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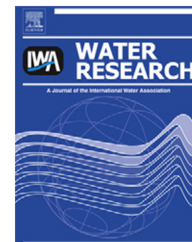

Review

A review of the use of sonication to control cyanobacterial blooms

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ABSTRACT

The development of cyanobacterial blooms in water bodies imparts undesirable characteristics to the water such as odours, tastes and the potential presence of toxins. Several chemical and physical methods have been used to control the blooms, but have limitations in terms of pollution and application on a large scale. A more recent approach has been the use of sonication in the control of cyanobacteria (also referred to as blue–green algae). This paper reviews current advancements in research on using sonication to control cyanobacteria, particularly *Microcystis aeruginosa*, as it is a prevalent and a major bloom-forming toxic species. The impact of sonication on the structure and function of *M. aeruginosa* is discussed, including the influence of sonication parameters such as power intensity, frequency and exposure time. Alternate strategies of cyanobacterial control in combination with sonication are also reviewed.

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1. Introduction

Cyanobacteria are abundant in water with a neutral or slightly alkaline pH. They are commonly 3–10 μm in size, depending on the species, and many contain gas vacuoles which are sensitive to variation in light intensity and aid in buoyancy regulation of the cells. They have a cell wall composed of peptidoglycan and lipopolysaccharide layers inside which resides the plasma membrane that surrounds the cytoplasm. Like other prokaryotes, they lack a nucleus and distinct organelles, and they are characterised by the presence of phycobiliproteins (Ressom et al., 1994). The members of the genus *Microcystis* are the most prevalent cyanobacteria worldwide, and the species *Microcystis aeruginosa* is the major bloom-forming organism (Carmichael, 1992; Willen and Mattsson, 1997; Silva, 2003; Sömek et al., 2008; Giannuzzi et al., 2011). *M. aeruginosa* can produce the hepatotoxin microcystin, a possible carcinogen (Mankiewicz et al., 2003), of which there are multitudinous congeners. Consequently, proliferation of this organism can interrupt the supply of drinking water, or recycled water should it occur in treatment lagoons. Cyanobacterial blooms can also be a financial burden to the operators of water treatment facilities where the cells can clog filters, leading to the use of significant amounts of chemicals for treatment, and deterioration of water quality (Lee et al., 2000a). The current treatment options such as filtration, coagulation, clarification, flotation, algicides, ozone and photolysis (Svrcek and Smith, 2004) are expensive, complex, and may cause further pollution (Lee et al., 2000a).

Ultrasound (sound waves of a frequency higher than 20 kHz) can lead to structural and functional disruption of cyanobacterial cells (Phull et al., 1997) and its use as a treatment option to control cyanobacterial blooms has been under consideration in recent decades. Ultrasonic radiation in water causes a series of compression and rarefaction cycles leading to the generation of cavitation bubbles (acoustic cavitation). Millions of these bubbles implode causing localised temperatures as high as 5,000 °C, pressures of up to 100 MPa and free radicals (Suslick, 1990). This has led to the application of acoustic cavitation to control cyanobacterial blooms in eutrophic water (Lee et al., 2000a).

Some studies have shown that sonication inhibits the growth rate of cyanobacteria by rupture or collapse of gas vesicles owing to cavitational effects causing disruption of the cell wall and membrane, interruption of photosynthetic activity, and inhibition of the cell division and cell cycle. The extent of damage and thus control is dependent on parameters such as frequency, intensity and duration of exposure (Nakano et al., 2001; Lee et al., 2002; Ahn et al., 2003; Tang et al., 2004; Zhang et al., 2006a; Purcell, 2009; Rajasekhar et al., 2012). The major advantage of sonication is that it is environmentally friendly compared with treatment strategies such as the use of algicides. Other treatment strategies such as minimizing nutrient loading are very difficult to implement within agricultural communities, and techniques such as aeration/artificial mixing, using filters embedded with activated carbon, or barley straw are relatively difficult to apply on a larger scale (Harper, 1992). On cell lysis, *M. aeruginosa* releases microcystins (Ressom et al., 1994) and as sonication has been reported to be effective for degrading microcystin-LR (Song et al., 2005), this could be an added advantage to its use in the control of the growth of this cyanobacterium. The purpose of this paper is to review the relevant studies to date on the use of sonication as a means of controlling cyanobacterial blooms, with a particular focus on *M. aeruginosa*.

2. Sonication parameters

The power or intensity, frequency and duration of exposure play an important role in controlling algal growth by sonication.

Frequency is a very important parameter that controls the behaviour of cavitation. At lower frequencies, the cavitation bubble has sufficient time to grow to its peak negative pressure. This results in a stronger shockwave when it implodes or is ruptured. At higher frequencies, less time is available for creating the cavitation bubble as the rarefaction cycle will be shorter. Generally speaking, higher intensities increase the sonochemical effects. Thus as the frequency increases, comparatively greater sound intensities have to be employed to attain cavitation. For example, 10 times more power is required to induce cavitation in water at 400 kHz than at a frequency of 10 kHz. Thus it may be more cost efficient to employ lower frequencies (Mason and Lorimer, 2002; Khanal et al., 2007).

Ultrasonic density is the power supplied per unit volume of the medium, expressed as W/L, kW/L or W/mL. It relates to the power input to the volume of medium, but does not account for duration (Khanal et al., 2007). *Ultrasonic dosage* is the amount of energy supplied per unit of volume of medium and is expressed as Ws/L (Khanal et al., 2007). This can be related to units of electricity or power consumed by representing it in terms of kWh. *Ultrasonic intensity* is the power supplied to the medium per unit of converter area, expressed as W/cm^2. It reflects the power generating capacity of the converter. Higher amplitudes imply higher ultrasonic intensity production (Lee et al., 2000a). Like frequency and intensity, the *duration of exposure* plays an important role in effective sonication. Increased duration means more rarefaction and compression cycles leading to added cavitation effects, and thus increased sonochemical effects (Suslick, 1990).

