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Evaluation of Power Ultrasonic Effects on Algae Cells at a Small Pilot Scale

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Abstract: It has been recognized for several years that power ultrasound can effectively inactivate algae cells at a laboratory scale. However, although ultrasonic inactivation shows great potential, there are few reports of its use when applied on a large scale. In this study, we have investigated the uses of two types of ultrasonic equipment at a small and medium laboratory scale for the control of algae blooms which are commercially available in similar configurations for industrial scale operation. The following equipment was tested using cultured algae suspension: (a) Dual Frequency Reactor (DFR) operating on 1 L in batch mode and 3.5 L in recirculating mode with two resonating plates at different frequencies of 16 and 20 kHz (Advanced Sonic Processing Systems, USA); (b) Sonolator operating in a flow mode treating 5 L using hydrodynamic cavitation (Sonic Corporation, Stanford, CA, USA). The most effective inactivation was obtained using the DFR in batch mode at 60% power setting for 10 min which resulted in a reduction of 60% of the original concentration (measured using optical density OD). In a recirculating loop mode, the treatment of 3.5 L algae suspension with a DFR for 15 min resulted in a reduction of 46% (OD). Ultrasonic treatment of 5 L suspension in a recirculating loop using the Sonolator over 5 h resulted in a reduction of 30% (OD). This study is the first to explore the use of two commercially available ultrasonic systems (DFR and Sonolator) both capable of direct scale-up to industrial levels for the control of algae. It demonstrates that sonication in a recirculating process has the potential to be effective in the treatment of algal cells on a large scale.

Keywords: algae blooms; ultrasound; pilot scale; industrial process

1. Introduction

There is a great deal of interest in methods for the control of algae blooms due to increasing environmental concerns relating to the contamination of drinking water resources [1]. According to a WHO report, approximately 60% of algae samples contain toxins [2]. Illness resulting from toxic algae has been documented as skin irritation, fever and liver damage. When cell numbers reach 10^5 per mL in drinking water, it can cause long-term illness, in particular liver cancer [3]. Current filtration systems are unable to cope with such blooms because algae can grow on filters, thus reducing their efficiency and sometimes resulting in filter blockage. Furthermore, odours, algal metabolites, toxins and other complex organic contaminants caused by algae activity are difficult to remove using conventional water treatment processes. While an increase in biocide concentration, e.g., chlorine will increase the amount of algae killed, this can be accompanied by the formation of harmful by-products as a result of interactions with the biocide [4]. Chemical methods also suffer from limitations due to potential non-algae specific effects, i.e., harm to other life-forms. The use of UV irradiation as a physical technique is promising but it becomes less effective when the water is turbid due to the build-up of algae or the presence of a suspension of particulate matter. In such turbid water, the light is scattered and becomes less effective. In addition, dissolved organic matter from algal suspension

can reduce the effectiveness of UV disinfection. Thus, there remains a need for another effective and safe control for the control of algae blooms.

The use of ultrasound has been suggested as an alternative and innovative technology for the inhibition of algal growth [5]. This type of treatment is environmentally friendly since it does not involve the use of chemical biocides and is almost unaffected by any turbidity in the water. We have reported that under laboratory conditions there are several ultrasonic parameters which can influence algal growth including frequency, intensity and treatment time [6]. The effects of ultrasound are based on the formation and collapse of acoustic cavitation bubbles [7–9]. The overall effects of acoustic cavitation on algae cells can be summarized as follows [6,10,11]: (1) High power low frequency ultrasound results in a reduction of algal cell numbers through the mechanical rupture of algal cell walls through cavitation bubble collapse; (2) Low power ultrasound can induce declumping of flocs of algae into single cells, thus making them more susceptible to chemical or sonochemical treatment; (3) Higher frequency ultrasound, although producing less powerful mechanical effects of cavitation collapse, generates more free radicals from the decomposition of water than low frequency ultrasound. Free radicals reduce cell numbers via chemical attack on the cell walls.

It has been shown that power ultrasound is effective on a lab scale for the inactivation of microorganisms [12,13], the removal phyto- and zooplankton in water [14] and the reduction of algae cell numbers [14]. There are however few commercially available ultrasonic treatments for the control of algae that have been examined and tested on a larger scale.

