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Effects of arsenic on growth and photosystem II (PSII) activity of *Microcystis aeruginosa*

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ABSTRACT

Effects of arsenic on growth, pigments content, oxygen evolution and photosystem II (PSII) activity of *Microcystis aeruginosa* were investigated in the present study. Various concentrations of As(III) did not show significant effects on growth and total carotenoids content within 24 h of treatment. After 48 h of treatment, 10 mg L^{-1} As(III) significantly inhibited the growth and synthesis of carotenoids of *M. aeruginosa*, while As(III) at concentrations ranging from 0 to 1 mg L^{-1} showed no significant inhibition. Chlorophyll *a* synthesis, oxygen evolution and chlorophyll fluorescence were more sensitive to As(III) exposure than carotenoid synthesis and growth. Chlorophyll *a* content, fast fluorescence rise transients and fluorescence decay kinetics appeared to be affected after the cells were exposed to 1 and 10 mg L^{-1} As(III) for more than 24 h. Treatment with 10 mg L^{-1} As(III) for 24 h or longer led to flattening of the fluorescence transient and drastic decrease of amplitude of fast phase of Q_A^- reoxidation kinetics. Exposure to As(III) mainly inhibited the quantum yield for primary photochemistry, density of reaction centers and photosynthesis performance index, and increased the dissipated energy. The decrease in amplitude of the fast and middle phases further revealed that once electron transfer from Q_A^- to Q_B was inhibited by As(III), more Q_A^- was reoxidized via $S_2(Q_AQ_B)^-$ charge recombination. As(III) stress may result in an increased stability of the S_2QB^- and S_2QA^- recombination.

1. Introduction

Arsenic (As) is a ubiquitous toxic element which occurs naturally in the earth's crust. Arsenic compounds have been used in various industries such as medicine, electronics, agriculture and metallurgy (Nriagu and Azcue, 1990). Human activities such as mining, burning of fossil fuels and pesticide application have caused arsenic pollution, although there is geological origin of some arsenic pollution (Bissen and Frimmel, 2003a,b).

The concentration of arsenic is usually less than 2 μg L^{-1} in seawater and 1–10 μg L^{-1} in unpolluted surface water and groundwater (Ng, 2005). But high levels of arsenic may occur in various water bodies. For example, 17–980 μg L^{-1} in well water in south–west Finland (Kurttio et al., 1998), 1–48000 μg L^{-1} in drinking water in western United States of America (Welch et al., 1988), and up to 1354 μg L^{-1} in well water in Inner Mongolia, China (Guo et al., 2001) were detected.

High levels of arsenic in water pose a great risk to animal and human health. Inorganic arsenic species are more toxic than organic forms to living organisms (Goessler and Kuehnett, 2002; Meharg and Hartley-Whitaker, 2002; Ng, 2005). Several previous studies showed the effects of arsenic on growth, nutrition or oxidative stress of higher plants (Carbonell et al., 1998; Rahman et al., 2007), green algae (Creed et al., 1990) or cyanobacteria (Gong et al., 2009a; Sorsa, 1983). Since aquatic microorganisms served as essential primary producers in the ecosystem, the study of the effects of arsenic on aquatic microorganisms will provide important information for understanding the risk of arsenic to the environment and ecosystem. Sorsa (1983) found that As could inhibit growth, photosynthesis and ATP formation of algae. While some other studies found that the growth and chlorophyll *a* (chl *a*) content of *Microcystis aeruginosa* exposed to arsenate ranging from 10^{-8} to 10^{-4} mol L^{-1} showed no difference from that of the control (Gong et al., 2009a,b). But the effects of As(III) on growth and photosynthesis of phytoplankton is still unclear.

M. aeruginosa was considered as one of the most common cyanobacteria (Oliver and Ganf, 2000; Qiu and Gao, 2002). The microcystin production (De Figueiredo et al., 2004; Oh et al., 2000) and the application of *M. aeruginosa* in bioremediation (Parker et al., 1998) or risk assessment of pollutants (Mitchell et al., 2002; Peterson et al., 1994) had been widely studied. *M. aeruginosa* also often served as an ideal material for studying

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the effects of contaminants on photosynthesis because of its similarity of the chloroplast to that of higher plants (Lang, 1968; Zhou et al., 2006).

Photosystem II (PSII) has been demonstrated to be very sensitive to various pollutants (Mohapatra et al., 1997, 2010; Pan et al., 2008, 2009; Qian et al., 2009, 2010; Li et al., 2010), which can be reliably probed by chlorophyll fluorescence. But the knowledge of the effects of As(III) on the photosynthetic apparatus, e.g. PSII, of phytoplankton is limited. This study aimed at using *M. aeruginosa* as material to investigate the effects of As(III) on growth, pigments content, oxygen evolution and PSII activity of cyanobacteria.

