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Effects of Sb(V) on Growth and Chlorophyll Fluorescence of *Microcystis aeruginosa* (FACHB-905)

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Abstract In this study, effects of antimony Sb(V) on growth, pigments content, oxygen evolution, and photosystem II (PSII) activity of *Microcystis aeruginosa* were investigated. JIP-test, Q_A^- reoxidation kinetic test and S-state test were used in this study to study the energy distribution and electron transport in PSII. Treatment with Sb(V) at various concentrations ranging from 5 to 100 mg/l had long-term effects on growth, pigments content, and oxygen evolution of *M. aeruginosa*. Low concentration of Sb(V) had no significant inhibition of the biomass production and PSII activity but inhibited the pigment synthesis. Growth, pigments content, oxygen evolution, and PSII activity were seriously inhibited when treated by high concentration of Sb(V) (100 mg/l). The target sites of Sb(V) toxic effect on the PSII of *M. aeruginosa* were mainly on the donor side and the apparatus in the light-dependent reaction. The quantum yield for photochemistry, density of reaction centers and photosynthesis performance index decreased, whereas the dissipated energy increased. PSII activity of *M. aeruginosa* was promoted when exposure to 50 mg/l Sb(V) by increasing the density of active reaction centers and electron transport after Q_A^-.

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Introduction

Microcystis aeruginosa was considered as one of the most common bloom-forming cyanobacteria. It often served as an ideal material for studying the effects of contamination on photosynthesis for the similarity with the chloroplast of higher plants [36].

Antimony (Sb) is widely used in various industries as an important component of integrated circuits and optoelectronic devices [2], coloring matter, brake lining, cable covering, and flame retardant [26]. A large amount of Sb has been released into the environment during these utilizations. High levels of Sb were detected in soil and water around smelters, industrial, and mining areas. For example, Sb concentrations in wastewater, river waters and polluted well water are 1.33–21.79, 0.063–0.037, and 24.02–42.03 mg/l, respectively [8]. Because Sb has potentially toxicity at very low concentrations, Sb pollution has become a growing environmental concern.

The toxicity of Sb is relevant to its species [26]. Sb exists mainly in 3 and 5 in soil, water, biological, and mineral samples. Sb(III) compounds are about ten times more toxic than the Sb(V) specie [7]. Sb_2O_3 has been proven to be carcinogenic by sufficient evidence [7, 10, 11]. Sb was listed as a priority pollutant by the United States Environmental Protection Agency [32] and the Council of the European Communities [4].

Knowledge about toxicity of Sb to plants and microorganisms was limited. A few studies showed that exposure to Sb could exert adverse influences on seed germination, plant growth, and chlorophyll synthesis [8, 21]. Recently, Zhang et al. [35] reported that photosystem II (PSII) activities of *Synechocystis* sp. were inhibited by Sb(III). But the knowledge of effects of Sb(V) on the PSII of cyanobacteria is unclear.

In this study, PSII activity of *M. aeruginosa* was detected by the chlorophyll *a* (chl *a*) fluorescence measurement, which could provide information on the absorption and distribution of the energy, and the transportation of electron in PSII of the intact algae cells [22, 25]. This study aimed at investigating the effects of Sb(V) on growth, pigments content, oxygen evolution, and PSII activity of *M. aeruginosa*.

Materials and Methods

Culture of *Microcystis aeruginosa*

The cyanobacterium *M. aeruginosa* (FACHB-905) was purchased from Freshwater Algae Culture Collection of Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China), and cultured in BG-11 medium [27] at 25 °C under continuous fluorescent white light (30 μmol photons/m^2/s) 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 UV2800 spectrophotometer (Unico, Shanghai, China). The cells in exponential growth phase were harvested for the following experiments.

Chemicals

Sb(V) was applied in the form of analytical-grade potassium pyroantimonate (Aladdin Reagent Database Inc., Shanghai, China). The Sb(V) solutions were prepared by dissolving potassium pyroantimonate in sterilized BG-11 medium to get the desired concentrations. The Sb(V) solutions were stored at 4 °C until use.

Treatments

Exponentially grown cells used for measurements of growth, pigments content, and oxygen evolution were cultured in flasks with BG-11 medium or prepared Sb(V) solutions to make the final Sb concentrations to 0, 5, 50, and 100 mg/l, respectively. The cells used for the chlorophyll fluorescence measurement were cultured in 10 × 10 mm^2 glass cuvettes with same volume. BG-11 medium or Sb(V) solutions at various concentrations were added into the cuvettes in same volume to make the final Sb(V) concentrations to 0, 5, 50, and 100 mg/l, respectively. The sample without Sb(V) was used as the control.

