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Damaging effects of ultrasonic treatment on the photosynthetic system of *Microcystis aeruginosa*

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ABSTRACT

Harmful algal blooms have become a global environmental problem. Ultrasonic technology was found to effectively inhibit the algal growth and reproduction of cyanobacteria in eutrophic water. Given that cyanobacterial blooms are difficult to treat, and treatment methods may cause problems leading to secondary pollution, a preventive control was proposed. In this research, algal cell sedimentation was performed to analyze the effects of ultrasonic technology on algae. The photosynthetic system of algae and the optimal combination of the ultrasonic removal of *Microcystis aeruginosa* were also studied. Low-frequency ultrasound (20, 40 and 100 kHz) was selected to analyze the changes in phycobiliprotein content, the expression level of the key gene in photosynthesis, and the settlement index after treatment with different ultrasonic frequencies and power densities. Results showed that the phycobiliprotein content and gene expression level decreased after ultrasonic treatment, and a large quantity of cells settled. The inhibition effectiveness at 40 kHz was much better than that at 20 and 100 kHz. When the power density of 0.02 W/mL decreased and was applied at a frequency of 40 kHz, the relative content of phycobiliprotein decreased significantly, and the phycobiliprotein level increased by 44%–45% compared with that of the control group after 10 d. Relative quantifications (RQs) of the *rbcL* gene (encoding RUBISCO protein) and *cpc* gene (encoding phycocyanin protein) were inhibited with a frequency of 40 kHz. The RQ value decreased by more than 40%. When the power density remained constant and was applied with a frequency of 40 kHz, both the algal cell sedimentation index and the inhibitory effect on algal growth increased. Thus, ultrasound with frequency of 40 kHz and intervals of 4–6 d is suggested when dealing with algal cells to prevent the algal biomass below the level of algal blooms from achieving preventive restraint, which is the ability of algal cells to self-repair and reproliferate.

Keywords: Ultrasound; *Microcystis aeruginosa*; Preventative restrain; Photosynthesis system; Settling

1. Introduction

Cyanobacterial blooms have become a global environmental problem [1] and become progressively severe in China. Cyanobacterial blooms occur frequently in all kinds of water bodies and may lead to imbalances of ecological systems, resulting in death of large number of aquatic organisms, such as aquatic plants and fishes [2].

A radical solution for cyanobacterial blooms is to reduce the input of exogenous nutrients and the levels of nitrogen and phosphorus in water because algae cannot propagate

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without these factors [3,4]. Traditional treatment methods can easily cause algae to release algal toxins, resulting in secondary water pollution. By extruding the management via preventive restrain and growth inhibition of algae, the biomass is kept at a certain level to avoid cyanobacterial blooms and their adverse effects. Ultrasonication is a new type of environmental technology [5]. Zhang et al. [6] found that low-frequency ultrasound cannot release microcystins. Ultrasonic cavitation can inhibit the formation potential of trihalomethanes, disinfection by-products, persistent organic pollutants, endocrine disruptors, algal toxins and harmful algal blooms, as well as the harmful refraction of organic compounds. This method is widely used for water treatment and presents a mild reaction to conditions, as well as rapid effects. Ultrasonication can reduce the risk of secondary pollution and prevent and control cyanobacterial blooms. Unlike in the past, ultrasonic treatment is used as a general method to remove algae. By observing the changes of indicators for a period, this research analyzes the inhibition degree of algal growth and suggests the mechanism of inhibition.

Cyanobacterial growth depends on the conversion of material to energy, which is the core of photosynthesis. Ultrasonic treatment can damage the phycobiliprotein [6–8]. This phenomenon can affect the function of the photosynthetic system and hinder the conversion of light energy into chemical energy, thus inhibiting cyanobacterial growth for a long period.

Lee et al. [9] applied ultrasonic treatments with short treatment times to blue-green algae harvested from Lake Senba. Chlorophyll-a concentration decreased in blue-green algae. A chlorophyll fluorometer showed that the chlorophyll fluorescence of *Microcystis aeruginosa* decreased after the ultrasonic treatment. An increase in power led to a decrease in photosynthetic activity [10]. Ultrasonic technology was also used on the blue-green algae in Taihu of China, and the chlorophyll-a concentration decreased [11]. Hao et al. [8] found that few days after treatment, the chlorophyll-a concentration in the experimental group was consistently lower than that in the control group. This finding showed that the ultrasonic treatment reduced the synthesis rate of chlorophyll-a. Zhang et al. [6] found that the maximum net photosynthesis rate of treated cells decreased after treatment. The fluorescence spectra of phycobiliproteins also changed. Light energy is restricted to phycobiliproteins; hence, phycobilisomes cannot be transferred to chlorophyll-a, and further photosynthesis processes were inhibited. As reported by Lee et al. [12], the fluorescence spectrometer scanning results showed that the fluorescence peaks of phycobiliproteins were indicated at 665 nm and decreased after ultrasonic treatment. The effects of ultrasonic treatment on the photosynthetic activity of algal cells were mainly focused on the concentration of chlorophyll-a and the content of phycobiliproteins.

D1 protein is located in photosystem II (PS II), which refers to the chloroplast gene *psbA* encoding the 32 kDa protein; D1 consists of the key parts of the primary photochemical reaction and electron transfer; D1 is also the main electron transport chain component of PS II [13]. RUBISCO protein is the key enzyme in photosynthetic carbon assimilation; this protein performs two kinds of catalytic activities, namely, carboxylase and oxygenase activities, which can connect carbon assimilation with light respiration [14]. Phycocyanin (PC) is a protein formed by phycobiliprotein. PC can receive the light energy of phycoerythrin (PE) and deliver light energy to chlorophyll-a. The main function of phycobilisomes in cyanobacteria cells is to capture light energy. They are attached to the external surface of thylakoid lamella. The absorbed light energy can be transferred to the chlorophyll for photosynthesis. Therefore, phycobilisomes are the antenna complexes of the photosynthetic system [15]. Phycobilisomes are the polymers of phycobiliproteins. The phycobiliproteins of *M. aeruginosa* can be divided into three types: PE, PC and allophycocyanin (APC) [16]. Light energy is captured by PE and then transferred to PC and APC. Afterward, light energy passes through lipid-soluble pigments and chlorophyll-a levels. Finally, light energy passes through the D1 protein, which is carried through the photochemical reaction. Thus, phycobiliproteins are important in the photosynthesis of algae. However, the long-lasting effects of different ultrasound parameters on the content of phycobiliproteins still require further study.

