Desalination and Water Treatment
www.deswater.com

doi:10.5004/dwt.2017.20218

68 (2017) 70–79
March

Preventive inhibition mechanism of algae by ultrasound based on analysis of physiological characteristics

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Received 11 June 2016 ; Accepted 5 September 2016

ABSTRACT

Blooms of cyanobacteria cause ecological imbalance, killing a large number of water plants, fish and other aquatic organisms. In addition to easily cause algae toxin emission, the traditional processing method has drawbacks of short period of time and high energy consumption. Ultrasonic technology, as the environmental and effective method, is put forward to control the algae biomass. However, most studies based on inhibitory effect of ultrasound irradiation on algae were conducted at the algae photosynthesis system, the physiological activity of other indicators was not in-depth studied, and most of which were the short-term effects with unknown timeliness. The objective of this paper was to explore the suitable ultrasonic parameters to remove algae and the influence of ultrasonic frequency and power on physiological activity both from physiological activity and biomass control of algae cells. The results showed that the physiological and metabolic activities of algae were lower and the number of intact cells decreased after ultrasonic radiation. The proportion of damaged cells was proportional to the ultrasonic power density. DNA fragments were not detected after ultrasonic irradiation and DNA chain structure remained complete. The effects of ultrasonic parameters on algae density were also studied. When the power density was 0.02 W/mL, the effect of treatment under ultrasonic frequency of 40 kHz was better than that of 20 kHz and 100 kHz. It is suggested to use ultrasonic treatment with the frequency of 40 kHz and power density of 0.0245 W/mL to treat the blue-green algae.

Keywords: Ultrasound; *Microcystis aeruginosa*; Esterase activity; Cells integrity; Inhibition ratio

1. Introduction

Cyanobacterial bloom refers to the phenomenon that the algae which is of rapid growth in the surface water and eventually becomes the dominat species [1]. Cyanobacterial bloom will cause ecological imbalance, killing a large number of water plants, fish and other aquatic organisms [2,3]. The radical measures in response to cyanobacterial bloom is to reduce the input of exogenous nutrients and the content of nitrogen salt and phosphate salt, which will reduce the amount of algae for its lack of necessary nutrients [4].

The traditional processing method easily leads to algae toxin emission, causing the secondary water pollution [5–7], therefore, the method of preventive inhibition for cyanobacteria using ultrasound technology [8] was put forward, by which the algae biomass could be controlled at a low certain level to avoid the occurrence of cyanobacterial bloom and its adverse effects through inhibiting the growth of algae. Ultrasonic technology is a new type of environmental technology [9] which is developing rapidly in recent years and widely used in the field of water treat-

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ment, ultrasonic cavitation can remove the trihalomethanes precursors, the disinfection by-products, persistent organic pollutants, algae toxin, harmful algae blooms and harmful refractory organic compounds etc., and thus can be used as a preventive technology to control cyanobacterial bloom. Considering that the previous treatment usually takes the ultrasonic processing as a general method to remove the algae, the study of the growth inhibition of algae and its inhibitory mechanisms by observing the changes of every index for a period of time is quite necessary.

Predecessors made some efforts on the research of the effects of ultrasound on the physiological activity of algae cells. Lee et al. [10] used the flow cytometry (FCM) to detect cell activity, the *Microcystis aeruginosa* whose gas vacuoles were collapsed after ultrasonic irradiation presented different lateral scattering diagram and fluorescence spectrum. Through the study of photosynthetic activity of Spirulina platensis, Tang et al. [11] found that the collapse of gas vacuoles could damage the enzyme in the thylakoid membrane, which affected the synthesis of chlorophyll-a and thus made photosynthetic activity weakened. Nakano et al. [12] found that the photosynthetic activity of *Microcystis aeruginosa* decreased with the increase in ultrasonic power. Zhang et al. [13] researched the influence of ultrasonic treatment on photosynthesis system of *Microcystis aeruginosa* and reported that the photosynthetic pigment content decreased and the growth of algae cells was slowed down after the ultrasonic irradiation. By using fluorimetry and flow cytometry, Wu et al. [14] also found the phycobiliprotein content decreased and the cell activity reduced after ultrasonic irradiation. The research of Wan et al. [15] showed that the Microcystis aeruginosa cells subsidence after ultrasonic irradiation, which reduced the photosynthetic activity of algae cells. Hao et al. [8] observed that the ultrasonic treatment with low power and high frequency could destroy the key components of the antenna complexes and thus inhibit the photosynthesis and the growth of algae cells. It was found by Tang et al. [16] that the damage of gas vacuoles could result in reduction of the photosynthetic activity of algae cells effectively. Moreover, a more detailed study operated by Purcell et al. [17] showed that up to 60% of photosynthetic organelles were destroyed by ultrasound at a frequency of 862 kHz. From above study, it could be concluded that the changes of physiological activity of algae cells would inevitably occur after the ultrasonic irradiation, however, most studies were conducted at the algae photosynthesis system, the physiological activity of other indicators was not in-depth studied, and most of which were the short-term effects with unknown timeliness. The mechanism of physiological activity needs to be explored by investigating the physiological activity of enzyme activity, cell integrity, and chain structure of DNA.

