Improved Cyanobacteria Removal from Harmful Algae Blooms by Two-Cycle, Low-Frequency, Low-Density, and Short-Duration Ultrasonic Radiation

Haocai Huang 1,2, Gang Wu 1, Chaowu Sheng 1, Jiannan Wu 1, Danhua Li 1 and Hangzhou Wang 1,*

1 Ocean College, Zhejiang University, Zhoushan 316021, China; hchuang@zju.edu.cn (H.H.); gangwu@zju.edu.cn (G.W.); 21634068@zju.edu.cn (C.S.); 21834066@zju.edu.cn (J.W.); 21434101@zju.edu.cn (D.L.)
2 Laboratory for Marine Geology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266061, China
* Correspondence: hangzhouwang@zju.edu.cn

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Abstract: Harmful algae blooms (HAB) in eutrophic lakes and rivers have become serious water quality problems that are difficult to eliminate using common methods. Previous research has demonstrated that powerful ultrasound can somewhat control cyanobacteria in HABs; however, effective and energy-efficient settings for ultrasonic parameters have not yet been rigorously determined. The results of this study showed that the effect of cyanobacteria removal was enhanced with ultrasonic frequencies, densities, and radiation durations of 20–90 kHz, 0.0005–0.1 W/mL and 0.5–10 min, respectively. Our analyses further demonstrated that the effective distance of ultrasound decreased with increasing frequency, and that damaged algae cells were able to repair themselves at low ultrasonic densities. To address the high energy consumption and small effective distance of conventional ultrasonic radiation treatments, we proposed a new cyanobacteria removal method based on two applications of low-frequency, low-density and short-duration ultrasonic radiation. We defined the energy effectiveness factors of ultrasonic radiation for algae removal as the algae removal rate divided by ultrasonic dosage. This method yielded an 87.6% cyanobacteria removal and the highest energy effectiveness factor, suggesting that two cycles of treatment provide a low-energy method for enhancing existing algae-removing technologies used in large bodies of water.

Keywords: algae removal; water treatment; Microcystis aeruginosa; sonication parameters comparison; ultrasonic radiation

1. Introduction

The occurrence of surface harmful algae blooms (HABs) caused by anthropogenic eutrophication has long been a serious water safety problem [1–3]. Most lakes in China have undergone eutrophication and suffer from HABs [4], which are considered to be very disruptive to sensitive ecosystems as they can significantly alter the physical, chemical and biological characteristics of water bodies [5,6]. Microcystis (e.g., cyanobacteria) pose a major threat to drinking and irrigation water supplies, as well as the general environment, primarily because many strains are capable of producing microcystins of a neurotoxic nature [7]. Large-scale outbreaks of cyanobacteria reduce the concentration of dissolved oxygen in the water; this accelerates the decomposition of anaerobic microorganisms and kills other aquatic organisms [8].
Numerous studies have proven that pre-treatment with pre-oxidants such as chlorine, chlorine dioxide, ozone or permanganate can control the massive growth of HABs [9]. However, some pre-oxidants stimulate the release of microcystins from algae cells into water, while others may accelerate the formation of disinfection by-products [10]. Some studies have shown that ultrasonic irradiation can break down gas vesicles in algae cells owing to cavitational effects, disrupting the cell wall and membrane, interrupting photosynthetic activity, and inhibiting the cell division and the cell cycle, thereby controlling algal growth [11–13]. Ultrasonic irradiation at 640 kHz has been demonstrated to cause the rapid degradation of microcystin-LR (a cyanobacterial toxin) [14]. Such treatments are considered environmentally friendly and have undergone rapid development in recent years [15]; however, the literature would seem to suggest there are key factors that should be considered when it comes to selecting ultrasonic parameters: the energy consumption, effective distance, and subsequent microcystin release.

