China). Experiments were carried out in 250 mL conical flasks containing 99.9 mL BG\(_{11}\) liquid medium, and 100 \(\mu\)L NA solutions in dimethyl sulfoxide (DMSO) were spiked into conical flask to obtain final NA concentrations of 0, 0.5, 1, 2, 4 mg L\(^{-1}\), respectively. DMSO was substituted by methanol (0.01% v/v) in nitrogen uptake experiment, because DMSO interfere the detection of NO\(_3\) when using the spectrophotometric method. Prior to the present experiments, we already observed that 0.2% (v/v) DMSO and 0.015% (v/v) methanol had no obvious effect on the growth and photosynthetic processes of *M. aeruginosa* PCC 7806. Each treatment was replicated three times, and the initial cellular concentration was 6.1 \times 10\(^2\) cell \(\mu\)L\(^{-1}\). All treatments were cultured in the same conditions mentioned above. The growth of *M. aeruginosa* was counted at interval of 24 h for 7 days using a hemacytometer. The growth rate was calculated as in Eq. 1.

\[
\text{Growth rate} = (\ln N_t - \ln N_i)/ (t_2 - t_1) \tag{1}
\]
Uptake Kinetics of P and N

*M. aeruginosa* PCC 7806 cells were starved in P free BG11 medium and N free BG11 medium, respectively, before nutrient uptake experiments, and the cultures were completely starved when cell density was constant or slightly decreased (Baldia et al., 2007). For P uptake, HK$_2$PO$_4$ was added to P free BG11 medium to obtain the concentrations of 2, 3, 6, 10, 20 μmol P L$^{-1}$. For N uptake, NO$_3$-N was added to N free BG11 medium to obtain the concentrations of 10, 20, 50, 100, 200 μmol N L$^{-1}$. Time course experiments were conducted prior to nutrient uptake experiments. The uptake rates were fairly constant during 1 h after inoculation for N uptake and 1.5 h for P uptake. From the results, 1 h after inoculation was applied for nutrient uptake experiments. PO$_4^{3-}$ and NO$_3^-$ uptake were measured by the rate of depletion from medium using spectrophotometric methods (MEPPRC, 2002). The total protein contents of *M. aeruginosa* PCC 7806 were also determined by Coomassie Blue-dye Binding Assay (Bradford, 1976) at 1 h after inoculation. The protein contents were obtained using Lineweaver-Burk linear regression line (2) which transformed from Michaelis-Menten Equation (3).

$$1/v = 1/V_{\text{max}} + K_m/V_{\text{max}} \times 1/[S] \quad \text{Lineweaver-Burk (2)}$$

$$v = (V_{\text{max}} \times [S])/(K_m + [S]) \quad \text{Michaelis-Menten (3)}$$

**Intracellular Microcystin-LR Determination**

Intracellular microcystin extracted with 75% methanol at 4°C in darkness (Fastner et al., 1998). The microcystin-LR was determined using HPLC. The chromatographic system consisted of a Waters Alliance HPLC equipped with a 2695 separation module with an online degasser, Waters 2996 photo diode array detector, and Waters Empower chromatography software (Waters). The concentration of microcystin-LR was determined by comparing the peak area at 238 nm with that of microcystin-LR standard and its separation was accomplished under a reversed-phase isocratic condition with a Hypersil BDS (base deactived silica) C$_{18}$ column (5 μm, 250 mm × 4.6 mm; Thermo Hypersil, Runcon, Cheshire, UK) in a run with 68% solution A (100% methanol v/v) and 32% solution B (0.05% aqueous trifluoroacetic acid v/v) over 20 min. The flow rate was set at 1 mL min$^{-1}$ and the column temperature was maintained at 30°C.

**Statistics**

Significant differences were determined by one-way ANOVA followed by LSD post-hoc test, differences were considered to be significant at $P < 0.05$.