The ultrasonic treatment may cause the secondary pollution risk. Lee et al.'s study [12] showed that ultrasonic irradiation would lower algae toxin emission. Wu et al. [18] found that ultrasonic treatment could be used to removal algae while also reduce the release of algae toxins. Zhang et al. [13] discovered that controlling processing time could avoid the release of algae toxins. Qiu et al. [19] found that ultrasonic irradiation with low frequency could effectively reduce the release of algae toxins. Research results from Rajasekhar et al. [20] suggested that the ultrasonic can decontaminate the algae toxins and the concentration of algae toxins in the solution was decreased through extending the time of irradiation. The above researches mainly evaluated secondary pollution risk in terms of the amount of release of algae toxins. Tang et al. [11] found that the cell membrane permeability of *Microcystis aeruginosa* and *Synechococcus*'s increased after ultrasonic irradiation, and ultrasound may cause lipid peroxidation, which resulted in change of the cell membrane structure. In order to solve the secondary pollution problems which caused by the algae toxins, this research explored the effect of ultrasonic parameters on the release of cell contents from levels of cell membrane damage.

As the essential material of the metabolism process of algae cells, esterase which is contained in algae cells can catalyzed the hydrolysis and synthesis of ester compounds and be used to reflect the metabolism of cells [21]. Jamers et al. [22] found the esterase activity of green algae decreased by the effect of cadmium. Hong et al. [23] discovered that EMA could reduce the esterase activity of *Microcystis aeruginosa*. Chemical agents added to cell suspension could change esterase activity of algae, but little works about the effect of ultrasonic irradiation on esterase activity of the *Microcystis aeruginosa* have been published.

Algae which was induced by external stimulus might lead to an apoptosis of cell, the rupture of chain structure in DNA that resulted in small molecular DNA fragments is one of the main characteristics of cell apoptosis [24]. Sakai et al. [25] found that the amount of pyrimidine dimer in cell DNA of *Microcystis aeruginosa* was increased with the time of UV radiation, in addition, the amount of pyrimidine dimer was associated with the DNA fragment length, the increase of the amount of pyrimidine dimer meaned that DNA chain structure had changed, however, the DNA molecular structure gradually recovered after UV radiation. DNA molecular structure can affect the growth of cells, thus the reason that cell growth was inhabited which be inhibited can be explored by scientific investigation of the effect of ultrasonic irradiation on DNA chain structure.

In this paper, *Microcystis aeruginosa* was chosen as the cyanobacteria species, the combination of the ultrasound parameters which is suitable for inhibiting the growth of cyanobacterial blooms was screened out through the study of the effect of ultrasound on esterase activity, cell integrity and DNA chain structure of algae cells. In addition, a comparison of the control effect of different ultrasonic parameters on the biomass of algae was made to provide the theoretical foundation for large-scale application of ultrasound technology for inhibiting algae in water body.

2. Materials and methods

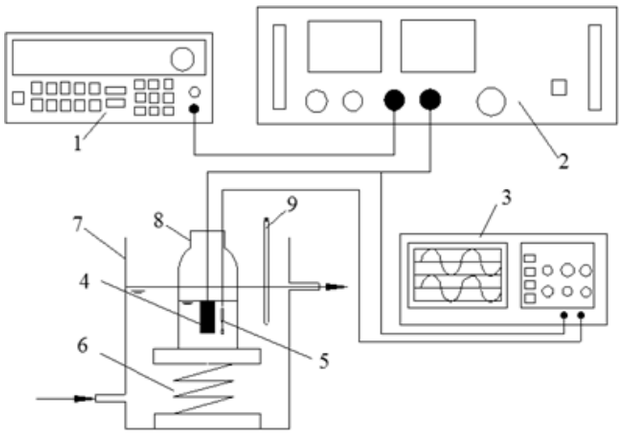
2.1. Algae species and ultrasonic processing

The sample of the experiment was *Microcystis aeruginosa* separated from the Dianchi Lake and numbered FACHB-905, which was purchased from Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB). The frequency of 20 kHz, 40 kHz and 100 kHz with power density fixed to 0.02 W/mL were carried out, and the power density of 0.0140 W/mL, 0.0245 W/mL and 0.0560 W/mL with the frequency fixed to 40 kHz

were employed, respectively, to treat *Microcystis aeruginosa* suspension. The schematic diagram of ultrasonic device is shown in Fig. 1. The apparatus for all experiments were bath type. Ultrasonic irradiation time of each group was 5 min. Algal cell solution volume is approximately 100 mL. It should be pointed out that the greater the algae density, the shorter the cell repair time. The growth of algal cells in the logarithmic phase holds better resistance to the ultrasonic. As for the *Microcystis aeruginosa*, it was more sensitive to sonication during the logarithmic phase. Most researchers usually focus on the algae during this growth phase. Therefore, initial algal density was controlled approximately to 8.3×10^6 cells/mL. The algae cells of the control group and experimental group were cultured for 6–10 d under appropriate condition. The enzyme activity of ester, the integrity of algae cell membrane and DNA chain structure was detected at a certain time. The curves of algae density and inhibition rate was drawn by using blood count plate to count.