Ultrasonic parameters, including frequency, density and irradiation duration, also play an important role in controlling the growth of algae by sonication [12,16]. Some studies have examined the influence of different parameters of ultrasonic irradiation during the removal of HABs [16,17]; however, these focused only on the effects of ultrasonic factors on algae removal in single ultrasonic irradiation treatment experiments, and tried to obtain the best combination of parameters. They did not study the continuous effect of multiple ultrasonic treatments on algae removal. Previous studies show a trend of higher frequencies and densities being more effective for inhibiting cyanobacteria than lower frequencies and densities [18,19]. However, higher frequency ultrasound declines faster with distance, and higher densities consume more power and are less cost efficient [20]. For a cyanobacteria, a long exposure duration will lead to greater exposure to cavitation effects and eventually cell lysis, which may not be desirable as it can lead to the release of toxins, such as microcystin for Microcystis aeruginosa [16]. Therefore, we consider the use of low-frequency, low-density, short-duration ultrasound to treat algae-containing water bodies. Collapsed gas vesicles can undergo self-recovery after destruction by a single ultrasonic irradiation treatment [21]; consequently, multiple treatments are needed to prevent the recovery of gas vesicles, but few studies have researched this.

This study proposes an algae removal method using low-frequency, low-density, short-duration ultrasound based on two cycles of irradiation; an initial (i.e., primary) treatment followed by a second identical (i.e., secondary) treatment after a given time has elapsed. The effects of various ultrasonic frequencies, densities and radiation durations on the degree to which the alga Microcystis aeruginosa could be removed was evaluated. We also studied the effects of different time intervals between the primary and secondary ultrasound radiation cycles on enhancing algae removal. The ultrasound dosage and energy effectiveness factor of sonication in the experiments were calculated. By comparing the results of experiments using one-cycle (i.e., primary only) and two-cycle (i.e., primary and secondary) ultrasound treatments, we obtained the optimal energy-saving and algae-removing parameters using two ultrasonic radiation cycles.

2. Materials and Methods

2.1. Alga Species and Culture Conditions

Microcystis is the most dominant colonial bloom-forming genus responsible for toxic, food-web disrupting, hypoxia-generating blooms in nutrient-enriched lakes worldwide [22]. In this study, M. aeruginosa, provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology of Chinese Academy of Sciences (FACHB-905, Wuhan, China), was selected as the research object; it was cultured in non-axenic standard BG-11 medium in an incubator. The temperature was set to 25 °C and the light intensity was set to 2300 lx with a light/dark cycle of 12/12 h. The experiment was performed when the M. aeruginosa culture solution reached the required capacity and algal density, and growth was in the logarithmic phase. Before the experiment, the algae-containing liquid was thoroughly mixed to ensure equal algae densities in all experimental groups.
2.2. Ultrasonic Algae Removal Device and Experimental Method

The experimental ultrasonic devices are shown in Figure 1. When the devices begin operating, the ultrasonic signal generator (SDG1025, Siglent Technologies Co., Ltd., Shenzhen, China) emits an electrical signal of a specific frequency (20–90 kHz). The electrical signal is amplified in a linear manner using a power amplifier and transmitted to an ultrasonic transducer (T235, Neptune Sonar Ltd., East Yorkshire, UK) that converts the signal into ultrasound power and releases it into the water body. The electric detection signal is transmitted to an oscilloscope (TDS1012C-EDU, Tektronix Inc., Beaverton, OR, USA) through the output detection port of the power amplifier so as to enable detection of the efficiency of the former. The voltage and current of the power amplifier output electrical signal are calculated according to the detection signal, and the output waveform of the power amplifier is detected to determine its working condition. A hydrophone (D300, Neptune Sonar Ltd., East Yorkshire, UK) receives the acoustic signal from the transducer in the algal liquid and converts it into an electrical signal for transmission to the oscilloscope. The oscilloscope converts the electrical signals transmitted from the hydrophone into images and measures the acoustic signal in the algal liquid.

![Experimental ultrasonic devices](image)

**Figure 1.** Experimental ultrasonic devices. 1. Signal generator; 2. Oscilloscope; 3. Power amplifier and matching circuit; 4. Transducer; 5. Hydrophone; 6. Algal liquid.