2. Materials and methods

2.1. Culture of microcystis aeruginosa

M. aeruginosa (FACHB-905, Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences) was cultured in BG-11 medium (Stanier et al., 1971) at 25 °C under continuous fluorescent white light (30 μmol photons $m^{-2} s^{-1}$) with a 12:12 h light–dark cycle. The growth of cultures was monitored every day by measuring cell optical density at 680 nm (OD_{680}) with a spectrophotometer (UV2800, Unico, Shanghai, China). The cells in exponential growth phase were harvested for the following experiments.

2.2. Chemicals

The chemicals used for As(III) treatments were applied in the form of analytical-grade arsenic trioxide. The As(III) solutions were prepared by dissolving arsenic trioxide in sterilized BG-11 medium and then diluted to desired concentrations. The As(III) solutions were stored at 4 °C until use.

2.3. Treatments

Exponentially grown cells used for measurements of growth, pigments content and oxygen evolution were cultured in flasks with BG-11 medium or prepared As solutions to make the final As(III) concentrations of 0.01, 0.1, 1, and 10 mg L^{-1}. The cells used for the chlorophyll fluorescence measurements were cultured in 10×10 mm^2 plastic cuvettes with same volume. BG-11 medium or prepared As(III) solutions at different concentrations were added into the cuvettes to make the final As(III) concentrations of 0.01, 0.1, 1, and 10 mg L^{-1}. The sample without As(III) was used as the control.

All samples were processed in triplicate and cultured at 25 °C under continuous fluorescent white light (30 μmol photons $m^{-2} s^{-1}$) with a 12:12 h light–dark cycle. The measurements were carried out at 2, 4, 6, 12, 24 and 48 h after treatment.

2.4. Measurement of growth

After As exposure for 2, 4, 6, 12, 24 and 48 h, growth of the *M. aeruginosa* cells was determined by measuring the optical density at 680 nm (OD_{680}) with the spectrophotometer (UV2800, Unico, Shanghai, China).

2.5. Measurement of pigments content

Exponentially grown cells were harvested by centrifugation at 8000 r min^{-1} for 5 min after exposure to various concentrations of As(III) for 2, 4, 6, 12, 24 and 48 h. Then chl *a* and total carotenoids content were determined by recording the absorbency of the supernatant, which was derived after centrifugation at 8000 r min^{-1} for another 5 min, with a spectrophotometer (UV2800, Unico, Shanghai, China) after the extraction of the cells in 80% acetone for 24 h at 4 °C in the dark, and then calculated according to Lichtenthaler and Wellburn (1983).

2.6. Measurement of oxygen evolution

Oxygen evolution was measured with a Clark-type oxygen electrode (Oxygraph, Hansatech Instruments Ltd., King's Lynn, Norfolk, England). During each measurement, 3 mL of sample was added into the reaction cuvette for 5 min oxygen evolution. The samples added into the reaction cuvette were kept at 25 °C by water bath with the help of the module of the measure system. A white light provided illumination at about 500 μmol photons $m^{-2} s^{-1}$ on the surface of the cuvette.

2.7. Measurement of PSII activity

PSII activity of *M. aeruginosa* was detected by chl *a* fluorescence measurement, which can provide information about photosynthetic apparatus, the absorption and distribution of the energy, and the transportation of electron in PSII of the intact algae cells (Zhou et al., 2006; Perales-Vela et al., 2007).

A double-modulation fluorometer (FL3500, PSI, Brno, Czech) was used for measuring the polyphasic fast fluorescence induction and Q_A^- reoxidation kinetics. The cells used for these chl *a* fluorescence measurements were exposed to various concentrations of As(III) in cuvettes as described above and dark-adapted for 3 min before each test.

2.7.1. Polyphasic fast fluorescence induction and JIP-test

The chl *a* fluorescence transients were recorded up to 1 s on a logarithmic timescale with the saturating flash intensity as 60% of the power. Data were acquired every 10 μs for the first 2 ms and every 1 ms thereafter. The polyphasic fluorescence induction kinetics was analyzed according to the JIP-test analysis (Strasser and Strasser, 1995). Some selected JIP-test parameters quantifying PSII behavior were derived from the fluorescence induction kinetics according to Strasser et al. (2004): φ_{Po}, maximum quantum yield for primary photochemistry (at $t=0$); Ψo, probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A (at $t=0$); φ_{Eo}, quantum yield of electron transport (at $t=0$); φ_{Do}, quantum yield of energy dissipation (at $t=0$); ABS/RC, TRo/RC, ETo/RC and DIo/RC, absorption flux, trapped energy flux, electron transport flux and dissipated energy flux per reaction center (at $t=0$) respectively; ABS/CSo, TRo/CSo, ETo/CSo and DIo/CSo, absorption flux, trapped energy flux, electron transport flux and dissipated energy flux per cross section (at $t=0$) respectively; RC/CSo, density of reaction centers; PI_{ABS}, photosynthesis performance index on absorption basis.