2.2. Esterase activity

The fluorescein diacetate (FDA) was used to measure cell activity of esterase referring to the method of Manuela D. Machado et al. (Machado and Soares, 2013) FDA is a non-polar and hydrophobicity compounds without fluorescent, it can permeate through cell membrane and be hydrolyzed by nonspecific esterase in the cell. For instance, the hydrolysates of lipase and acyltransferase [26] were acetic acid molecules and fluorescein, the fluorescence intensity of fluorescein at 525 nm was found to be the largest. Decreases in the fluorescence intensity indicated that the esterase activity was decreased. Therefore, the fluorescence intensity can be used to characterize the esterase activity of algae cells. The relative activity of esterase can be calculated according to the ratio of fluorescence intensity of experimental group to control group. Computation formula is as follows:



1. Signal Generator; 2. Power Amplifier; 3. Oscilloscope;
4. Transducer; 5. Hydrophone; 6. Telescopic stand;
7. Cooling device; 8. Glass flask; 9. Thermometer

Fig. 1. Schematic diagram of ultrasonic device.

$$\text{The relative activity of esterase} = \frac{\text{Mean fluorescence intensity}_{\text{experimental group}}}{\text{Mean fluorescence intensity}_{\text{control group}}} \quad (1)$$

2.3. Cell integrity and DNA fragmentation testing

Cell membrane integrity of algae was analyzed by using FACSCalibur flow cytometry and the excitation wavelength was detected at 488 nm. Collecting PI fluorescence through FL3 channels and collecting 50000 cells from FL3 channels at each interval, then Kaluze Analysis software (Beckman Coulter company, USA) was used to collect and analyze signals, from which the ultrasonic parameters that inhibit the growth of algae while offer a much less membrane damage can be determined.

Through the method of agarose gel electrophoresis, gel separated after electrophoresis was placed on electrophoresis imager (Bio-Rad Imaging System) in the UV light to be imaged, and then the image was analyzed and outputted by Image Lab 3.0 software to detect the DNA fragmentation of algae cells by ultrasonic irradiation.

2.4. Algae density and growth inhibition ratio

Ultrasonic wave with the frequency of 20 kHz, 40 kHz and 100 kHz were used to treat *Microcystis aeruginosa* suspension, respectively. Then the algae cells which was trained to the specific time after ultrasonic irradiation was picked up and fixed by Lugo reagent. After that, each sample was counted at least three times using blood count plate (1 mm × 1 mm × 0.1 mm) and the mean was taken to eradicate any discrepancies.

The inhibition rate (I_r) [27] which is based on the growth rate of algae cells can be used to evaluate the inhibition effect of ultrasonic irradiation on the growth of algae cells. Computation formula is as follows:

$$I_r(\%) = \frac{V_c - V_T}{V_c} \times 100 = \left(1 - \frac{\ln N_{T_i} - \ln N_{T_o}}{\ln N_{C_i} - \ln N_{C_o}}\right) \times 100 \quad (2)$$

where I_r is inhibition rate which is based on the growth rate of algae cells, V_c is the cell growth rate of control group, V_T is the cell growth rate of experimental group, N_{Ti} is the algae density of experimental group that has been treated for i min, N_{To} is the initial algae density of experimental group, N_{Ci} is the algae density of control group that has been treated for i min, N_{C0} is the initial algae density of control group.

3. Results and discussion

3.1. Effect of ultrasonic irradiation on physiological activity of algae cells

3.1.1. Enzyme activity

3.1.1.1. Effect of ultrasonic frequency on esterase activity of algae cells

The change of relative esterase activity under ultrasonic irradiation with different frequency is shown in Fig. 2a. The changes of esterase activity after ultrasonic treatment with the ultrasonic frequency of 20 kHz, 40 kHz and

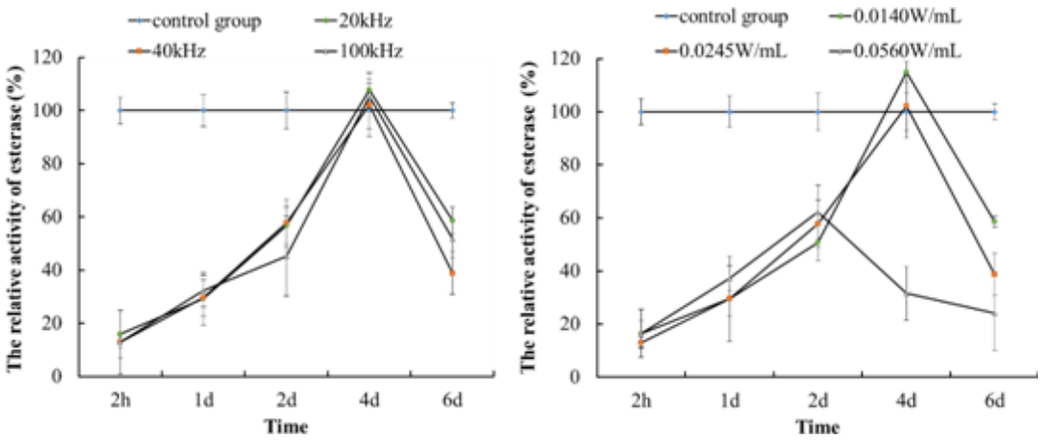


Fig. 2. The relative activity of esterase after ultrasonic irradiation with different frequency and fixed power density of 0.02 W/mL (A); with different power density and fixed frequency of 40 kHz (B).

