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# Growth Inhibition of Cyanobacteria by Ultrasonic Radiation: Laboratory and Enclosure Studies

CHI-YONG AHN,<sup>†</sup> MYUNG-HWAN PARK,<sup>†</sup>  
SEUNG-HYUN JOUNG,<sup>†</sup> HEE-SIK KIM,<sup>†</sup>  
KAM-YONG JANG,<sup>‡</sup> AND HEE-MOCK OH<sup>\*,†</sup>

Environmental Biotechnology Laboratory,  
Korea Research Institute of Bioscience and  
Biotechnology, Daejeon 305-333, Korea, and  
Clean World Hi-Tech Company, Daejeon 306-010, Korea

The growth of *Microcystis aeruginosa* UTEX 2388 was repressed by ultrasonic radiation and resulted in an increased chlorophyll *a* content and cell size, suggesting the inhibition of cell division. However, growth was recovered immediately after the interruption of ultrasonication. In addition to the disruption of gas vesicles, other mechanisms of growth inhibition were also investigated. Although free radicals were produced by ultrasonication and hydrogen peroxide, the resulting lipid peroxidation in the cells was not comparable, indicating minimal damage by the free radicals. Ultrasonic radiation late in the day was found to be most effective in reducing the growth rate of *M. aeruginosa*, and this timing also corresponded to the phase of daily cell division. In an enclosure experiment, ultrasonic radiation reduced the pH, DO, total nitrogen, and total phosphorus, whereas it increased the water temperature, conductivity, and orthophosphate concentration. The algal cell density and chlorophyll *a* concentration drastically decreased after 3 d of ultrasonication, plus the cyanobacterial proportion was selectively reduced as compared to other algal species. Accordingly, ultrasonic radiation would appear to have considerable potential as an effective control method for cyanobacterial blooms.

## Introduction

Eutrophication and algal blooms in lakes and reservoirs are worldwide problems. Summer blooms in eutrophic lakes are usually composed of cyanobacteria, and this competitive dominance of cyanobacteria is fostered by their resistance to grazing (1), buoyancy regulation (2, 3), and massive accumulation of nutrients (4). The proliferation of cyanobacteria leads to increased water treatment costs, a degraded recreational value (5), bad taste/odor (6), and sometimes toxin production (7).

A variety of methods have already been developed and applied to cope with the problems related to cyanobacterial blooms, including nutrient diversion, artificial destratification, hypolimnetic aeration/withdrawal, sediment oxidation/removal, phosphorus precipitation, and biomanipulation (8). Even though such methods are often effective, many of them are very expensive and sometimes give rise to secondary

pollution. Consequently, more economical and pollution-free methods are still required, particularly in developing countries.

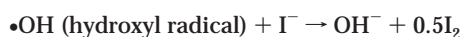
Ultrasound has not been recognized as a control method for cyanobacterial blooms until quite recently. Ultrasound concentrates the diffuse energy of sound through cavitation (i.e., the compression, rarefaction, and implosive collapse of bubbles in a liquid) (9). This collapse by cavitation produces intense local heating (ca. 5,000 °C) and high pressure (ca. 500 atm) with a very short lifetime. The heating and cooling rates are greater than  $10^9$  K s<sup>-1</sup>. Ultrasonic radiation at a high intensity is known to induce the disruption of gas vesicles (10, 11), inhibition of photosynthesis (11), production of free radicals (12), and destruction of membranes. The main effect of ultrasound on bloom control has been focused on the sedimentation of cyanobacteria through the breakdown of gas vesicles (13, 14). Sedimented cyanobacteria are unable to photosynthesize as the light does not reach down to the sediment. However, the disrupted gas vesicles regenerate in a relatively short time (10), plus the application of ultrasound to a lake has not yet produced satisfactory results (14).

Accordingly, the current study investigated other aspects related to the effect of ultrasound on cyanobacteria that have not been given sufficient attention until now, for example, damage due to free radicals, efficient timing of treatment, and selective control of cyanobacteria based on both laboratory and field studies.

## Materials and Methods

**Measurement of Cyanobacterial Growth after Ultrasonication.** *Microcystis aeruginosa* UTEX 2388 was cultivated in a GL medium (15) under the conditions of 26 °C, 100 rpm, 140 μmol m<sup>-2</sup> s<sup>-1</sup>, and light/dark (L/D) 14 h/10 h cycles. The ultrasonic radiation was created using an ultrasonic processor equipped with a 13-mm horn and 5-mm microtip (model VC600, Sonics and Materials Inc., Danbury, CT), and applied twice a day for 2 min at 09:00 and 18:00. The power and frequency of the processor were 600 W and 20 kHz, respectively. The ultrasonic settings were output 5 and 50% duty. The growth of *M. aeruginosa* was monitored with ultrasonic radiation for 6 d and then without ultrasonication. The cell number was counted using a particle counter (Coulter Z1, Coulter Corp., Miami, FL). Chlorophyll *a* (Chl-*a*) was extracted with a chloroform:methanol mixture (2:1 v/v), and the fluorescence was measured using a fluorometer (Turner 450, Barnstead/Thermolyne, Dubuque, IA) according to the method of Wood (16).