**RESULTS**

**Growth Characteristics**

Figure 1 showed the growth curve of *M. aeruginosa* PCC 7806 in BG11 medium under the conditions mentioned above. The effects of NA on the growth rates of *M. aeruginosa* PCC 7806 were shown in Figure 2. Compared with the control, the growth rate of *M. aeruginosa* PCC 7806 was significantly inhibited during first 3 days exposed to all NA treatments. The inhibitory effect on growth rate was only observed at over 2 mg L$^{-1}$ NA on 4th day. The growth rate was recovered on 5th, 6th, and 7th days.

**Photosynthetic Pigments Contents**

Figures 3 and 4 illustrated the change of Chl a and carotenoid, PC and APC contents per cell in response to NA stress. There was little difference in Chl a contents per cell both after 2- and 7-day exposure to all NA treatments, and carotenoid contents in the treatments of 0.5 and 1 mg L$^{-1}$ were slightly boosted in NA stress after 2-day exposure. PC and APC contents per cell showed almost no difference at

![Fig. 2. Effects of NA on the growth rate of *M. aeruginosa* PCC 7806 in BG11 medium. The horizontal axis is the exposure time of *M. aeruginosa* PCC 7806 in BG11 medium with different doses of NA. The error bars represent average ± standard deviation. *P < 0.05 (LSD).](image-url)
the concentrations of no more than 2 mg L\(^{-1}\) NA on 2nd day, but both PC and APC decreased significantly when the concentrations reached 4 mg L\(^{-1}\). The contents of Chl \(a\), carotenoid, PC, and APC per cell showed little difference in all treatments on 7th day.

**Oxygen Evolution**

The oxygen evolution of *M. aeruginosa* PCC 7806 in response to NA stress was shown in Figure 5. Compared with control, oxygen evolution of *M. aeruginosa* PCC 7806 was significantly inhibited after 2 days exposure in NA stress. Compared with the control, there was no significant difference in all treatments after 7-day exposure.

**Uptake of P and N**

With the respect to the uptake kinetic parameters, \(V_{\text{max}}\) is the maximum uptake rate at substrate saturation, \(K_{\text{m}}\) is the Michaelis-Menten constant, \(V_{\text{max}}/K_{\text{m}}\) is an index describing uptake rates versus substrate concentration at very low substrate concentrations, with higher values suggesting high efficiency in nutrients uptake (Healey, 1980). P and N uptake kinetics parameters of *M. aeruginosa* in NA stress were shown in Figures 6 and 7 and Tables I and II. \(K_{\text{m}}\) for P uptake had not significantly changed in all treatments, while the \(V_{\text{max}}\) and \(V_{\text{max}}/K_{\text{m}}\) under NA stress were significantly higher than those of the control at 2 and 4 mg L\(^{-1}\) (Table I). For NO\(_3\^-\)N, the \(K_{\text{m}}\), \(V_{\text{max}}\), and \(V_{\text{max}}/K_{\text{m}}\) were relatively consistent in all treatments (Table II). These parameters suggested that there were no effects on uptake of NO\(_3\)^-N, but could stimulate PO\(_4\)^3- uptake for *M. aeruginosa* in NA stress.

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**Fig. 3.** Chl \(a\) and carotenoid (Car) contents of *M. aeruginosa* PCC 7806 under the NA stress. Chl \(a\) (2 d) and Chl \(a\) (7 d) in the horizontal axis are the Chlorophyll \(a\) contents of *M. aeruginosa* PCC 7806 after 2 days and 7 days exposure in different doses of NA, respectively. Car (2 d) and Car (7 d) in the horizontal axis are the carotenoids contents of *M. aeruginosa* PCC 7806 after 2 days and 7 days exposure, respectively. The error bars represent average ± standard deviation. "\(^*\)P < 0.05 (LSD)."

**Fig. 4.** PC and APC contents of *M. aeruginosa* PCC 7806 under the NA stress. PC (2 d) and PC (7 d) in the horizontal axis are the phycocyanin contents of *M. aeruginosa* PCC 7806 after 2 days and 7 days exposure in different doses of NA, respectively. APC (2 d) and APC (7 d) in the horizontal axis are the allophycocyanin contents of *M. aeruginosa* PCC 7806 after 2 days and 7 days exposure, respectively. The error bars represent average ± standard deviation. "\(^*\)P < 0.05 (LSD)."