100 kHz were similar, which presented the trend that gradually increased firstly and then decreased on the whole. The esterase activity of each experimental group that has been treated for 2 h decreased by about 85%. Unifies the data of algae density which showed that the algae density decreased by 14 –22% two hours after ultrasound irradiation. It can thus be seen that the apparent reduction of the esterase activity is associated with the death of algae cells. Thereafter, the relative esterase activity of the experimental group gradually increased and reached the highest value in 4th day. The highest value was slightly higher than the esterase activity level of the control group, the relative esterase activities of the experimental group under ultrasonic frequency of 20 kHz, 40 kHz and 100 kHz were 1.07 times, 1.02 times and 1.05 times of controls, respectively.

The relatively high esterase activity of the algae cells may be related to the ability of some damaged cells to repair themselves. In this stage, the biochemical reaction rate is accelerated, which results in demands for more esterase to keep catalytic reactions going and thus adapt to the adverse environmental conditions and eventually avoid the death of algae cells. Similar results were also obtained by Jamers et al. who studied the effects of cadmium on the esterase activity of green algae [22]. From Fig. 2a we can also find that the relative esterase activity of the experimental group was decreased rapidly four days later. The relative esterase activities of the experimental group under ultrasonic frequency of 20 kHz, 40 kHz and 100 kHz were decreased to 58.7%, 38.8% and 51.7% respectively in sixth day, from which we can see that the esterase activity of algae cells under ultrasonic frequency of 40 kHz was the lowest. The metabolic activity of algae cells increased at first and then decreased, which indicated that a series of effects of ultrasonic irradiation could inhibited the growth and recovery of algae cells, leading to the process of cell death. The metabolic rate was then slowed down and the esterase activity was reduced.

3.1.1.2. Effect of ultrasonic power density on esterase activity of cells

The curve of change of the relative esterase activity under ultrasonic irradiation with different power density and fixed frequency of 40 kHz is shown in Fig. 2b. After ultrasonic irradiation with the frequency of 40 kHz for 5 min, the relative esterase activity of algae cells also showed the trend to become higher at first and then get lower. However, it must be pointed out that the time points of the sudden fall were different under ultrasonic irradiation with different power density. The relative esterase activity of each experimental group that has been treated for 2 h decreased by about 85%. The apparent reduction of the esterase activity is associated with the death of algae cells. The relative esterase activity increased gradually from 2 h to 2 d after ultrasonic irradiation. In addition, the relative esterase activity the experimental group with the ultrasonic power density of 0.0560 W/mL was slightly higher than the other two groups with smaller ultrasonic power density. The analysis of the results above showed that the greater the power density, the more severe the lesion of algae cells, which resulted in demands for more esterase to keep catalytic reactions going and thus promote cell repair. The esterase activity of the experimental group with the power density of 0.0560 W/ mL was decreased four two days later, while the treatment groups with the power density of 0.0140 W/ mL and 0.0245 W/ mL began to decrease after the fourth day. The different time points of the sudden fall indicated that the ultrasonic intensity would affect the process of cell repair. Some of the algae cells suffered permanent damage and triggered an inability to restore breaks, leading to the process of cell death, in addition, some algae cells experienced decreased growth and the esterase content compared to the cells of control group. The esterase activity of experimental group with the power density of 0.0140 W/ mL and 0.0245 W/mL at the fourth day was higher than that in the control group, which was related to the cellular stress response. On the sixth day, the esterase activity of experimental group was decreased to 58.6%, 38.3% and 24.2% respectively. The greater the ultrasonic power density, the lower the relative esterase activity.

3.1.2. Cell integrity

3.1.2.1. Effect of ultrasonic frequency on the integrity of the cell membrane

The proportion of living cells and damaged cells of *Microcystis aeruginosa* under ultrasonic irradiation with

different frequencies is shown in Fig. 3a. The proportion of damaged cells increased with the treatment of various ultrasonic frequencies. The damaged cells of experimental group with the frequency of 20 kHz, 40 kHz and 100 kHz increased by 17.3%, 25.9% and 25.2%, respectively, compared to control group, which indicated that the structure of cell membrane was destroyed by ultrasonic irradiation, resulting in the increase of the number of cells that damaged in the membrane structure. The cavitation effect of ultrasonic in water can produce the hydroxyl radical (·OH). In this part of experiment, ultrasonic irradiation was acted on algae suspension with the volume of 240 mL under the frequency of 40 kHz, producing about 0.5 μmol/L hydroxyl radical. Actually, the hydroxyl radical has a strong oxidation, which results in induction of peroxidation reaction of unsaturated fatty acid on the cell membrane and the increase of the cell membrane permeability. Wan Li et al. [28] found that organic matter exudation of *Microcystis aeruginosa* was increased after ultrasonic irradiation, which was due to the increase of the permeability of cell membrane. Mason et al. [29] suggested that the higher the ultrasonic frequency, the more hydroxyl radical generated by cavitation. The results from this paper also showed that ultrasonic irradiation with the frequency of 40 kHz and 100 kHz were more destructive to the cell membrane compared to the lower frequency of 20 kHz. In addition, the proportion of living cells in the control group was only 47.5%. Similar results were obtained by Wu et al. [14] who used flow cytometry to detect cell activity, this is due to the fact that the living cell samples also contains a part of the cells that die natural deaths.