**Measurement of Free Radical Production and Lipid Peroxidation.** The free radicals were detected using the method of Hart and Henglein (12). Iodide ions (I<sup>-</sup>) react with free radicals, and the resulting iodine molecules (I<sub>2</sub>) can be detected at an absorbance of 350 nm:



The production of free radicals was measured in a 0.2 M KI solution after ultrasonication for 1, 2, and 5 min. The production of free radicals after 2 min of ultrasonication was also measured with the addition of H<sub>2</sub>O<sub>2</sub> at concentrations of 1, 10, 100, and 1000 μM.

The lipid peroxidation was measured based on the method of thiobarbituric acid reactive substances (TBARS) with certain modifications (17). Sonicated samples of 4 mL were mixed sequentially with 0.1 mL of 20% sodium dodecyl sulfate (SDS), 0.3 mL of 2% butyl hydroxytoluene, 40 μL of 200 mM ethylene bis(oxyethylenetriole)tetraacetic acid (EGTA), and

\* Corresponding author e-mail: heemock@kribb.re.kr; telephone: +82-42-860-4321; fax: +82-42-860-4598.

<sup>†</sup> Korea Research Institute of Bioscience and Biotechnology.

<sup>‡</sup> Clean World Hi-Tech Company.

2 mL of 0.67% 2-thiobarbituric acid in a 10% trichloroacetic acid solution. The mixture was then reacted at 90 °C for 20 min. After being cooled to room temperature, 4 mL of butanol was added, and the absorbance of the supernatant was measured at 532 nm. Ultrasonic radiation with H<sub>2</sub>O<sub>2</sub> at concentrations of 1, 10, and 100 µM was also assayed for lipid peroxidation.

#### Determination of Efficient Timing for Ultrasonication.

Initially, the division timing of *M. aeruginosa* was measured. Under the conditions of 26 °C, 100 rpm, 140 µmol m<sup>-2</sup> s<sup>-1</sup>, and L/D 14 h/10 h cycles, *M. aeruginosa* was cultivated and sampled to measure the cell density every 4 h. The light phase started at 08:00 (L0), while the dark phase started at 22:00 (L14 = D0). The instantaneous growth rate was determined by the following equation:

$$\mu = \frac{\ln N_t - \ln N_0}{t}$$

where  $\mu$  is the instantaneous growth rate (d<sup>-1</sup>),  $N_t$  is the cell density at time  $t$ ,  $N_0$  is the cell density at time 0, and  $t$  is the time interval.

The effects of ultrasonic radiation on growth were compared after a light phase of 1 (L1), 5 (L5), 10 (L10), and 13 h (L13). The ultrasonication was applied using an ultrasonic processor for 3 min a day at the designated time. The conditions of treatment were 50% duty and output 5. The specific growth rate was calculated as the slope of the "natural log of the cell density (cells mL<sup>-1</sup>) versus time (d)".

**Field Study Based on Enclosure Experiments.** Cylindrical plastic enclosures that were 0.6 m in diameter, 0.7 m deep, and contained approximately 200 L of pond water were constructed in a small eutrophic pond in Jeonmin-dong, Daejeon, Korea. From September 3 to September 12, 2002, ultrasonic radiation was applied in an upward direction from a depth of 40 cm using a custom-made ultrasonic apparatus (USP-s, Morko Co., Daegu, Korea). The power and frequency of the ultrasonic machine were 630 W and 22 kHz, respectively, and the period of ultrasonic operation and break was 40 and 210 s, respectively. The power and frequency of the USP-s ultrasonic processor was not much different from those of the VC600 ultrasonic processor, but the former was much bigger and had a disk-shaped transducer with a diameter of 170 mm for the field application. Samples of the surface water were collected every 3 d after mild stirring and used to determine the cell count and nitrogen, phosphorus, and Chl-*a* contents.

**Analyses of Water.** The water temperature, pH, and conductivity were all measured in situ using a YSI meter (63/100 FT, YSI Inc., Yellow Springs, OH). The dissolved oxygen (DO) was measured with a DO meter (95/100 FT, YSI Inc., Yellow Springs, OH).

The total nitrogen (TN) and total phosphorus (TP) were determined after persulfate oxidation to nitrate (18) and orthophosphate (19), respectively. The resulting nitrate was then determined by a second-derivative method (20), while the orthophosphate was determined using the ascorbic acid method (21). The total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were determined after filtering the water sample through a GF/C filter (Whatman Ltd., Maidstone, UK) and persulfate oxidation. The total particulate nitrogen (TPN) and total particulate phosphorus (TPP) were obtained by subtracting TDN from TN and TDP from TP, respectively.

The algal cell numbers were counted using a Fuchs-Rosenthal counting chamber (Paul Marienfeld GmbH & Co., Lauda-Königshofen, Germany) under an optical microscope (Microphot-FXA, Nikon Corp., Tokyo, Japan). The precipita-