2.7.2. Measurement of Q_A^- reoxidation kinetics

Q_A^- reoxidation kinetics curves after a single turnover flash were measured in the 200 μs to 10 s time range. Both actinic (30 μs) flashes and measuring (2.5 μs) flashes were provided by red LEDs. The measuring flash intensity was set as 80% of the power. The Q_A^- reoxidation kinetics data were recorded with eight data points per decade. The Q_A^- reoxidation kinetics curves were fitted by the three component exponential equation to calculate the amplitudes and time constants as described by Li et al. (2010).

2.8. Statistics

Means and standard deviation (SD) were calculated for each treatment. The significance of differences between control and various treatments were determined using Student's *t*-test. Statistical significance was accepted when the *P* value was less than 0.05.

3. Results

3.1. Effects on growth

Effects of various concentrations of As(III) on the growth of *M. aeruginosa* were shown by OD_{680} in Fig. 1. The growth of the cells showed slight decrease with increasing As(III) concentration within 24 h. When treated by various concentrations of As(III) for 48 h, cells growth was inhibited. Significant differences were shown between control and treatments with 0.01, 0.1 and 10 mg L^{-1} As(III) ($P < 0.05$). OD_{680} of the cell suspension showed significant gap between the cells treated by 10 mg L^{-1} As(III) and the control ($P < 0.05$). When treated by high concentration (10 mg L^{-1}) of As(III) for more than 24 h, the density of the cells began to decrease, while the density of the cells in other treatments kept increasing. It was observed that the green cells turned into yellow after 48 h of treatment with 10 mg L^{-1} As(III), indicating the cells were severely damaged.

3.2. Effects on pigments content

Fig. 2 showed the effects of As(III) on the contents of chl *a* (Fig. 2a) and total carotenoids (Fig. 2b) of *M. aeruginosa*. Chl *a* synthesis of *M. aeruginosa* was more seriously inhibited by As(III) than carotenoids (Fig. 2). The contents of chl *a* and total

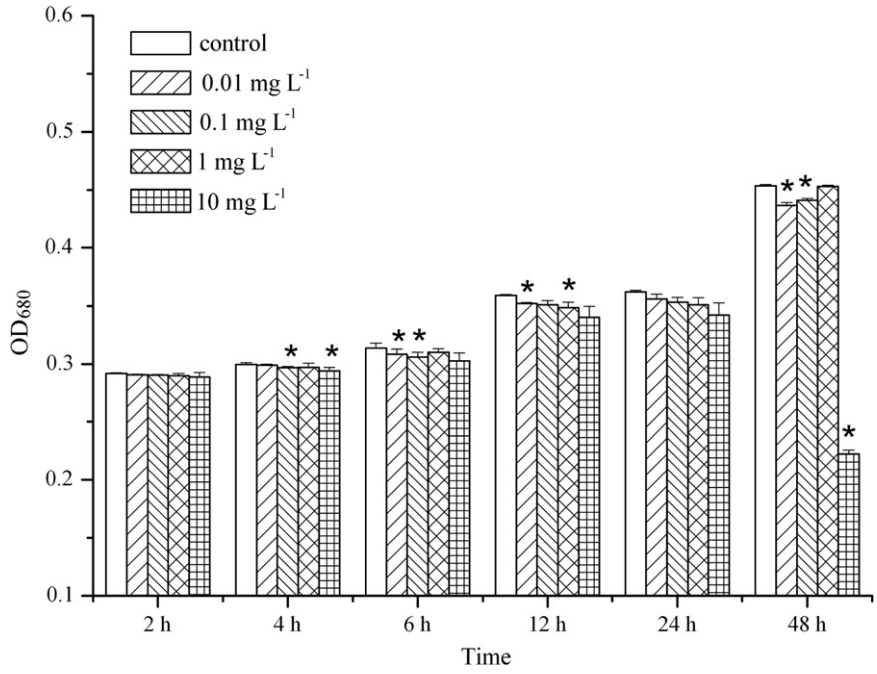

Fig. 1. Growth of *Microcystis aeruginosa* at various As(III) concentrations expressed as optical density at 680 nm (OD_{680}). Data were means ± SD ($n=3$). Significant levels between control and treatments were indicated by asterisks ($P<0.05$).

carotenoids of cells treated with 10 mg L^{-1} As(III) for 48 h were significantly different from control ($P<0.05$), 55.84% and 82.96% of that of control, respectively. The chl *a* content decreased after exposure to 10 mg L^{-1} of As(III) for 48 h, whilst the content of carotenoids kept increasing regardless of various concentrations of As(III) during the experiment.

3.3. Effects on oxygen evolution

The O_2 evolution of *M. aeruginosa* treated with 10 mg L^{-1} As(III) decreased significantly ($P<0.05$) after 2 h and nearly dropped to zero at 48 h. The O_2 evolution of the cells treated with 0.01, 0.1 and 1 mg L^{-1} As(III) showed no significant differences between the control, which was similar to the response pattern of OD_{680} (Fig. 3).