3.1.2.2. Effect of ultrasonic power density on the integrity of the cell membrane

The proportion of live cells and damaged cells of *Microcystis aeruginosa* with different power density and fixed frequency of 40 kHz is shown in Fig. 3b. The increment of the proportion of damaged cells after ultrasonic irradiation was positively correlated with the power density. The correlation coefficient is 0.92. The proportion of damaged cells were increased by 11.6%, 25.8% and 34.9%, which implied that the integrity of cell membrane and the content of biliproteins was the lowest with the power density of 0.0560 W/ mL. The poorer content of biliproteins under higher power density might be related to lipid peroxidation. In fact, the phycobiliprotein of polymer-phycobilisomes attached to the thylakoid membrane [30] of which lipid constituents contained polyunsaturated fatty acids [31]. The comprehensive detection indexes showed that the suitable ultrasonic parameters of the power density in the practical application was about 0.0245 W/mL, to which the number of membrane damaged cells corresponded were relatively small compared with the experimental group with the power density of 0.0560 W/mL, leading to low energy consumption and a relatively small risk of the release of cellular contents, such as toxins and harmful metabolites, to the water body. The cells inhibition which induced by the ultrasonic irradiation with the power density of 0.0245 W/mL was significantly increased compared with the experimental group of the power density of 0.0140 W/mL, which result in better control effect on algae biomass.

3.1.3. Chain structure of DNA

DNA ladder detection can reflect the change of DNA chain structure of DNA. The result of electrophoresis of the control group and the experimental group of DNA is shown in Fig. 4. The left-most strip was Marker DNA, which was used to estimate the size of the DNA fragment. During the period of cultivation, the DNA samples of the control group and the experimental group always maintained a bright band. However, the band of the experimental group was relatively dark, which was likely due to the decrease of the algae cell density after ultrasonic treatment, resulting in smaller concentration of the isolated DNA. The molecular weights of DNA were similar between the experimental group and control group, in addition, the fragment length of DNA was a little more than 8000 bp and did not present the apoptotic DNA ladder pattern, which indicated that chain structures of DNA in algae cells remained intact after ultrasonic irradiation with the frequency of 20 kHz, 40 kHz and 100 kHz. Tao Yi et al. [32] used UV-C irradiation to treat *Microcysits aerugisona*, result of DNA electrophoresis also showed that the chain structure of DNA remained complete and DNA molecules did not damaged after UV-C irradiation. Pescheck et al. [33] found that the cyclobutane pyrimidine dimers (CPD) appeared in the structure of DNA was likely to hinder the replication and transcription of DNA

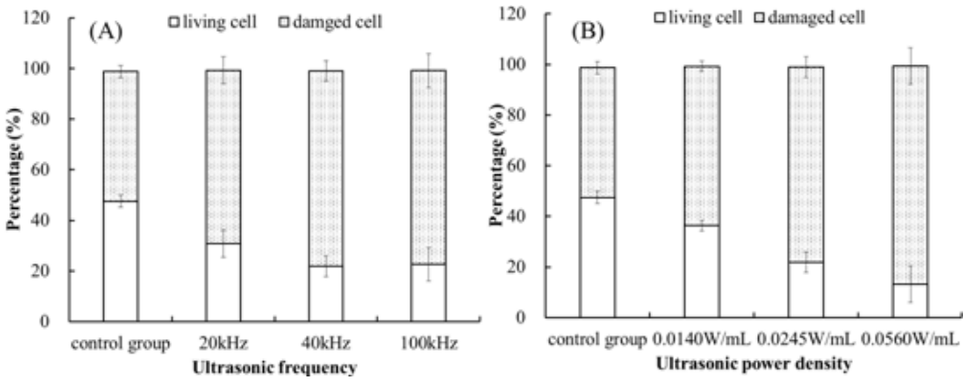
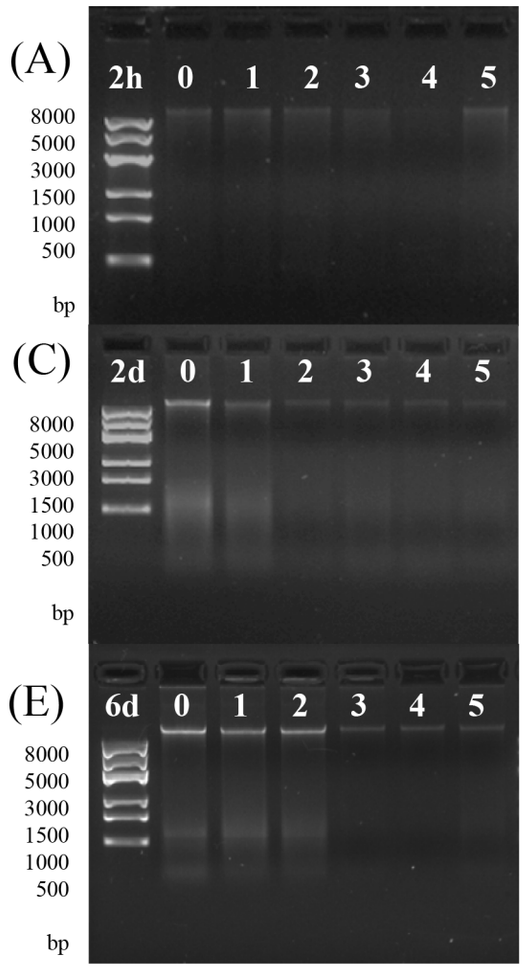


Fig. 3. The proportion of the cells after ultrasonic irradiation with different frequency and fixed power density of 0.02 W/mL (A); with different power density and fixed frequency of 40 kHz (B).