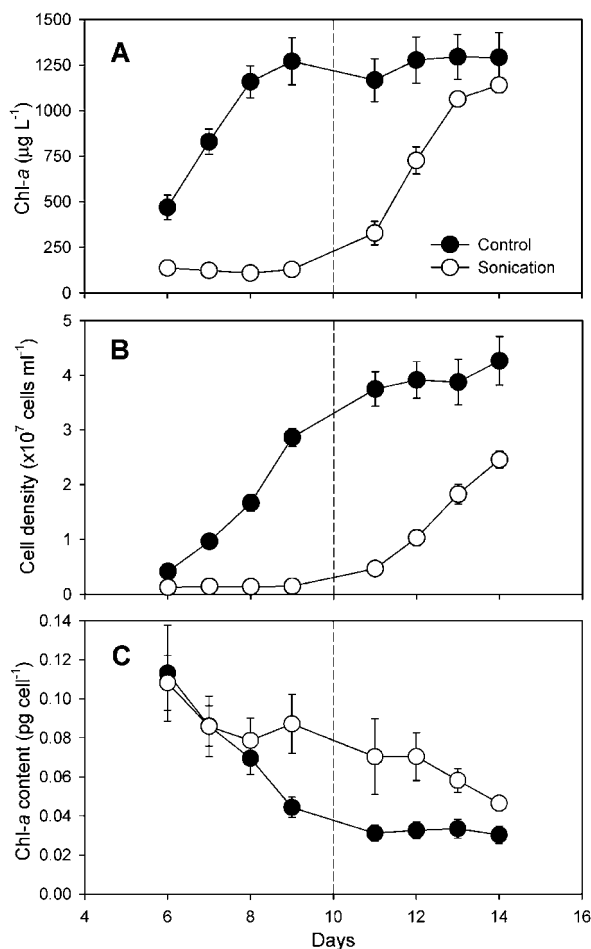


FIGURE 1. Growth inhibition of *Microcystis aeruginosa* UTEX 2388 by ultrasonication, expressed by Chl-*a* concentration (A), cell density (B), and Chl-*a* content per cell (C). The *M. aeruginosa* was sonicated for 6 d, from day 4 to day 9. The vertical dashed lines indicate the interruption of ultrasonication. The vertical bars indicate the SD.

tion and daily irradiance data were obtained from the Korea Meteorological Administration.

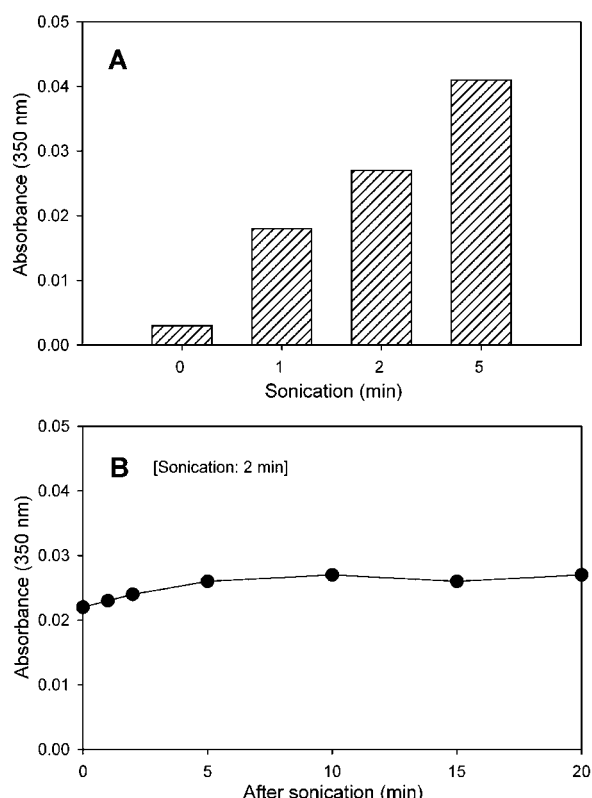
## Results

### Effect of Ultrasonic Radiation on Growth of Cyanobacteria.

A liquid culture of *M. aeruginosa* UTEX 2388, Cyanophyceae, was treated with ultrasonic radiation for 6 d. The Chl-*a* concentration and cell density of the sonicated *M. aeruginosa* remained nearly constant, while untreated cells grew exponentially during the same period (Figure 1A,B). However, the Chl-*a* and cell density of the sonicated cyanobacteria started to increase shortly after the ultrasonication was interrupted. The differences in the Chl-*a* and cell densities between the sonicated and untreated groups corresponded exactly to the duration of the ultrasonication.

The Chl-*a* content in the untreated *M. aeruginosa* decreased during the exponential phase, yet the sonicated cells did not show a comparable decrease (Figure 1C). When the untreated cells reached the stationary phase, the Chl-*a* content decreased to a minimal level of about 0.03 pg cell<sup>-1</sup>. After ultrasonication stopped, the Chl-*a* content in the sonicated cells also started to decrease. In general, the size of the sonicated cells was larger than that of the untreated cells, which seemed to reflect the difference in the Chl-*a* content between the two groups.

**Free Radical Production and Lipid Peroxidation.** One of the damage mechanisms resulting from ultrasonic radia-



**FIGURE 2.** Production of free radicals by ultrasonication. The effect of the ultrasonic duration (A) was determined along with the residual effect after ultrasonication (B). Free radicals react with iodide ions ( $I^-$ ), producing iodine molecules ( $I_2$ ), which were measured at an absorbance of 350 nm.