All the samples were cultured at 25 °C under continuous fluorescent white light (30 μmol photons/m^2/s) with a 12:12-h light–dark cycle.

Measurement of Growth

After exposure to Sb(V) for 2, 4, 6, 12, 24, 48, and 72 h, cell growth was determined by measuring the optical density at 680 nm (OD_{680}) with a UV2800 spectrophotometer (Unico, Shanghai, China).

Measurement of Pigments Content

Exponentially growing cells were harvested by centrifugation at 8,000 r/min for 5 min after treated with various Sb concentrations for 2, 4, 6, 12, 24, 48, and 72 h. After the extraction of the cells in 80 % acetone for 24 h in the dark at 4 °C, chl *a* and total carotenoid contents were determined by testing the absorbency of the supernatant (after centrifugation at 8,000 r/min for another 5 min) with a UV2800 spectrophotometer (Unico, Shanghai, China) and then calculated in the formulae according to Lichtenthaler and Wellburn [15].

Measurement of Oxygen Evolution

Oxygen evolution was measured at 25 °C with a Clark-type oxygen electrode (Hansatech Instruments Ltd., King's Lynn, Norfolk, England). During each measurement, 3 ml of cells sample was added into the reaction cuvette for 5 min oxygen evolution. A white light provided illumination on the surface of the cuvette at about 500 μmol photons/m^2/s.

Measurement of Chlorophyll Fluorescence

A double-modulation fluorometer FL3500 (Photon Systems Instruments spol. s r.o., Brno, Czech) was used for measuring the polyphasic fast fluorescence induction, Q_A^- reoxidation kinetics, and the proportion of active and inactive reaction centers [20, 22, 23, 35]. The cells used for these chlorophyll fluorescence measurements were cultured and exposure to various concentrations of Sb(V) in cuvettes as described above. All the samples were dark-adapted for 3 min before each test.

Polyphasic Fast Fluorescence Induction and JIP-Test

The chlorophyll 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 [29]. The following data were directly obtained from the fast rise kinetic curves: F_O, the initial fluorescence, was measured at 50 μs when all reaction centers (RCs) were open; F_J and F_I are the

fluorescence intensity at J step (at 2 ms) and I step (at 30 ms); F_m, the maximal fluorescence, was the peak fluorescence when all RCs were closed after illumination. Selected JIP-test parameters quantifying PSII behavior were calculated from the above original data according to Strasser et al. [30]: φ_{Po}, maximum quantum yield for primary photochemistry (at $t = 0$); Ψ_o, 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, TR_o/RC, ET_o/RC, and DI_o/RC, absorption flux, trapped energy flux, electron transport flux, and dissipated energy flux per reaction center (at $t = 0$); ABS/CS_o, TR_o/CS_o, ET_o/CS_o, and DI_o/CS_o, absorption flux, trapped energy flux, electron transport flux, and dissipated energy flux per cross section (at $t = 0$); RC/CS_o, density of reaction centers; PI_{ABS}, performance index on absorption basis.

Measurement of Q_A^- Reoxidation Kinetics

The Q_A^- reoxidation kinetics curves after a single turnover flash were measured in the 200 μs–10 s time range. Both actinic flashes (30 μs) and measuring flashes (2.5 μs) 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 described by Li et al. [14].

S-State Test of Inactive PSII Centers ($PSII_X$)

In active PSII centers ($PSII_A$) the oxidation of Q_A^- is rapid, whereas in inactive centers ($PSII_X$) the oxidation of Q_A^- is much slower [3]. The proportion of inactive RCs in the whole cells can be measured by the S-state test. The measuring flash intensity was set as 60 % of the power. After each actinic flash, the fluorescence decay is measured. The population of $PSII_X$ centers was estimated by the difference between the fluorescence level at 110 ms after the fourth flash and F_O according to the formula described by Pan et al. [20].

Statistics

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

Results

Effects of Antimony on Growth

Figure 1 shows the effects of exposure to various concentrations of Sb(V) on the growth of *M. aeruginosa*. Compared to the control, growth of *M. aeruginosa* were not significantly affected by Sb(V) during the first 24 h. During 24–72 h, cell growth were affected little after treatment with 5 and 50 mg/l Sb(V) but were markedly inhibited by 100 mg/l Sb(V) ($P < 0.05$).