In four sets of experiments, multiple groups of 500 mL *M. aeruginosa* were prepared with an initial density of $1.20 \times 10^6$ cells/mL at an initial pH of 7.3. In each experimental set, one variable was explored while all other conditions were held constant and the samples were exposed to ultrasonic radiation, as follows: ultrasonic frequency was explored at four levels (20–90 kHz), and the effects on algae removal rate and chlorophyll *a* were obtained; duration of ultrasonic radiation was explored at four levels (0.5–10 min), and the effects on algae removal rate and chlorophyll *a* were obtained; ultrasonic density was explored at five levels (0.0005–0.1 W/mL), and the effects on algae removal rate and chlorophyll *a* were obtained; and finally, based on optimal frequency, duration and density conditions obtained from the first three experimental sets, the effects of the time interval between primary and secondary treatments of the two-cycle treatment were explored at three levels (12–36 h) plus a control level which featured only the primary treatment and no secondary application. For all experiments, the water temperature was maintained at 20 °C and the *M. aeruginosa* were continuously cultured for more than 72 h after the experiment began. The concentrations of chlorophyll *a* and the algae cell densities in all groups were sampled every 12 h. To reduce measurement errors, each sample was measured three times and the average values were reported.

2.3. Analysis and Measurement Methods

The concentrations of chlorophyll *a* and algal cell densities were considered to be indicators of the growth of *M. aeruginosa*. The former was detected using a laboratory fluorometer (Trilogy, Turner Designs, Inc., San Jose, CA, USA) while the latter was detected using a hemocytometer with the aid of
an optical microscope (Motic BA310, MOTIC MICROSCOPES, San Antonio, TX, USA). By comparing the algal densities and concentrations of chlorophyll $a$ before and after the ultrasonic experiments, we were able to evaluate the effect of the ultrasonic radiation on algae removal. $M. \text{aeruginosa}$ were deprived of the ability to adjust their buoyancy by the destruction of their gas vesicles and sank to the bottom of the container. Therefore, samples were uniformly taken from the ~2–3 cm layer below the surface of the water during the detection period. The algal cell density was calculated as follows:

$$C = \frac{A}{A_C} \times m \times y \times 10^4$$

where $C$ represents the cell density (cells/mL), $A$ is the area of the counting box (mm$^2$), $A_C$ is the counting area (mm$^2$), $m$ is the number of cells in the counting area, $y$ is the dilution factor of the algal samples, and the volume of the counting box is 0.1 mL.

The algae removal rate, calculated as follows, was used to characterize the removal effect of the ultrasonic radiation:

$$RR = \frac{C_c - C_t}{C_c} \times 100\%$$

where $RR$ represents the removal rate (%), and $C_c$ and $C_t$ denote the algal cell densities of the control group and the experimental (test) group at the same time, respectively. The control group was used as a baseline due to the influence of cell proliferation caused by cell multiplication after sonication. The removal rate was applicable when the initial conditions of the control and experimental groups, such as the algal density and chlorophyll $a$ concentration, were the same. Algae continued to grow during the experiment; therefore, we used the final algal cell density in the control group as the comparison value.

The ultrasonic dosage is the amount of energy supplied per unit of volume of medium and is a practical method of expressing the energy input for the algae removal on a volume basis [20]. It was calculated as follows:

$$DOS = DE \times d \times n$$

where $DOS$ represents the ultrasonic dosage (Ws/mL), $DE$ is the ultrasonic density (W/mL), $d$ is the ultrasonic duration (s), and $n$ denotes the number of cycles of ultrasonic radiation in the experiment.

In a previous study, the energy effectiveness factor of the ultrasonic control of algae was defined as the percentage of algal cell reduction divided by ultrasonic density [19]. However, ultrasonic dosage can be used instead of ultrasonic density. This relates most of the ultrasonic parameters, i.e., ultrasonic power, irradiation duration and volume of solution, with the algae removal rate achieved. It may give a better overview of the effect of ultrasonic radiation [15]. In this study, the energy effectiveness factor of ultrasonic radiation was calculated as follows:

$$E = \frac{RR}{DOS}$$

3. Results and Discussion

Many factors affect the efficiency of removing algae via ultrasonic radiation. During the actual utilization of the removal device, factors such as the species of algae and water pH, flow and temperature are uncontrollable [23]. However, the ultrasonic frequency, density, radiation duration, and the time intervals between the primary and secondary cycles are controllable via the device used. We studied these four factors using single-factor experiments that provided a basis for optimizing them.