One system that has been used for large-scale applications of sonochemistry is the Dual Frequency Reactor (DFR, Advanced Sonic Processing Systems) which has also been employed in environmental applications for the degradation of organic contaminants in wastewater [15]. The DFR consists of opposing plates operating at 16 and 20 kHz. The use of dual frequencies introduces interference in the active volume between the plates and accentuates the cavitation activity. For the processing of algae suspensions, this ensures complete and thorough treatment for all of the cells inside the active volume. Another system for flow treatment is the Sonolator (Sonic Corporation, Stanford, CA, USA) which employs hydrodynamic cavitation to produce its effects by forcing water through a small aperture. This commercial system has been mainly used in mixing processes. Xu et al., have used hydrodynamic cavitation to inhibit algal growth, a 6 L suspension of algae was recirculated through the system for a period of over 114 h [16]. Hydrodynamic cavitation is less powerful than acoustically generated cavitation and so it requires longer exposure times generally via recirculation for use in environmental protection [17].

We have investigated the potential for scale-up of these two commercial cavitation devices for algae cell removal. A Dual Frequency Reactor (DFR) with an active volume between the plates of 1 L was used to treat algae suspensions in both a static mode (1 L) and in a recirculating system involving a total volume of 3.5 L. These results were compared with those obtained with a Sonolator recirculating a volume of 5 L of the algae suspension over a period of 5 h. Both of these systems in recirculating configurations may be scaled up for commercial operation.

2. Material and Methods

2.1. Algae and Culture Conditions

Microcystis aeruginosa used in this study was purchased from Culture Collection of Algae and Protozoa (CCAP—strain number 1450/15). It was cultured using blue-green medium (BG11-CCAP). Algal suspensions were placed in a plant growth room and incubated at 25 °C while being exposed to 12-h cycles of incandescent lights and darkness to reproduce natural day and night cycles (diurnal). The algae employed in this research were by a logarithmic growth phase.

2.2. Ultrasonic Equipment

2.2.1. Dual Frequency Reactor (Advanced Sonic Processing Systems, USA)

The Dual Frequency Reactor (DFR, Advanced Sonic Processing Systems, Oxford, MS, USA) (Figure 1A) has two vertical metal plates each vibrating at a different frequency (16 and 20 kHz). Each plate is 12 × 50 cm (0.06 m^2) and is driven by a magnetostrictive transducer, with a gap of 5 cm providing an active volume of 1 L. Employing a combination of two different frequencies (16 and 20 kHz) has the advantage over two plates at the same frequency in that the cavitation zone is far more active [18]. The reactor was operated at a power setting of 40% and 60% of maximum power (at higher power settings it was not possible to control the temperature of the algae suspension). Ultrasonic treatment was employed for 10 min on a volume of 1 L algal suspension (~0.2 OD at 680 nm) retained in the volume between the two plates, i.e., in a "batch" mode. The normal configuration for this system is a "recirculating" mode that involved the use of a pump to drive liquid through the reactor set in a vertical position from the bottom (Figure 1B). For algae treatment, 3.5 L of the algae suspension was continuously recirculated back through the pump and DFR from a reservoir.

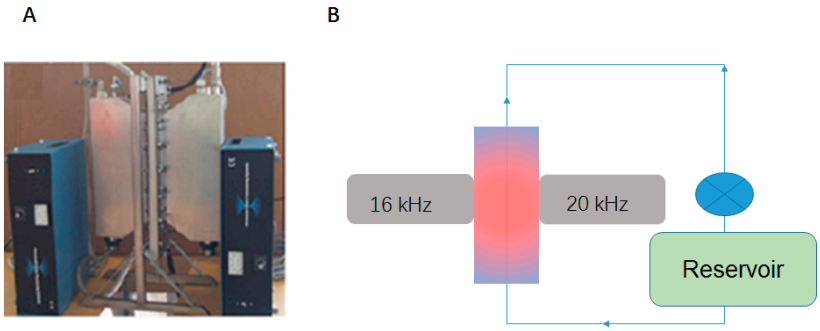

Figure 1. (**A**) 16 and 20 kHz Dual Frequency Reactor (DFR, Advanced Sonic Processing Systems, 1 L batch and 3.5 L circulating); (**B**) Representation of the DFR reaction chamber in recirculating mode.