3. Effects of ultrasound on *cyanobacteria*

3.1. *Rupture of gas vacuoles*

The gas vacuoles within cyanobacteria provide buoyancy, aiding in regulating the position in the water column and thus their exposure to light, an important factor in determining the extent of their growth (Reynolds, 1972). The gas vacuoles are made up of stacks of cylindrical gas vesicles which are closed by conical ends (Bowen and Jensen, 1965; Walsby, 1994). The majority of the literature has discussed the impact of sonication on *M. aeruginosa* in terms of its disrupting or collapsing the gas vacuoles. It was shown by three German scientists, Ahlborn, Klebahn and Strodtmann, in 1895, that after applying pressure to an algal suspension the gas vesicles collapsed (Hammer, Cork and Bottle experiment) and the cells sank to the bottom of the vessel (Walsby, 1972). In contrast, the kinetics of assembly of gas vacuoles in *M. aeruginosa* was studied by using sonication as a means of collapsing the gas vacuoles (Lehmann and Jost, 1971). These studies inspired the idea of using ultrasonic radiation to damage the gas vacuoles of the algal cells, causing them to sink within the water column and so reducing their access to sunlight, thus helping to control algal blooms (Lee et al., 2000a). Lee et al. (2000a) observed that after sonication of cyanobacteria at 120 W input power and 28 kHz for just 3 s, 80% of the algal suspension settled, and almost all settled after 30 s exposure. Transmission electron microscopy of the cells showed that the gas vacuoles were intact before sonication and collapsed after sonication (Lee et al., 2000a). These results were in accordance with another study of the effect of 1.7 MHz ultrasound (intensity of 0.6 W cm^{-2}) on a strain of *M. aeruginosa* with gas vacuoles and a cyanobacterium devoid of gas vacuoles (*Synecochoccus PCC7942*) by Tang et al. (2004). Repeated sonication of the former inhibited growth severely and increased generation time, whereas the latter was not affected and this was attributed to the absence of gas vacuoles.

Once a gas bubble is disturbed from its state of rest by a compression or rarefaction, it will pulsate at its natural frequency provided that there is no further interference or applied force. If an acoustic wave forces the bubble to pulsate at this frequency, the bubble resonates, i.e., it exhibits high amplitude oscillations or pulsations in response to the force. The frequency at which there is a maximum response or vibrational amplitude is known as the resonance frequency of that bubble (Ainslie, 2010). The resonance frequency of free bubbles can be estimated by the following equation (Phillips et al., 1998):

$$f = \frac{1}{2\pi a}\sqrt{\frac{3\gamma}{\rho}\left(p_0 + \frac{2\sigma}{a}\right) - \frac{2\sigma}{a\rho}}$$

where f is the ultrasound frequency, γ is the ratio of heat capacities of the gas at constant pressure and volume (1.39 for air), a is the radius of the bubble, p_0 is the ambient pressure (=105 Pa), ρ is the density of the surrounding medium (=10^3 kg/m^3), σ is the surface tension of the surrounding medium, which is ignored.

Similarly, for a given ultrasound frequency the size of the bubbles that will resonate, or the resonance size of the free bubbles, can be estimated from the above equation (Phillips et al., 1998; Zhang et al., 2006b). When the assumed values in the brackets are used, the actual resonance frequency of an air bubble in water is 0.166 mm at 20 kHz and 2.47 μm at 1320 kHz (Zhang et al., 2006b). Gas vesicle size in cyanobacteria appears to be determined by the balance between effective provision of buoyancy and the strength required by the gas vesicle to withstand hydrostatic pressure, where the strength is determined by the stiffness of the gas vesicle wall material and ratio of wall thickness to cylinder radius (Walsby, 1971, 1992). Zhang et al. (2006b) considered the gas vesicles in *M. aeruginosa* to be up to 1 μm in length. To make use of the above equation, the cylindrical gas vesicle is approximated to be a sphere, due to its very small size. By calculation it can be seen that the resonance size of gas vacuoles is of the same order of magnitude as the cavitation bubbles, thus undergoing acoustic cavitation and eventually collapsing. Therefore the gas vesicles are more likely to resonate at higher rather than lower sound frequencies (Zhang et al., 2006b).

In contrast, studies on gas vesicle structure suggested that the maximum gas vesicle size measured for *M. aeruginosa* after 12 h growth was 600 nm or 0.6 μm (Lehmann and Jost, 1971; Walsby, 1994) and the mean length was 430.2 nm (Walsby, 1994). The use of the value of 1 μm in the above calculations may be because some literature reports generalize the length of cyanobacterial gas vesicles to be up to 1 μm (Bowen and Jensen, 1965; Walsby, 1994). Hao et al. (2004b) calculated the resonance frequency to be approximately 6.5 MHz by assuming the diameter and length of the gas vesicle to be 1 μm. As previously noted, the use of the above equation for calculating the resonance size of gas vesicles required the small cylinder to be approximated to a sphere. However, the length to diameter ratio (l:d) of the gas vesicle based on the values obtained from Walsby (1994) is approximately 7:1 (for ease of calculation, length of gas vesicle was approximated to 500 nm, diameter, d = 65.2 nm) (Walsby, 1994). Clearly, if the l:d ratio approached 1, the assumption of approximating the cylindrical gas vesicle to a sphere would be more accurate. These assumptions could lead to a significant margin of error in calculating the resonance size of a gas vesicle for cyanobacteria.