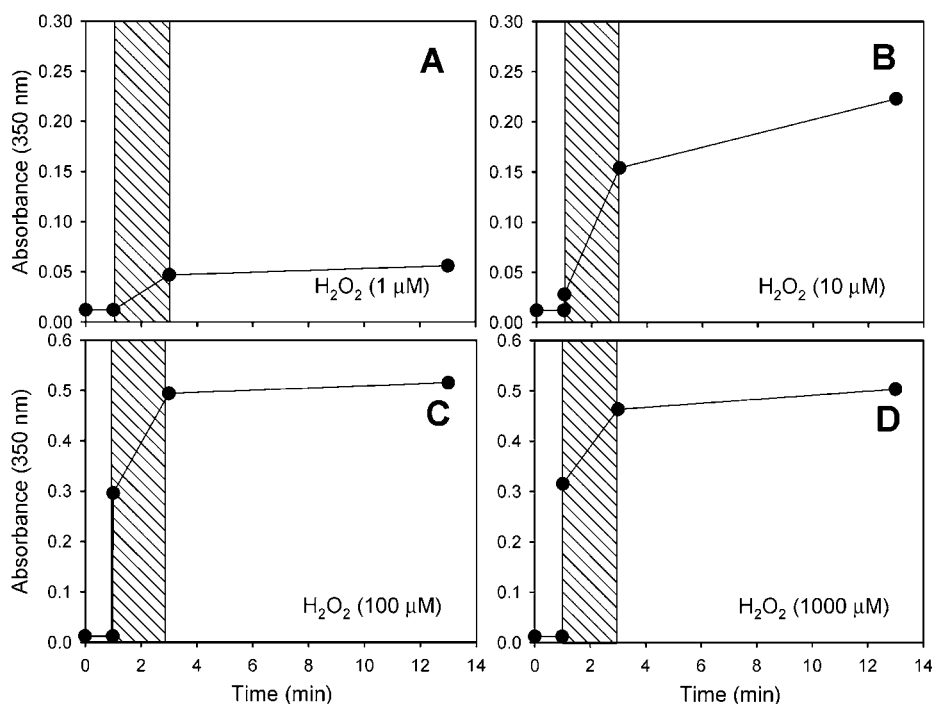
tion is the production of free radicals that destroy cells by inhibiting photosynthesis, damaging membranes, and lipid peroxidation. Free radical production increased when the

duration of the ultrasonication was prolonged (Figure 2A). However, the production of free radicals did not continue when the ultrasonication was interrupted (Figure 2B).

The addition of  $H_2O_2$  promoted the production of free radicals (Figure 3). Yet, without ultrasonication, free radicals were only produced with the addition of  $H_2O_2$  at a concentration above  $10 \mu M$ . The proportion of free radicals produced by  $H_2O_2$  increased with a higher  $H_2O_2$  concentration; however, there was no significant difference between  $H_2O_2$  concentrations of 100 and  $1000 \mu M$  (ANOVA,  $P = 0.608$ ). The number of free radicals produced with  $100 \mu M$   $H_2O_2$  was about 10 times higher than that produced with  $1 \mu M$ . As such, the addition of  $H_2O_2$  above a concentration of  $100 \mu M$  was determined to be sufficient to maximally enhance the production of free radicals.

The most predominant damage caused by free radicals is lipid peroxidation. The relative degree of lipid peroxidation was measured using two ultrasonic devices (Figure 4). Although lipid peroxidation increased with the duration of ultrasonic radiation, the increase was not remarkable. Moreover, it was much lower as compared to the production of free radicals, indicating that the free radicals did not induce a comparable lipid peroxidation in *M. aeruginosa*. In addition, the lipid peroxidation did not increase equivalently, even with the addition of  $H_2O_2$ . The two ultrasonic devices exhibited the same trend and did not differ significantly in their effect. The lipid peroxidation caused by the VibraCell ultrasonic generator was generally higher than that due to the Morko generator.

**Efficient Timing for Ultrasonic Radiation.** In natural environments, the cell division of algae occurs at a specific time during the day. *M. aeruginosa* only divided late in the light phase (L10–L12) under a cycle of 14 h of light and 10 h of dark (Figure 5). Since the susceptibility of algae to ultrasonic radiation was supposed to vary based on the cell cycle, the effect of ultrasonic timing was investigated. The growth of the sonicated cells was retarded, regardless of the timing of the ultrasonic radiation ( $3 \text{ min d}^{-1}$ ). However, just-divided cells (L13) were the most sensitive to ultrasonication, as the specific growth rate of L13 cells was 30% lower than



**FIGURE 3.** Combinational effect of hydrogen peroxide with ultrasonication on production of free radicals. The hatches indicate the period of ultrasonication.