2.2.2. Sonolator (Sonic Corporation, Stanford, CA, USA)

The Sonolator (Sonic Corporation, Stanford, CA, USA) is an in-line homogenizing device that provides hydrodynamic cavitational energy by forcing a liquid under pressure through a small orifice and on to a metal blade set in its path (Figure 2A) [19]. The rapid flow of fluid across the blade produces hydrodynamic cavitation in an active volume of approximately 50 mL (Figure 2B). This equipment is generally used for mixing, homogenization, emulsification and dispersion. In our work, the Sonolator was connected through a reservoir to provide continuous recirculation of 5 L of algae suspension through the cavitation zone.

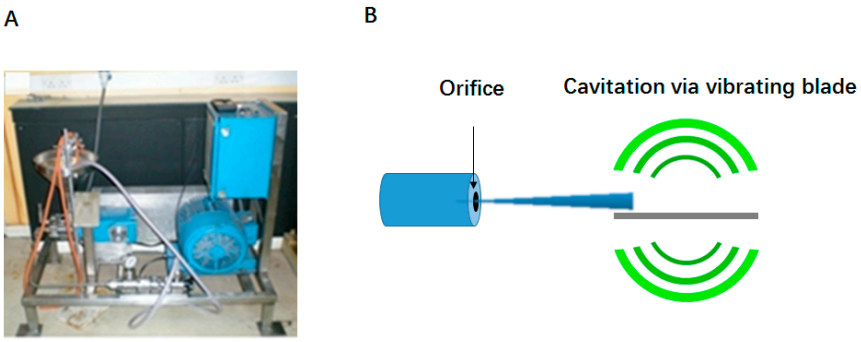

Figure 2. (**A**) Sonolator (Sonic Corporation); (**B**) representation of the "liquid whistle" effect generating hydrodynamic cavitation as liquid is forced through an orifice and across a blade.

2.3. Ultrasonic Treatment

2.3.1. Sonication of Algae Using 16 and 20 kHz Dual Frequency Reactor (DFR)

The DFR is normally used in a recirculation mode but for these experiments a batch mode was also investigated where 1 L of the algae suspension was held in the active zone of the DFR between the two resonating plates. In both batch and recirculating modes, two power settings were employed of 40% and 60%. Higher powers could not be used in this work because it proved difficult to maintain a steady temperature under higher power conditions due to the heat generated by the transducers. In the batch mode, the reaction volume within the DFR was 1 L and the Intensity calculated by calorimetry was 0.0177 W/cm^3 at 40% power and 0.0256 W/cm^3 at 60% power. The DFR was filled with a suspension of *Microcystis aeruginosa* (OD of 0.25 at 680 nm) and sonicated for 10 min. It was not possible to withdraw samples for analysis from the cavitation zone of the DFR and so samples were taken after 0, 1, 2, 3, 5 and 10 min by turning off the equipment and draining to allow removal of a sample then refilling the DFR and continuing until the next sample time. Using this method, the temperature was kept below 30 °C throughout measurements at both powers.

The recirculating mode involved sonicating 3.5 L standard suspension (OD of 0.15 at 680 nm) of *Microcystis aeruginosa* at 40% and 60% power for 15 min. Samples were taken from the suspension outside of the reactor after 0, 1, 2, 5, 10 and 15 min treatment. Following 15 min treatment, the total exposure time in the ultrasonic reactor was calculated as 5.67 min at a flow rate of 1 L/min; the temperature during recirculating treatment did not rise above 25 °C at 40% power and 30 °C at 60% power.

2.3.2. Sonication of Algae Using the Sonolator for 5 h

A suspension of *Microcystis aeruginosa* (5 L, optical density (OD) of 0.2 at 680 nm) was sonicated for 5 h recirculating with a flow rate of 4.6 L/min. The suspension temperature was maintained below 25 °C using a cooling system (FL300, Julabo, Seelbach, Germany). Samples were taken after 0, 1, 2, 3, 4 and 5 h ultrasonic treatment. In this system, the actual time of exposure of the 5 L suspension in the active cavitation zone (50 mL) was calculated to be 1.09 min over a 5 h treatment. At this pumping speed, the frequency generated in the orifice with vibrating plate was 30 kHz (according to manufacturer's information). It was not possible to measure the intensity of cavitation in this system.