Effects of Sb(V) on Pigments Content

Figure 2 shows the effects of Sb(V) on the contents of chl *a* (Fig. 2a) and total carotenoids (Fig. 2b) of *M. aeruginosa*. The contents of chl *a* (Fig. 2a) and total carotenoids (Fig. 2b) decreased with the increase of Sb(V) concentration. Sb(V) inhibited chl *a* more seriously than total carotenoids. When treated by 100 mg/l Sb(V) for 72 h, the content of chl *a* showed no more increase than 48 h. Exposure to 100 mg/l of Sb(V) for 72 h, the contents of chl *a* and total carotenoids were just 47.37 and 74.6 % compared to control, respectively.

Effects of Sb(V) on Oxygen Evolution

Oxygen evolution of *M. aeruginosa* were not markedly affected by various concentrations of Sb(V) during first 12 h (Fig. 3). O_2 evolution of cells treated by high concentration of Sb(V) (100 mg/l) decreased gradually after 12 h. Exposure to 50 and 100 mg/l Sb(V) for 48 and 72 h significantly reduced the O_2 evolution ($P < 0.05$).

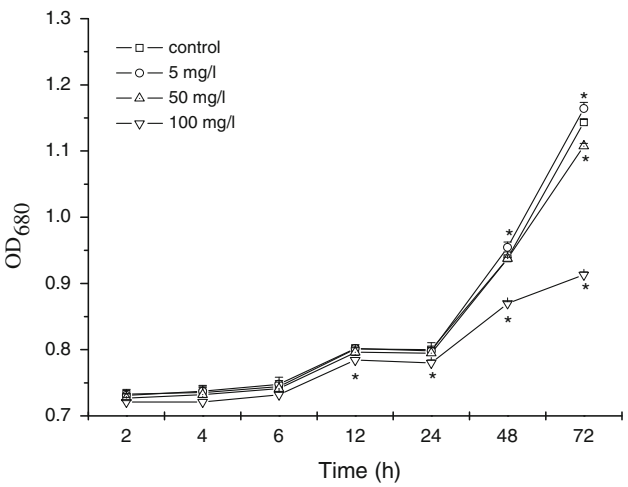

Fig. 1 Growth of *Microcystis aeruginosa* at various Sb(V) concentrations expressed as optical density at 680 nm (OD_{680}). Data were mean ± SD ($n = 3$). Significant levels between control and treatments were indicated by *asterisks* ($P < 0.05$)

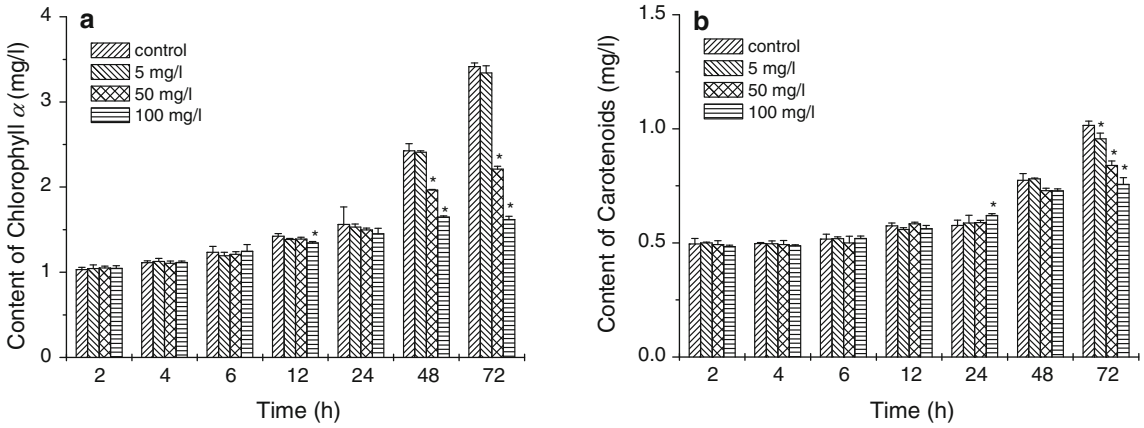

Fig. 2 Contents of chlorophyll *a* (**a**) and total carotenoids (**b**) of *Microcystis aeruginosa* at various Sb(V) concentrations. Data were mean ± SD ($n = 3$). Significant levels between control and treatments were indicated by *asterisks* ($P < 0.05$)