3.4. Effects on the fast fluorescence rise

Fig. 4 showed the fast fluorescence induction kinetics of the cells treated with various concentrations of As(III) for 24 h. The fluorescence intensities of the OJIP curves of cells exposure to 1 and 10 mg L^{-1} As(III) dropped clearly. The OJIP curve for the 10 mg L^{-1} As(III) treatment lost the typical OJIP shape, indicating the serious damage of high concentration of As(III) (10 mg L^{-1}) to PSII. More information was obtained from the JIP-test parameters (Table 1). No JIP-test parameters were calculated for 10 mg L^{-1} As(III) treatment because the OJIP curve leveled off for this treatment.

Most of the JIP-test parameters did not have significant differences between the control and treatments with low concentrations of As(III) (0.01, 0.1 mg L^{-1}). Treatments with 0.1 and 1 mg L^{-1} As(III) resulted in decreases of the maximum quantum yield for primary photochemistry (φ_{Po}), quantum yield of electron transport (φ_{Eo}), density of reaction centers (RC/CSo) and performance index on absorption basis (PI_{ABS}), the increase of quantum yield of energy dissipation (φ_{Do}), absorption flux per reaction center (ABS/RC), dissipated energy flux per reaction center (DIo/RC) and dissipated energy flux per cross section (DIo/CSo). The probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A (Ψo) and quantum yield of electron transport (φ_{Eo}) did not show significant difference between each treatment and the control. For 1 mg L^{-1} As(III) treatment, φ_{Eo} decreased slightly, φ_{Po} and ABS/CSo decreased significantly while DIo/RC and DIo/CSo increased significantly ($P<0.05$). RC/CSo and PI_{ABS} showed significant decrease for 0.1 and 1 mg L^{-1} As(III) treatments ($P<0.05$).

3.5. Effects on Q_A^- reoxidation kinetics

The effects of As(III) on the Q_A^- reoxidation curves of *M. aeruginosa* were shown in Fig. 5 and the Q_A^- reoxidation kinetic parameters summarized in Table 2. The curves and the calculated parameters were obtained from the cells exposure to various concentrations of As(III) for 24 h. The amplitudes of the Q_A^- reoxidation kinetics curves decreased with increasing of As(III) concentration at 24 h, and the lowest curve (treatment with 10 mg L^{-1} As(III)) showed significant gap with the control (Fig. 5).

The relative amplitude of the fast phase (A_1) ranged from 94.42% to 92.09%, which dominated the reoxidation kinetics of both untreated and treated samples. The fast phase decreased significantly at high concentration of As(III) (10 mg L^{-1}). The relative amplitudes of middle phase (A_2) and slow phase (A_3) were lower compared to the fast phase (A_1). The amplitudes of middle phase (A_2) showed significant decrease at 1 and 10 mg L^{-1} As(III) ($P<0.05$). However, the amplitude of slow phase (A_3) slightly increased at 0.01, 0.1 and 1 mg L^{-1} As(III) and increased significantly at 10 mg L^{-1} As(III) ($P<0.05$).

The time constant of fast phase, middle phase and slow phase (T_1, T_2 and T_3) did not show significant difference between control and treatment with 0.01, 0.1 and 1 mg L^{-1} As(III). However, the time constant of middle phase and slow phase (T_2 and T_3) increased significantly when treated with 10 mg L^{-1} As(III) ($P<0.05$).