**Fig. 5.** Oxygen evolution of *M. aeruginosa* PCC 7806 under the NA stress. The horizontal axis is the exposure time of *M. aeruginosa* PCC 7806 in BG11 medium containing different doses of NA. The error bars represent average ± standard deviation. "\(^*\)P < 0.05 (LSD)."

**Fig. 6.** Lineweaver-Burk plots showing the effects of NA on phosphorus uptake of *M. aeruginosa* PCC 7806 under different doses of NA. The error bars represent average ± standard deviation.
Intracellular Microcystins Content

Since microcystins are mainly accumulated within the cell (Juttner and Luthi, 2008), we determined intracellular microcystin-LR content after 14 days exposure. Figure 8 showed the intracellular microcystin-LR contents after 14-day exposure in NA stress. The results showed that the contents of intracellular microcystin-LR had not been affected in all treatments.

DISCUSSION

NA belongs to a medium-chain monocarboxylic acid, and the stress caused by medium-chain monocarboxylic acids could affect the spatial organization of the plasma membrane, and interferes functions of the membrane as a matrix for enzymes and as a selective barrier, thereby leading to increase of the passive flow of protons through the plasma membrane and loss of plasma lemma integrity (Stevens and Hofemyer, 1993). All the aforementioned features exhibited that NA may act as a membrane active substance, which contributes for the inhibitory effects to M. aeruginosa.

Regarding the effect of NA on the photosynthetic processes, oxygen evolution of M. aeruginosa was inhibited at 0.5 mg L\(^{-1}\) NA, which may be explained by two possible reasons: The first is that NA stress affected phycobilisomes in which PC and APC contents decreased dramatically at higher NA concentration (Figure 4). As the light harvesting protein complex, phycobilisome transfers light energy to chlorophyll and then to the reaction center (Grossman et al., 1993), and the decrease of PC and APC contents would lead to the drop in light harvesting, and then influence the efficiency of oxygen evolution. The second is that NA, as a medium-chain fatty acid, may be integrated into thylakoid membranes and interferes the fluent of membranes, eventually affects the balance of electron flowing in photosynthetic processes, since it was reported that fatty acids could inhibit electron transport in Photosystem II (Venediktov and Krivoshejeva, 1983; Warden and Csatorday, 1987). Chl \(a\) contents did not show significant influence in all NA stress treatments, indicating that Chl \(a\) was not the target of NA to M. aeruginosa.

As for the carotenoids, only the treatments of 0.5 and 1 mg L\(^{-1}\) were significantly higher than control and the treatments of 2 and 4 mg L\(^{-1}\) failed to reach the significantly different level. This was beyond what we expected. A further check of the \(P\) value of LSD \(\text{post-hoc}\) test when compared with control (0.036 of 0.5 mg L\(^{-1}\), 0.019 of 1 mg L\(^{-1}\), 0.071 of 2 mg L\(^{-1}\), and 0.064 of 4 mg L\(^{-1}\)) showed that these \(P\) values of NA stress treatments were fluctuated around the level of 0.05, and statistic analysis showed that the carotenoid contents among all NA stress treatments had no significant difference. All these suggested that NA stress may increase the contents of carotenoid in M. aeruginosa, but the degree of the increase was so slight that the \(P\) values fluctuated around the level of 0.05. The reason for higher carotenoid contents at 0.5 and 1 mg L\(^{-1}\) NA (not 2 and 4 mg L\(^{-1}\)) may be explained as random errors due to small samples of the present experiments.

Carotenoids are essential structural and functional components of the photosynthetic antenna and reaction center complexes (Li and DellaPenna, 2004), and they can prevent the generation of singlet oxygen by quenching and dissipating the energy as heat (Frank and Cogdell, 1996). Many