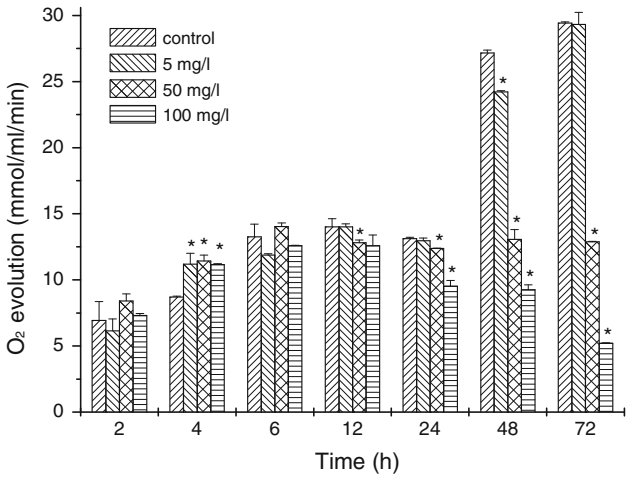

Fig. 3 O_2 evolution of *Microcystis aeruginosa* at various Sb(V) concentrations. Data were mean ± SD ($n = 3$). Significant levels between control and treatments were indicated by *asterisks* ($P < 0.05$)

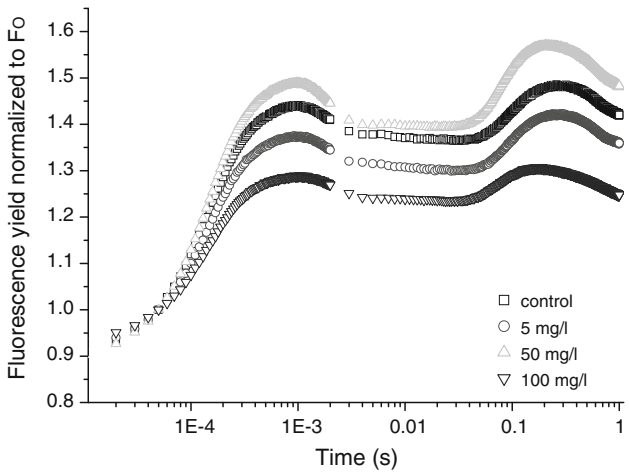

Fig. 4 Fast fluorescence rise transient of *Microcystis aeruginosa* treated with various concentrations of Sb(V) for 48 h. Each value represented the mean of three replicates

Treatments with 50 and 100 mg/l Sb(V) for 72 h led to 56.25 and 82.28 % inhibition of O_2 evolution, respectively.

Effects of Sb(V) on the Fast Fluorescence Rise

Figure 4 showed the polyphasic fast fluorescence induction test of the cells treated by various concentrations of Sb(V) for 48 h. The fluorescence intensities of the OJIP curve decreased when treated by 5 and 100 mg/l Sb(V), but increased at the concentration of 50 mg/l. F_J, F_I, and F_m decreased with increasing Sb(V) concentration except 50 mg/l. The fluorescence intensity of the OJIP curve decreased drastically and the shape of the curve became flattened when treated by 100 mg/l Sb(V).

More information could be obtained from the JIP-test parameters shown in Table 1. The JIP-test parameters did not have significant difference between control and the treatment with 5 mg/l Sb(V). However, most of the parameters were changed significantly when treated by 50 and 100 mg/l Sb(V) ($P < 0.05$). Treatment with 50 mg/l Sb(V) resulted in an increase of the maximum quantum yield for primary photochemistry (φ_{Po}), and significant increase of probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A (Ψ_o), quantum yield of electron transport (φ_{Eo}), absorption flux and electron transport flux per reaction center (ABS/CS_o and ET_o/CS_o), and density of reaction centers (RC/CS_o). While the treatment with 100 mg/l Sb(V) resulted in significant decrease of maximum quantum yield for primary photochemistry (φ_{Po}), quantum yield of electron transport (φ_{Eo}), absorption flux and electron transport flux per reaction center (ABS/CS_o and ET_o/CS_o) and significant increase of quantum yield of energy dissipation (φ_{Do}), absorption flux and dissipated energy flux per reaction center (ABS/RC and DI_o/RC) and dissipated energy flux

Table 1 The JIP-test parameters of *Microcystis aeruginosa* cells after 48 h of different treatments