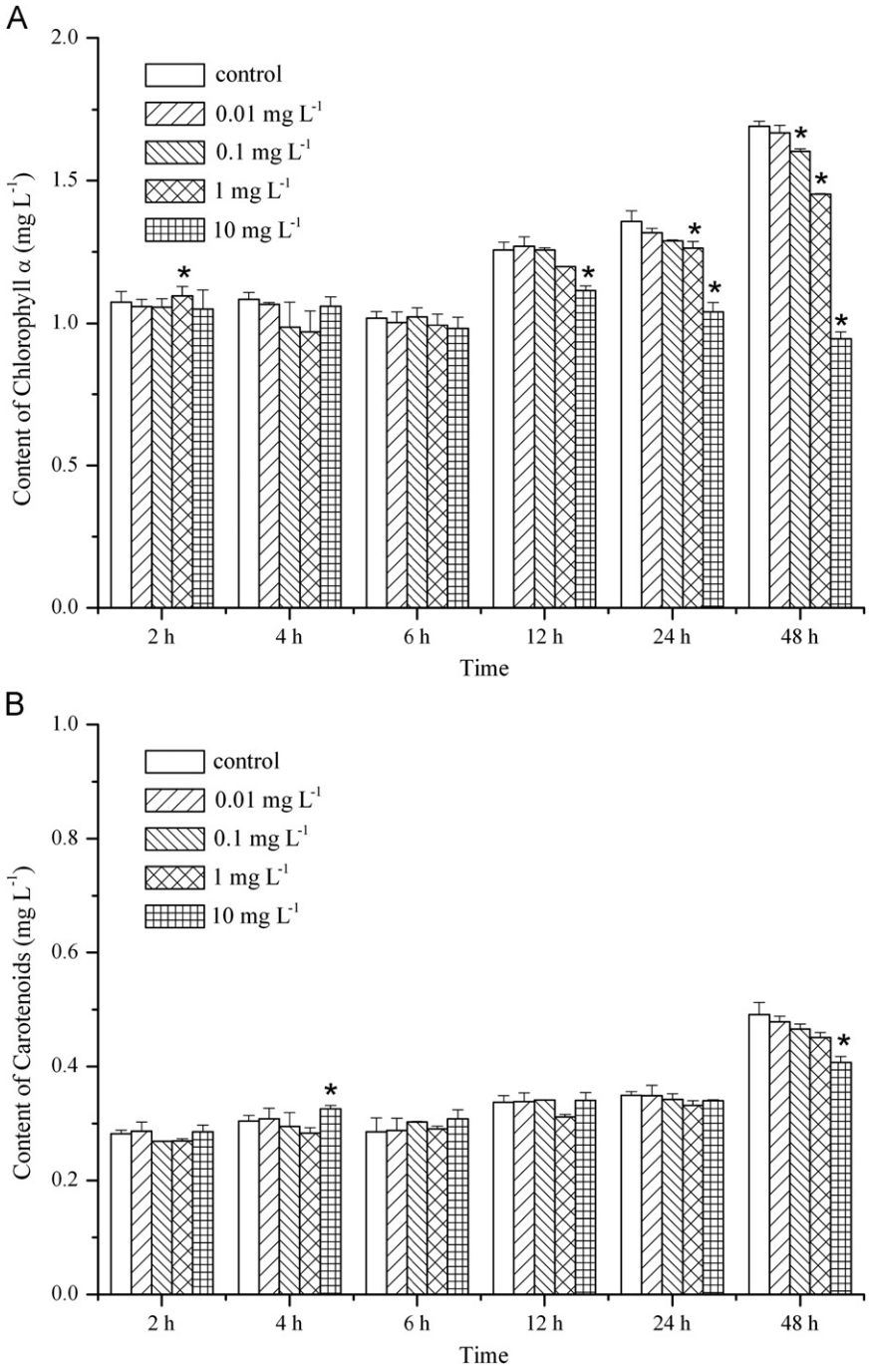

Fig. 2. Contents of chlorophyll *a* (A) and total carotenoids (B) of *Microcystis aeruginosa* at various As(III) concentrations. Data were means ± SD ($n=3$). Significant levels between control and treatments were indicated by asterisks ($P<0.05$).

4. Discussion

In the present study, effects of As(III) on growth, pigments content, oxygen evolution and photosystem II (PSII) activity of *M. aeruginosa* were investigated.

Growth of the cells treated by 10 mg L^{-1} As(III) for more than 48 h was significantly reduced ($P<0.05$). However, growth of *M. aeruginosa* showed tolerance to low concentration of As(III) (0.01, 0.1, and 1 mg L^{-1}) as cell growth kept increasing during the experiment (Fig. 1).

The contents of chl *a* and total carotenoids showed significant differences between the cells treated with 10 mg L^{-1} As(III) for 48 h and the control. The synthesis of chl *a* was more vulnerable to exposure to As(III) than total carotenoids. Growth and chl *a* content began to decrease after 12 h of exposure to high concentration of As(III) (10 mg L^{-1}). But the content of total carotenoids (Fig. 2b) of *M. aeruginosa* was less sensitive to As(III) comparing to the changes of growth and chl *a* content.

Some studies show that growth of some algae responds more sensitively to environment stress than chlorophyll synthesis, for example, the response of *Scenedesmus incrassatulus* to Cu^{2+} (Perales-Vela et al., 2007). However, in the present study, chl *a* synthesis of *M. aeruginosa* was more susceptible to As(III). When the cells were treated with 10 mg L^{-1} As(III), chl *a* content was significantly reduced at 12 h, which was earlier than the time when significant inhibition of cell growth was observed. This was