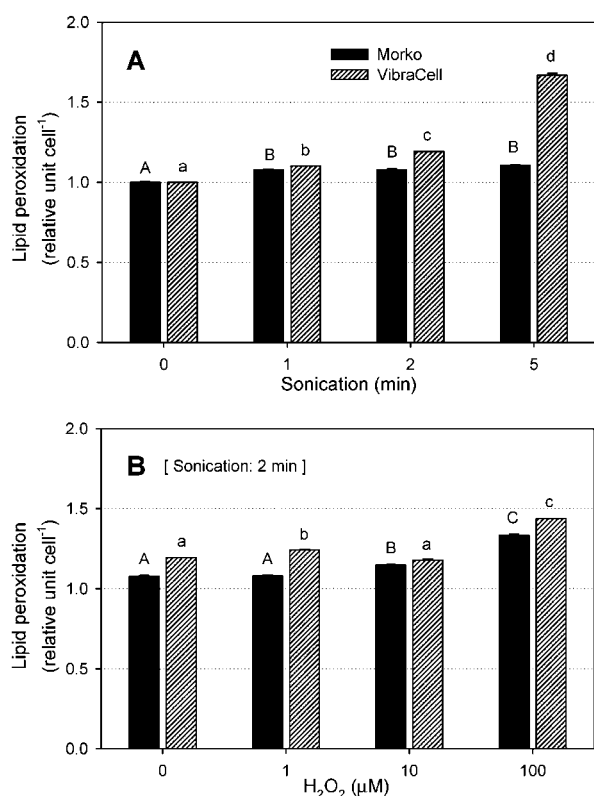


FIGURE 4. Effect of ultrasonication on lipid peroxidation with various treatment times (A) and additions of hydrogen peroxide (B). The different letters indicate significant differences at  $P < 0.01$ . The vertical bars indicate the SD.

that of L1 cells (Figure 6). On the sixth day of incubation, the cell density of L1 cells was 72% higher than that of L13 cells. Thus, it would appear that economical control of cyanobacterial bloom is possible, if the appropriate time of treatment is known.

**Enclosure Experiments.** An enclosure experiment was conducted to test the applicability of ultrasonic radiation. The water temperature and conductivity were about 3 °C and 50  $\mu\text{S cm}^{-1}$  higher, respectively, in the sonicated enclosure as compared with those of the control (Figure 7). In contrast, the pH and DO declined abruptly in the sonicated enclosure during the first 3 d. The increased water temperature decreased DO seemed to be intercorrelated. Such physical and chemical changes are typical effects of ultrasonic radiation. The decreased pH and DO indicated that the ultrasonication inhibited photosynthesis by the algae. When the volume of the enclosure was increased, the rise in the water temperature was smaller.

The TN and TDN were much lower in the sonicated enclosure (Figure 8). In contrast, the decrease in the TP was minimal with sonication. The decrease in the TDP and orthophosphate was much slower in the sonicated enclosure.

While a floating scum of cyanobacteria was observed on the surface water in the control enclosure, none was formed in the sonicated enclosure. The Chl-*a* concentration decreased to one-fourth ( $32.5 \pm 2.8 \mu\text{g L}^{-1}$ , mean  $\pm$  SD) of the initial level ( $111.3 \pm 0.8 \mu\text{g L}^{-1}$ ) after 3 d of ultrasonic radiation (Figure 9A). In contrast, the Chl-*a* concentration almost doubled during the same period from  $127.1 \pm 1.4$  to  $244.2 \pm 2.1 \mu\text{g L}^{-1}$  in the control enclosure. However, the Chl-*a* concentration in the control dropped rapidly during the next 3 d. This abrupt decline was mainly due to sedimentation of the proliferated algae on the bottom of the enclosure. The sampling practice also contributed to the rapid decline of

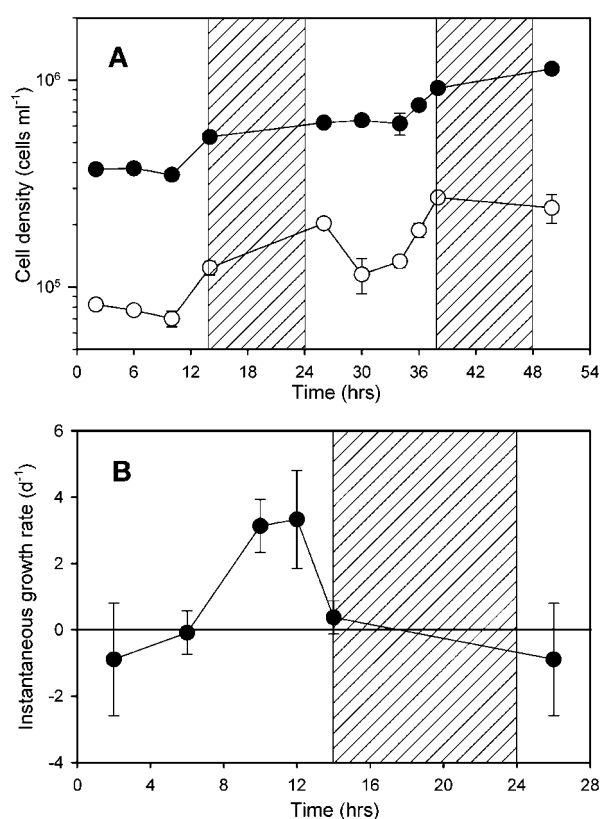


FIGURE 5. Timing of cell division (A) and instantaneous growth rate (B) of *Microcystis aeruginosa* UTEX 2388 under L/D cycles of 14 h/10 h. The hatches indicate the dark phases. The vertical bars indicate the SD.