Considering that the *psbA* and *rbcL* genes are the key genes of the light system II and carbon assimilation, their levels of expression can be selected as the evaluation indexes for photosynthesis [17]. Haifeng et al. [17] found that cadmium demonstrates a significant dose relationship with *psbA* and *rbcL* genes. Under high concentration of cadmium, the magnitude of gene expression of *rbcL* and *psbA* decreased significantly. Hou et al. [18] studied the effects of copper and cadmium on soluble proteins and photosynthetic pigment contents in duckweed and also found significant dose relationships. Xiong et al. [19] used real-time fluorescence quantitative polymerase chain reaction (PCR) to analyze *rbcL* gene transcription changes and found that after 120 h of exposure to plumbum(II), the relative abundances of *rbcL* in *Chlorella protothecoides* and *Chlorella vulgaris* decreased by 43.93% and 43.91%, respectively, compared with those in the control group. The effect of the expression level of the photosynthetic key protein gene mainly depends on different Pb ion concentrations. Toxic heavy mental ions from water can easily pollute the environment, but ultrasound, which is an environmentally friendly technology, is widely used in water treatment and will not cause secondary pollution to the environment. The effects of ultrasound on gene expression need to be studied to further understand the influence of ultrasound on *M. aeruginosa*.

M. aeruginosa contains a gas vacuole, which is composed of hollow cylindrical vesicles [20]. Gas can penetrate through the gas vacuole membrane [21]. Therefore, the cell buoyancy can be regulated, and the position of the cells in the water column can be adjusted. When the pressure in the gas vacuole exceeds the critical value, the structure of the gas vacuole will collapse and flatten [22]. Klebahn's "Hammer and bottle cork" test found that a certain pressure on the algal suspension can destroy the gas vacuole; as such, algae will settle on the bottom of the container. As early as 1971, Lehmann and Jost [23] have used ultrasound to produce a series of reactions damaging the gas vacuole inside *M. aeruginosa.* The rate constant of algal removal is related to ultrasonic frequency [24]. Compared with *Synechococcus*, which does not contain a gas vacuole, the biomass increment of *M. aeruginosa* decreased by 65% and required a long regeneration time.

This phenomenon showed that the resonance effect influenced the growth of algae with gas vacuoles [25]. Rajasekhar et al. [26] also confirmed that the growth of algae with gas vacuoles was significantly inhibited by ultrasound. Research about the effect of ultrasound on the gas vacuoles mainly involved algal cells with gas vacuoles as subjects. We found that the destruction of the gas vacuoles by ultrasound can cause deposition of algal cells.

Phycobiliproteins and the expression level of the key photosynthetic protein gene were detected in algae cells after ultrasonic treatment with different parameters. These experiments were determined to analyze the effect of ultrasound on the cell photosynthesis system of *M. aeruginosa* and reveal the mechanism underlying the growth inhibition of cyanobacterial blooms by ultrasonication. The control effects of different parameters of ultrasonic treatment on algal biomass were compared through experiments based on the mechanism study. The theoretical basis would be provided for the application of ultrasonic preventive technology in the water remediation.

2. Materials and methods

2.1. Algae species and ultrasonic treatment

The algae species used in the experiment is *M. aeruginosa* (number FACHB-905; isolated from Dianchi, 1998), which was acquired from the wild biological germplasm collection of Chinese Academy of Sciences—FACHB. Ultrasonic frequencies of 20, 40 and 100 kHz were used for the cell suspension of *M. aeruginosa* with the power density of 0.02 W/mL. Different power densities were used for the cell suspension of *M. aeruginosa* with different ultrasonic frequencies of 20, 40 and 100 kHz. Each ultrasound treatment time was set to 5 min [24,25]. The algal cell solution volume was approximately 100 mL. The initial algal density was approximately 8.3×10^6 cells/mL. After ultrasonic treatment, the cells were fostered under a 14/10 h light/dark cycle at 30°C for 8 d. The phycobiliprotein content was sampled and examined for 2 h and then for 1, 2, 4, 6, 8 and 10 d [27]. The transcription levels of the genes encoded were sampled and examined with D1, RUBISCO and PC within 2 h and then after 1, 2, 4, 6, 8 and 10 d to determine the change curve of settlement index.

2.2. Phycobiliprotein

We detected the relative amounts of PE, PC and APC [28]. The specific operation steps are described as follows: (1) Up to 3 mL of algae solution was taken to a 10 mL centrifuge tube. (2) Algae cells were collected and centrifuged at 5,000 rpm for 10 min, and supernatant was discarded. (3) For phosphoric acid buffer washing, 1.5 mL of phosphate-buffered saline (PBS) was added, and the mixture was centrifuged at 5,000 rpm for 10 min; the supernatant was discarded and centrifuged again. (4) For freeze thawing, 1.5 mL of PBS was added, and the mixture was frozen for 4 h at –20°C; afterward, the mixture was oscillated to melt at 20°C in the dark for 30 min; this step was repeated twice. (5) The mixture was then centrifuged at 5,000 rpm for 10 min, and the supernatant was collected. (6) For photometry checking, the supernatant was detected for A_{565}, A_{620} and A_{650} as follows [27]:

$$\mathrm{PE}\left(\mathrm{mg}\cdot\mathrm{L}^{-1}\right)=\frac{A_{565}-2.8\times\mathrm{PC}-1.34\times\mathrm{APC}}{1.27} \quad (1)$$

$$\mathrm{PC}\left(\mathrm{mg}\cdot\mathrm{L}^{-1}\right)=\frac{A_{620}-0.7\times A_{650}}{7.38} \quad (2)$$

$$\mathrm{APC}\left(\mathrm{mg}\cdot\mathrm{L}^{-1}\right)=\frac{A_{650}-0.19\times A_{620}}{5.65} \quad (3)$$

$$\mathrm{PB}=\mathrm{PE}+\mathrm{PC}+\mathrm{APC} \quad (4)$$

where A_{565} represents the absorbance at 565 nm, A_{620} represents the absorbance at 620 nm and A_{650} represents the absorbance at 650 nm.

2.3. Gene expression level

Real-time fluorescence quantitative PCR was used to determine the expression levels of the four following genes in the photosynthetic system of algal cells: *psbA* gene encoding D1 protein, *rbcL* gene encoding RUBISCO protein, *cpc* gene encoding PC and 16s rRNA gene encoding reference proteins.

The primer sequences of four kinds of target genes as summarized in Table 1 (Primer Synthesis, Sangon Biotech (Shanghai) Co., Ltd., Shanghai) [27].