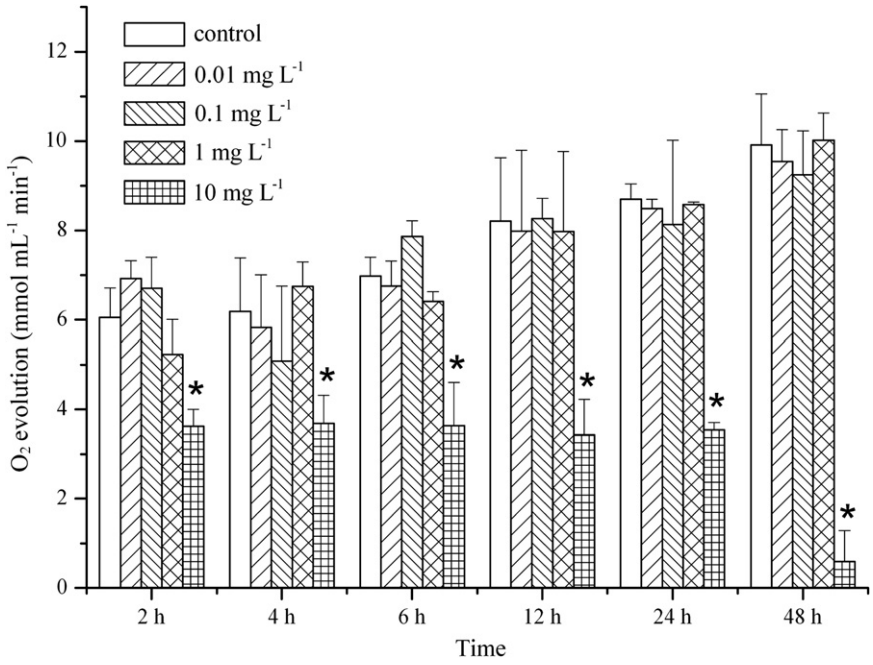

Fig. 3. O_2 evolution of *Microcystis aeruginosa* at various As(III) concentrations. Data were means ± SD ($n=3$). Significant levels between control and treatments were indicated by asterisks ($P<0.05$).

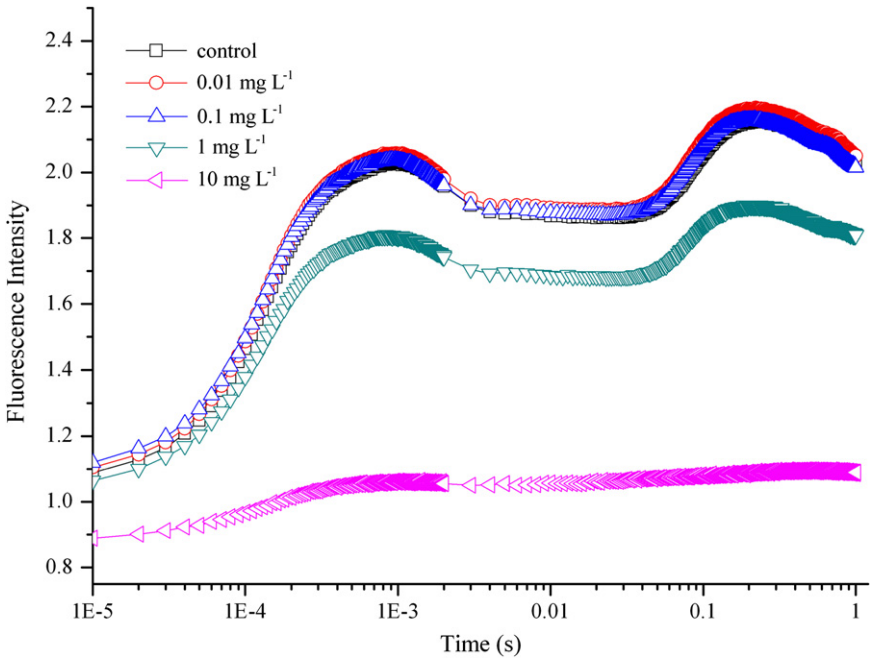

Fig. 4. Fast fluorescence rise transient of *Microcystis aeruginosa* treated with various concentrations of As(III) for 24 h. Each value represented the mean of 3 replicates.

in agreement with the sensitivity of chl *a* to some other environment stresses such as heavy metals (Küpper et al., 2002; Han et al., 2008).

The inhibition of high concentration of As(III) (10 mg L^{-1}) on growth led to the decrease of the number of the cells. It was observed that the green cells solutions began to turn into yellow after 48 h of treatment with 10 mg L^{-1} As(III), suggesting the cells were severely affected and might be destroyed. Since As(III) may disrupts adenosine triphosphate (ATP) production through several mechanisms, and therefore such interferences could lead to cell death. These effects may also be the reason for the inhibition of As(III) on the growth and the synthesis of chl *a* of *M. aeruginosa*.

Oxygen evolution and chl *a* fluorescence were found to be more sensitive to As(III) toxicity than cell growth or pigments. For example, the oxygen evolution dropped to 6% of the control after exposure to 10 mg L^{-1} As(III) for 48 h. This suggests that photosynthetic apparatus, especially the oxygen-evolving complex, was a main target of As(III) exposure.