<table>
<thead>
<tr>
<th>NA concentration (mg L(^{-1}))</th>
<th>(K_m) ((\mu)M)</th>
<th>(V_{\text{max}}) ((\mu)m/h(^{\times})mg total protein)</th>
<th>(V_{\text{max}}/K_m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg L(^{-1})</td>
<td>14.74 ± 7.21 (a)</td>
<td>0.24 ± 0.07 (b)</td>
<td>0.0174 ± 0.0032 (b)</td>
</tr>
<tr>
<td>0.5 mg L(^{-1})</td>
<td>15.30 ± 2.56 (a)</td>
<td>0.28 ± 0.04 (b)</td>
<td>0.0180 ± 0.0003 (b)</td>
</tr>
<tr>
<td>1 mg L(^{-1})</td>
<td>19.45 ± 9.28 (a)</td>
<td>0.34 ± 0.13 (ab)</td>
<td>0.0186 ± 0.0030 (b)</td>
</tr>
<tr>
<td>2 mg L(^{-1})</td>
<td>9.34 ± 2.91 (a)</td>
<td>0.25 ± 0.05 (b)</td>
<td>0.0276 ± 0.0028 (a)</td>
</tr>
<tr>
<td>4 mg L(^{-1})</td>
<td>18.77 ± 5.95 (a)</td>
<td>0.50 ± 0.11 (a)</td>
<td>0.0274 ± 0.0025 (a)</td>
</tr>
</tbody>
</table>

Data in table are means ± SD, different letters (in parenthesis) after data means significant difference (\(P < 0.05\)) (LSD).
researches demonstrated the contents of carotenoids increased in oxidant stress (Schafer et al., 2005, 2006; Srivastava et al., 2005; Rath and Adhikary, 2007). Our results showed that carotenoid contents were slightly boosted after 2 days to expose in NA stress, suggesting that NA may induce oxidant stress up on *M. aeruginosa*. Since NA itself is not an oxidant material, one possible explanation is that NA stress interfere the balance of electron flowing in photosynthetic processes which produced reactive oxygen. To quench reactive oxygen, more carotenoids were supposed to be formed in *M. aeruginosa* under NA stress.

There is hardly information about the effect of medium-chain fatty acids on P uptake in cyanobacteria/algae, but Kasemets et al. (2006) showed that the addition of low concentrations of medium-chain monocarboxylic acids (octanoic and decanoic acids) increased *Q*\textsubscript{ATP} (specific ATP production rate) and *m*\textsubscript{e} (maintenance energy requirement for growth) in *Saccharomyces cerevisiae*. It means that the *Saccharomyces cerevisiae* needed more energy to cope with the stress of the two medium-chain monocarboxylic acids. *M. aeruginosa* PCC 7806 cells underwent starvation treatment before P uptake experiment, and intracellular free PO\textsubscript{4}\textsuperscript{3–} was nearly exhausted when the experiment started. We speculate that *M. aeruginosa* PCC 7806, similar to *Saccharomyces cerevisiae*, needs more energy to cope with NA stress, and more high-energy phosphate bonds were required to synthesize under different doses of NA stress, which may explain that NA stress stimulated phosphorus uptake of *M. aeruginosa* in this study since PO\textsubscript{4}\textsuperscript{3–} is a substance in high-energy phosphate bond synthesis.

Many factors have been observed to affect the synthesis of microcystins in *Microcystis* species. Iron deficiency resulted in the increase of microcystin-LR content relative to total protein (Beatriz et al., 2006), and high light (Kaebernick et al., 2000) increased the transcription of *mcyB* and *mcyD*, two genes involved in the synthesis of microcystins, and H\textsubscript{2}O\textsubscript{2} stress (Schatz et al., 2007) also could up-regulate transcription of *mcyB*. One experiment in our lab also proved that pyrogallol could up-regulate the transcription of *mcyB* in *M. aeruginosa* PCC 7806 (unpublished data). Our result here showed that there was no difference in microcystin contents per cell between the control and the treatments under NA stress.

As described by Nakai et al. (2005), the total inhibitory of the *Myriophyllum spicatum* culture solution to *M. aeruginosa* could not be accounted for by NA, because culture solution of *Myriophyllum spicatum* with higher density (100 g wet wt L\textsuperscript{–1}) produced NA as \(~\)50 µg L\textsuperscript{–1}, which is much lower than EC\textsubscript{50} (0.5 mg L\textsuperscript{–1}). The physiological features of *M. aeruginosa* under NA stress, such as growth rate, oxygen evolution, and pigment content, exhibited difference between 2nd and 7th day exposure: most of them were sensitive to NA stress after 2 days but showed little difference between 2nd and 7th day exposure: most of them were sensitive to NA stress after 2 days but showed little difference.