RNA extraction was performed using EASYspin Plus Plant RNA Purification Kit (Aidlab Biotechnologies Co., Ltd., Beijing). The OD value of RNA was measured using a Thermo Scientific NanoDrop2000c spectrophotometer (Thermo Fisher Scientific, USA), and the RNA concentrations were calculated. RNA integrity was monitored in gel electrophoresis.

RNA reverse-transcription reaction was performed using HiScript Q RT SuperMix for qPCR Kit (Vazyme, USA). Up to 16 μL of RNase-free ddH_2O, 4 μL of 4× gDNA wiper Mix and 200 ng of template RNA were added into the RNase-free centrifuge tube by using an H-1 micro-mixer (Shanghai Kang Wo Photoelectric Instrument Co., Ltd., Shanghai) to ensure that the liquid was uniformly mixed. The reaction was then processed in S1000 Peltier Thermal Cycler (Bio-Rad Laboratories, USA) at 42°C for 2 min. A 4 μL volume of 5× qRT SuperMix II was added to the first step reaction

Table 1
Primer sequences of four target genes

Target genes	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
cpc	TTGACCGAAAAAGCCAATTC	TTTTCTTTACCGCGTTGGTC
rbcL	GCGGGGTTAATGACTGAGAA	AAAAGTTGCCGACGATGTTC
psbA	GATCGCCTTTAGGGTCTTCC	CATGCAGGTGTATGGAATGC
16s rRNA	CTGCTGTCAAATCAGGTTGC	CACCGATGTTCTTCCCAATC

tube, incubated stepwise at 50°C for 15 min, and then at 85°C for 2 min. The first chain of cDNA was used for the reaction immediately or stored at –20°C.

The samples of each gene group (*cpc*, *rbcL* and *psbA*) were detected by SYBR Green I in an ABI 7500 Fast Real-Time PCR System (Applied Biosystems, USA). Each PCR reaction liquid and reaction condition were prepared as described in Table 2. The PCR products were then subjected to melting curve analysis (Fig. S1).

Relative quantification (RQ) was performed with the $2^{\Delta\Delta Ct}$ method, and the RQ of genes was calculated as follows:

$$\mathrm{RQ} = 2^{-\Delta\Delta Ct} \tag{5}$$

$$\Delta\Delta Ct = \left(Ct^{T2} - Ct^{C2}\right) - \left(Ct^{T1} - Ct^{C1}\right) \tag{6}$$

where Ct^{C1} is the target gene, and Ct is the value of the control group. Ct^{T2} is the target gene, and Ct is the value of the experimental group. Ct^{C2} is the reference gene, and Ct is the value of the experimental group.

2.4. Sedimentation of algal cells

We followed the experimental methods of Rodriguez-Molares et al. [29] and quantified the settlement degree of *M. aeruginosa* caused by ultrasonic waves. Ultrasonic treatment removed the algae solution in 10 mL glass tube. Both the control and experimental groups were cultured under the same environmental conditions. By comparing the densities of the 5 mL algae in the test tubes, we observed the level of the algae cells in the experimental group compared with that in the control group, which was expressed by the settlement index as follows:

$$SI = \left(1 - \frac{C^S}{C^c}\right) \times 100\% \tag{7}$$

where C^S is the value of the experimental group, with algae density in the upper layer of the 5 mL algae sample (in cell/mL); C^C is the value of the control group, with algae density in the upper layer of the 5 mL algae sample (in cell/mL). Without any treatment in the control group, some of the cells settled because of death. The proposed method was used to deduct the settlement caused by non-ultrasonic action and compared the effects of ultrasonic treatment on the sedimentation of *M. aeruginosa*.

Table 2
PCR reaction liquid and reaction condition

Reactants	Volume (μL)	Reaction temperature (°C)	Time (s)
qPCR SYBR Green Master Mix	10.0	95	300
PCR Forward Primer (10 μM)	0.5	95	10
PCR Reverse Primer (10 μM)	0.5	60	30
50× Rox Reference Dye II	0.4	Followed by 40 cycles of 95°C for 10 s and 60°C for 30 s	
cDNA Template	1.0		
ddH_2O	Added to 20		

3. Results and discussion

3.1. Effect on phycobiliprotein

3.1.1. Effect of ultrasonic frequency on phycobiliprotein accumulation

The power density used with the three frequencies (20, 40 and 100 kHz) in this experiment is 0.02 W/mL. The changes in relative contents of PE, PC and APC in each group are shown in Fig. 1. The relative contents of PE, PC and APC first decreased and then increased. The contents decreased to the lowest after 2 h of ultrasound treatment, and the relative contents were approximately the same (40%). The effect of the same ultrasound treatment on the degree of immediate damage of phycobiliprotein was also similar. Subsequently, each experimental group showed different changes. The content of phycobiliprotein in the experimental group (100 kHz) increased the fastest and reached the maximum (more than 100%) within 4 d; the maximum value obtained was more than the phycobiliprotein content of the control group. The algal cells might achieve a stress response to external disturbances. Their protein synthesis ability was higher than that of the normal state. After 4 d, the relative content of the experimental group gradually decreased. Within 4–10 d, the relative content of the experimental group (20 kHz) was stable at 85%–92%. The absolute content of the experimental group (40 and 100 kHz) increased continuously, but the relative content decreased by 10%–16%. This finding was ascribed to the decrease of phycobiliprotein content, which can capture and deliver light energy during the early stages of ultrasound treatment. Such decrease affected the photosynthetic carbon assimilation on algae cells because these cells lacked the necessary organic compounds for growth and division (such as protein and carbohydrates). Therefore, the growth rate of the experimental group was lower than that of the control group.

3.1.2. Effect of ultrasonic power density on phycobiliprotein accumulation

The relative contents of phycobiliprotein with different ultrasonic power densities at a frequency of 40 kHz are shown in Fig. 2.