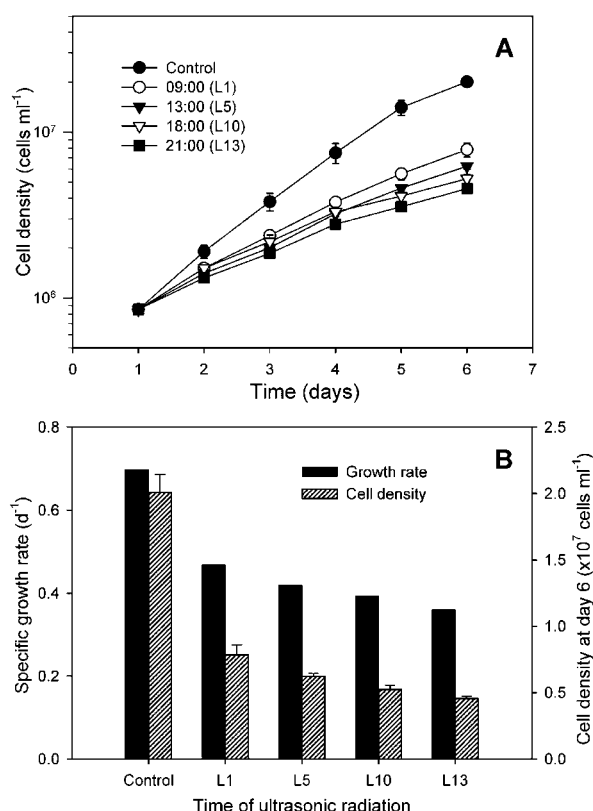
Chl-*a*, as only surface water was withdrawn. The overall pattern of the Chl-*a* concentration was very similar to those of total standing crops and Cyanophyceae (Figure 9B). The composition of the algal community was also influenced by ultrasonic radiation. The percentage of Cyanophyceae was 13% on average in the sonicated enclosure, while that in the control was 47%, implying that ultrasonication selectively inhibited cyanobacteria rather than other algae.

## Discussion

The major adverse effect of ultrasonication on cyanobacteria has been attributed to the disruption of the gas vesicles, thereby leading to sedimentation on the bottom of lakes and the prevention of photosynthesis (13, 14). However, this kind of growth inhibition is irrelevant in laboratory cultivation as there is no disadvantage for cells without gas vesicles as regards receiving light for photosynthesis. Therefore, in the current experiments, the growth of *M. aeruginosa* was inhibited by other mechanisms. The prompt resumption of growth right after the interruption of ultrasonication (Figure 1) would seem to suggest that the growth was only temporarily restrained. In effect, the cell cycles of *M. aeruginosa* were suppressed from running, and the higher Chl-*a* content and larger cell size of the sonicated cells also supported this inference. Indeed, the Chl-*a* content and cell size started to decrease simultaneously with the interruption of ultrasonication.

In addition to the restraint of the cell cycle, damage by free radicals was also considered as another inhibitory effect on the cyanobacterial cells. As the duration of the ultrasonic radiation increased, the production of free radicals also increased. Plus, the addition of H<sub>2</sub>O<sub>2</sub> at concentrations of up to 100  $\mu\text{M}$  further stimulated the production of free radicals

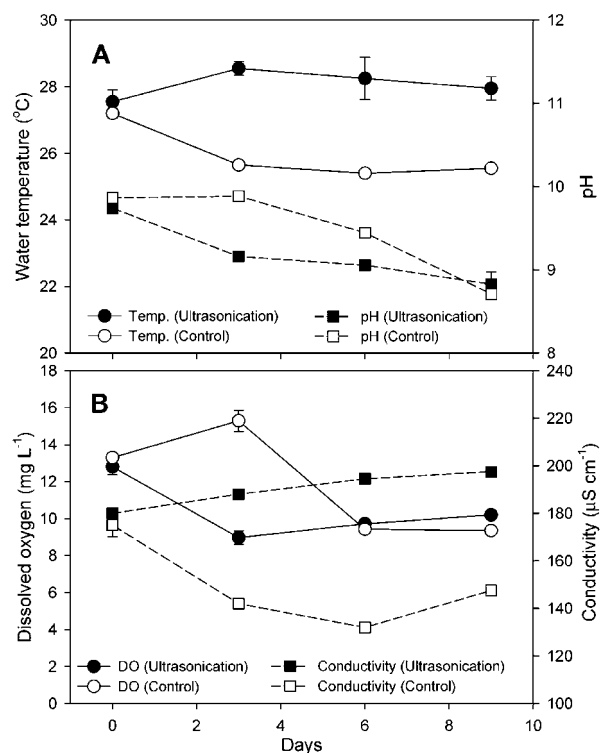




**FIGURE 6.** Effect of different ultrasonic timings on growth of *Microcystis aeruginosa* (A) and specific growth rate (B). The ultrasonication was conducted for 3 min at the designated time. The light phase started at 08:00 (L0) and ended at 22:00 (L14). The vertical bars indicate the SD.

(Figure 3). An increased presence of free radicals destroys cellular constituents and functions by various mechanisms, the most fatal being lipid peroxidation. However, contrary to expectation, there was no significant increase in lipid peroxidation with prolonged sonication or the addition of H<sub>2</sub>O<sub>2</sub> (Figure 4). Rather, the increase in lipid peroxidation was quite small relative to the large production of free radicals, suggesting that the produced free radicals did not directly induce lipid peroxidation in the cyanobacterial cells. It is postulated that the scavenging of free radicals by phycobiliproteins may have been responsible for the small increases in lipid peroxidation, as phycobiliproteins exhibit antioxidant activities (22, 23). In general, the free radicals produced by ultrasonication appeared to be unable to attack the cells efficiently due to the cyanobacterial defense mechanisms. Consequently, the growth inhibition by ultrasonic radiation was not considered to result from free radicals.