Sb (mg/l)	φ_{Po}	Ψ_o	φ_{Eo}	φ_{Do}	ABS/RC	DI_o/RC	ABS/CS_o	ET_o/CS_o	DI_o/CS_o	RC/CS_o	PI_{ABS}
Control	0.327 ± 0.027	0.155 ± 0.027	0.051 ± 0.008	0.673 ± 0.027	10.892 ± 0.968	7.347 ± 0.946	1.473 ± 0.028	0.075 ± 0.019	0.991 ± 0.049	0.136 ± 0.011	0.009 ± 0.001
5	0.297 ± 0.036	0.185 ± 0.033	0.056 ± 0.016	0.703 ± 0.036	12.206 ± 1.676	8.619 ± 1.640	1.446 ± 0.032	0.080 ± 0.022	1.107 ± 0.068	0.120 ± 0.014	0.008 ± 0.004
50	0.364 ± 0.012	0.222 ± 0.005*	0.081 ± 0.004*	0.636 ± 0.012	10.043 ± 0.356	6.390 ± 0.343	1.573 ± 0.036*	0.127 ± 0.005*	1.001 ± 0.038	0.157 ± 0.004*	0.016 ± 0.002*
100	0.235 ± 0.034*	0.121 ± 0.050	0.028 ± 0.009*	0.765 ± 0.034*	15.197 ± 2.092*	11.669 ± 2.418*	1.829 ± 0.037*	0.054 ± 0.027*	1.400 ± 0.090*	0.122 ± 0.013	0.003 ± 0.002*

* Data were mean ± SD ($n = 3$) and significant levels between control and treatments were indicated by asterisks ($P < 0.05$)

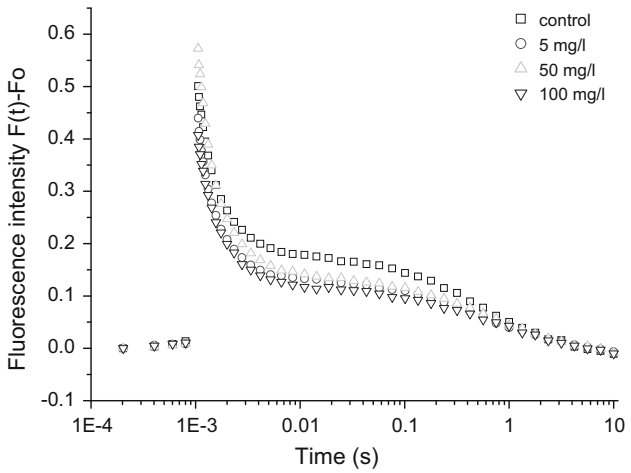

Fig. 5 Q_A^- reoxidation kinetic curves of *Microcystis aeruginosa* treated with various concentrations of Sb(V) for 48 h. Each value represented the mean of three replicates

per cross section (DI_o/CS_o). The performance index on absorption basis (PI_{ABS}) increased by 77.78 % compared to control when the cells were treated with 50 mg/l Sb(V), but decreased by 66.67 % when treated with 100 mg/l Sb(V).

Effects of Sb(V) on Q_A^- Reoxidation Kinetics

Figure 5 showed the effects of various concentration of Sb(V) on the Q_A^- reoxidation of *M. aeruginosa*. The amplitude of the variable fluorescence increased after exposure to 50 mg/l Sb(V) for 48 h, but decreased when the cells were treated with 5 and 100 mg/l Sb(V). The Q_A^- reoxidation kinetic parameters were summarized in Table 2. The relative amplitude of the fast phase (A_1) ranged from 92.7 to 94.69 %, which dominated the reoxidation kinetics of both the untreated and Sb-treated samples. The fast phase decreased slightly at 100 mg/l Sb(V). The relative amplitudes of middle phase (A_2) and slow phase (A_3) were lower compared to the fast phase (A_1). The amplitude of middle phase (A_2) changed slightly after exposure to various concentration of Sb(V). However, the amplitude of slow phase (A_3) decreased a little at 50 mg/l Sb(V) and increased at 100 mg/l Sb(V). There were significant differences in the time constant of fast phase and middle phase (T_1 and T_2) between control and 100 mg/l Sb(V) treatment ($P < 0.05$). The time constant of slow phase were significant increased due to 50 and 100 mg/l Sb(V) treatment ($P < 0.05$).