2.3.3. Measurement of the Effect of Cavitation on Algae Suspensions

Optical density measures the amount of light absorbed by an algal suspension and the absorbance at 680 nm can be used as an estimate of algae concentration. All experiments were performed in triplicate and the results were reported as an average percentage reduction, calculated using the following equation:

$$\% \text{ cell remaining} = (C_t/C_0) \times 100\%$$

where C_t represents the concentration measurement at time = t and C_0 at time zero.

3. Results and Discussion

3.1. Sonication of Algae Using the Dual Frequency Reactor (DFR)

3.1.1. Batch Mode

Heating of an algae suspension may result in the killing of cells and so in these studies the temperature was controlled to under 30 °C during ultrasonic treatment to minimise any heating effects. At 40% power setting (1 L, Intensity 0.0177 W/cm^3) for a 10 min batch treatment on 1 L algae suspensions, a continuous reduction in optical density was observed over time with a final value for the remaining cell percentage of 92% (Figure 3). At 60% power setting (1 L, Intensity 0.0256 W/cm^3), a much higher inactivation was achieved such that after 10 min batch treatment the remaining cell

percentage was 39.17% by optical density. The DFR system was developed originally to introduce high power ultrasound into flow systems from small to large scale and so it offers a potential method for water treatment [19]. The major advantage of the DFR in batch mode is that it allows the whole of the algal suspension to be in the cavitation zone of the reactor during sonication whereas in a flow system the residence time would depend on the flow rate. At the low frequencies employed in this system (16 and 20 kHz), acoustic cavitation leads mainly to mechanical effects, i.e., high shear forces generated can lead to the direct rupture of cells and thus immediate cell death. It has been shown that both ultrasonic frequency and intensity have a significant effect on *Microcystis aeruginosa*. At low intensities, sonication can cause declumping together with some inactivation and the proportion of which of these predominates depends on the conditions used [10,13]. Figure 3 shows that, in the case of the DFR system, an increase of ultrasonic intensity leads to an improvement in inactivation. The results were compared with other conventional methods which show that DFR sonication in static mode is effective within a relative short period (Table S1).

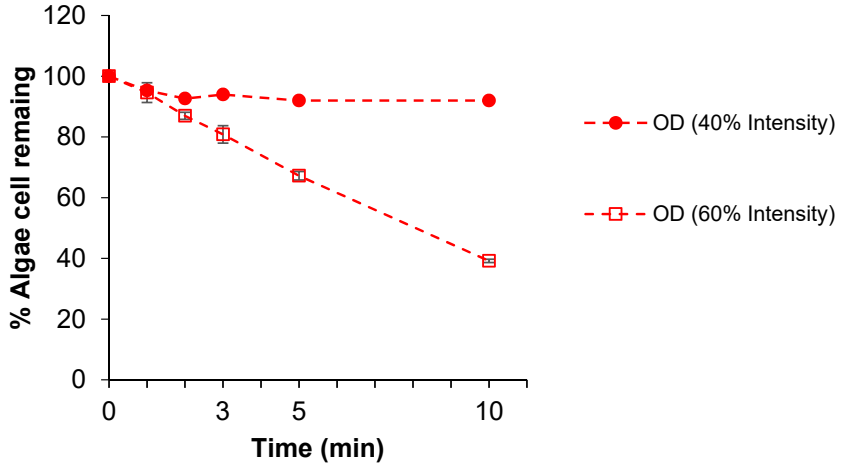

Figure 3. Sonication of 1 L of *Microcystis aeruginosa* suspension in batch mode at 40% and 60% power settings (OD = optical density at 680 nm).