Changes in the relative contents of PE after ultrasonic treatment are shown in Fig. 2(A). Within 2 h, the relative content of PE decreased rapidly, and the levels of PE, which were at power densities of 0.0140 and 0.0245 W/mL, were 43% and 43% lower than those of the control group; the relative content of PE, which was at power density of 0.0560 W/mL, was nearly zero. After 1 day, the relative content of PE began to increase until 4 d; the relative content of PE, which was at power density of 0.0140 W/mL, was 94% and almost the same as the control group. After 4 d, the relative content of PE increased slowly, and this trend can reflect the inhibitory

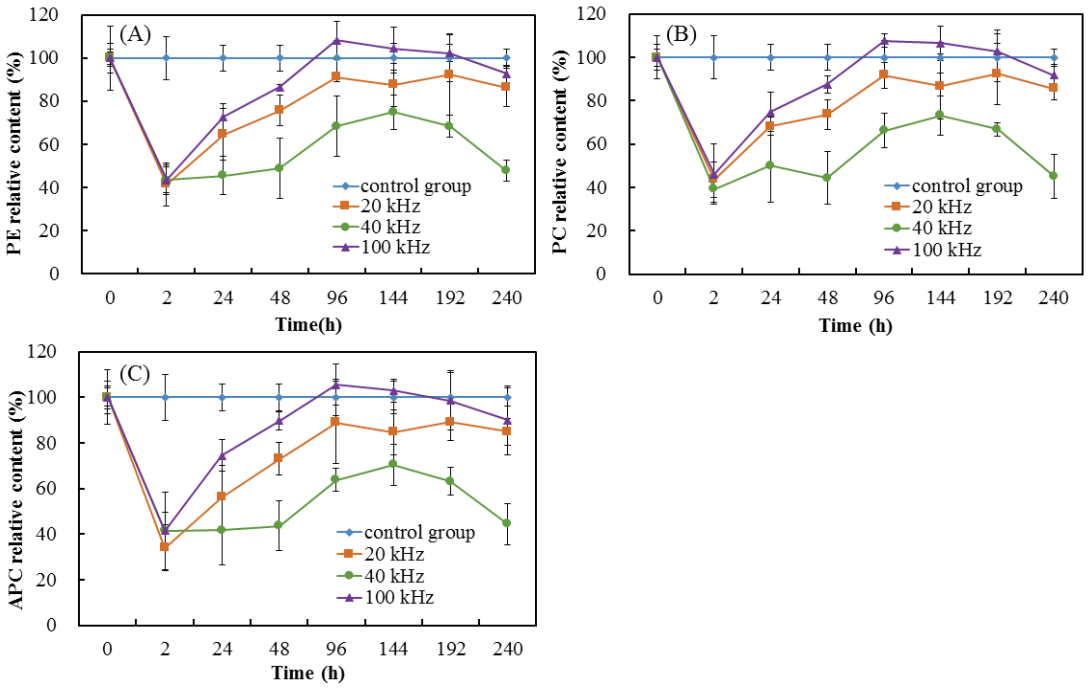

Fig. 1. Effect of frequency on the relative contents of PE (A), PC (B) and APC (C).

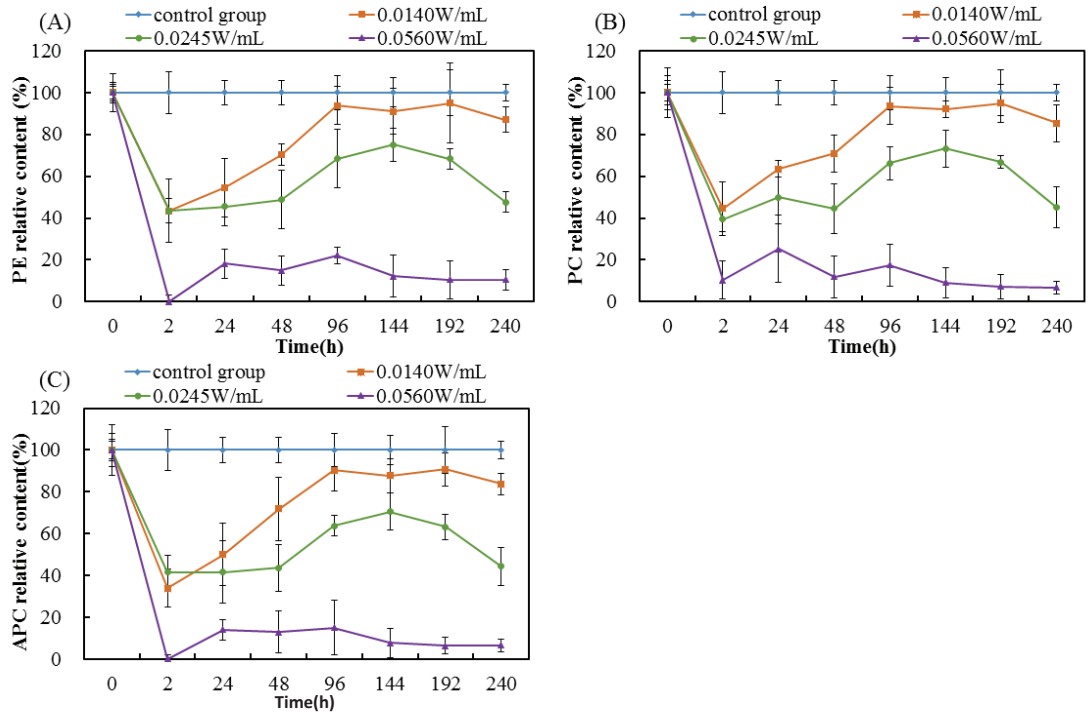

Fig. 2. Effect of power density on the relative contents of PE (A), PC (B) and APC (C) when the frequency was 40 kHz.

effect of ultrasonic treatment on the growth of algae. The biomass of the experimental group was lower than that of the control group, and the absolute content of PE was minimal because of the removal of few algal cells. Nevertheless, the algal cells could repair but cannot repair completely because of the damage; therefore, the ability of cell growth and division in the experimental group was lower than that in the control group. The results indicated that PE was damaged by ultrasound, and the ability of algal cells to capture and transfer light energy decreased.

Changes in the relative contents of PC after ultrasonic treatment are shown in Fig. 2(B). Within 2 h, the relative content of PC in the experimental group significantly decreased; when the ultrasonic power increased, the content decreased.

The relative content of PC, which was at a power density of 0.0560 W/mL, was not totally corrupted. The ability of PC to resist ultrasonic destruction was higher than that of PE and APC. The experimental group (0.0140 W/mL) first entered the repair stage. After 4 d, the relative content of PC, which was at power density of 0.0140 W/mL, was 94%. The contents of PC in both groups showed a decreasing trend, which suggested the inhibitory effect of ultrasonic treatment. The results indicated that PE was damaged by ultrasound, and the ability of algal cells to capture light energy decreased.

