Ultrasonic selectivity on depressing photosynthesis of cyanobacteria and green algae probed by chlorophyll-a fluorescence transient
Zhipeng Duan, Xiao Tan and Niegui Li

ABSTRACT
Ultrasound can inhibit cyanobacterial growth through rupturing cells, but this pathway frequently has the risk to release intercellular toxin (e.g., microcystin). Depressing photosynthesis without cell disruption may provide a new strategy to control cyanobacterial blooms using ultrasound, especially Microcystis blooms. In this work, Microcystis aeruginosa (toxic cyanobacteria) and Chlorella pyrenoidosa (typical green algae) were chosen as model microalgae to verify this hypothesis. Results showed that ultrasound has the ability to inhibit cyanobacterial photosynthesis significantly and selectively. Specifically, sonication damaged QA, a tightly bound one-electron acceptor, and blocked electron flow at QB, a two-electron acceptor, in the photosystem II (PSII) of M. aeruginosa when it was exposed for 60 s (35 kHz, 0.043 W/cm²). Moreover, 44.8% of the reaction centers (RCs) in the PSII of M. aeruginosa were transferred into inactive ones (RCsis), and the cell concentration decreased by 32.5% after sonication for 300 s. By contrast, only 7.9% of RCsis occurred in C. pyrenoidosa, and cell concentration and chlorophyll-a content reduced by 18.7% and 9.3%, respectively. Differences in both species (i.e., cell structures) might be responsible for the varying levels to sonication. This research suggests that cyanobacteria, especially Microcystis, could be controlled by ultrasound via damaging their PSIIs.

Key words | energy fluxes of PSII, JIP-test, Microcystis blooms, ultrasound

INTRODUCTION
Compared with nutrient diversion, ecological engineering methods and addition of algaecides, ultrasonic irradiation has been reported as an effective method to cope with cyanobacterial blooms (Purcell et al. 2013; Dehghani 2016). Previous examinations revealed that the potential mechanisms in controlling cyanobacteria using ultrasound are the following: (a) rupturing cells through high-power acoustic cavitation (Wang & Yuan 2016) and (b) hazarding photosynthetic activity (PA) or cell viability, i.e. enzyme activity (Zhang et al. 2006; Wu et al. 2012), eventually resulting in programmed algal cell death (Wu et al. 2011). Indeed, the latter is appropriate in scale-up applications of ultrasound to address such blooms because of the lower risk of releasing harmful intracellular components (i.e., microcystin).

Previous reports have suggested that ultrasound might degrade the cyanobacterial photosynthetic antenna directly and then weaken their PAs. Based on an enclosure experiment, Ahn et al. (2003) concluded that ultrasonication depressed photosynthesis of algae, indicated by the declining dissolved oxygen concentration and pH during the ultrasonic treatment. The effect of sonication on PA of Microcystis sp. has been analyzed by Fan et al. (2014), who suggested that ultrasonic treatment caused instant damage to PA. A more detailed study showed that irradiation for 5 min (25 kHz, 0.32 W/cm²) decreased the chlorophyll-a content, the phycocyanins (PC) absorbance and the oxygen evolution rate in M. aeruginosa by 21.3%, 44.8% and 40.5%, respectively (Zhang et al. 2006). The authors thought that the ‘empty-rod’ structure of PC could result in the higher sensitivity of PC than chlorophyll-a to sonication. This might be one of the reasons why the ultrasonic irradiation selectively inhibited the growth of cyanobacteria in a field investigation implemented by Ahn et al. (2007). Similar research conducted by Tang et al. (2005) found...
that the maximum net photosynthesis rate of *Spirulina (Arthrospira) platensis* (indicated by monitoring oxygen evolution) dropped by 49% compared with the control after exposure for 5 min (1.7 MHz, 0.6 W/cm²). However, little information is known about the effects of ultrasound on the structures, compositions and functions of cyanobacterial photosynthetic apparatus, especially the energy fluxes of photosystem II (PSII), which are the basic process of photosynthesis.