3.1. Impact of Ultrasonic Frequency

We prepared five groups of 500 mL of $M. \text{aeruginosa}$; one served as the control and the others were exposed to ultrasonic radiation held at a constant energy (density of 0.01 W/mL) for a fixed duration of 5 min at frequencies of 20, 28, 40 and 90 kHz. The change in the growth of $M. \text{aeruginosa}$ at different ultrasonic frequencies is shown in Figure 2, which depicts samples of each experimental group. The
effects of these frequencies on the removal rate and chlorophyll a concentration of *M. aeruginosa* are shown in photographs (Figure 3).

Figure 2. Growth of *M. aeruginosa* after treatment for 5 min at different ultrasonic frequencies.

Figure 3 shows that the ultrasonic radiation treatment had a significant effect on algae removal. At equal radiation durations, the removal rates at ultrasonic frequencies of 20, 28, 40 and 90 kHz were 60.9%, 65.9%, 74.6% and 90.0%, respectively, after 48 h. The removal rate improved with increasing frequency. The effect of algae removal tends to stabilize approximately 36 h after ultrasound irradiation.

When an ultrasonic wave propagates in the algae-containing water body, the sound wave attenuates as the propagation distance increases. The sound intensity \( I \) and the pressure \( p \) from the source, at the distance \( d \), can be expressed as follows:

\[
I = I_0 \exp(-\alpha d)
\]


\[ p = p_e \exp(-\alpha d) \] (6)

where \( p_e \) represents the original ultrasonic pressure, \( I_0 \) represents the original ultrasonic intensity, and \( \alpha \) is the ultrasonic absorption coefficient.

Ultrasound absorption is caused by the dissipation of ultrasonic energy, which converts acoustic energy into heat energy. Sound wave attenuation is divided into scattering, absorption and diffusion modes based on the degree of attenuation of the sound intensity. Ultrasonic absorption attenuation is mainly a result of thermal conduction, viscosity, and the relaxation effect caused by microscopic changes to the medium. Ultrasonic diffusion attenuation arises from the expansion of the surface area of the plane wave as it propagates through the medium. Finally, ultrasonic scattering attenuation is caused mainly by the change in the direction in which the ultrasonic radiation is propagated, as determined by obstruction from algal cells. The latter does not have much effect in algae-containing waters. In summary, the ultrasonic absorption coefficient can be expressed as follows:

\[ \alpha = \frac{2\pi^2 f^2}{c^3 \rho} \left( \frac{4}{c_p} \right) \left( \frac{\gamma - 1}{c_p} \right) k \] (7)

where \( f \) represents the ultrasonic frequency (Hz), \( c \) is the ultrasonic velocity (m/s), \( \rho \) is the density of water (kg/m³), \( \eta \) is the viscous coefficient of the medium, \( \gamma \) is the specific heat ratio, \( k \) is the thermal conductivity of the medium, and \( c_p \) is the specific heat capacity at a constant pressure. On the one hand, this formula indicates that, theoretically, higher frequencies correspond to a greater absorption of ultrasonic energy, a faster attenuation in water, and smaller propagation distances. On the other hand, Fan’s simulation using COMSOL Multiphysics proves that a low-frequency ultrasonic radiation has a larger effective distance [24]. Thus, it can be inferred that low-frequency ultrasonic radiation may have a larger impact distance and can therefore be considered for application in large bodies of water.

### 3.2. Impact of the Duration of Ultrasonic Radiation

To determine the impact of the duration of the ultrasonic radiation, its frequency was fixed at 20 kHz, ultrasonic density was set to 0.015 W/mL, and radiation duration was set to 30 s and 1, 5 and 10 min. The effects of radiation duration on the removal rate and chlorophyll \( a \) concentration of \( M. \ aeruginosa \) are shown in Figure 4.