Changes in the relative contents of APC after ultrasonic treatment are shown in Fig. 2(C). The changes in relative contents of APC in the experimental group were similar to those in PE and PCs. Within 2 h, the relative content of APC in the experimental group with the power density of 0.0140, 0.0245 and 0.0560 W/mL under ultrasonic frequency of 40 kHz decreased by 34%, 41% and 0%, respectively. The stage of restoration of algal cells occurred from 2 h to 4 d. The relative content of APC was restored gradually; when the power density increased, the rate of repair of APC increased. Ultrasound can inhibit the growth of algal cells, resulting in a decreasing trend in the content of APC. Tao et al. [30] found that the reduction of photosynthetic pigment protein content after UV-C treatment would lower the ability to capture and transfer light energy of algal cells, because the repair and the synthesis mechanism of photosynthetic pigments were damaged. From this, it can indicated that APC of algal cells was damaged, the light energy transfer chain was cut off, and the ability to capture and transfer light energy decreased after ultrasound treatment.

3.2. Effect of the expression level of the key protein gene

3.2.1. Effect of ultrasonic frequency on gene expression level

Three frequencies (20, 40 and 100 kHz) were used in this experiment, with a power density of 0.02 W/mL. The gene expression level of samples after treatment with different ultrasonic frequencies is shown in Fig. 3.

The *psbA* gene encodes the D1 protein, and the synthesis of D1 protein is affected by the level of its expression [31]. Fig. 3 shows that ultrasonic treatment did not affect the relative expression level of the *psbA* gene. Within 2 h, the RQ value of the experimental group, which was at a frequency of 20 kHz, decreased by 96.7% compared with that of the control group. The rates of *psbA* gene expression, which were at frequencies of 40 and 100 kHz, increased by 5.9% and 12.3%. The expression level of *psbA* mRNA increased. This increase is a potential response to D1 protein denaturation, which was caused by stress, and can further increase the synthesis of D1 protein and repair the structure of the PS II reaction center.

The *rbcL* gene encodes the RUBISCO protein. The content or carboxyl activity of the *rbcL* gene was closely related to the photosynthetic rate. The expression level of the experimental group at frequencies of 20 and 100 kHz increased by 18.2% and 14.4%, respectively; this finding was associated with a protective reaction. The transcriptional level of the *rbcL* gene significantly decreased after ultrasonic treatment at a frequency of 40 kHz. The RQ value was 43.6% higher than that of the control group. The expression level of the *rbcL* gene was significantly inhibited.

The *cpc* gene encodes the PC protein. The content of PC can affect the transfer of light energy in the photosynthetic system. The expression of the *cpc* gene was similar to that of the *rbcL* gene in terms of ultrasonic frequency. The relative expression levels of the experimental groups at frequencies of 20, 40 and 100 kHz were 127.7%, 48.2% and 94.4%, correspondingly. Ultrasonic treatment at frequency of 100 kHz slightly influenced the transcription of the *cpc* gene. By contrast, ultrasonic treatment at frequency of 40 kHz significantly inhibited the expression of the *cpc* gene.

3.2.2. Effect of ultrasonic power density on gene expression level

The relative expression levels of the target gene under different power densities with a frequency of 40 kHz are shown in Fig. 4. The expression of the *psbA* gene was slightly influenced. The RQ value of the experimental group at a power density of 0.0140 W/mL was 90.7% higher than that of the control group. The RQ values at power densities of 0.0245 and 0.0560 W/mL increased. The transcription levels of the *rbcL* and *cpc* genes were significantly inhibited compared with those of the *psbA* gene. The RQ value decreased by more than 40%. Compared with the RQ values in the experimental group with power densities of 0.0245 and 0.0140 W/mL, the expression levels of the *rbcL* and *cpc* genes were not significantly different. These results indicated that the effects of ultrasonic treatment on gene expression were similar to those

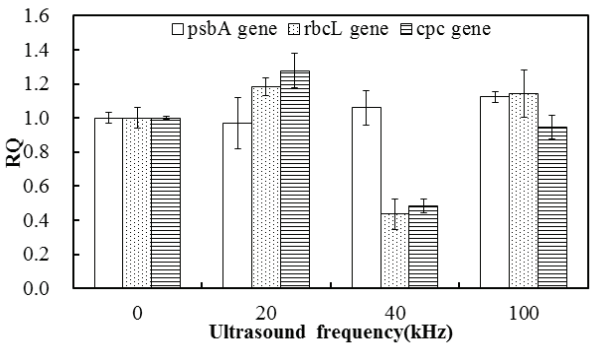

Fig. 3. Effect of frequency on the relatively quantitate expression level of genes.

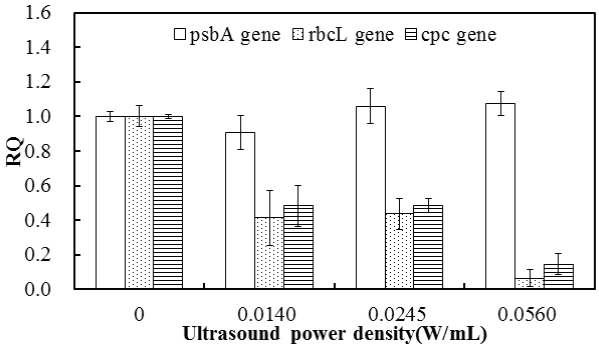

Fig. 4. Effect of power density on the relatively quantitate expression level of genes.

under relatively low power density. When the power density was increased to 0.0560 W/mL, the RQ value significantly decreased, and the *rbcL* and *cpc* gene expression levels were only 6.3% and 14.5%. The photosynthetic rate of algal cells also significantly decreased. The content of PC treated with the same ultrasonic parameters decreased to 10.3% within 2 h. The transfer process of the *cpc* gene was blocked by ultrasonic treatment, and the content of PC also decreased.

3.3. Effect on sedimentation of algal cells

By analyzing the changes in the photosynthesis system of algal cells after ultrasonic treatment, three frequencies can damage the structure and function of algal cells. Among these frequencies, 40 kHz significantly damaged the photosynthesis system and physiological activity. To achieve the purpose of preventive inhibition, the effect of different parameters on the long-term inhibition of algae biomass was investigated. In this study, the density and sedimentation indexes of *M. aeruginosa* were compared, the effects of ultrasonic treatment on algae were obtained directly, and the control effect of 40 kHz on algae biomass was verified.

Algal cells produced sedimentation when the algal cells containing gas vacuoles were treated with ultrasonic waves. The algal cells of the control group would grow and settle naturally without any treatment, and some aging cells settled slowly. Given that the structures of gas vacuoles [7,12] were damaged, some of the algal cells in the experimental group gradually settled down to the bottom of the tube. Hence, the sedimentation rate and quantity are related to the ultrasonic parameters. The effects of frequency and power density on the sedimentation of *M. aeruginosa* are mainly discussed in this study.