Number	Ultrasonic parameters
0	Control
1	20 kHz, 0.0210 W/mL
2	40 kHz, 0.0140 W/mL
3	40 kHz, 0.0245 W/mL
4	40 kHz, 0.0560 W/mL
5	100 kHz, 0.0210 W/mL

Fig. 4. Gel electrophoresis of DNA (A - 2 h; B - 1 d; C - 2 d; D - 4 d; E - 6 d).

and eventually result in the death of cells after the UV-C irradiation. However, a further study is needed on the influence of ultrasound irradiation on the molecular composition of DNA in algae cells.

3.1.4. Mechanism of physiological activity

By investigating the physiological activity of enzyme activity, cell integrity, and chain structure of DNA, it seems fair to presume the mechanism of physiological activity, which is presented as follows. The metabolic activity of algae cells began to decrease after ultrasonic irradiation for four days with the frequency and power density of 40 kHz and 0.0245 W/mL, respectively, in addition, the metabolic activity fell to 61.9% on the sixth day, which suggested that a series of effects of ultrasonic irradiation could inhibited the growth and recovery of algae cells, leading to the process of cell death. The metabolic rate was then slowed down and the esterase activity was reduced. The damaged cells increased by 25.9% after ultrasonic irradiation for 2 h, which indicated that the structure of cell membrane was destroyed by ultrasonic irradiation, resulting in the increase of the number of cells that damaged in the membrane structure. The cavitation effect of ultrasonic in water can produce the hydroxyl radical. In this part of experiment, ultrasonic irradiation was acted on algae suspension with the volume of 240 mL under the frequency of 40 kHz, producing about 0.5 μmol/L hydroxyl radical. Actually, the hydroxyl radical has a strong oxidation, which results in induction of peroxidation reaction of unsaturated fatty acid on the cell membrane and the increase of the cell membrane permeability. The molecular weights of DNA were similar between the experimental group and control group, the fragment length of DNA was a little more than 8000 bp and did not present the apoptotic DNA ladder pattern, which indicated that chain structures of DNA in algae cells remained intact after ultrasonic irradiation with the frequency of 40 kHz. A further study is needed on the influence of ultrasound irradiation on the molecular composition of DNA in algae cells.

3.2. Control effect of algae biomass

Through the study of the effect of ultrasonic irradiation on the physiological activity of algae cells, it was found that ultrasonic with the frequency of 40 kHz had relatively great influence on the physiological activity of algae cells.

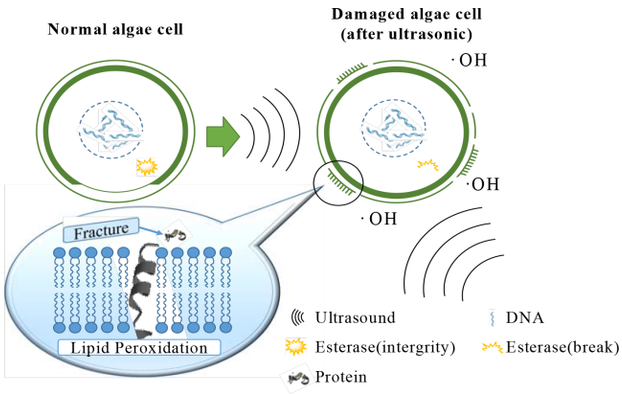


Fig. 5. Mechanism of physiological activity of algae cells.

In order to prove the reliability of this parameter, the control effect of algae biomass was studied.

The World Health Organization's Guidelines for Safe Recreational Waters [34] pointed out that the maximum limit value of algae density in recreational waters was 2×10^4 cells/mL, which was considered as the warning level of cyanobacterial bloom and had little adverse effect on health. We can control the cells density at the warning level to avoid the occurrence of cyanobacterial bloom and its adverse effects through preventive inhibition for cyanobacteria using ultrasound technology. In this part of the experiment, the inhibitory effect of different ultrasonic parameters on *Microcystis aeruginosa* was studied and the growth of algae after ultrasonic irradiation was analyzed in order to explore the effect of ultrasonic power density and frequency on the growth inhibition of *Microcystis aeruginosa.*

3.2.1. Algae density

The curve of change of algae density under ultrasonic irradiation with different power density and fixed frequency of 20 kHz is shown in Fig. 6a. The algae density in each experimental group that has been treated for 2 h decreased by 10%, 24% and 44%, respectively, the algae density in some experimental groups increased gradually after a certain period of culture.

The curve of change of algae density under ultrasonic irradiation with different power density and fixed frequency of 40 kHz is shown in Fig. 6b. The algae density in each experimental group that has been treated for 2 h decreased by 12%, 21% and 43%, respectively. The greater the ultrasonic power density, the lower the algae density is.