![Figure 4](image-url)

**Figure 4.** (a) Algae removal rate and (b) concentration of chlorophyll \( a \) after ultrasonic radiation treatment of different durations.

Figure 4 shows that the removal rates with ultrasonic irradiation durations of 0.5, 1, 5 and 10 min were 68.8%, 74.4%, 81.2% and 90.6% 48 h after irradiation, respectively (Figure 4). This illustrates that longer radiation treatment durations corresponded to higher removal rates. In experimental
groups with radiation durations of <5 min, the algae removal rate changed by <4%, indicating that increases in the algae removal rate by increasing the ultrasonic radiation duration in this range were not economical. The concentrations of chlorophyll a in the experimental groups with shorter radiation durations (≤1 min) were below those of the experimental groups with longer radiation durations (>1 min), because the latter corresponded to the rupture of an increasing number of M. aeruginosa cells, leading to increasing amounts of intracellular chlorophyll a entering the water. Similarly, it would follow that algal cell lysis may lead to the release of microcystin toxins for M. aeruginosa. To counter this, the ideal settings for the sonication parameters would likely cause the collapse of gas vesicles, but not cause cell lysis, and thereby may not contribute to the possibility of toxin release into the water. An excessively long ultrasonic treatment thus causes adverse effects on aquatic organisms and consumes more energy than a shorter treatment period. Therefore, during actual ultrasonic algae removal operations, the radiation duration must be considered seriously from the perspective of reducing energy consumption and ensuring environmental protection.

3.3. Impact of Ultrasonic Density

The conditions during tests of the impact of ultrasonic density were slightly different than those for the other tests. Here, six groups of 500 mL of M. aeruginosa were prepared, one serving as the control while the other five were exposed to ultrasonic radiation at 20 kHz at the beginning of the experiment. The duration of the radiation was fixed at 5 min and the radiation densities were set to 0.0005, 0.0025, 0.015, 0.05 and 0.1 W/mL. The change in the growth of M. aeruginosa at different ultrasonic densities is shown in Figure 5. The effects of ultrasonic density on the removal rate and chlorophyll a concentration of M. aeruginosa as observed in the samples extracted are shown in Figure 6.

![Figure 5](image_url)

**Figure 5.** Growth of M. aeruginosa after treatment at different ultrasonic densities.

The removal rates at ultrasonic densities of 0.0005, 0.0025, 0.015, 0.05 and 0.1 W/mL were 40.6%, 58.0%, 81.2%, 92.0% and 92.8% 48 h after ultrasonic irradiation, respectively, when all other factors were held constant. The removal rate increased with the increasing radiation density in this range. For ultrasonic radiation densities >0.015 W/mL, the algae removal rates were >80%; in these cases, the ultrasonic radiation densities were greater than the cavitation threshold, and algae removal was mainly due to ultrasound-generated cavitation [25]. For ultrasonic radiation densities of ≥0.05 W/mL, the algae removal rate difference was <1%, indicating that the effect of ultrasonic radiation density...
eventually became saturated. At low ultrasonic densities, the ultrasound mainly produced mechanical effects in the water, yielding lower algae removal rates. As shown in Figure 6, the removal rates at ultrasonic densities of 0.0005 and 0.0025 W/mL began to decrease 48 h after treatment, showing that the algae cells mechanically damaged by low-density ultrasound radiation were able to repair themselves.

In summary, we infer that low-density ultrasonic radiation (i.e., that below the cavitation threshold) achieves algal control mainly through mechanical impacts on water. The algae removal rate is relatively low and the damage to the algal cells can be repaired. However, high-density ultrasonic radiation (that above the cavitation threshold) achieves a higher algae removal rate due to the combined effect of cavitation and mechanical agitation, with cavitation being predominant. The damage caused to algae by high-density ultrasonic radiation is permanent and cannot be repaired. Although lower-density ultrasound consumes less power, it is necessary to increase the algae removal rate and prevent the algae cells from self-repairing; this can be achieved by multiple treatment cycles.