Another study (Tang et al., 2004) used the size of the gas vacuole instead of the vesicle in the equation, since gas vacuoles are made up of the cylindrical gas vesicles (Walsby, 1994). The radius of the vacuole in *M. aeruginosa* was taken to be 3–5 μm and the resonance frequency was calculated to be 1.3–2.16 MHz. The authors investigated the resonance effects of gas vesicles by performing sonication on cyanobacteria with and without gas vacuoles. Change in electrical conductivity was used as an indirect indicator of cavitation yield in the algal suspension. There was a sharp increase in electrical conductivity of the *M. aeruginosa* after sonication at 1.7 MHz, 0.6 W/cm^2, which supported the theory of resonance effects on gas vacuoles (Tang et al., 2004). The above-mentioned studies point towards the use of higher frequencies for controlling cyanobacterial blooms through vacuole rupture. In spite of higher frequencies having lower cavitation intensities at the same power intensities, there is effective inhibition of the cells compared with lower frequencies due to

their proximity to the resonance frequency of gas vesicles/vacuole (Hao et al., 2004b).

Despite potential errors associated with the assumptions and the differences between studies, there is a general consensus that the resonance effects on gas vacuoles/vesicles in cyanobacteria during sonication are a major cause of gas vesicle/vacuole collapse and effective growth inhibition (Hao et al., 2004b; Tang et al., 2004; Zhang et al., 2006b).

It is possible for gas vacuoles to regenerate after sonication, this depends on the environmental conditions. After sonicating *M. aeruginosa* at 1.75 W/mL, 28 kHz for 30 s, Lee et al. (2000b) investigated the rate of regeneration of gas vacuoles under different incubation conditions of light and aeration. Flow cytometry was used to differentiate sonicated and unsonicated cells via the presence of intact or collapsed gas vacuoles, based on the amount of side scatter light. Four different conditions of illumination and aeration were used for culturing the sonicated cyanobacteria: aerated (1 L/min) and illuminated (27 $\mu E/m^2 s$), non-aerated and illuminated, non-aerated and partially illuminated ($\sim 5.5\ \mu E/m^2 s$), and non-aerated and non-illuminated. They found that when aerated and illuminated, the sonicated cyanobacteria reached the same cell concentrations as the control culture within 24 h, whereas under non-aerated and illuminated conditions it took 36 h; under non-aerated and partially illuminated conditions the sonicated cyanobacteria reached 87% of the cell concentration of the control in 60 h. Under conditions of no illumination and no aeration, the sonicated cyanobacteria did not regenerate their gas vacuoles. Thus the authors concluded that the rate of gas vacuole regeneration of sonicated cyanobacteria was mainly dependent on the intensity of illumination.

3.2. *Effect on photosynthesis*

Algae and plants utilise antenna complexes to capture the photons from sunlight effectively. The two major antenna complexes are chlorophyll–protein complexes within the cell membrane (i.e., water soluble) and phycobiliproteins (phycobilisomes or PBS) outside the cell membrane (water insoluble). Unlike other bacteria, cyanobacteria possess chlorophyll *a* which absorbs light at both 430–440 nm and 660–680 nm, as well as PBS which absorbs over 470–650 nm, making them oxygenic photosynthetic organisms (Ho and Krogmann, 1982).

One study of the effects of sonication on cyanobacterial photosynthesis focused on the impact on the photosynthetic pigments and oxygen evolution rate. Lee et al. (2000a) utilized the change in chlorophyll fluorescence and concentration of chlorophyll over time at different power settings as a measure of the effect on photosynthetic activity. All the power settings tested showed that ultrasonic irradiation inflicted immediate damage on photosynthetic activity, and the degree of damage was dependent on input power and frequency (Lee et al., 2000a). Another study on the effect of sonication on another bloom-forming cyanobacterium, *Spirulina platensis*, showed that there was a greater effect on phycocyanin than on chlorophyll *a* (Tang et al., 2003). The authors also showed that the collapse/implosion of gas vesicles, which can yield very high and localized temperatures, decreased the photosynthetic activity.

A more detailed study by Zhang et al. (2006a) showed that treatment of 250 mL cyanobacterial suspension at a frequency of 25 kHz and power density of 0.32 W/mL for 5 min led to reductions in chlorophyll *a* (by 21.3%), phycocyanin absorbance (44.8%), photoactivity (40.5%) and cell concentration (10.8%). These changes showed that the photosynthetic components were significantly damaged, resulting in inhibition of photosynthesis and reduction of cell growth rate (Fig. 1). Zhang et al. (2006a) explained that the phycocyanin was impacted more than chlorophyll *a* because of its empty rod structure which is prone to collapse during cavitation, and/or chlorophyll *a* being within the cell membrane and so protected against cavitation. Purcell (2009) showed a greater reduction in photosynthetic activity in comparison to corresponding decrease in cell number for *M. aeruginosa* at a frequency of 862 kHz (60% cf. <20%). However, weaker effects on chlorophyll *a* were noted in other studies: with settings of 80 W at 80 kHz, initial chlorophyll *a* concentration decrease was slower than the decrease in cell concentration and stabilised after 5 min (Zhang et al., 2006b). A similar trend was noted in a field study at Lake Senba, Japan (Nakano et al., 2001). The results for this very shallow lake (mean depth of 1.0 m) are discussed in detail in Section 7 on alternate strategies of cyanobacterial control.