Additionally, it is a fact that, various eukaryotic algae, such as green algae, live in eutrophic water bodies with cyanobacteria. Both phytoplankton are the basic rocks of primary productivity in aquatic ecological systems. Therefore, one should be careful about promoting ultrasound for controlling cyanobacteria blooms in field aquatic systems before a good understanding of its effects on eukaryotic algae. Although PAs of cyanobacteria can be damaged by sonication, the responses of PSII in eukaryotic algae to sonication are not well studied. Normally, dramatic differences exist between eukaryotic algae and cyanobacteria in terms of the architectures of cell and the structures and/or components of PSII. For instance, gas vacuoles were discovered routinely in cyanobacteria but rarely found in eukaryotic algae (Lee 2011). In addition, the light-harvesting complex of the PSII (LHCII) in eukaryotic algae typically consists of some 200–300 chlorophyll molecules (mainly chlorophyll-a coupled with approximately 30% of chlorophyll-b) (Liu et al. 2004). Due to the shortage of chlorophyll-b and the light-harvesting chlorophyll-proteins in cyanobacterial cells, by contrast, they rely instead on the phycobiliproteins, assembled in bodies known as phycobilisomes, which are mainly PC (Reynolds 2006). Therefore, influences of ultrasound on PSIIIs of eukaryotic algae would differ from cyanobacteria. In this study, alterations in the energy fluxes of PSII in cyanobacteria (*M. aeruginosa*) and eukaryotic algae (*C. pyrenoidosa* as the subject) induced by ultrasound were evaluated in order to verify whether ultrasound is useful to inhibit cyanobacteria selectively through depressing their PSII.

**MATERIALS AND METHODS**

Microalgae culture

*Microcystis aeruginosa PCC 7806* and *Chlorella pyrenoidosa FACHB-5* were purchased from Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences, and then cultured in 250 mL Erlenmeyer flasks with BG11 medium in a sterile illumination incubator at 25 ± 0.5 °C under 30 μmol (photons) m⁻² s⁻¹ light intensity with a light: dark cycle of 12 h:12 h.

**Ultrasonic equipment and exposure**

An ultrasonic transducer (35 kHz; bath-type; DAS Corp., Hangzhou China) was employed in this work. A cooling system was used to maintain the temperature of algal suspension around 25 °C (Figure 1). The acoustic power of the ultrasonic system was accurately measured using the calorimetry method (Wu et al. 2012). Briefly, 200 mL of water was sonicated for 15 min without operating the cooling system, and the temperature was recorded every 5 min. This measurement was carried out in triplicate and then the average was calculated. The acoustic intensity was computed as the following:

\[
AI = \left( \frac{dT}{dt} C_p M \right) / V
\]

where AI is the acoustic intensity (W/cm³); V is the volume of treated liquid (cm³); T is the temperature (°C) and t is the processing time (s). \(C_p\) indicates the heat capacity of water at 25 °C (J Kg⁻¹ °C⁻¹) and M is the mass of water (kg). After measurement and calculation, it was found that AI was 0.043 W/cm³.

Two hundred milliliters of algae suspension was placed in a 500 mL beaker and then sonicated for 5, 30, 60, and 300 s. Meanwhile, the untreated sample was set as control. Three indicators were measured immediately after sampling: (a) cell concentration was measured by cell counting using a hemocytometer (HAE); (b) chlorophyll-a content was measured following the method used in Hao et al. (2004);
and (c) PC absorbance was determined according to the method described in Zhang et al. (2006). All experiments were performed in triplicate and the average was shown. To monitor the changes of chlorophyll-a fluorescence transient, all of the samples (treated with different exposure durations and untreated) were cultured for 24 h in a sterile incubator with the cultural conditions mentioned above.

**Measurement of the chlorophyll-a fluorescence transient and data analysis**

Algal samples (2 mL) were taken from the treated and untreated samples which were incubated for 0, 1, 5, 6, and 24 h after sonication, respectively. These samples were kept in darkness for 20 min before chlorophyll-a fluorescence transients were recorded using a FluorPen fluorometer (AquaPen-C AP-C 100, Photon Systems Instruments, Czech Republic) at room temperature. Illumination in the equipment was provided by a PIN photodiode with 667 to 750 nm bandpass filters, with the saturating light of 3,000 μmol (photons) m⁻² s⁻¹. Fluorescence induction curves were plotted on a logarithmic time scale and kinetics exhibited a polyphasic rise over time known as O-J-I-P transients (Strasser & Srivastava 1995). The following data were directly obtained from the kinetic curves: the fluorescence yield at 50 μs is considered as the initial fluorescence (Fo), in which all reaction centers (RCs) in PSII are open; the fluorescence intensities at step J (2 ms) and at step I (60 ms) are recorded as Fj and Fi, respectively, while the J-I phases are caused by the gradual reduction in primary electron acceptors, QA and QB; Fm (maximal fluorescence) is the peak of the fluorescence induction curve at the step P, where all RCs are closed, connecting to accumulation of QA/QB⁻ (Strasser 1992).