3.4. Impact of Time Interval between Primary and Secondary Ultrasound Radiation Cycles

Given that high-frequency ultrasonic radiation has a small impact distance within the water, the ultrasonic frequency should be low for application in large bodies of water. We tested algae removal with two cycles of ultrasonic irradiation at a low frequency, low density, and short duration to determine whether this two-cycle mode could improve the algae removal rate. The effects of different time intervals between the primary and secondary ultrasound radiation cycles on the algal removal rate were also investigated.

For these experiments, five groups of 500 mL of *M. aeruginosa* were prepared with initial densities of $1.20 \times 10^6$ cells/mL and an initial pH of 7.3. The experimental groups were labelled A–D. These experiments used a frequency of 20 kHz, a density of 0.0025 W/mL, and an irradiation duration of 1 min. The experiment began with the primary ultrasonic irradiation treatment. For groups B, C and D, secondary ultrasound radiation treatments were performed 12, 24 and 36 h after the primary ultrasound radiation treatment, respectively. The effects of the different time intervals between the treatments in terms of the removal rates and chlorophyll *a* concentrations of *M. aeruginosa* are shown in Figure 7.

![Figure 6](image-url)
We calculated the ultrasonic dosage and the factor of the energy effectiveness of the experimental groups. The algal removal rates in groups A, B, C and D were 53.0%, 73.0%, 79.9% and 86.7% 96 h after the primary irradiation, respectively. Although the reduction effect of the secondary treatment is not immediately visible after the time of application, the cumulative effect is maintained over time. We conclude that *M. aeruginosa* cells complete cell repair within 36–48 h after low-frequency and low-density ultrasonic irradiation, at which point the chlorophyll *a* concentration begins to increase (Figures 3 and 6). Therefore, our results suggest that a secondary sonication time close to 36 h after the primary sonication offers the best conditions for algae removal and durable algae control.

3.5. Comparison of One-Cycle and Two-Cycle Ultrasound Radiation

The highest algae removal rate in the two-cycle ultrasound radiation mode was 86.7% in Section 3.4. We calculated the ultrasonic dosage and the factor of the energy effectiveness of the experimental groups with a similar algal removal rate in previous sections. By comparing these indicators, we could observe the most cost-efficient method for controlling algal growth. The ultrasonic parameters, dosage and energy effectiveness factors of the experimental groups are listed in Table 1. The latter will determine the optimal ultrasound radiation mode in terms of economy in energy consumption, as well as effectiveness. Using two-cycle ultrasound radiation appears to consume less energy and be more efficient.

| Cycle | Frequency (kHz) | Density (W/mL) | Duration (s) | Removal Rate (%) | Dosage (Ws/mL) | Energy Effectiveness Factor |
|-------|-----------------|----------------|--------------|------------------|----------------|----------------------------|
| 1     | 40              | 0.01           | 300          | 74.6             | 3              | 24.9                       |
| 1     | 90              | 0.01           | 300          | 90.0             | 3              | 30.0                       |
| 1     | 20              | 0.015          | 60           | 74.4             | 0.9            | 82.7                       |
| 1     | 20              | 0.015          | 300          | 81.2             | 4.5            | 18.0                       |
| 1     | 20              | 0.015          | 600          | 90.6             | 9              | 10.1                       |
| 1     | 20              | 0.015          | 300          | 81.2             | 4.5            | 18.0                       |
| 1     | 20              | 0.05           | 300          | 92.0             | 15             | 6.1                        |
| 1     | 20              | 0.1            | 300          | 92.8             | 30             | 3.1                        |
| 2     | 20              | 0.0025         | 60           | 86.7             | 0.3            | 289.0                      |

4. Conclusions

In this study, we fabricated an experimental device for removing the alga *M. aeruginosa* via ultrasonic irradiation. The frequency, density and duration of ultrasonic irradiation all affected the efficacy of algae removal; single-factor experiments showed that ultrasonic radiation at high frequency,