3.3. *Free radical reactions*

Free radical reactions are a vital part of sonochemistry. It is believed that they destroy cyanobacterial cells by inhibiting photosynthesis and causing lipid peroxidation, with most of the damage due to lipid peroxidation resulting in damaged cell membranes. However, there does not seem to be a consensus on whether free radicals really do damage *M. aeruginosa* cells or whether it is sufficient to be significant. In one study, hydrogen peroxide was added to increase the number of free radicals produced during sonication, but the resultant increase in lipid peroxidation was small (Ahn et al., 2003). It has been postulated that free radicals are not directly involved in lipid peroxidation in cyanobacterial cells and also that the antioxidant activity of phycobiliproteins protected the cell (Hirata et al., 2000; Estrada et al., 2001; Ahn et al., 2003).

Tang et al. (2004) observed a different response to sonication by cyanobacteria with and without gas vacuoles.

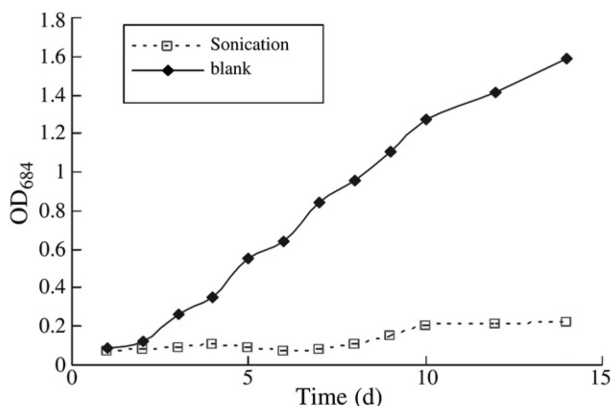

Fig. 1 – Growth inhibition of *Microcystis aeruginosa* by sonication, 25 kHz, 0.32 W/mL, 5 min (Zhang et al., 2006a).

Malondialdehyde (MDA) is a byproduct of lipid peroxidation and its concentration is used as a measure of this reaction using the thiobarbituric acid test (Gutteridge, 1986; Halliwell and Chirico, 1993). Cell membrane damage increases membrane permeability which can be determined by measuring electrolyte leakage. Sonicated *M. aeruginosa* had 60% greater membrane permeability than the cyanobacterium *Synecocchus* which does not possess gas vacuoles. Increase in both MDA and membrane leakage showed that lipid peroxidation occurred in *M. aeruginosa* during sonication and was more pronounced than in the other cyanobacterial species (Tang et al., 2004).

In another study, carbonate was used as a scavenger to remove free radicals, making them unavailable for cell damage. It was observed that addition of carbonate did not affect algal cell inactivation efficiency by ultrasound (Zhang et al., 2006b). Sonication studies on endothelial cells have shown that the mechanical, rather than the sonochemical, effects dominate the fundamental mechanism of increased membrane permeability (Lawrie et al., 2003; Kudo et al., 2009). Thus the increase in membrane leakage in the study of Tang et al. (2004) can be attributed mainly to the mechanistic effects of sonication rather than just lipid peroxidation. For cyanobacteria, the ultrasonic disruption of gas vesicles and subsequent loss of buoyancy would thus be considered as the main cause of algal cell removal, rather than the results of free radical reactions. However the impact of free radical reactions, although not very pronounced, cannot be completely ruled out (Zhang et al., 2006b).

3.4. *Effect on cell cycle, cell division and cellular morphology*

Using a 14/10 h light/dark cycle, it was found that *M. aeruginosa* divides late in the light cycle. Even though sonication was effective regardless of its time of application, the specific growth rate of cells late in the light cycle was 30% lower than in the first hours of the light cycle. Suppression of the cell cycle resulted in higher chlorophyll *a* content and larger cell size. Thus *M. aeruginosa* is more susceptible to sonication late in the light cycle or just before sunset (Ahn et al., 2003).

Algal growth can be divided into lag, exponential growth, declining growth rate, stationary and death phases (Fogg and Thake, 1987). Zhang et al. (2006b) reported that *M. aeruginosa* was more sensitive to sonication during the exponential growth stage, although the difference was relatively small. Sonication studies on filamentous cyanobacterial species such as *Anabaena circinalis* (Rajasekhar et al., 2012) and *Aphanizomenon flos-aquae* (Purcell, 2009) showed relatively greater inhibition in comparison to the unicellular algae studied. The results indicated that sonication affects these filamentous algae by disrupting their filamentous structure and/or cell wall.

4. Influence of sonication parameters on cyanobacterial bloom control

The majority of the studies investigating the impact of sonication on cyanobacteria have focused on *M. aeruginosa* due to its worldwide prevalence. Table 1 provides a summary of the major studies on the use of sonication to control cyanobacterial blooms.

4.1. *Power and frequency*

Examination of the literature shows that a range of frequency, power or intensity, and exposure times has been used in sonication studies on *M. aeruginosa*. Frequency ranges from 20 kHz to 1.7 MHz were used (Lee et al., 2000a, b; Ahn et al., 2003; Tang et al., 2004; Zhang et al., 2006a, b; Joyce et al., 2010; Rajasekhar et al., 2012). The cavitation intensity is dependent on both power and frequency; lower frequency settings are able to induce more powerful cavitation, which accelerates sonochemical reactions. As previously noted, it is known that algal cell reduction is due to acoustic cavitation (generation and collapse of cavitation bubbles) during sonication. As the frequency of sonication increases, less time is available during the rarefaction cycle to create the cavitation bubble, thus requiring greater sound intensities or power to achieve cavitation (Mason and Lorimer, 2002).

The results obtained by Zhang et al. (2006b) were consistent with this observation as the highest frequency of 1.32 MHz at 80 W for 10 min gave the highest cell removal. It took only 20 min to reach 90% cell removal efficiency for 1.32 MHz, whereas 102 min were required at 20 kHz. When selecting frequencies and power settings, energy consumption, exposure time, subsequent microcystin release and the end use of the treated water, need to be taken into consideration. The effects of sonication on microcystin release are discussed in Section 4.3.