3.1.2. Recirculating Mode

In order to apply high power sonication to the treatment of a larger volume of liquid, the method of choice is normally a flow system in which the mixture to be treated is pumped from a reservoir through an intense sonication zone and then either to the next step (a flow through system) or back to the reservoir in a continuous cycle (flow loop system) [19]. In our studies, the DFR system was used in the latter type of loop system to treat 3.5 L of suspension (OD 0.2 at 680 nm) at a flow rate of 1 L/min. At 40% power over 15 min, the results from optical density measurements showed very little effect in terms of algae cell reduction (Figure 4). This suggests that under very mild sonication conditions there is little inactivation. This is in accord with previous observations [6,10]. However, at 60% power, the effect of sonication over 15 min was significant with the final percentage reduced to 54.31% (Figure 3). The contrast in sonochemical treatment between batch and flow loop conditions is made clear when the actual "residence time" of the suspension in the cavitation zone is calculated [20]. Batch conditions involved a total of 15 min exposure of 1 L suspension in the active zone between the plates. In contrast, in the recirculating mode involving 3.5 L of suspension at a flow rate of 1 L/min over 15 min, the actual time of the flowing suspension in the 1 L active zone is 4.29 min.

Table 1 shows that the inactivation effects on algae from such a dual frequency ultrasonic field operating in a flow system at 60% power is similar to that of the single 20 kHz probe at 0.0403 W cm^{-3} (200 mL) with the remaining cell percentages of 54.31% and 50.82% (OD at 680 nm) respectively over 30 min [10]. These results suggest that the DFR system has great promise for providing effective

treatment of surface water with algae blooms because; unlike the probe system, it can be directly scaled up with the same configuration. In such a configuration, a substantially increased flow rate could provide adequate cooling to allow the device to operate at much higher powers. The inhibition effect of sonication on algae was assessed by culturing the cells remaining after sonication for 48 h. Compared with control samples, the cell numbers did not increase rapidly, indicating an inhibition effect (Figure S2). The results demonstrated that powerful sonication treatment has great potential as a method for algae control and removal.

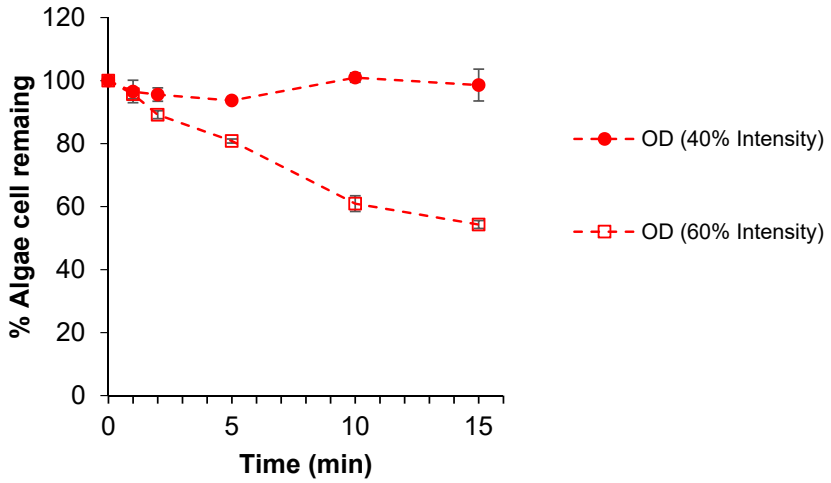

Figure 4. Sonication of 3.5 L of *Microcystis aeruginosa* suspension using the DFR in recirculating mode at 40% and 60% power setting (OD = optical density at 680 nm).

Table 1. The effects of ultrasound on *Microcystis aeruginosa* algal suspensions using 20 kHz probe and the DFR system.

Ultrasonic Treatment	Intensity	Volume (Litre)	Residence Time (Minutes)	% Cell Remaining (OD)
20 kHz Probe [10]	0.0403	0.2	30	50.82
DFR (Static, 40% power setting)	0.0177	1	10	92
DFR (Static, 60% power setting)	0.0256	1	10	39.17
DFR (Recirculating, 40% power 15 min)	-	3.5	4.29	98.60
DFR (Recirculating, 60% power 15 min)	-	3.5	4.29	54.31