3.3.1. Effect of ultrasonic frequency on the sedimentation of M. aeruginosa

The changes of settlement index after different frequencies with a power density of 0.02 W/mL are shown in Figs. 5 and 6. The settlement index rose the fastest within 24 h under ultrasonic treatment for 5 min at three frequencies. The results showed that the algal cells achieved the most rapid deposition rate, with settlement indexes of 39%, 65% and 63%. Approximately 39%–65% of all the algal cells produced sedimentation after the ultrasonic treatment. As shown in Fig. 5, we observed that large number of algal cells sank to the bottom of the container.

The settlement index of the experimental group reached saturation within 48 h and remained stable between 48 and 144 h, to some extent. Furthermore, the *M. aeruginosa* began to repair the damaged gas vacuoles and synthetize new gas vacuoles. The number of suspended algal cells increased gradually. The settlement indexes of the experimental groups at frequencies of 40 and 100 kHz were similar. The settlement index of the experimental group under 40 kHz frequency was slightly higher than that of the experimental group under 100 kHz frequency. The settlement index of the experimental group with a frequency of 20 kHz was the least and began to decrease after 144 h. The biomass increased significantly at that time. Rodriguez-Molares et al. [29] treated *M. aeruginosa* with a frequency of 21.5 kHz and power density of 0.0137 W/mL. The sedimentation did not reach saturation in 48 h but lasted for nearly 1 week. This finding may be related to the initial algal density. The algal density used by Rodriguez-Molares et al. [29] was approximately 13.3×10^6 cells/mL. The algae underwent different growth periods when the initial algal density was varied.

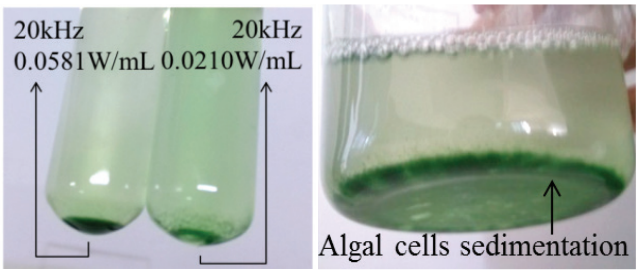

Fig. 5. Algal cell sedimentation after ultrasonic treatment.

3.3.2. Effect of ultrasonic power density on sedimentation of M. aeruginosa

The change of curve of the settlement index after different power densities, with frequencies of 20, 40 and 100 kHz, are shown in Figs. 6(B)–(D), respectively.

3.3.2.1. Settlement variation at frequency of 20 kHz With frequency of 20 kHz, the settlement index increased the fastest within 24 h and showed that the sedimentation rate of algal cells was the fastest. The experimental groups at power densities of 0.0210 and 0.0581 W/mL were basically saturated for 48 h and remained stable for 48–144 h, to some extent. At this stage, gas vacuoles began to repair, and algal cells started to divide and proliferate. The settlement indexes of the experimental groups at power densities of 0.0210 and 0.0581 W/mL began to decrease 144 h later. Cells stopped settling, and the biomass began to increase. The settlement index of the experimental group, which was at power density of 0.1029 W/mL, began to increase after 48 h and reached 100% within 192 h. The intensity of ultrasound caused the non-recoverable damage on algal cells. As the power density increased, the settlement index of each time point increased, and the number of settling cells was large.

3.3.2.2. Settlement variation at frequency of 40 kHz With frequency of 40 kHz, the settlement index increase was the fastest within 24 h, with different variation rules. The settlement index of the experimental group, which was at power density of 0.0140 W/mL, first increased and then decreased. This finding showed that the cell sedimentation quantity was low during the initial stage of ultrasound; some cells did not sink, but the physiological activity was affected, and the number of cells increased slowly during the late stage. The settlement index of the experimental group, which was at power density of 0.0245 W/mL, was saturated within 48 h at approximately 76.5%. The settlement index of the experimental group, which was at power density of 0.0560 W/mL, reached nearly 100% after 96 h. Algal cells were almost all off. However, the suitable light and temperature cannot be

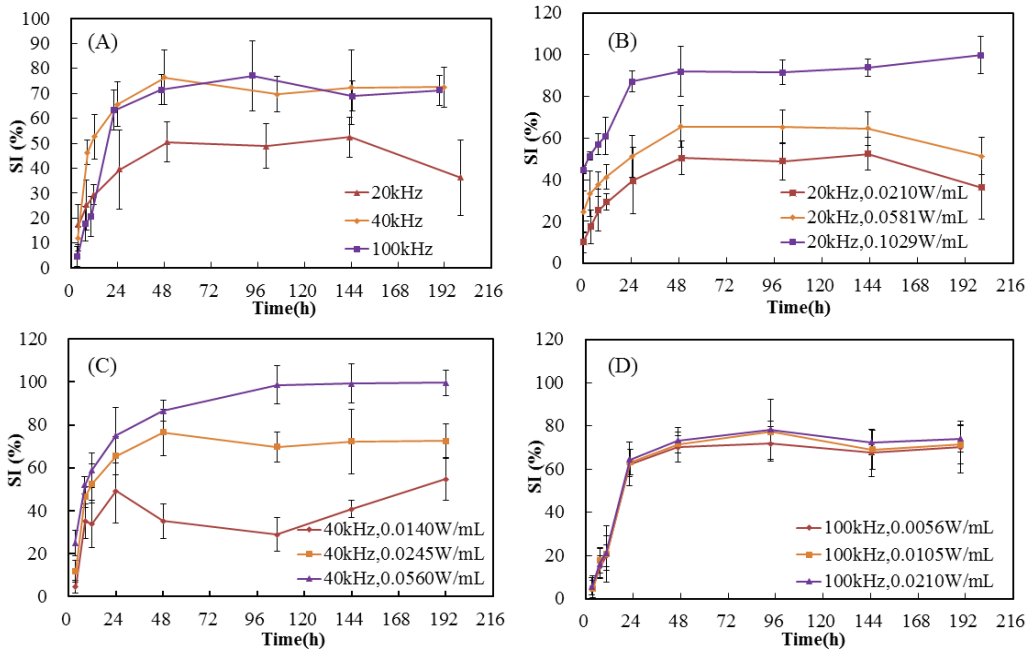

Fig. 6. Effect of frequency on sedimentation index (SI) (A). Effect of power density on sedimentation index (SI) when the frequency was 20 kHz (B), 40 kHz (C) and 100 kHz (D), respectively.

restored. When the power density increased, the number of settling cells also increased.