Joyce et al. (2010) used 20, 40, 580, 864 kHz and 1.146 MHz at intensities of 0.0178, 0.0213, 0.0018, 0.0042 and 0.0026 W/cm^3, respectively, for 30 min. Although 864 kHz gave maximum algal cell reduction, in terms of efficiency (percentage algal inactivation divided by intensity) 580 kHz was the most efficient. In this study, the timeframe of the whole experiment was comparatively short, i.e., 30 min, which gave only the immediate effects of sonication on *M. aeruginosa*. The power settings were comparatively lower than other studies. Interestingly in this study, sonication at lower frequencies (20 kHz and 40 kHz) led to a decrease in algal cell concentration, followed by an apparent increase after 20 min. The authors explained that two effects occurred during sonication. The first effect was initial ‘inactivation’ of the algal cells during the first 20 min of sonication, leading to a decrease in their concentration. The inactivation of cells refers to the algal cell reduction by the collapse of the gas vacuoles and sinking of the cells. The second effect was that further sonication after 20 min caused declumping and produced more individual cells which explained the apparent rise in algal cell concentration.

Although there have been several studies of sonication at different frequency and power settings, to date there is no recommended combination. As mentioned earlier, increase in frequency requires greater sound intensity or power to attain cavitation. Low frequencies are desirable due to relatively lower power consumption (Mason and Lorimer, 2002). However, previous studies show a trend of higher frequencies being more effective for cyanobacterial inhibition than lower

Table 1 – Summary of the sonication studies.

Algal species	Frequency	Sonication power/density	Duration of exposure	Reference
Microcystis viridis and Microcystis aeruginosa	28 kHz	40 W[a], 120 W[a], 12 W/mL	0, 3, 5, 10, 15 and 30 s	Lee et al. (2000a)
Microcystis spp.	28 kHz	700 W[a]	30 s	Lee et al. (2000b)
Naturally occurring cyanobacteria in Lake Senba, Japan	10 sonication units installed along lake, 200 kHz	100 W[a] each	5 s (experiment ran for 2 years)	Nakano et al. (2001)
Microcystis spp., Naturally occurring cyanobacteria in Lake Senba, Japan	10 sonication units installed along lake, 200 kHz	100 W[a]	Same as above	Lee et al. (2002)
M. aeruginosa	20, 22 kHz	Lab studies: 600 W[a]; Field Study (200 L pond): 630 W, 0.00315 W/mL	Lab studies: 2 times a day for 2 min; Field Study: 40s sonication and 210 s break	Ahn et al. (2003)
Spirulina platensis	Horn system: 20 kHz Beaker system: 200 kHz	0.025, 0.05, 0.075, 0.01 W/mL 0.05 W/mL	5 min each	Hao et al. (2004a)
Spirulina (Arthrospira) platensis	20 kHz 1.7 MHz	0.0875 W/mL 0.0175 W/mL	5 min 0, 1, 3, 5, 7, 9 min	Hao et al. (2004b)
M. aeruginosa	25 kHz	0.32 W/mL	5 min	Zhang et al. (2006a)
M. aeruginosa	Frequency effects studied at: 20, 80, 150, 410, 690 and 1320 kHz	Power effects studied at: 0.16, 0.24, 0.32, 0.4 W/mL at 80 kHz	10 min	Zhang et al. (2006b)
Naturally occurring cyanobacteria	22 kHz	630 W in 9000 m^3 pond	Cyclic sonication: 85 s with 30 s break for a period of nearly 47 days	Ahn et al. (2007)
M. aeruginosa	20 kHz 40 kHz 580 kHz 864 kHz 1146 kHz	0.0714 W/mL 0.0213 W/mL 0.0018, 0.0210, 0.049 W/mL 0.0042 W/mL 0.0026 W/mL	30 min sonication (sampling at 0, 5, 10, 20, 30 min)	Joyce et al. (2010)
M. aeruginosa, *Anabaena circinalis* and *Chlorella* spp.	20 kHz	0.043, 0.085, 0.139, 0.186, 0.32 W/mL	5, 10, 15 and 20 min sonication	Rajasekhar et al. (2012)

a Volumes not mentioned in the study.

frequencies (Phull et al., 1997; Hao et al., 2004a; Zhang et al., 2006b; Joyce et al., 2010). This is in spite of the fact that lower frequency produces more intense cavitation effects (Mason and Lorimer, 2002). The reason higher ultrasonic frequencies were more effective was due to its proximity to the resonance frequency of the gas vesicles/vacuoles in cyanobacteria, thus making them more likely to undergo acoustic cavitation and collapse (Tang et al., 2004; Hao et al., 2004a; Zhang et al., 2006b). Furthermore, higher frequencies increase the free radical production within the solution (Petrier et al., 1992). Purcell (2009) conducted sonication of filamentous algal species at different frequencies (20, 582, 862 kHz and 1.144 MHz). The filamentous cyanobacterium *Aphanizomenon flos-aquae* was best inhibited at higher frequencies, whereas the filamentous diatom *Melosira* sp. was best inhibited at lower frequencies. This is due to the difference in cellular characteristics as the former contains gas vacuoles and has a flexible cell wall leading to pronounced sonication effects at higher frequencies due to resonance effects and disruption of the filament structure. *Melosira* sp. has a comparatively more rigid exoskeleton (composed of silica) and lower frequencies produce intense cavitation effects to disrupt its cell wall/frustule.