High concentration of As(III) (10 mg L^{-1}) led to significant inhibition of growth, pigments synthesis and photosynthetic activities including oxygen evolution. The fluorescence transients test is a useful method to detect the energy fluxes and the transportation of electron in PSII (Strasser and Govindjee, 1992; Zhou et al., 2006; Perales-Vela et al., 2007). In the present study,

Table 1
The JIP-test parameters of *Microcystis aeruginosa* cells after 24 h of different treatments. The JIP-test parameters shown above were derived from the cells exposed to 0, 0.01, 0.1 and 1 mg L^{-1} As(III). Because the fluorescence transient of *M. aeruginosa* exposed to 10 mg L^{-1} As(III) lost its shape, no relative parameters were calculated from this group. Each value represented the mean of 3 replicates. Data were means ± SD ($n=3$). Significant levels between control and treatments were indicated by asterisks ($p<0.05$).

As (mg L^{-1})	φ_{Po}	Ψo	φ_{Eo}	φ_{Do}	ABS/RC	DIo/RC	ABS/CSo	ETo/CSo	DIo/CSo	RC/CSo	PI_{ABS}
control	0.423 ± 0.003	0.223 ± 0.028	0.094 ± 0.011	0.577 ± 0.003	8.757 ± 0.225	5.054 ± 0.155	1.247 ± 0.003	0.118 ± 0.014	0.720 ± 0.005	0.142 ± 0.003	0.024 ± 0.003
0.01	0.423 ± 0.011	0.237 ± 0.033	0.100 ± 0.012	0.577 ± 0.011	8.874 ± 0.364	5.119 ± 0.302	1.267 ± 0.046	0.126 ± 0.012	0.731 ± 0.030	0.143 ± 0.008	0.026 ± 0.004
0.1	0.408 ± 0.009	0.232 ± 0.042	0.095 ± 0.019	0.592 ± 0.009	9.267 ± 0.251*	5.485 ± 0.220	1.281 ± 0.028	0.121 ± 0.021	0.758 ± 0.027	0.138 ± 0.004*	0.023 ± 0.006*
1	0.367 ± 0.004*	0.227 ± 0.011	0.083 ± 0.005	0.633 ± 0.004*	10.46 ± 0.228*	6.628 ± 0.181*	1.204 ± 0.012*	0.100 ± 0.007	0.763 ± 0.003*	0.115 ± 0.004*	0.016 ± 0.002*

fluorescence transients and the JIP-test parameters changed drastically under stress of As(III), indicating that PSII was a primary target of As(III) (Baker, 1991; Gong et al., 2008).

The leveling off of the fluorescence transients curves at 1 and 10 mg L^{-1} As(III) indicates that part of PSII centers were inactivated and the reduction of PQ was inhibited (Strasser et al., 1995). The decrease in fluorescence intensities at J–I–P steps were normally explained with the inhibition of the electron transport at the donor side of PSII, which resulted in the accumulation of P_{680}^{+}, a strong fluorescence quencher (Govindjee, 1995; Zhang et al., 2010). The impacts of As(III) on O_2 evolution and chl *a* also indicate the inhibitory effects of As(III) on the donor side of PSII. This was in accordance with the quenching effect of variable fluorescence yield at J–I–P steps, which was due to the deregulation of the water-splitting system (Strasser, 1997; Zhang et al., 2010).

The JIP-test parameters further indicate that As(III) has inhibitory effects on the donor side of PSII (Table 1). This can be concluded from the decrease in the maximum quantum yield for primary photochemistry (φ_{Po}) when the cells were treated with 1 mg L^{-1} As(III). However, there was no significant change of Ψo and φ_{Eo}. This suggests the inhibition of PSII by As(III) was more due to light dependent reaction, presented by φ_{Po}, rather than dark reaction after Q_A^-, presented by Ψo. This agrees with the results of Perales-Vela et al. (2007). It implies that As(III) inhibits the primary charge separation.

The treatment with 1 mg L^{-1} As(III) increased the quantum yield of energy dissipation (φ_{Do}) and dissipated energy flux (DIo/RC and DIo/CSo; Table 1), which means that excess excitation energy has been converted to thermal dissipation in order to keep the energy balance between absorption and utilization, and minimize the potential of photooxidative damage (Müller et al., 2001; Zhang et al., 2010). More energy was dissipated to protect or maintain the cellular homeostasis, which may be used to explain the decrease of the cell growth under various environment stresses (Hagemeyer, 1999; Perales-Vela et al., 2007). Exposure to As(III) also decreased ABS/CSo and RC/CSo. The above changes caused the drastic decrease of the overall photosynthesis performance index (PI_{ABS}), which was a much sensitive parameter to environment stress such as heavy metals.