Algal cell divisions are confined to a specific time of day (24, 25). A variety of other physiological activities of algae also exhibit rhythmic oscillations under L/D cycles including photosynthesis (26), nitrogen fixation (27), luminescence (28), amino acid uptake (29), and phosphate uptake (30). These rhythmic changes in cellular physiology enable algae to anticipate periodic changes in the environment and respond appropriately. Therefore, certain processes that are very sensitive to light or oxygen occur exclusively at night (31). For example, the survival rate of *Chlamydomonas reinhardtii* exhibits a rhythm against UV radiation, and its phase of sensitivity corresponds to the time of nuclear division at the transition point from light to dark (32). In the current study, *M. aeruginosa* was most susceptible to ultrasonic radiation late in the light phase (Figure 6), immediately after cell division (Figure 5). Although the differences in the growth



**FIGURE 7.** Variations in water temperature, pH (A), dissolved oxygen, and conductivity (B) resulting from ultrasonication in enclosure experiment. The vertical bars indicate the SD.

rates were not significant with various ultrasonic timings, the differences in the cell densities gradually increased. The growth rate of L1 treated cells was 30% greater than that of L13 treated cells. Meanwhile, the cell density of L1 treated cells was 72% higher than that of L13 treated cells on the sixth day, and this difference continued to increase. Although it is unclear which mechanism is mainly associated with the higher sensitivity at a specific time, the current finding is still very useful for the effective application of ultrasonication in controlling cyanobacterial blooms. As such, ultrasonic radiation just before sunset would appear to be most efficient for controlling *Microcystis* blooms.

When the applicability of ultrasonic radiation was tested in enclosure experiments, ultrasonication was found to increase the water temperature in the enclosure, which can favor cyanobacterial growth. However, decreases in the pH and DO showed that this was not the case, as these reductions reflected lower photosynthesis and the suppression of algal growth. Plus, the increase in the water temperature would be more insignificant as the volume of the enclosure increases.

The larger decreases in the TN, TP, Chl-*a*, and algal density in the sonicated enclosure were considered to have resulted from faster sedimentation of the cyanobacterial cells resulting from destroyed gas vesicles. The TPN and TPP, which make up the algal cell composition, also showed a decreasing pattern and were lower in the sonicated enclosure. However, the TPN and TPP also declined in the control enclosure, although their decreases were slower. This retardation in algal sedimentation was correlated with the initial cyanobacterial proliferation in the control enclosure and late sedimentation of overgrown algae.

The higher concentrations of TDP and inorganic orthophosphate in the sonicated enclosure were attributed to ultrasonic effects, such as the dissolution and dissociation of loosely adsorbed phosphate from the particles. In contrast, the TDN in the sonicated enclosure remained constant

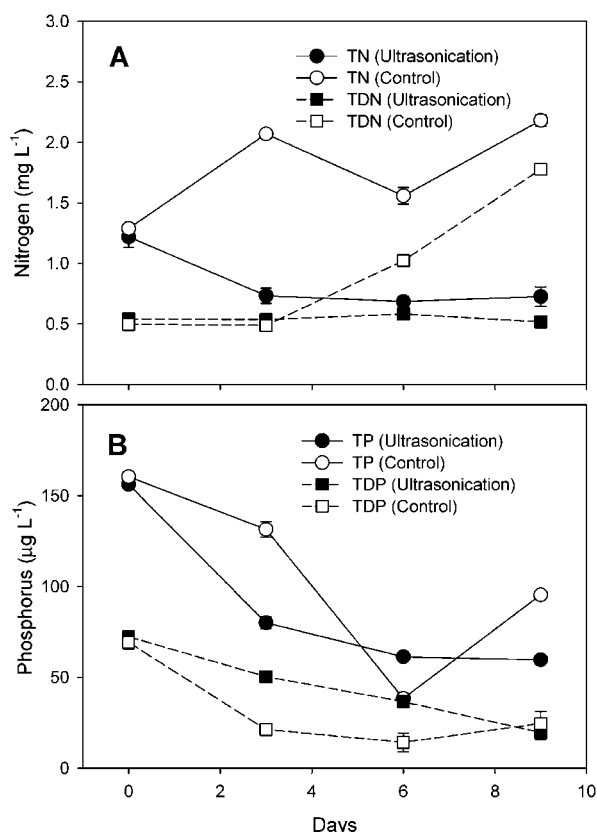


FIGURE 8. Changes in total nitrogen (TN), total dissolved nitrogen (TDN) (A), total phosphorus (TP), and total dissolved phosphorus (TDP) (B) resulting from ultrasonication in enclosure experiment. The vertical bars indicate the SD.

because TDN is principally composed of ammonia, nitrite, and nitrate, which are not usually associated with other inorganic or organic particles. The collapse of the cyanobacterial cells was improbable because no release of microcystin has been observed with ultrasonic radiation (11).