3.3.2.3. Settlement variation at frequency of 100 kHz

Compared with the effect of ultrasonic treatment of 20 and 40 kHz, given the power density of each experimental group with only a small difference to a frequency of 100 kHz, the settlement index of the experimental group presented a small difference during the culture period, and the change trends were similar. With a frequency of 100 kHz, the settlement index increased the fastest within 24 h. This finding showed that the sedimentation rate of algal cells was the fastest and stable between 48 and 192 h, to some extent. At this stage, gas vacuoles began to repair, the damaged cells floated and started to divide and proliferate, and the biomass increased gradually. When power density increased, the settlement index also increased. This result indicated that the number of algal cells with damaged gas vacuoles in structure was large.

During the initial stage of ultrasound, the gas vacuoles of *M. aeruginosa* were damaged by cavitation and cannot provide buoyancy for cells. Algal cells can reabsorb and synthesize proteins needed for the ruptured gas vacuoles and create new gas vacuoles. Each gas vacuole was shaped like a small double cone originally, and then the shape changed to an oval gradually. This process probably occurred within 48 h. Cells can reconstruct large gas vacuoles to prevent settlement. After 96–144 h, the buoyancy provided by the gas vacuoles can stop settling. Ultrasound can destroy the gas vacuoles and was conducive to remove *M. aeruginosa* in actual water by ultrasonic treatment. Moreover, *M. aeruginosa* colonially existed in the form when the number of algal cells was the same and the moving friction of congeries were small; the sedimentation velocity was great, and the settlement was enhanced by ultrasonic treatment. When the algal cells sank to the water layer with less light, cells failed to carry out photosynthesis without regenerating gas vacuoles.

4. Conclusions

Ultrasonic treatment can damage the photosynthetic pigments, including PE, PC and APC of *M. aeruginosa*. The ability of algal cells to capture light energy was reduced, and the light energy transfer chain was cut off. At the same time, ultrasonic treatment inhibited the transcription of the encoding gene of D1 protein, RUBISCO and PC. Such treatment can affect the synthesis of proteins and repair mechanisms. Under the same frequency and increased power density, the sedimentation index increased, and the number of sedimentation cells also increased. Therefore, we recommend the use of ultrasound with frequency of 40 kHz to control the ultrasonic intensity and avoid cell break because algal cells possess the ability of self-repair and reproliferation.

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References

[1] G. Fan, D. Liu, G. Zhu, Q. Lin, L. Chen, Influence factors in kinetics during removal of harmful algae by ultrasonic irradiation process, Desal. Wat. Treat., 52 (2014) 7317–7322.

[2] D.M. San, R.V. Azanza, C.L. Villanoy, G.S. Jacinto, Eutrophic waters, algal bloom and fish kill in fish farming areas in Bolinao, Pangasinan, Philippines, Mar. Pollut. Bull., 57 (2008) 295–301.
[3] D.W. Schindler, Evolution of phosphorus limitation in lakes, Science, 195 (1977) 260–262.
[4] S.K. Choi, J.Y. Lee, D.Y. Kwon, K.J. Cho, Settling characteristics of problem algae in the water treatment process, Water Sci. Technol., 53 (2006) 113–119.
[5] G. Fan, J. Luo, J. Chen, Q. Lin, L.C.R. Lin, Influence of ultrasonic intensity on *Microcystis* sp. viability: a flow cytometric analysis, J. Appl. Microbiol., 8 (2014) 519–524.
[6] G. Zhang, P. Zhang, H. Liu, B. Wang, Ultrasonic damages on cyanobacterial photosynthesis, Ultrason. Sonochem., 13 (2006) 501–505.
[7] H. Hao, M. Wu, Y. Chen, J. Tang, Q. Wu, Cavitation mechanism in cyanobacterial growth inhibition by ultrasonic irradiation, Colloids Surf., B, 33 (2004) 151–156.
[8] H. Hao, M. Wu, Y. Chen, J. Tang, Q. Wu, Cyanobacterial bloom control by ultrasonic irradiation at 20 kHz and 1.7 MHz, J. Environ. Sci. Health, Part A, 39 (2004) 1435–1446.
[9] T.J. Lee, K. Nakano, M. Matsumare, Ultrasonic irradiation for blue-green algae bloom control, Environ. Technol., 22 (2001) 383–390.
[10] Y. Qiu, F. Rong, F. Yang, J. Li, H. Long, W. Wu, Ultrasound frequency impacts on the removal of indigenous blue-green algae taken from Lake Taihu, Adv. Mater. Res., 383–390 (2012) 3758–3762.
[11] J. Li, H. Long, C. Song, W. Wu, T.O. Yeabah, Y. Qiu, Study on the removal of algae from lake water and its attendant water quality changes using ultrasound, Desal. Wat. Treat., 52 (2014) 4762–4771.
[12] T.J. Lee, K. Nakano, M. Matsumura, A new method for the rapid evaluation of gas vacuoles regeneration and viability of cyanobacteria by flow cytometry, Biotechnol. Lett., 22 (2000) 1833–1838.
[13] J. Caspermeyer, Getting to the origins of photosynthesis, Mol. Biol. Evol., 32 (2015) 1658–1659.
[14] K. Gao, Algal Carbon Fixation-Basis, Advances and Methods, Science Press, Beijing, 2014.
[15] W. Mai, M. Ikeuchi, Phycobilisome: architecture of a light-harvesting supercomplex, Photosynth. Res., 116 (2013) 265–276.
[16] I.N. Stadnichuk, P.M. Krasilnikov, D.V. Zlenko, Cyanobacterial phycobilisomes and phycobiliproteins, Microbiology, 84 (2015) 131–143.
[17] Q. Haifeng, L. Jingjing, P. Xiangjie, J. Haiyan, S. Liwei, F. Zhengwei, Photoperiod and temperature influence cadmium's effects on photosynthesis-related gene transcription in *Chlorella vulgaris*, Ecotoxicol. Environ. Saf., 73 (2010) 1202–1206.
[18] W. Hou, C. Xiao, G. Song, Q. Wang, C.C. Chang, Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (*Lemna minor*), Plant Physiol. Biochem., 45 (2007) 62–69.
[19] B. Xiong, W. Zhang, L. Chen, K. Lin, M. Guo, W. Wang, X. Cui, H. Bi, B. Wang, Effects of Pb(II) exposure on *Chlorella protothecoides* and *Chlorella vulgaris* growth, malondialdehyde, and photosynthesis-related gene transcription, Environ. Toxicol., 29 (2014) 1346–1354.
[20] C.C. Bowen, T.E. Jensen, Blue-green algae: fine structure of the gas vacuoles, Science, 147 (1965) 1460–1462.
[21] A.E. Walsby, The permeability of blue-green algal gas-vacuole membranes to gas, Proc. R. Soc. London, Ser. B, 173 (1969) 235–255.
[22] P.K. Hayes, A.E. Walsby, The inverse correlation between width and strength of gas vesicles in cyanobacteria, Brit. Phycol. J., 21 (1986) 191–197.
[23] H. Lehmann, M. Jost, Kinetics of the assembly of gas vacuoles in the blue-green alga *Microcystis aeruginosa*, Arch. Microbiol., 79 (1971) 59–68.
[24] G. Zhang, P. Zhang, B. Wang, H. Liu, Ultrasonic frequency effects on the removal of *Microcystis aeruginosa*, Ultrason. Sonochem., 13 (2006) 446–450.
[25] J. Tang, Q. Wu, H. Hao, Y. Chen, M. Wu, Effect of 1.7 MHz ultrasound on a gas-vacuolate cyanobacterium and a gas-vacuole negative cyanobacterium, Colloids Surf., B, 36 (2004) 115–121.
[26] P. Rajasekhar, L. Fan, T. Nguyen, F.A. Roddick, Impact of sonication at 20 kHz on *Microcystis aeruginosa*, *Anabaena circinalis* and *Chlorella* sp., Water Res., 46 (2012) 1473–1481.
[27] Y. Tao, X. Mao, J. Hu, H.O.L. Mok, L. Wang, D.W.T. Au, J. Zhu, X. Zhang, Mechanisms of photosynthetic inactivation on growth suppression of *Microcystis aeruginosa* under UV-C stress, Chemosphere, 93 (2013) 637–644.
[28] M.P. Padgett, D.W. Krogmann, Large scale preparation of pure phycobiliproteins, Photosynth. Res., 11 (1987) 225–235.
[29] A. Rodriguez-Molares, S. Dickson, P. Hobson, C. Howard, A. Zander, M. Burch, Quantification of the ultrasound induced sedimentation of *Microcystis aeruginosa*, Ultrason. Sonochem., 21 (2014) 1299–1304.
[30] Y. Tao, X. Zhang, D.W.T. Au, X. Mao, K. Yuan, The effects of sub-lethal UV-C irradiation on growth and cell integrity of cyanobacteria and green algae, Chemosphere, 78 (2010) 541–547.
[31] P. Mulo, I. Sakurai, E.M. Aro, Strategies for psbA gene expression in cyanobacteria, green algae and higher plants: from transcription to PSII repair, Biochim. Biophys. Acta, 1817 (2012) 247–257.