### TABLE II. Nitrogen uptake kinetic parameters of *M. aeruginosa* in NA stress

<table>
<thead>
<tr>
<th>NA concentration (mg L\textsuperscript{–1})</th>
<th><em>K</em>\textsubscript{m} (µM)</th>
<th><em>V</em>\textsubscript{max} (µm/h*mg total protein)</th>
<th><em>V</em>\textsubscript{max}/<em>K</em>\textsubscript{m}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>109.74 ± 56.40 (a)</td>
<td>4.01 ± 1.95 (a)</td>
<td>0.0295 ± 0.0118 (a)</td>
</tr>
<tr>
<td>0.5</td>
<td>65.92 ± 21.93 (a)</td>
<td>2.57 ± 0.48 (a)</td>
<td>0.0403 ± 0.0167 (a)</td>
</tr>
<tr>
<td>1</td>
<td>57.71 ± 10.83 (a)</td>
<td>2.19 ± 0.82 (a)</td>
<td>0.0381 ± 0.0078 (a)</td>
</tr>
<tr>
<td>2</td>
<td>49.16 ± 4.31 (a)</td>
<td>1.94 ± 0.98 (a)</td>
<td>0.0352 ± 0.0076 (a)</td>
</tr>
<tr>
<td>4</td>
<td>91.35 ± 65.82 (a)</td>
<td>2.11 ± 1.71 (a)</td>
<td>0.0268 ± 0.0097 (a)</td>
</tr>
</tbody>
</table>

Data in table are means ± SD; different letters (in parenthesis) after data means significant difference (*P* < 0.05) (LSD).

Fig. 8. Intracellular microcystin-LR of *M. aeruginosa* PCC 7806 under the NA stress. The error bars represent average ± standard deviation.

Fig. 9. Effects of NA on the growth of *M. aeruginosa* PCC 7806 in BG11 medium containing different doses NA. The horizontal axis is the time of exposure of *M. aeruginosa* PCC 7806 in BG11 medium with different doses NA. The error bars represent average ± standard deviation. *P* < 0.05 (LSD).
after 7 days. The remaining NA content in the medium was analyzed, and result showed that only 10.3% NA vaporized at the concentration of 4 mg L$^{-1}$ NA after 7 day exposure. Therefore, vaporization seems not the reason accounting for the physiological difference between 2nd and 7th day after exposure to NA stress. DAPI staining result indicated that there was no obvious cell death in NA stress in the 7-day time course experiments even at the concentration of 4 mg L$^{-1}$. These results implied the inhibitory effect of NA on Microcystis worked through a mild way, and M. aeruginosa would soon get adapted to NA stress.

From Figure 9, it is shown that even the cell numbers in NA stress treatments were significantly lower than those of the controls, but another important point we should notice here is that the cell numbers in NA stress treatments increased along the culture times. The cell number on 7th day at the concentration of 4 mg L$^{-1}$ was 10 times of the initial number on inoculation day. As shown in Figure 2, difference in cell numbers between treatments and control after 7-day exposure was mainly caused by the inhibition of growth rate within first 4 days, and the growth rate would be recovered since 5th day. These results indicated that it is still difficult to completely control M. aeruginosa at the concentration of less than 4 mg L$^{-1}$ NA, even though the EC$\text{50}$ of NA to the growth of M. aeruginosa is 0.5 mg L$^{-1}$. Furthermore, the result from our lab experiments showed that M. aeruginosa in BG$\text{11}$ medium was not completely eliminated even at NA concentration of 16 mg L$^{-1}$ which is far beyond the solution of NA in water. From the aforementioned points, it is important to perform reevaluation of allelochemicals like NA, based on multiple studies of algal responses such as physiological and further molecular features before applying them in bloom control. According to the results obtained in the present study, from the view of physiology of M. aeruginosa PCC 7806, it is suggested that NA is not a potential practicable chemical in Microcystis bloom control in natural waters.

REFERENCES