The JIP-test is usually used to analyze the polyphasic fluorescence induction curves (Stirbet & Govindjee 2011), which can provide adequate information about the structures, conformations and functions of their photosynthetic apparatus (Strasser & Srivastava 1995). Some useful parameters for quantifying the energy fluxes of photosystem II or PSII's behavior calculated from the original data described above are shown in Table 1.

**Table 1 | Formulae or terms used in the JIP-test (Force et al. 2003)**

<table>
<thead>
<tr>
<th>Formulae or terms</th>
<th>Explanations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vj = (Fj – Fo)/(Fm – Fo)</td>
<td>Relative variable fluorescence at 2 ms or the proportion of RCs closed at 2 ms (unconnected PSIs only)</td>
</tr>
<tr>
<td>Mo = 4(F300s身后 – Fo)/(Fm – Fo)</td>
<td>Approximated initial slope (in ms⁻¹) of the fluorescence transient V = f(t)</td>
</tr>
<tr>
<td>TRo/ABS = [1 – (Fo/Fm)]</td>
<td>Maximum quantum yield of primary photochemistry, [Fv/Fm ratio]</td>
</tr>
<tr>
<td>ETo/TRo = (1 – Vj)</td>
<td>Probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond QA</td>
</tr>
<tr>
<td>ABS/RC = [Mo(1/Vj)]/(Fv/Fm)]</td>
<td>Absorption flux per RC</td>
</tr>
<tr>
<td>TRo/RC = Mo(1/Vj)</td>
<td>Maximal trapping energy flux per RC</td>
</tr>
<tr>
<td>ETo/RC = Mo(1/Vj)(1 – Vj)</td>
<td>Electron transport from QA to QB⁻ flux per RC</td>
</tr>
<tr>
<td>DTo/RC = (ABS/RC) – (TRo/RC)</td>
<td>Dissipated energy flux per RC</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

**Effects of sonication on cell concentration and algal pigments**

Sonication time plays an important role in controlling algae growth. Previous studies showed that less than 5 min of exposure could be an appropriate ultrasonic duration, because the content of algal toxin in suspension did not increase. Therefore, the sonication time was set to no more than 5 min in this work. After sonication, the changes of algae cell concentration, chlorophyll-a content and PC absorbance were measured, and the results are exhibited in Table 2.
For *M. aeruginosa*, cell concentration, chlorophyll-\(\alpha\) content and PC absorbance were not influenced significantly (\(P < 0.05\)) when no more than 60 s of exposure was implemented. However, when sonication time was prolonged to 300 s, cell concentration, Chl-\(\alpha\) content and PC absorbance reduced by 32.5%, 27.8% and 31.1%, respectively. It should be noted that the difference between the reductions in these indicators was not significant (\(P > 0.05\)). In other words, the lost Chl-\(\alpha\) content and PC absorbance could have resulted from the algal cell disruption or the release of intercellular components. A similar study revealed that the PC absorbance and the Chl-\(\alpha\) content of *M. aeruginosa* reduced by 44.8% and 21.3%, respectively, after sonication for 300 s (25 kHz, 0.32 W/cm\(^2\)) (Zhang et al. 2019). Cell concentration (measured by optical density at 684 nm (OD684) of cell suspension), however, dropped slightly by 10.8%, which was considerably lower than that of this work. This difference might be due to the gaps between methods used to measure the cell disruption. Indeed, the OD of algal suspension was an overall measure of cell concentration (disrupted cells were still embodied, because their cellular inclusions remained in the suspension), but just intact cells were included when cell counting was used to measure the cell disruption in this case. Wu et al. (2012) also found that the reduction in cell concentration was different with different measurements. In comparison, *C. pyrenoidosa* was highly resistant to sonication (Table 2). After 300 s of sonication, cell concentration and Chl-\(\alpha\) content decreased only by 18.7% and 9.3%, which were much lower than those of *M. aeruginosa*.