Q_A^- reoxidation kinetics test was done to detect the effects of As(III) on the function of the acceptor side of PSII of *M. aeruginosa* (Crofts and Wraight, 1983). There was significant decrease of amplitude of the fast phase and increase of amplitude of the slow phase (Table 2), implying that the contribution of $S_2(Q_AQ_B)^-$ charge recombination to Q_A^- reoxidation was increased (shown by A_3) while electron transfer from Q_A^- to Q_B (shown by A_1) was inhibited (Vass et al., 1999; Pan et al., 2008). The decrease in amplitude of the fast and middle phases further revealed that once electron transfer from Q_A^- to Q_B was inhibited by As(III), more Q_A^- was reoxidized via $S_2(Q_AQ_B)^-$ charge recombination. As(III) may result in an increased stability of the S_2QB^- and S_2QA^- recombination. These changes may largely be due to modification of the oxygen-evolving complex, especially affecting the charge transfer characteristics of the S_2 state, which agrees well with the result that oxygen-evolving complex was a main target of As(III) toxicity.

In summary, our study shows that high concentration of As(III) (10 mg L^{-1}) has serious inhibitory effects on *M. aeruginosa*. Growth, pigment synthesis, oxygen evolution and PSII activity were seriously inhibited when *M. aeruginosa* were treated with 10 mg L^{-1} As(III). Exposure to As(III) mainly inhibited the quantum yield for primary photochemistry, density of reaction centers and photosynthesis performance index, but increased the dissipated energy. Whether lower concentration of As(III) has effects on *M. aeruginosa* or other microalgae need further study as the

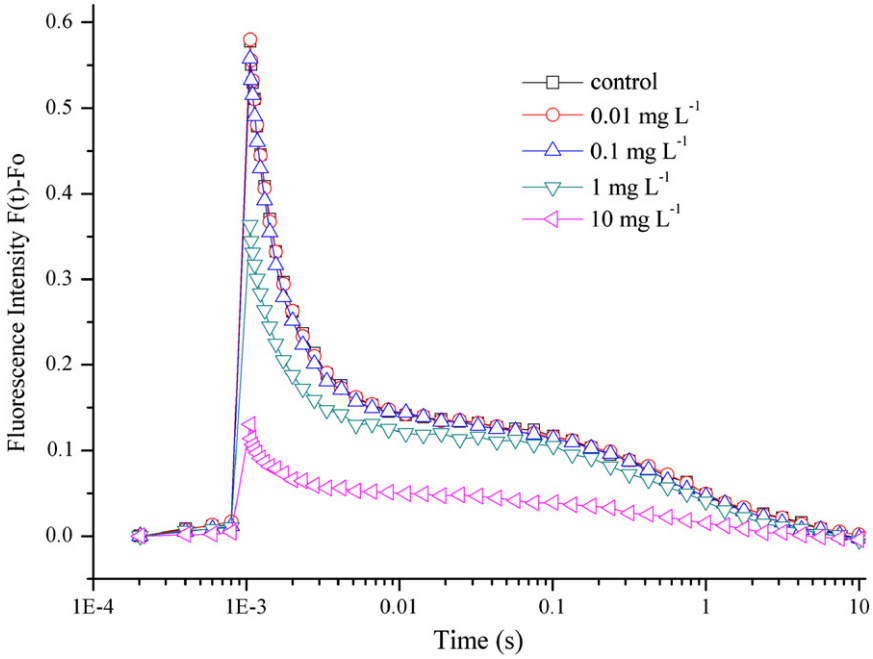

Fig. 5. Q_A^- reoxidation kinetic curves of *Microcystis aeruginosa* treated with various concentrations of As(III) for 24 h. Each value represented the mean of 3 replicates.

Table 2
Kinetic deconvolution of fluorescence decay kinetics of *M. aeruginosa* cells after 24 h of different treatments. A1–A3 were the amplitudes, and T_1–T_3 were the time constants. Data were means ± SD (n=3). Significant levels between control and treatments were indicated by asterisks ($P<0.05$).

Treatment	Fast phase		Middle phase		Slow phase	
As (mg L^{-1})	A_1 (%)	T_1 (μs)	A_2 (%)	T_2 (ms)	A_3 (%)	T_3 (s)
control	94.42 ± 0.87	355.73 ± 19.37	3.72 ± 0.60	2.37 ± 0.14	1.86 ± 0.31	0.79 ± 0.04
0.01	94.01 ± 1.94	351.82 ± 38.83	3.89 ± 0.88	2.45 ± 0.21	2.10 ± 1.06	0.81 ± 0.03
0.1	93.72 ± 1.79	375.54 ± 27.53	3.77 ± 0.81	2.64 ± 0.28	2.50 ± 1.01	0.78 ± 0.04
1	94.02 ± 1.74	278.80 ± 57.90	3.21 ± 0.58*	2.09 ± 0.24	2.44 ± 0.70	0.75 ± 0.03
10	92.09 ± 0.16*	392.59 ± 25.12	1.91 ± 0.57*	11.53 ± 0.25*	6.00 ± 0.73*	0.91 ± 0.09*

toxicity of arsenic may have long-term effects. The results in the present study confirmed that the chl *a* fluorescence test is a useful method to detect the effects of pollutants on photosynthesis of organisms. And it is a powerful means to assess the risk of pollutants in environments.

Acknowledgments

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