3.2. Sonication of Algae (5 L) Using the Sonolator in Recycling Mode for 5 h

The Sonolator was equipped with a circulating pump to drive 5 L *Microcystis aeruginosa* suspension (OD 0.2 at 680 nm) through the hydrodynamic reactor zone (50 mL) at a flow rate of 4.6 L/min. The residence time in the reactor zone is only 3 min over the period of 5 h. Following ultrasonic treatment with the Sonolator, the concentration of algae by optical density steadily decreased to 72.86% of the original value (Figure 5). Our tests demonstrate that hydrodynamic cavitation as generated in the Sonolator can reduce algal concentration although the treatment required a large number of passes through the active zone of the equipment. There have been a few reports of the use of hydrodynamic cavitation devices for algae control but in general they do not produce such an immediate effect as direct sonication with power ultrasound [16]. As with acoustic cavitation, it is possible that hydrodynamic cavitation can break up flocs of algae into dispersions of single cells thus providing a similar competition between declumping and inactivation with the result depending on the conditions and length of treatment.

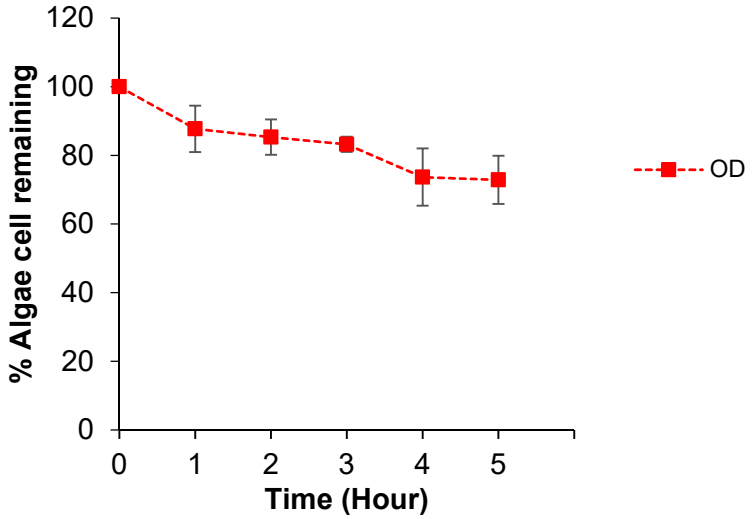

Figure 5. Inactivation of 5 L of *Microcystis aeruginosa* suspension using the Sonolator system (OD = optical density at 680 nm).

4. Conclusions

A 60% inactivation (by OD) of 1 L *Microcystis aeruginosa* suspension in batch mode was obtained using acoustic cavitation generated by a Dual Frequency Reactor operating at 60% power (Advanced Sonic Processing Systems, USA). It was not possible to use higher powers in this configuration due to overheating of the suspension. Using a recirculating mode, the temperature could be controlled and at 60% power 3.5 L suspension was treated for 15 min to achieve 45% reduction (OD). The difference between static and recirculating mode is that in the static mode the whole sample is in the ultrasonic zone of the reactor for the entire time whereas in the circulating mode a larger volume was used and only part of the suspension was in the active zone of the reactor at any one time. At a flow rate of 1 L/min, the exposure time over 15 min circulation was only 4.29 min.

Hydrodynamic cavitation using a Sonolator (Sonic Corporation) over 5 h recirculation of 5 L suspension at 4.6 L/min achieved 27% inactivation (OD). Under these conditions, the ultrasonic frequency was 30 kHz and the length of exposure in the 50 cm^3 reaction zone was only 3 min. These results indicate that hydrodynamic cavitation can control algae in water but it does require recycling through the reactor and a substantial treatment time.

Overall, these results show that with further scale-up and optimization, similar systems could be developed and made available for very large-scale algae treatment.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4441/9/7/470/s1, Figure S1: Heat of 1 L of *Microcystis aeruginosa* suspension at 30 °C for 15 min and culture for 48 h (OD = optical density at 680 nm), Figure S2: Resistance test on Microcystis aeruginosa for 48 h (OD = optical density at 680 nm), Table S1: Review of conventional removal methods on *Microcystis* cells.

Author Contributions: X. Wu and T. Mason conceived and designed the experiments; X. Wu performed the experiments; X. Wu and T. Mason. analyzed the data; X. Wu contributed reagents/materials/analysis tools; X. Wu and T. Mason wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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