### Effect of sonication on energy fluxes of PSIIIs

Figure 2 shows the rapid rise fluorescence kinetics of *M. aeruginosa* and *C. pyrenoidosa*. The untreated sample displayed a typical OJIP fluorescence induction curve

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cell concentration (10(^6) cells/mL)</th>
<th>Chl-(\alpha) content ((\mu)g/mL)</th>
<th>PC absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>M. aeruginosa</em></td>
<td><em>C. pyrenoidosa</em></td>
<td><em>M. aeruginosa</em></td>
</tr>
<tr>
<td>Control</td>
<td>40.3 ± 3.2a</td>
<td>50.7 ± 1.7a</td>
<td>0.162 ± 0.010a</td>
</tr>
<tr>
<td>5 s</td>
<td>42.6 ± 9.6a</td>
<td>49.9 ± 3.3a</td>
<td>0.149 ± 0.006a</td>
</tr>
<tr>
<td>30 s</td>
<td>39.9 ± 3.2a</td>
<td>52.3 ± 3.5a</td>
<td>0.149 ± 0.017a</td>
</tr>
<tr>
<td>60 s</td>
<td>43.5 ± 4.8a</td>
<td>48.3 ± 4.2a</td>
<td>0.150 ± 0.016a</td>
</tr>
<tr>
<td>300 s</td>
<td>27.2 ± 3.3b</td>
<td>41.2 ± 2.8b</td>
<td>0.117 ± 0.005b</td>
</tr>
<tr>
<td>1-(Ct/C0)(^a)</td>
<td>0.67</td>
<td>0.81</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Values are shown as mean ± SD (\(n = 3\)). Significant difference is marked with different letters (\(P < 0.05\)).

\(^a\)1-(Ct/C0)\(^b\) indicates the reduction of treated samples for 300 s (Ct) over the control (C0).

![Figure 2](https://iwaponline.com/wst/article-pdf/76/8/2085/449594/wst076082085.pdf)
It was found that the effect of ultrasound on the polyphasic fast-phase fluorescence kinetics was exposure time dependent. As seen in Figure 2, *M. aeruginosa* was affected seriously and its relative fluorescence kinetic decreased substantially with an increasing sonication. As a result, the shape of the J-I-P phases became flat gradually, and the Fm value reduced dramatically. These changes could be explained by the inhibition of the electron transport at the donor side of PSII (Zhang et al. 2010). In comparison, the fluorescence yield of *C. pyrenoidosa* was influenced slightly by ultrasound (Figure 2).

JIP-test parameters, generally, are expressed as percentages of control, which are calculated from the fluorescence kinetic. And PSII's energy fluxes in algal cells, including absorption, trapping, electron transport and dissipation, can be effectively monitored by this test (Perron & Juneau 2011; Perron et al. 2012).

Results of the JIP-test on *M. aeruginosa* revealed that an increasing exposure time caused the rapid decreases in the trapping probability (TRo/ABS) and the electron transport probability (ETo/TRo). Meanwhile, significant increases in the effective antenna size of an RC (ABS/RC), the initial net reduction rate of QA (Mo), and the effective dissipation (Dlo/RC) were observed (Figure 3(a)). In agreement with the fluorescence kinetics, however, the results of JIP-test parameters except for TRo/ABS, ABS/RC and Dlo/RC suggested that *C. pyrenoidosa* was highly resistant to sonication (Figure 3(b)).

![Figure 3](https://iwaponline.com/wst/article-pdf/76/8/2085/449594/wst076082085.pdf)

**Figure 3** | JIP-test parameters expressed as percentages of the control when *M. aeruginosa* (a) and *C. pyrenoidosa* (b) were exposed with different durations. Significant difference compared with control is marked as *P < 0.05*. Error bars indicate forward SD.
Non-Q\textsubscript{A}-reducing reaction centers (or silent RCs) caused by sonication

The parameter TRo/ABS (or Fv/Fm) is related to the maximum quantum yield of primary PSII photochemistry, or the trapping probability that an absorbed photon will be trapped by an RC to reduce Q\textsubscript{A}. And it has been used frequently to evaluate the effect of chemical or physical pressures on PA of plant or algae (Stirbet & Govindjee 2007).