![Figure 7. (a) Algae removal rates and (b) concentrations of chlorophyll *a* under different modes of ultrasound radiation treatment for algae removal.](image-url)
high density and long duration had the best algae-removal effects. However, high ultrasonic frequencies and long irradiation durations caused the greatest rupture of algal cells, which would likely also have a negative effect on other aquatic organisms. Although low-frequency ultrasonic radiation had a larger effective distance than high-frequency radiation, the algae removal rate was lower under the former. To address this problem, we proposed a new mode in which two cycles of low-frequency, low-density ultrasonic radiation were used over a short duration. To test this, we performed ultrasonic radiation at 20 kHz and 0.0025 W/mL twice for 1 min; 36 h was determined to be the optimal time interval between the first and second radiation cycles. The algae removal rate during the two-cycle mode under optimal conditions was 86.7%. We calculated the dosage and the factor of the energy effectiveness of ultrasound in the experimental groups, and found that the new mode was the most efficient. The new algae removing method has advantages including a low cost, improved environmental protection (i.e., less harm to aquatic species), and increased impact distance. For independent algae removal devices, lower energy consumption often corresponds to longer operation periods. Therefore, considering the need for long-term algal removal and the inhibition of cell growth in large bodies of water, this new algae removing method can be applied to the design of a stand-alone floating device to remove algae using less energy than the method currently used in similar devices.

Future research should include studies of the effects of ultrasonic radiation treatments on other harmful algae species, the in situ application of the experimental device to an actual HAB, the effect of increased ultrasonic radiation cycles on algae removal, the possible risks for other organisms in the aquatic ecosystem, and the release of toxins from Microcystis cells.

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References
1. Hudnell, H.K. The state of U.S. freshwater harmful algal blooms assessments, policy and legislation. *Toxicon* 2010, 55, 1024–1034. [CrossRef]
2. Olalekan, A.A.; Malik, K. Application of Giovanni for rapid assessment of harmful algal blooms in the Arabian Gulf. *Arab. J. Geosci.* 2015, 8, 8767–8775. [CrossRef]
3. Jasim, S.Y.; Saththasivam, J. Advanced oxidation processes to remove cyanotoxins in water. *Desalination* 2017, 406, 83–87. [CrossRef]
4. Qian, Y.; Xu, N.; Liu, J.; Tian, R. Inhibitory effects of *Pontederia cordata* on the growth of *Microcystis aeruginosa*. *Water Sci. Technol.* 2018, 2017, 99–108. [CrossRef]
5. Al Shehhi, M.R.; Gherboudj, I.; Ghedira, H. An overview of historical harmful algal blooms outbreaks in the Arabian Seas. *Mar. Pollut. Bull.* 2014, 86, 314–324. [CrossRef]
6. Wang, K.; Saththasivam, J.; Yiming, W.; Loganathan, K.; Liu, Z. Fast and efficient separation of seawater algae using a low-fouling micro/nano-composite membrane. *Desalination* 2018, 433, 108–112. [CrossRef]
7. Wu, F.; Kong, H.; Shang, Y.; Zhou, Z.; Gu, Y.; Liu, Q.; Hu, M. Histopathological alterations in triangle sail mussel (*Hyriopsis cumingii*) exposed to toxic cyanobacteria (*Microcystis aeruginosa*) under hypoxia. *Aquaculture* 2017, 467, 182–189. [CrossRef]
8. Dalu, T.; Wasserman, R.J. Cyanobacteria dynamics in a small tropical reservoir: Understanding spatio-temporal variability and influence of environmental variables. *Sci. Total Environ.* 2018, 643, 835–841. [CrossRef]
9. Shen, Q.; Zhu, J.; Cheng, L.; Zhang, J.; Zhang, Z.; Xu, X. Enhanced algae removal by drinking water treatment of chlorination coupled with coagulation. *Desalination* 2011, 271, 236–240. [CrossRef]
10. Liang, H.; Nan, J.; He, W.; Li, G. Algae removal by ultrasonic irradiation-coagulation. Desalination 2009, 239, 191–197.