The ideal power and frequency setting would be one that causes collapse of gas vacuoles, damage to the membrane/cell wall (but does not cause cell lysis and hence release of toxins into the water), inhibits photosynthesis (via pigment destruction), and is energy efficient (Phull et al., 1997; Lee et al., 2000a,b; Ahn et al., 2003; Tang et al., 2004). Many of the previous studies analysed the immediate effect of sonication on the algal cells, but this does not indicate whether it was only a temporary effect and if the algal cells are really repressed or not. Future studies should focus more on the longer term effects of sonication.

It is also worth noting that Joyce et al. (2010) pointed out that the notations for power used by previous authors, for example, a power of 40 W at different frequencies of 20 kHz, 200 kHz and 1.7 MHz, are a problem as it is very difficult to achieve the same output power at different frequencies. It seems that many studies may have stated the input power to the transducer rather than the power produced. So, it is much more accurate to use intensity in W/cm^3 or W/mL, rather than simply state power in W. This parameter gives the actual ultrasonic power dissipated into the solution and thus experienced by the cyanobacterial cells.

4.2. *Exposure time*

The exposure time primarily depends on the frequency, power, desired effect and also the scale of study. For inducing a required cavitation intensity, the higher the frequency, the more power that is required (Mason and Lorimer, 2002). For cyanobacteria, a longer exposure time will lead to greater

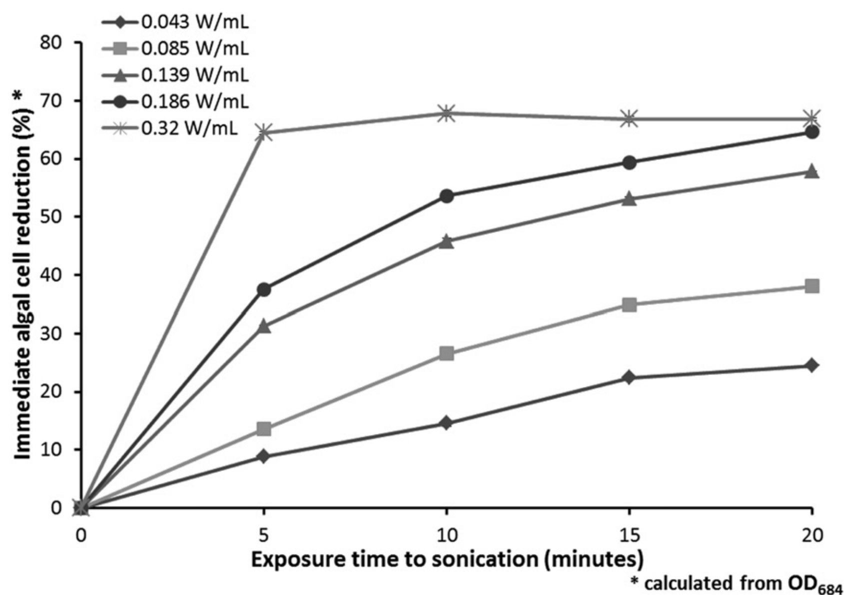

Fig. 2 – Immediate effect of sonication at 20 kHz on *M. aeruginosa* at different ultrasonic intensities and exposure times (Rajasekhar et al., 2012).

exposure to cavitation effects and eventually cell lysis (Fig. 2), which may not be desirable as it can lead to the release of toxins, such as microcystin for *M. aeruginosa*. Instead, repeated short exposure is recommended as it inhibits cell division and propagation (Tang et al., 2004). In laboratory scale studies, sonication was done in 5 s, 5 min to even 30 min increments over different frequency and power settings and the subsequent growth of cells was observed (Lee et al., 2002; Hao et al., 2004a, b; Zhang et al., 2006b; Joyce et al., 2010; Rajasekhar et al., 2012). In one field-based study, sonication was applied for a cycle of 40 s followed by a break of 210 s (Ahn et al., 2003).

4.3. *Impact of sonication on concentration of microcystin*

Control or inhibition of the growth of *M. aeruginosa* is the desirable end result of using sonication. However, as foreshadowed in the previous section, another important parameter that should be taken into consideration is the release of microcystins upon cell lysis. Thus, a combination of ultrasonic parameters that causes growth inhibition and does not increase microcystin concentration in the water is desirable. For example, Zhang et al. (2006b) recommended an ultrasonic power setting of below 0.24 W/mL (48 W) instead of 0.4 W/mL (80 W) (in spite of 80 W being more energy efficient) for treating drinking water supplies, as there was an increase in microcystin concentration when 80 W was used. Ma et al. (2005) showed that microcystins were degraded by sonication. They found 150 kHz to be the best frequency for microcystin removal, and increasing ultrasonic power and exposure times caused a proportional increase in their degradation. It was also seen that 5 min of sonication did not increase the concentration of free microcystins in *M. aeruginosa* suspensions at ultrasonic densities of 0.075, 0.15 or 0.225 W/mL at 20 kHz (Ma et al., 2005) or at 0.32 W/mL, 25 kHz (Zhang et al., 2006a). Another study on the effect of sonication on *M. aeruginosa* showed proportionately increased growth inhibition coupled with decrease in microcystin concentrations in response to higher sonication densities and exposure times (Rajasekhar et al., 2012).

5. Efficiency of sonication

Joyce et al. (2010) calculated efficiency of sonication as the percentage algal cell reduction divided by ultrasonic density (W/cm^3). Instead of using ultrasonic density, ultrasonic dosage can be used (Mason et al., 1994). This relates most of the ultrasonic parameters, i.e., the ultrasonic power, exposure time, volume of solution with the percentage algal cell reduction achieved. It may give a better overview of the effect of sonication.