For M. aeruginosa, TRo/ABS declined significantly as a result of sonication (Figure 3(a)), suggesting that its PA was depressed remarkably. This was consistent with previous studies showing that a decrease of oxygen evolution of cyanobacteria was observed after sonication for 5 minutes (Tang et al. 2003; Zhang et al. 2006). The decrease of TRo/ABS in M. aeruginosa was associated with a significant increase in the antenna size of PSII or absorption flux per RC (ABS/RC). It should be noted that ABS/RC is a measurement of the average absorption per active RC or of the average number of absorbing chlorophylls per active RC. For photosynthetic samples exposed to chemical or physical stresses, even though no absorptive changes in PSII occurred, probably, an apparent decrease of TRo/ABS and a stability of TRo/RC would be observed (Tsimilli-Michael et al. 1998). This might be due to the transition by which some of the active RCs turned to ‘silent RCs’ (RC\textsubscript{si}s). Generally, RC\textsubscript{si}s are characterized by the following (Lavergne & Lecointre 1993): (a) they can neither reduce Q\textsubscript{A} nor reverse exchange their excitation energy to the antenna; therefore, the variable fluorescence cannot be impacted by the corresponding PSII units, and all their excitation energy is then dissipated as heat or fluorescence; and (b) they will reactivate as soon as the stress that provoked the conformational modification is ceased. The percentage of treated algae’s RC\textsubscript{si} compared with the control samples can be calculated using the following equation (Strasser et al. 2004):

\[
RC_{si} = \left[ 1 - \frac{(ABS/RC)}{(ABS/RC)^c} \right] \times 100\% \]

\[
= \left[ 1 - \frac{[M_0/V_j]}{[M_0/V_j]} \times \frac{[1 - (F_o/F_m)]}{[1 - (F_o/F_m)]} \right] \times 100\% \quad (2)
\]

where RC is the reaction center (RC in the control sample is shown with a superscript of ‘c’, inactive or silent RC is shown with a superscript of ‘si’, and those RCs that remained active after sonication are depicted without superscript). For other labels in the formula, see the list of terms in Table 1.

Figure 4 shows that an increasing exposure time resulted in an increasing percentage of RC\textsubscript{si}. As can be seen, the RC\textsubscript{si} in M. aeruginosa rose sharply to 44.8% of control after sonication for 300 s. The increased RC\textsubscript{si} could explain the higher levels of D1o/RC (Figure 3(a)), because they dissipated most of their excitation energy. At the same time, the reduction in active RCs contributed to the increase in ABS/RC and the decrease of TRo/ABS (Perron & Juneau 2011). In comparison, RC\textsubscript{si} of C. pyrenoidosa increased slightly to 7.9% in the same conditions. Despite the slight change, the RC\textsubscript{si}s in C. pyrenoidosa might be responsible for almost all the alterations in its PSII under the ultrasonic pressure (Figure 3(b)).

Effect of sonication on electron transport chains in PSII

When M. aeruginosa was sonicated for more than 60 s, the rate at which an electron was trapped by an active RC (TRo/RC) increased substantially (Figure 3(a)), indicating that there was an increasing reduction rate of Q\textsubscript{A} to Q\textsubscript{B}. This was also confirmed by the approximation of the slope at the origin of fluorescence rise (Mo), which is a measure of the initial reduction rate of Q\textsubscript{A} (Force et al. 2003; Perron & Juneau 2011). The increases in TRo/RC and Mo, therefore, might be due to a decrease of the reducible Q\textsubscript{A} number. Further, the electron transport probability (ETo/TRo) that an electron residing on Q\textsubscript{A} would enter the electron transport chain went down dramatically when M. aeruginosa was exposed just for 5 s, indicating that sonication blocked the electron transport from Q\textsubscript{A} to Q\textsubscript{B} (Figure 3(a)), which was similar to DCMU, an inhibitor of the electron flow beyond...
Q absorption. This effect was also proved by the decrease of electron transport in RC (ETo/RC), which is an indicator of the re-oxidation of reduced QA via electron transport in an active RC (Force et al. 2003). For C. pyrenoidosa, however, there was no visible effect on electron transport chain in its PSII induced by ultrasound (Figure 5(b)).