The curve of change of algae density under ultrasonic irradiation with different power density and fixed frequency of 100 kHz is shown in Fig. 6c. The differences in the algae density in each experimental group that has been treated for 2 h was not large compared with the results under the ultrasonic frequency of 20 kHz and 40kHz, which was due to the much smaller ultrasonic power intensity with the frequency of 100 kHz and little difference of the power density of each experimental group, resulting in the similar density

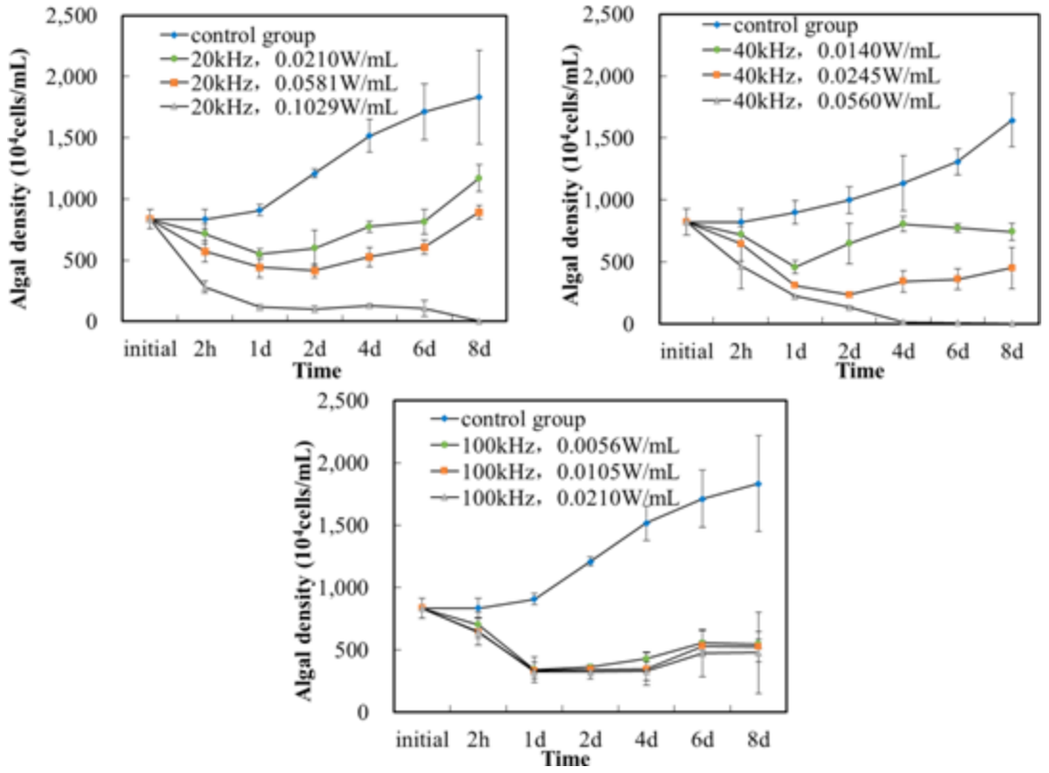


Fig. 6. The algae density after ultrasonic irradiation with different power density and fixed frequency of 20 kHz (A), 40 kHz (B) and 100 kHz(C).

and changing trends of algae cells. The algae density of the experimental group began to increase 48 h after ultrasonic irradiation. The algae density of experimental group was always lower than that of the control group and had still not been restored to the initial level in culture 8 d later-just about 60% of the initial algae density.

3.2.2. Inhibition ratio

3.2.2.1. Effect of ultrasonic frequency on the inhibition rate of algae cells

The diameters of the cavitation threshold and cavitation bubble depend on the ultrasonic frequency; thus frequency is an important ultrasonic parameter. The power density of the three different frequencies (20 kHz, 40 kHz and 100 kHz) used by the experiment was fixed to 0.02 W/ mL. The inhibition rate of each group is shown in Fig. 6. Variation of the inhibitory rate of algae cells after ultrasonic irradiation with different frequencies is shown in Fig. 7a. The inhibition rate presented a gradually decreasing trend on the whole after ultrasound treatment.

On first day, the inhibition rate with the ultrasonic frequency of 100 kHz which showed the maximum one was about 1172%, that is, the growth rate in control group was 11.72 times than that in experimental group. On the second day, the inhibition rate of each group was significantly lower than that of the first day and revealed a trend of gradual decrease, which indicated that the growth rate of the experimental group increased gradually. Actually, the repair mechanisms are activated by stimulation of external circumstances, resulting in the float of the bottom cell and the increase of the cell density. Two days after the ultrasound treatment, the inhibition rate of algae cells in experimental group with the frequency of 40 kHz was the highest, the second was treated by the frequency of 100 kHz and the lowest was treated by the frequency of 20 kHz. Based on the study mentioned above, the algae removal effect by ultrasonic irradiation was good with the frequency of 100 kHz in the early stage (within 1 day), however, its effect of inhibiting algae was less effective than that treated by the frequency of 40 kHz in the long run (two days after the ultrasonic irradiation).

3.2.2.2. Effect of ultrasonic power density on the inhibition rate of algae cells

Variation of the inhibitory rate of algae cells after ultrasonic irradiation with different power density and fixed frequency of 20 kHz is shown in Fig. 7b. The algae density of the experimental group with the ultrasonic power density of 0.0210 W/mL and 0.0581 W/mL began to increase at the first and second days, in addition, recovered to the initial biomass at the sixth and eighth days, respectively, and during that the experiment process, the algae density in the experimental group was always less than that in the control group. The algae density after ultrasonic irradiation