In place of using Ws/mL, it would make more sense if ultrasonic dosage were expressed in terms of power consumption (kWh/m^3), i.e., 1 Ws/mL = 0.2778 x kWh/m^3. This gives an indication of the electric power consumption as it is expressed in kWh. Thus, when calculating the efficiency of sonication in terms of kWh/m^3, the percentage algal reduction can be related to units of electric power consumed per cubic metre of solution. Rajasekhar et al. (2012) related the efficiency of sonication in relation to the algal cell reduction and showed how these parameters in combination can be used in selecting optimal conditions of sonication.

6. Selectivity of ultrasonic treatment

A potential advantage of ultrasonic treatment is its reported selectivity for cyanobacteria which possess gas vacuoles over microorganisms which do not. This was apparent in the different effect of sonication on *M. aeruginosa* (with gas

vacuole) and *Synechococcus PCC 7942* (without gas vacuole) observed by Tang et al. (2004). Sonication at 1.7 MHz and 0.6 W/cm^2 inhibited the cell division and propagation of the former, but had no effect on the latter. The cavitation yield in suspension was significantly high for *M. aeruginosa*, which was related to the resonance effect on the gas vacuoles. Also, as mentioned in Section 3.3, increased lipid peroxidation and membrane permeability as a result of sonication was distinguishable in *M. aeruginosa* (Tang et al., 2004).

The selectivity of sonication on cyanobacteria was demonstrated in a recent study (Rajasekhar et al., 2012) that compared the sonication effects on *M. aeruginosa* and *A. circinalis* with a green alga, *Chlorella* sp. It was found that both the cyanobacteria showed significant growth inhibition in comparison with the green algae. It was also shown that the filamentous *A. circinalis* showed a greater degree of inhibition than *M. aeruginosa* under similar sonication conditions which was attributed to weaker gas vacuoles of the former (Brookes et al., 1994) and the disruption of filament structure. Purcell (2009) reported that the filamentous cyanobacterium *Aphanizomenon flos-aquae* gave highest cell removal rates due to the presence of both gas vacuoles and its filamentous nature. However, it was also observed in this study that the filamentous diatom *Melosira* sp. was inhibited at lower frequencies. Filamentous algae are known to clog filters in water treatment plants, and algae such as *Anabaena* can cause both odour and taste problems as well (Hess et al., 2002), so sonication may be used as an effective treatment in this situation.

A similar result was observed in a field study by Ahn et al. (2007). A reduction in cyanobacterial growth resulted in the pond being dominated by green algae and diatoms, as evident from the decreased Shannon–Weaver species diversity index ($P < 0.05$). This lower diversity did not negatively affect the environment as the growth of the green algae ensured essential primary production to maintain a healthy ecosystem. All other aquatic organisms seemed to be unaffected by the sonication. In another field study, Klemencic and Griessler-Bulc (2010) analysed the effect of sonication combined with UV radiation and glass fibre filters in a closed loop water treatment system on a number of algal species in a fish farm. It was found that there were 50% fewer cyanobacterial species compared with the control, and sonication had a statistically significant influence on algal community structure. Contrary to the observation by Ahn et al. (2007), it was found that sonication did not seem to favour the growth of green algae, rather the control had a 27% higher proportion of green algae compared with the sonicated pond.

7. Combination of sonication with other treatment strategies for cyanobacterial control

The application of sonication in a large water body would probably require the appropriate placement of more than one sonicator so that all parts of the water body would receive equal exposure and that stagnant regions (which are favourable for algal growth) are prevented (Nakano et al., 2001; Ahn et al., 2007). Pumps may be used to ensure adequate exposure of the water to sonication, as in the following examples.

Klemencic and Griessler-Bulc (2010) used a combination of glass fibre filters, UV radiation and sonication for algal control in aquaculture. The water was passed through a roughing filter, followed by a glass fibre filter able to remove particles <0.1 mm at a maximum flow rate of 0.5 m^3/h, as well as two UV devices (40 W each, wavelength of 210–400 nm and average flow rate 4 m^3/h) running in parallel. It was found that there was a 2.4% reduction of chlorophyll *a* concentration and 87% decrease in the total number of algal species compared with the control. This technique may be applied to small ponds or lakes for control of cyanobacterial blooms.

Nakano et al. (2001) reported that cyanobacterial growth was inhibited by liquid flow of more than 5 cm/s. They conducted a field study with a sonicator (100 W, 200 kHz) coupled with a water jet circulator to provide intense sonication and efficient mixing. Ten units were placed along the lake (Lake Senba, China), so that stagnant regions were avoided as much as possible. Ahn et al. (2007) utilized an apparatus with a combination of sonicator (22 kHz and 630 W) and two water pumps (flow rate: 300 L/min and power of 750 W) to treat a whole pond (2 m depth and 9,000 m^3). The advantage of that study was that a neighbouring pond just 200 m away of the same size and trophic state could be used as a control. In both studies there was a significant decrease in the concentration of dissolved oxygen and chlorophyll *a*, which indicated inhibition of photosynthesis. The study by Nakano et al. (2001) on Lake Senba showed successful reduction in peak concentrations of chlorophyll *a* from 200 μg/L to 130 μg/L, and in the study by Ahn et al. (2007) the chlorophyll *a* concentrations dropped to approximately 10 μg/L compared with the control lake for which the concentration was 20–87 μg/L. The positive effect of sonication was evident from the change in chlorophyll *a* concentration which peaked after cessation of sonication and then decreased after resumption of operation of the apparatus (Ahn et al., 2007).