**Effect of hydrogen peroxide on energy fluxes of PSII**

Sonication can induce acoustic cavitation in liquid, which is the dominant mechanism for chemical activation in sonochemistry (Luo et al. 2011). Free radical reaction, a chemical effect of the cavitation, may destroy algal photosystems and induce lipid peroxidation of cell membranes (Tang et al. 2004). The free radical effect, therefore, could cause the alternation in energy fluxes of PSII, and result in the varied levels of sensitivity between strains to sonication. In order to verify this hypothesis, M. aeruginosa and C. pyrenoidosa were exposed in a hydrogen peroxide solution (100 μM) for 2 min, and then their chlorophyll-a fluorescence was measured. The JIP-test results are shown in Table 3. After the treatment, all the selected JIP-test parameters were changed dramatically in both strains, indicating that algal PSII were destroyed severely by oxidation. Especially, the values of ETo/TRo and ETo/RC reduced to near zero, suggesting that the electron transport chain was blocked completely by hydrogen peroxide. Ahn et al. (2005) also sonicated M. aeruginosa in H2O2 solution (100 μM) for 2 min and then measured the lipid peroxidation of the cell membranes. Their results showed that the increased production of free radicals did not cause marked lipid peroxidation. It suggested that the predominant damage of free radicals induced by cavitation might be inactivating photosynthesis of algal cells through altering the energy fluxes in PSII (i.e., silencing the reaction centers, damaging the QA, and blocking the electron transport chain).

Interestingly, however, unlike ultrasound, hydrogen peroxide damaged the PSII of C. pyrenoidosa more seriously than that of M. aeruginosa, indicated by all the selected JIP-test parameters (Table 3). This suggested that there were no specific structures or functions in the cells of C. pyrenoidosa to prevent their PSII from suffering from oxidation compared with M. aeruginosa. Thus, the high resistance of C. pyrenoidosa to sonication might be due to the lower level of free radicals produced in the suspension than that of M. aeruginosa in the same ultrasonic conditions. The shortage of gas vacuoles in C. pyrenoidosa might be responsible for the low production of free radicals. Previous studies involving the influences of cavitation on cyanobacterial growth inhibition have suggested that gas vacuoles in algae cells played a positive role of cavitation nuclei, which can lower the threshold of cavitation and then promote the production of free radicals (Luo et al. 2011).

**Recovery of inhibited PA after sonication**

The recovery of PA in C. pyrenoidosa was not measured, because it was highly resistant to sonication. For the counterpart, this process is shown in Figure 5. According to the analysis above, TRo/ABS, ABS/RC and DIO/RC were mainly connected with the changes of RC in PSII. The initial rate of reduction from QA to QB at the beginning. On the whole, in this work, all the selected JIP-test parameters roughly recovered to the control samples’ levels after cultivation for 6 hours, except for ETo/TRo and ETo/RC, which took 24 hours to regain their initial levels after sonication (Figure 5(b)).

### Table 3: Effects of hydrogen peroxide (100 μM; exposure for 2 min) on energy fluxes of PSII in algal cells

<table>
<thead>
<tr>
<th>JIP-test parameters</th>
<th>M. aeruginosa</th>
<th>C. pyrenoidosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control  (%)</td>
<td>100 μM (%)</td>
</tr>
<tr>
<td>TRo/ABS</td>
<td>0.29 ± 0.01</td>
<td>0.19 ± 0.00</td>
</tr>
<tr>
<td>ABS/RC</td>
<td>12.0 ± 0.19</td>
<td>20.7 ± 0.42</td>
</tr>
<tr>
<td>TRo/RC</td>
<td>3.5 ± 0.00</td>
<td>4.0 ± 0.03</td>
</tr>
<tr>
<td>Mo</td>
<td>2.9 ± 0.03</td>
<td>3.9 ± 0.01</td>
</tr>
<tr>
<td>ETo/TRo</td>
<td>0.18 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>ETo/RC</td>
<td>0.65 ± 0.03</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>DIO/RC</td>
<td>8.5 ± 0.19</td>
<td>16.7 ± 0.32</td>
</tr>
</tbody>
</table>

Values were shown as mean ± SD (n = 3).
capacities (Figure 5). This illustrated that RC\textsuperscript{silent}, inhibition of reducible QA, and impediment of electron flow at the Q\textsubscript{B} binding site in PSII induced by ultrasound disappeared gradually as soon as the stress was removed. It agreed with one of the characteristics of RC\textsuperscript{silent} described above (in the section Non-Q\textsubscript{A}-reducing reaction centers (or silent RCs) caused by sonication). Furthermore, the effect of sonication on the whole electron transport chain was relatively durable.