11. Ahn, C.-Y.; Park, M.-H.; Joung, S.-H.; Kim, H.-S.; Jang, K.-Y.; Oh, H.-M. Growth Inhibition of Cyanobacteria by Ultrasonic Radiation: Laboratory and Enclosure Studies. Environ. Sci. Technol. 2003, 37, 3031–3037. [CrossRef] [PubMed]

12. Tang, J.; Wu, Q.; Hao, H.; Chen, Y.; Wu, M. Effect of 1.7 MHz ultrasound on a gas-vacuolate cyanobacterium and a gas-vacuole negative cyanobacterium. Colloids Surf. B Biointerfaces 2004, 36, 115–121. [CrossRef] [PubMed]

13. Zhang, G.; Zhang, P.; Liu, H.; Wang, B. Ultrasonic damages on cyanobacterial photosynthesis. Ultrason. Sonochem. 2006, 13, 501–505. [CrossRef]

14. Song, W.; Teshiba, T.; Rein, K.; O’Shea, K.E. Ultrasonically induced degradation and detoxification of microcystin-LR (Cyanobacterial Toxin). Environ. Sci. Technol. 2005, 39, 6300–6305. [CrossRef] [PubMed]

15. Rajasekhar, P.; Fan, L.; Nguyen, T.; Roddick, F.A. A review of the use of sonication to control cyanobacterial blooms. Water Res. 2012, 46, 4319–4329. [CrossRef] [PubMed]

16. Rajasekhar, P.; Fan, L.; Nguyen, T.; Roddick, F.A. Impact of sonication at 20 kHz on Microcystis aeruginosa, Anabaena circinalis and Chlorella sp. Water Res. 2012, 46, 1473–1481. [CrossRef] [PubMed]

17. Zhang, G.; Zhang, P.; Wang, B.; Liu, H. Ultrasonic frequency effects on the removal of Microcystis aeruginosa. Ultrason. Sonochem. 2006, 13, 446–450. [CrossRef]

18. Hao, H.; Wu, M.; Chen, Y.; Tang, J.; Wu, Q. Cyanobacterial bloom control by ultrasonic irradiation at 20 kHz and 1.7 MHz. J. Environ. Sci. Health Part A Toxic Hazard. Subst. Environ. Eng. 2004, 39, 1435–1446. [CrossRef] [PubMed]

19. Joyce, E.M.; Wu, X.; Mason, T.J. Effect of ultrasonic frequency and power on algae suspensions. J. Environ. Sci. Health Part A Toxic Hazard. Subst. Environ. Eng. 2010, 45, 863–866. [CrossRef]

20. Khanal, S.K.; Grewell, D.; Sung, S.; Van Leeuwen, J. Ultrasound applications in wastewater sludge pretreatment: A review. Crit. Rev. Environ. Sci. Technol. 2007, 37, 277–313. [CrossRef]

21. Howard, C.; Burch, M.; Zander, A.; Rodriguez-Molares, A.; Dickson, S.; Hobson, P. Quantification of the ultrasound induced sedimentation of Microcystis aeruginosa. Ultrason. Sonochem. 2014, 21, 1299–1304.

22. Fang, F.; Gao, Y.; Gan, L.; He, X.; Yang, L. Effects of different initial pH and irradiance levels on cyanobacterial colonies from Lake Taihu, China. J. Appl. Phycol. 2018, 30, 1777–1793. [CrossRef]

23. Park, J.; Church, J.; Son, Y.; Kim, K.T.; Lee, W.H. Recent advances in ultrasonic treatment: Challenges and field applications for controlling harmful algal blooms (HABs). Ultrason. Sonochem. 2017, 38, 326–334. [CrossRef] [PubMed]

24. Fan, G. Study on Ultrasound Controls Technology of Microcystis sp. in Water. Ph.D. Thesis, Faculty of Urban Construction and Environmental Engineering, Chongqing University, Chongqing, China, 2012.

25. Koda, S.; Miyamoto, M.; Toma, M.; Matsuoka, T.; Maebayashi, M. Inactivation of Escherichia coli and Streptococcus mutans by ultrasound at 500 kHz. Ultrason. Sonochem. 2009, 16, 655–659. [CrossRef] [PubMed]