Effects of Sb(V) on Inactive PSII Centers ($PSII_X$)

The fluorescence decay curves induced by a series of single-turnover flashes of *Microcystis aeruginosa* treated

Table 2 Kinetic deconvolution of fluorescence decay kinetics of *M. aeruginosa* cells after 48 h of different treatments

Treatment Sb (mg/l)	Fast phase		Middle phase		Slow phase	
	A_1 (%)[a]	T_1 (μs)[b]	A_2 (%)[a]	T_2 (ms)[b]	A_3 (%)[a]	T_3 (s)[b]
Control	93.74 ± 0.82	330.59 ± 22.88	3.44 ± 0.20	2.23 ± 0.23	2.82 ± 0.62	6.67 ± 0.33
5	94.20 ± 0.64	317.35 ± 19.76	3.43 ± 0.33	2.05 ± 0.22	2.37 ± 0.40	7.05 ± 0.14
50	94.69 ± 0.83	335.53 ± 25.80	3.08 ± 0.33	2.35 ± 0.20	2.23 ± 0.51	7.57 ± 0.19*
100	92.70 ± 0.97	388.73 ± 29.62*	3.46 ± 0.14	3.16 ± 0.22*	3.84 ± 0.83	7.75 ± 0.71*

A_1–A_3 the amplitudes, T_1–T_3 the time constants

* Data were mean ± SD ($n = 3$) and significant levels between control and treatments were indicated by asterisks ($P < 0.05$)

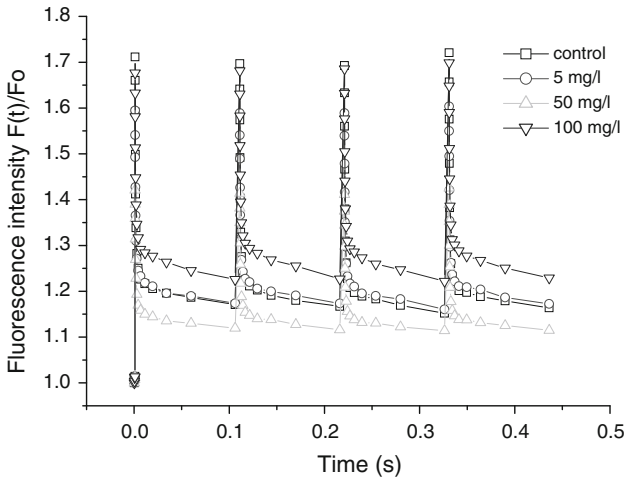

Fig. 6 Fluorescence decay *curves* induced by a series of single-turnover flashes of *Microcystis aeruginosa* treated with various concentrations of Sb(V) for 48 h. Each value represented the mean of three replicates

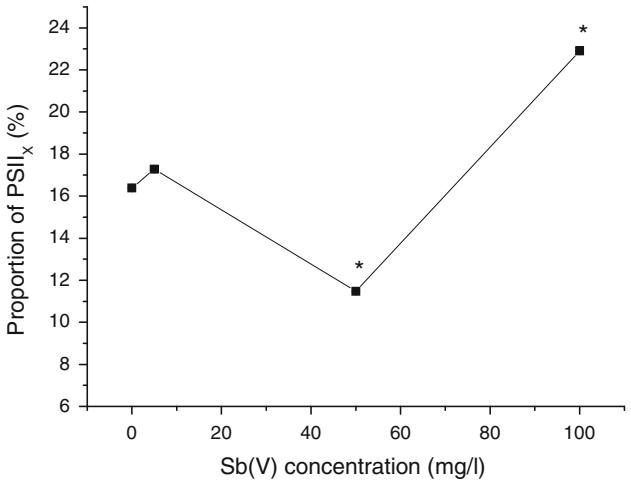

Fig. 7 The proportion of inactive PSII centers ($PSII_X$) of *Microcystis aeruginosa* treated with various concentrations of Sb(V) for 48 h. Data were mean ± SD ($n = 3$). Significant levels between control and treatments were indicated by *asterisks* ($P < 0.05$)

with various concentrations of Sb(V) for 48 h were shown in Fig. 6. The curves were similar between control and 5 mg/l Sb(V) treatment whereas there were differences between control and treatment with 50 and 100 mg/l Sb(V). Number of inactive PSII centers ($PSII_X$) were calculated and shown in Fig. 7. The number of $PSII_X$ changed slightly at 5 mg/l Sb(V). But the proportion of $PSII_X$ centers decreased from 16.39 % for the control to 11.48 % for 50 mg/l Sb(V)-treated sample and increased to 22.91 % for 100 mg/l Sb(V)-treated sample.