However, most of the inhibited PAs recovered rapidly (Figure 5). As a similar study, Lee et al. (2001) reported that PAs of the sonicated blue-green algae displayed an initially rapid decrease, and then roughly regained the original level. In addition, a fast recovery of PA might result in the weak inhibition of the growth of \textit{M. aeruginosa} after sonication for one time. Rajasekhar et al. (2012) reported that sonicated \textit{M. aeruginosa} (20 kHz, 0.043 W/cm\textsuperscript{3} for 5 min) was able to recover to the initial cell concentration after culture for approximately 12 hours (Figure 2(a) in the original paper). These results suggested that intermittent ultrasonication with an interval of several hours could be better for controlling Microcystis growth.

Different cell structures and compositions for explaining the various resistances to sonication

The findings of this work demonstrated that \textit{M. aeruginosa} was much weaker than \textit{C. pyrenoidosa} in the ultrasonic field regarding the reduction in cell concentration, the detriment of pigment-protein complexes and the alterations of PSII’s energy fluxes. This was similar to the findings revealed by Rajasekhar et al. (2012). Differences in cell structures or components of both species, especially in terms of gas vacuoles and PSII, might be responsible for their various responses to sonication.

Gas vacuoles in algal cells can be collapsed rapidly in an ultrasound field (Jachlewski et al. 2013). This collapse normally produces extremely high and localized temperature and pressure, as well as free radicals (Luo et al. 2014). The effects of the collapse might change the structure or conformation of pigment-protein complexes and/or other compositions in PSII. Moreover, they also might contribute to the disruption of algal cells, the peroxidation of algal lipid and the inhibition of cell division (Ahn et al. 2003). Tang et al. (2004) researched the responses of two species of cyanobacteria, gas-vacuole (\textit{M. aeruginosa} PCC 7806) and negative gas-vacuole (\textit{Synechococcus} PCC 7942), to acoustic cavitation. Their results revealed that when \textit{M. aeruginosa} was irradiated (1.7 MHz, 0.6 W/cm\textsuperscript{2}), biomass increment declined by 65% and relative malondialdehyde (MDA) content, a quantitative indicator of lipid peroxidation, increased by 65%. In contrast, sonicated \textit{Synechococcus} still grew as fast as the control. Only 9% of the increase in relative MDA content was gained. In the present experiment, the JIP-test results demonstrated that sonication damaged QA and blocked electron flow at the Q\textsubscript{B} binding site in PSII of \textit{M. aeruginosa}. Furthermore, the cell concentration decreased by 32.5% after exposure for 300 s (Table 2). In contrast, \textit{C. pyrenoidosa}, which does not have gas vacuoles in cells, was highly resistant to sonication. As a result, its cell concentration and

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure5.png}
\caption{The recovering process of photosynthesis activity of \textit{M. aeruginosa} after sonication.}
\end{figure}
Chl-a content reduced by only 18.7% and 9.3%, respectively, under the same conditions (Table 2).

On the other hand, differences in PSII’s energy fluxes induced by sonication might further reinforce the different degrees of sensitivity to sonication. The light-harvesting protein in M. aeruginosa is PC, which constitutes phycobilisome, a large antennae complex located in the stromatic space between thylakoid membranes, but C. pyrenoidosa produces instead chlorophyll-protein. Zhang et al. (2006) revealed that PC, due to its ‘empty-rod’ structure, was more vulnerable than chlorophyll-protein under an ultrasonic field. After sonication, moreover, the phycobilisomes were separated from the thylakoid membranes scanned by transmission electron microscopy (Wan et al. 2014).

**CONCLUSIONS**

- Photosynthesis of M. aeruginosa was depressed significantly and selectively by ultrasound.
- Most of the alterations in PSII’s energy fluxes induced by sonication might be explained by the conversion of active RCs to silent ones in C. pyrenoidosa.
- Sonication damaged QA and blocked electron flow at the QA binding site in the PSII of M. aeruginosa.
- For C. pyrenoidosa, there was no visible effect of sonication on the electron transport chain of its PSII after sonication for 300 s.
- Inhibited PA of M. aeruginosa was roughly recovered when it was cultured for 6 hours after sonication. Therefore, intermittent sonication with an interval of several hours could be better for controlling cyanobacterial populations.

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