Supporting information

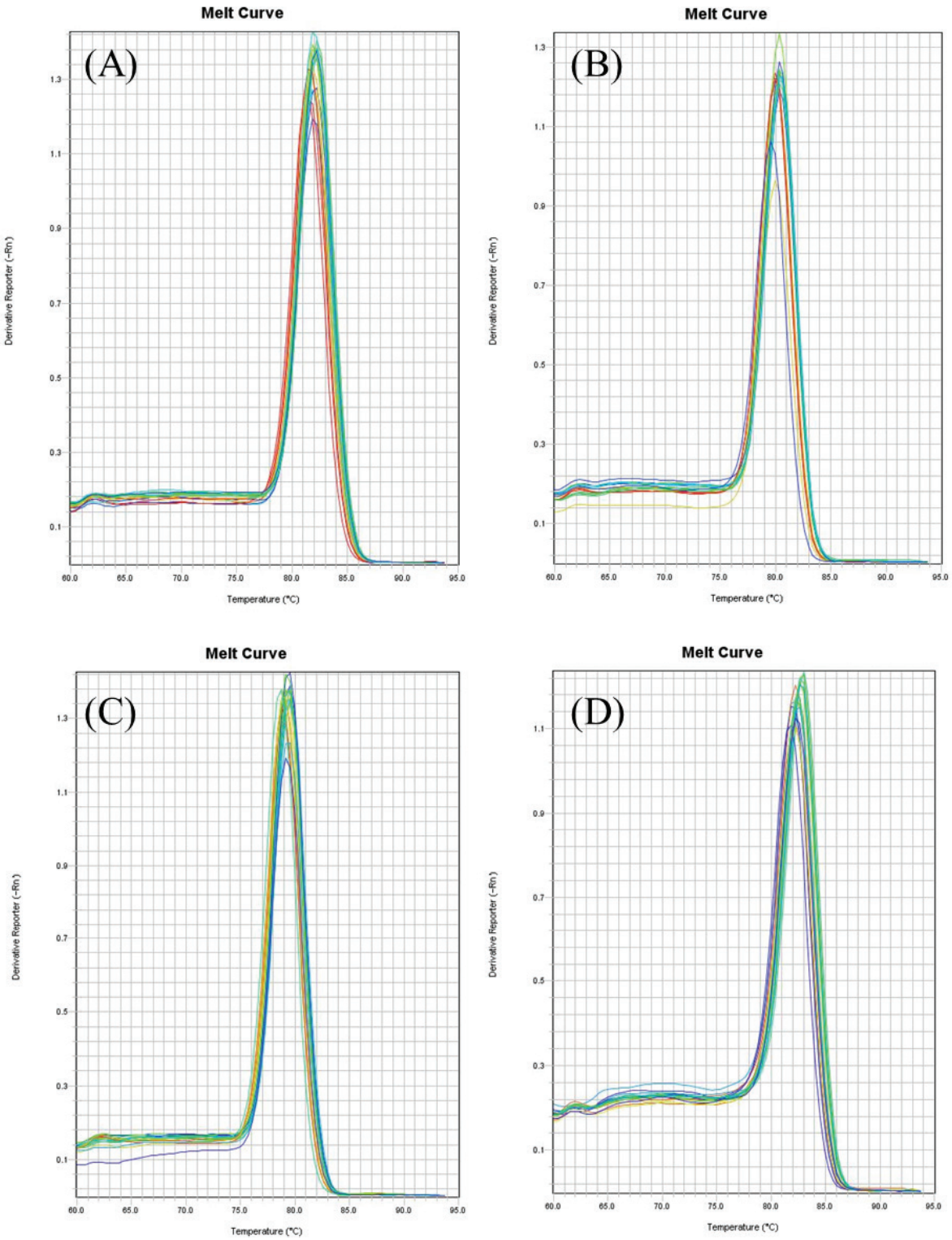

Fig. S1. The melting curve of four kinds of genes (A) 16s rRNA; (B) *psbA* gene; (C) *rbcL* gene and (D) *cpc* gene.