Discussion

This study shows that exposure to higher concentrations of Sb(V) for longer time has adverse effects on growth, pigments content, oxygen evolution, and PSII activity of *M. aeruginosa*. Growth, pigments content, and oxygen evolution were not changed significantly by Sb(V) until treated for more than 24 h. After treatments for more than 48 h, growth, pigments content, and oxygen evolution were markedly reduced by 50 and 100 mg/l Sb(V). However, low concentration of Sb(V) (5 mg/l) stimulated *M. aeruginosa* growth as cell number increased continuously and was significant higher than control during 72 h of exposure to 5 mg/l Sb(V) (Fig. 1).

The content of chl *a* decreased more than the total carotenoids when the cells were treated with 50 and 100 mg/l Sb(V). For example, exposure to 100 mg/l Sb(V) for 72 h caused the contents of Chl *a* and total carotenoids decreased to 47.37 and 74.6 % of the control, respectively (Fig. 2b). The content of chl *a* showed significant decrease when exposed to 50 and 100 mg/l Sb(V) for 48 h, whereas the content of total carotenoids did not show significant difference between different treatments and control. This suggests that Chl *a* is more sensitive to Sb(V) than carotenoids, which was consistent with some studies which reported that chl *a* showed greater susceptibility to heavy metal than carotenoids [6, 13]. These results showed the tolerance of carotenoids to Sb(V) toxicity, witch led to the increase of the ratio of total

carotenoids/chl *a*. The tolerance of carotenoids and the increase of the ratio of carotenoids/chl *a* were suggested as protection mechanism by some previous studies, as carotenoids were effective quenchers in dissipating excess excitation energy and antioxidant pigments [12, 16]. Pan et al. [21] reported that Sb could promote the formation of reactive oxygen species (ROS), and the activities of peroxidase (POD), superoxide dismutases (SOD) were inhibited at high concentrations of soil Sb. The tolerance of carotenoids and the increase of the ratio of carotenoids/Chl *a* in this study imply a protection mechanism had been built by antioxidant pigments in protecting against ROS within 48 h. Although carotenoids are more tolerable than Chl *a*, the content of total carotenoids also decreased significantly under high concentrations of Sb(V) (50 and 100 mg/l) after long time of exposure (72 h). The protection mechanism was damaged or reduced by long-time exposure to high concentration of heavy metal Sb(V).

At low concentration of Sb(V) (5 mg/l) the pigments decreased while the cell growth was stimulated (Figs. 1, 2). This means that low concentration of Sb(V) (5 mg/l) does not decrease the biomass production but can inhibit the pigment synthesis, which was in agreement with the effects of Cd on the growth and chlorophyll content [36].

O_2 evolution was seriously inhibited by Sb(V) after long-time exposure. O_2 evolution of *M. aeruginosa* decreased gradually after exposure to 100 mg/l Sb(V) for more than 12 h (Fig. 3). This indicates that O_2 evolution complex was one of the targets of Sb(V), which was similar to the toxic effect of Sb(III) [35].

Many studies showed that PSII was the primary target of environment stresses, such as heavy metal, high temperature and ultraviolet [1, 9, 33], and both the donor and acceptor side of PSII can be the site of heavy metal [19, 34]. In order to detect the effects of Sb(V) on the PSII activity of *M. aeruginosa*, a variety of chlorophyll fluorescence tests were applied. Treatments with various concentrations of Sb(V) for 48 h had significant effects on the polyphasic fast fluorescence induction curves. The fluorescence intensities increased due to treatment with 50 mg/l Sb(V), but decreased at 5 and 100 mg/l Sb(V) (Fig. 4). The similar trend was observed from the amplitudes of the variable fluorescence of Q_A^- reoxidation kinetics curves (Fig. 5). F_m decreased and the shape of curve flattened when treated by high concentration of Sb(V) (100 mg/l), indicating that part of PSII centers were inactivated and the reduction of PQ (nonphotochemical phase) was inhibited [29]. The decrease in fluorescence intensities at phase J, I, and P were normally explained as the inhibition of the electron transport at the donor side of PSII and subsequent accumulation of P_{680}^+, a strong fluorescence quencher [5, 35]. The inhibitory impacts of Sb(V) on O_2 evolution and content of chl *a* indicate the noticeable damages to water-splitting site and light harvesting chl *a*, which was in agreement with previous research that the quenching effect of variable fluorescence yield at J, I, and P was due to the deregulation of the water-splitting system [28, 35].