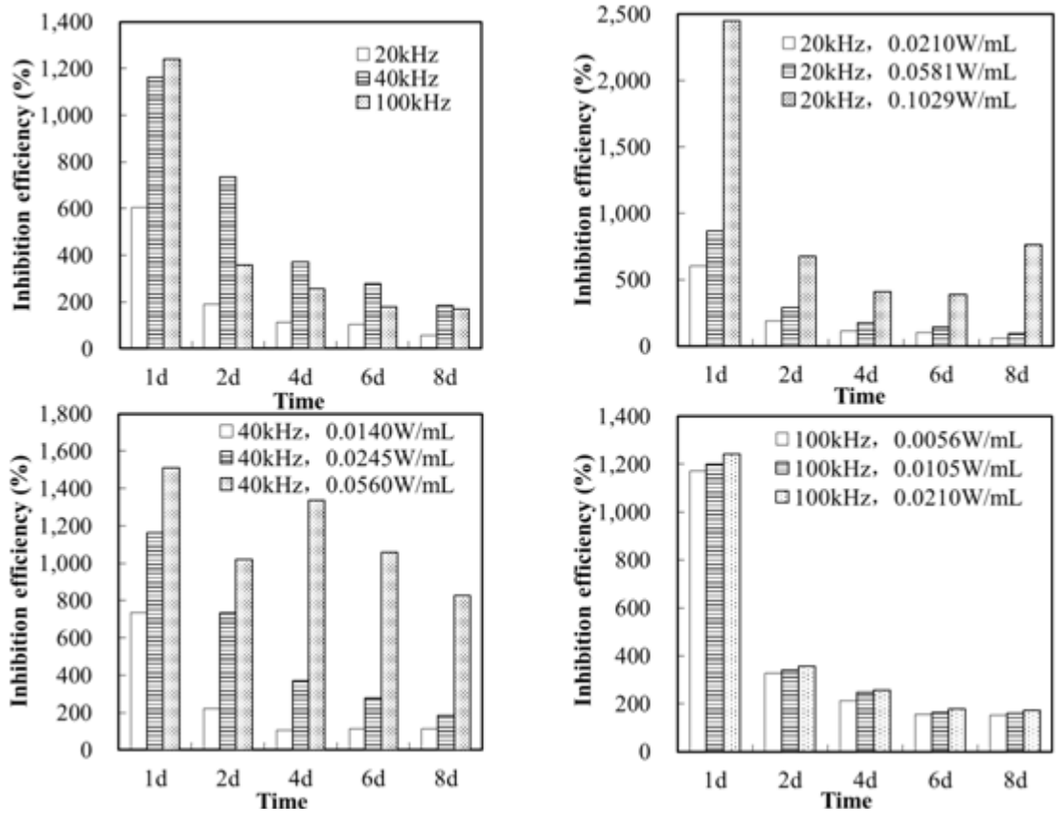


Fig. 7. The inhibition efficiency after ultrasonic irradiation with different frequency and fixed power density of 0.02 W/mL (A); with different power density and fixed frequency of 20 kHz (B), 40 kHz (C) and 100 kHz (D).

with power density of 0.1029 W/mL was only 14% of initial biomass and was decreased with time without the capacity to repair the damage. In fact, ultrasonic irradiation with the frequency of 20 kHz will not result in a high percentage of cell death in a very short time if the ultrasonic intensity is below a certain value, but lead to the damage of large part of the algae cells, inhibiting the growth of the cells and slowing down the growth rate of biomass.

Variation of the inhibitory rate of algae cells after ultrasonic irradiation with different power density and fixed frequency of 40 kHz is shown in Fig. 7c. The algae density of the experimental group with the ultrasonic power density of 0.0140 W/mL and 0.0245 W/mL decreased firstly and then increased again, in addition, began to increase at the first and second days, respectively. The algae cells treated by ultrasonic irradiation with the power density of 0.0140 W/mL recovered to the initial biomass at the fourth day and then the algae density was relatively stable. The algae cells treated by ultrasonic irradiation with the power density of 0.0245 W/mL gradually increased two days after ultrasonic irradiation and did not recovered to the initial biomass during the period of culture. The algae density of treated by ultrasonic irradiation with the power density of 0.0560 W/mL was consistently decreasing and approximated to zero at the fourth day, indicating that the algae cells suffered permanent damage and triggered an inability to restore breaks. It should be pointed out that the ultrasonic irradiation whose ultrasonic intensity was below a certain value was mainly responsible for the damage and inhibiting the growth of the cells, leading to slowing down the rate of cell division but not deadly effects on algae cells, which was similar to the effect of ultrasonic irradiation with the frequency of 20 kHz.

Variation of the inhibitory rate of algae cells after ultrasonic irradiation with different power density and fixed frequency of 100 kHz is shown in Fig. 7d. The differences in the inhibition rate of algae cells in each experimental group was not large, which was due to the much smaller ultrasonic power intensity with the frequency of 100 kHz and little difference of the power density of each experimental group.

4. Conclusions

The algae cells would experience self-repair processes of the esterase activity after ultrasonic irradiation, however, the esterase activity of experimental group was always lower than that of the control group. The structure of cell membrane was destroyed by ultrasonic irradiation, resulting in the decrease of the number of intact cells. The greater the ultrasonic power density, the lower the algae density after ultrasonic irradiation with fixed frequency. It is suggested to use ultrasonic treatment with the frequency of 40 kHz and power density of 0.0245 W/mL to treat the algae cells.

Acknowledgments

The work was supported by Natural Science Foundation of China (51308123), China Postdoctoral Science Foundation (2014M561856), China Scholarship Council, Opening Measuring Fund of Large Precious Apparatus of Fuzhou University (2016T041) and Academy of Integrative Medicine Fujian China.

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