Other indicators of water quality were also examined, such as suspended solids (SS), chemical oxygen demand (COD), transparency and total phosphate concentration. These were found to be significantly lower in the study at Lake Senba, which showed that the sonication complemented the flushing induction strategy in controlling the cyanobacterial bloom (Nakano et al., 2001). Ahn et al. (2007) found that turbidity of the pond increased due to the operation of the pump which promoted suspension of sediments and higher N and P concentrations. As mentioned previously, the presence of light and/or aeration can help in regeneration of gas vacuoles and thus the vertical migration of cyanobacterial cells to the surface. Nakano et al. (2001) observed that the chlorophyll *a* content did not decrease in accordance with the improved transparency and lower SS, this was attributed to the shallow nature of the lake (mean depth of 1 m) as there was a greater opportunity for sufficient illumination and growth recovery. This is the same situation for laboratory studies; there is a better chance of growth recovery due to the smaller scale and relatively shallow nature of the samples, compared with the natural conditions in a water body. Also, it should be noted that better ultrasonic power dissipation is possible in laboratory scale studies due to the smaller volume of the algal solutions utilised.

As described above, using sonication in combination with flushing and/or mixing seems to offer an effective strategy for cyanobacterial control. Another concept involved a study by Lee et al. (2002) of the feasibility of bacterially-assisted control of cyanobacteria by sonication using *Myxobacter* (a *Bdellovibrio*-like bacterium) which can lyse cyanobacteria and so lead to their death. *M. aeruginosa* culture samples were exposed to sonication or *Myxobacter*, or both, and cell numbers were monitored. Cells exposed to sonication only increased in number at a slightly lower rate than the controls (i.e., those not sonicated or exposed to *Myxobacter*). The *M. aeruginosa* culture samples exposed to *Myxobacter* only showed a slight initial increase in cell number followed by a decline at a greater rate. On the other hand, those subjected to both sonication and *Myxobacter* declined at a very rapid rate.

A study using an ultrasonic water treatment system (Sonoxide®, Ashland, Inc.) on a green alga, *Scenedesmus capricornutum*, utilised a combination of micro-bubbles and high-frequency, low power ultrasound. Air induced micro-bubbles were passed through an ultrasonic chamber where the algal cells were exposed to low power, high-frequency ultrasonic energy (1.5–2 MHz and below 10 W/cm^2) (Broekman et al., 2010). The synergy between micro-bubbles and ultrasound created shear stress which damaged and lysed the cells (Wu, 2002; Broekman et al., 2010). The presence of micro-bubbles lowers the threshold for cavitation, requiring only low power ultrasound (Ward et al., 1999; Broekman et al., 2010). It was found that this combination led to a significant decrease in the regeneration of algal cells compared with sonication alone. The advantage of using lower power with this technique is that larger volumes can be treated without the need for more costly high power ultrasonic technology (Broekman et al., 2010). This technique could potentially be applied to cyanobacterial control, but the lysing of cells may release microcystins which is undesirable.

Zhang et al. (2009) demonstrated that sonication enhances coagulation-based removal of *M. aeruginosa*. It was found that sonication for 5 s along with addition of the coagulant polyaluminium chloride improved the removal ratios of algal cells by 67% in comparison with adding the coagulant on its own. In addition, the percentage reduction of turbidity was greater than that of algal cell removal. The shorter duration of sonication is an attractive feature as it reduces power required for sonication and resultant costs.

8. Conclusions

Sonication as a means of cyanobacterial control has been under investigation for the past decade. The widely acknowledged effects of sonication on cyanobacterial growth inhibition are by the collapse of gas vesicles/vacuoles, membrane and/or cell wall disruption, and interruption of photosynthetic activity. It has been shown in many cases that in addition to disruption of cyanobacterial cells via these effects, sonication can potentially selectively remove cyanobacterial species such as *M. aeruginosa and A. circinalis*, and has the advantage of degrading microcystins.

Despite the advantages of sonication for cyanobacterial control, there are many concerns that should be addressed. It can be seen that the approximation of cylindrical gas vesicles to spheres, and assumption of gas vesicles to be 1 μm in length for *M. aeruginosa*, could potentially lead to errors in calculating the resonance frequencies. The laboratory-based studies have used relatively higher ultrasonic intensities for the investigation of cyanobacterial growth inhibition. These intensities cannot be implemented practically in larger lakes or ponds as there is significantly less power transmitted in larger volumes and thus relatively less impact on the cyanobacteria. Operation on larger scales may require more powerful ultrasonic transducers which can drive up the operating costs. Another matter of concern is the duration of the reported studies as the majority focused on the immediate effects of sonication on cyanobacteria. Although these may demonstrate that sonication inhibits cyanobacterial growth, they did not necessarily show to what extent and whether it is effective and practical over the longer term. Additionally, studying the effect of sonication on pure cultures does not necessarily indicate how cyanobacteria will behave in the natural environment. Generally they live in communities of different algae and organisms in which the effects of sonication could be different.

Sonication shows potential for use in cyanobacterial bloom control on a larger scale, but there has been no study that has determined an optimal or ideal combination of ultrasonic parameters. Parameters such as frequency, power density, exposure time should be correlated with the effects on algal cell inhibition, toxin (such as microcystin) concentration, chlorophyll content and water quality parameters such as N and P concentrations, and DOC or COD. Correlating the efficiency of sonication (kWh/m^3), algal cell reduction and microcystin concentration is one such example. Thus it is recommended that more field scale sonication studies be conducted under natural conditions such as in lakes or ponds to better reflect actual application. Correlation of the sonication parameters and the effects on algal cells gives further flexibility in optimizing the process such that the efficiency is maximised with maximal cyanobacterial cell reduction, minimal energy usage and minimal toxin release or concentration.

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