The general trend of Chl-*a* was very similar to that of the algal density in the enclosure experiment (Figure 9). The algae, particularly the cyanobacteria, decreased abruptly after 3 d in the sonicated enclosure, whereas they increased more than 2-fold in the control. The changes in the cyanobacterial percentage during the first 3 d were quite dramatic in both enclosures. The cyanobacterial percentage in the sonicated enclosure dropped from 66% to 0.3% within 3 d, while that in the control increased from 66% to 91%, indicating the possibility of selective control of cyanobacteria with a minimal effect on other algal species. The selective control of cyanobacteria was thought to be principally possible by sedimentation of cyanobacteria after collapse of gas vesicles, which was extensively studied by Lee et al. (10, 11) and Nakano et al. (14).

In addition to the earlier sedimentation of cyanobacteria, the fragmentation of large *Microcystis* colonies was considered as another possible reason. *Microcystis* places its position at an optimal depth within the water column by regulating buoyancy. Thus, it overcomes the vertical separation between light and nutrients. When it migrates up and down, the colony of larger size moves faster according to Stokes' law, finding its optimal depth more efficiently. As the ultrasound fragments large colonies of *Microcystis* into smaller ones even to individual cells, *Microcystis* actually loses its ability of vertical migration, a competitive advantage over other algae.

Besides other mechanisms must have worked through the destruction of physiological functions, such as the

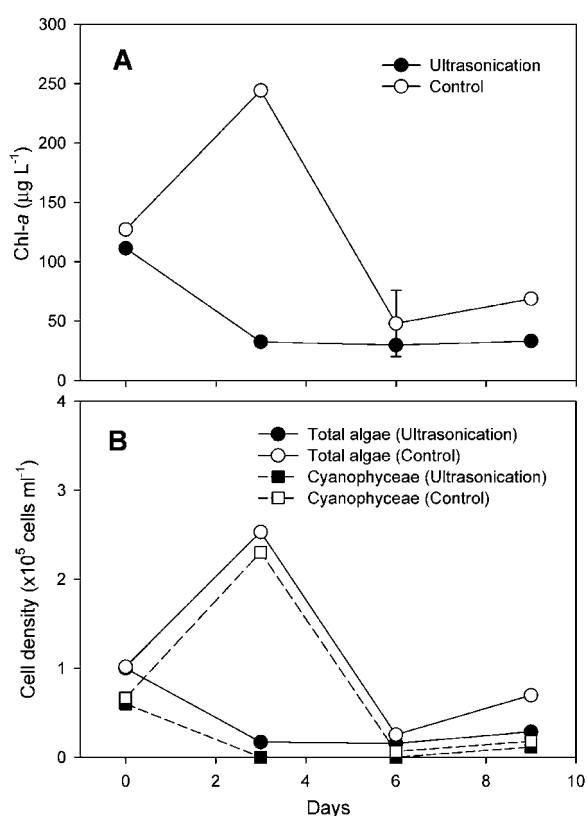


FIGURE 9. Changes in chlorophyll *a* concentration (A) and cell densities of total algae and Cyanophyceae (B) in enclosure experiment. The vertical bars indicate the SD.

prevention of cell cycle progression. However, it has not been fully investigated if those kinds of inhibition are also applicable to eucaryotic algae. They are waiting further researches.

The scum on the surface water in the control enclosure was mainly composed of cyanobacteria by virtue of their gas vesicles. In contrast, there was no scum formation throughout the experiments in the sonicated enclosure, representing an observable evidence for the selective control of cyanobacteria.

Nakano et al. (14) applied ultrasonic radiation to Lake Senba and monitored the changes in water quality over 2 yr. Ten ultrasonic systems with water jet pumps were installed throughout the lake, and major improvements were attained in the transparency, suspended solids, chemical oxygen demand, and TP yet not in the Chl-*a* concentration. It was difficult to clearly discriminate the effects by ultrasonication from those by increased flushing water. Particularly, the decrease in TP was explained by the improved circulation and aeration affected by water jet circulator (33). The weaker effect on Chl-*a* reduction may have been partly due to the low power (100 W) and high frequency (200 kHz) of the ultrasonic radiation, since ultrasound at a lower frequency has a greater effect as it induces more powerful explosions of larger cavitations (9). The minimal effect on controlling the cyanobacterial bloom suggests that the cyanobacteria rapidly recovered their buoyancy and resumed active growth even after sedimentation on the bottom of the lake due to ultrasonic radiation. In fact, the regeneration of gas vesicles was relatively rapid (10). Furthermore, cyanobacteria would appear to have a better chance of recovery if they settle in a shallow area, as sonicated *Microcystis* did not show any difference in growth with untreated cells under conditions of aeration and sufficient light (11). Lake Senba is a very shallow lake with a mean depth of 1 m, which further supports rapid recovery.

A rough estimation on installation and operation cost showed that the ultrasonic radiation was more economical than other chemical and physical methods such as alum treatment and hypolimnetic aeration (34). This technology was originally designed to affect the epilimnion because *M. aeruginosa* is usually floating near the surface. Compared with chemical treatments applied in whole depths, the ultrasonic treatment can be more cost-effective by focusing only on the surface water.

In conclusion, the effect of ultrasonic radiation was very fast and definitive in controlling cyanobacteria. In addition to disrupting the gas vesicles, ultrasonication was also found to disturb the cell cycle and cell division, while the variability of sensitivity to ultrasonic radiation during the day provides for more economical and efficient cyanobacterial growth inhibition. Finally, a field study based on an enclosure experiment confirmed the applicability of ultrasonic radiation to the control of cyanobacteria.

### Acknowledgments

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