The sharp decrease in the maximum quantum yield for primary photochemistry (φ_{Po}) under stress of 100 mg/l Sb(V) indicates that Sb(V) had inhibitory effects on the donor side of PSII (Table 1). These led to the decrease in the quantum yield of electron transport (φ_{Eo}) but no significant change in the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A (Ψ_o). This was in accordance with the results of Perales-Vela et al. [25]. It suggests that high concentration of Sb(V) (100 mg/l) inhibited the primary charge separation. Treatment with 100 mg/l Sb(V) increased the quantum yield of energy dissipation (φ_{Do}) and dissipated energy flux (DI_o/RC and DI_o/CS_o). This caused excessive excitation energy to be de-excited as thermal dissipation for minimizing the potential of photooxidative damage [18, 35]. When treated by 100 mg/l Sb(V), there were also decreases in electron transport flux per cross section (ET_o/CS_o) and density of reaction centers (RC/CS_o), which agreed with the increase of inactive PSII centers ($PSII_X$) (Fig. 7). The above changes finally led to the drastic decrease of the overall photosynthesis performance index (PI_{ABS}).

Q_A^- reoxidation kinetics test was done to detect the effects of Sb(V) on the function of the acceptor side of PSII of *M. aeruginosa* (Fig. 5). The parameters (A_1–A_3) generally did not show significant change after exposure to various concentrations of Sb(V), indicating the inhibition on the acceptor side was less than the inhibition on the donor side of PSII, (Table 2). The effects of 100 mg/l Sb(V) mainly on the donor side of PSII and the light dependent reaction but less on the electron transport after Q_A^-. However, there were a slight decrease of amplitude of the fast phase and an 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 slightly increased (shown by A_3) while electron transfer from Q_A^- to Q_B (shown by A_1) was slightly inhibited [20, 33]. These results were in agreement with the increased proportion of $PSII_X$ (Fig. 7) and inhibition of the electron transport from Q_A^- to Q_B [20, 35].

Low concentration of Sb(V) (5 mg/l) had little impact on PSII activity while 50 mg/l Sb(V)-enhanced photosynthetic ability of PSII. Under stress of 50 mg/l Sb(V) or more within 48 h, although chl *a* content and oxygen evolution were inhibited, the overall photosynthetic performance of *M. aeruginosa* was promoted. This can be the result of the increase of RC/CS_o and the decrease of the proportion of $PSII_X$ (Fig. 7). The increase of ABS/CS_o, ET_o/CS_o, and Ψ_o agree with the increase of the amplitude of fast phase obtained from the Q_A^- reoxidation kinetics test

(Table 2). These changes led to the increase of quantum yield of electron transport (φ_{Eo}) and performance index (PI_{ABS}). It suggests that the photosynthetic apparatus of *M. aeruginosa* was tolerable to 50 mg/l Sb(V) within 48 h. The improved photosynthetic ability was suggested to provide more energy to tolerate the stress of Sb(V). More energy may be used to protect the photosynthetic apparatus of *M. aeruginosa*, such as energy for production of metal-binding polypeptides [24], active transport [17] and the synthesis of enzymes and compounds related with oxidative stress [31]. But long-term effects of 50 mg/l Sb(V) may show inhibition on photosynthetic ability, as the antioxidant pigments carotenoids decreased significantly after treatment for more than 72 h, suggesting the tolerance and protection mechanism of *M. aeruginosa* were destroyed after long-time exposure to heavy metal.

In summary, our study shows that treatments with various concentrations of Sb(V) have long-term effects on growth, pigments content, and oxygen evolution of *M. aeruginosa*. Low concentration of Sb(V) (5 mg/l) causes no decrease in biomass production and little changes of PSII activity but inhibits the pigment synthesis. Growth, pigments content, oxygen evolution, and PSII activity were seriously inhibited when *M. aeruginosa* were treated with high concentration of Sb(V) (100 mg/l). The toxicity target sites of Sb(V) in the PSII of *M. aeruginosa* were mainly on the donor side and the apparatus in the light-dependent reaction, such as water-splitting system. The quantum yield for photochemistry, density of reaction centers, and photosynthesis performance index decreased, whereas the dissipated energy increased. PSII activity of *M. aeruginosa* was promoted when exposure to 50 mg/l Sb(V) by increasing the density of active reaction centers and electron transport after Q_A^-. The JIP-test, Q_A^- reoxidation kinetic test and S-state test used in this study were useful for investigating the effects of pollutants on photosynthetic organisms, such as microalgae and cyanobacteria.

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