



CONTROLLING CYANOBACTERIA WITH ULTRASOUND

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ABSTRACT

Cyanobacteria present a risk to drinking water supplies when large blooms occur in reservoirs in warmer months. High concentrations affect water quality, increase risk to public health, and present a significant financial liability to water utilities, which must meet stringent standards. The treatment of cyanobacterial blooms has traditionally been carried out using copper algicides, which also affect non-target species and can result in metal residue in the reservoir sediments. As an alternative, ultrasound treatment has been the subject of research, and treatment units are commercially available. This paper presents a brief review of the scientific literature and published field trial reports, a desktop analysis of patents and commercial brochures, and a review of the limitations that the technology is currently facing.

Keywords: Ultrasonic sound, Cyanobacteria. I-INCE Classification of Subjects Number: 64.1

1. INTRODUCTION

Despite their key role in Earth's ecosystem, cyanobacteria are seen as a nuisance by water authorities. This is because potable water reserves have become so rich in nutrients that they support high concentrations of these organisms, with adverse consequences in terms of taste, odour, and public health. It has therefore become necessary for water utilities around the world to develop processes that control algal blooms and remove the associated biomass prior to distributing the water in a potable form that meets WHO guidelines and customer expectations. The primary method of water treatment consists of dosing reservoirs with chemical compounds when blooms occur, followed by a range of filtration and purification processes at the treatment plant.

The most frequently used chemical is Copper Sulphate (CuSO_4), which is very effective against cyanobacteria, but also affects other life forms such as fish, and leaves a heavy metal residue that accumulates in the benthic zone, which could only be cleared by dredging and disposal on dry land. There is a need for alternative methods with a lesser environmental impact, such as aeration, water circulation, non-persistent chemical additives, thermal or mechanical treatments.

Treatments ranging from flocculation, filtration, to aeration ultraviolet and chemical dosing are at the disposal of water utilities, each with their drawbacks in terms of cost, or adverse side effects (1). Still yet to be demonstrated as feasible at an industrial level, ultrasound treatment is seen as an attractive alternative without any long term side effects, and its potential cost of implementation is yet to be ascertained.

This paper is focused on the use of ultrasound to disrupt the life cycle of *Microcystis aeruginosa*. A significant body of research in this domain has been published, a considerable number of patent applications have been lodged, and sonication units are commercially available, and marketed with numerous case study reports. However, there is often a disconnect between the research activities and the commercial products, and the authors of this paper were unable to find independent validation studies in the form of peer-reviewed scientific publications on field trials using commercial units, while laboratory-scale experiments are abundantly documented. The purpose of this paper is to discuss

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the apparent gap between laboratory experiments and commercial treatment solutions, by trying to articulate the manner in which these products are most likely to affect cyanobacteria, and assessing this against the scientific literature.

After a brief description of *Microcystis aeruginosa* and its buoyancy regulation mechanism, the effect of various control methods is briefly outlined before an overview of ultrasound treatments and their possible effect is provided. A review of the relevant patents and commercially available products is described. Finally, the discrepancies between the scientific work and commercial efforts, and recommendations for further work to bridge that gap, are made.

2. DESCRIPTION OF *MICROCYSTIS AERUGINOSA*

Cyanobacteria thrive in diverse environments and constitute a broad phylum. For the purpose of this paper, it is necessary to focus on the species of interest, *Microcystis aeruginosa*, although other genera such as *Anabaena* are of equal interest from the perspective of water quality. A Transmission Electron Microscopy (TEM) image of a *Microcystis aeruginosa* cell is presented in Figure 1. It shows a number of features, including the alveolar structure of gas vacuoles, which the algae uses to regulate cell flotation according to its needs in nutrients and light.

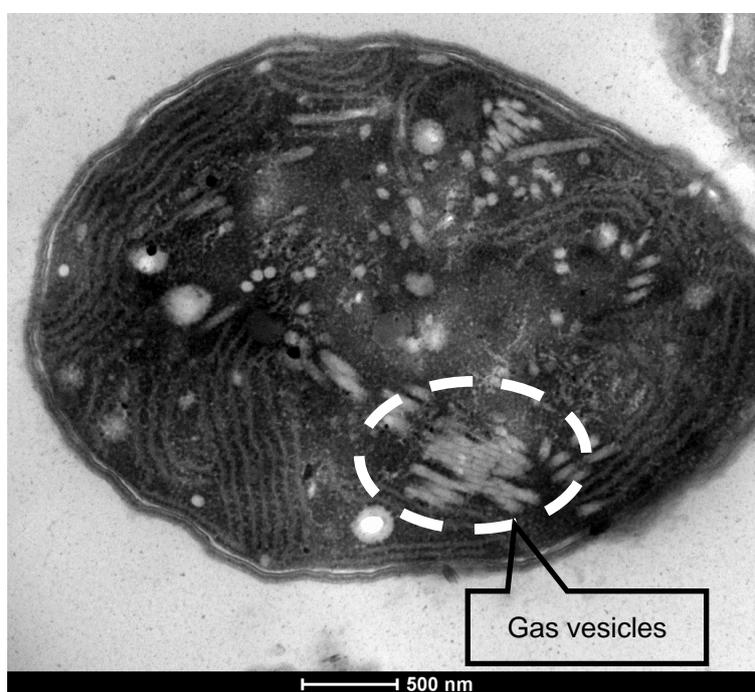


Figure 1 – TEM image of *Microcystis aeruginosa*, highlighting some of the gas vesicles.

As individual cells group into colonies, this flotation mechanism is reinforced against friction forces and the bacteria are able to migrate quite quickly, following a circadian regime driven by light and energy (2). From a mechanical perspective, these gas vacuoles present a weakness that can be exploited to control blooms. The daily vertical migration in the water column is a fundamental mechanism supporting gas vacuolated cyanobacteria, and if the buoyancy regulation mechanism can be disabled for a long enough period, the bacteria will sink to the bottom where light, temperature and nutrients are inadequate to sustain them. The cyanobacteria will become dormant or die, thereby preventing the formation of a bloom.

3. OPTIONS TO CONTROL CYANOBACTERIA

Gas vacuoles are rigid, honeycomb-like structures filled with air at ambient pressure (3). They are subjected to the internal pressure of the cell, the turgor pressure, in addition to the ambient water pressure (4). There are two possible mechanisms to collapse gas vesicles:

- Increase the ambient pressure to the point where it, in combination with turgor pressure, is sufficient to collapse the gas vesicles in a quasi-steady manner. Holland and Walsby (5) found that the pressure required to completely collapse the gas vesicles of *Microcystis* was 0.65–1.10

MPa where the cell turgor pressure was reduced to zero. The turgor pressure, which is determined by a range of environmental and functional parameters, was estimated to be of the order of 0.35 MPa in that experiment. It contributes to the total pressure on the gas vesicles, and thereby reduces the external pressure required to collapse them.

- Applying a pressure variation at a frequency that is close to the gas vacuole (or vesicle) structural resonance frequency, thus creating structural displacements of a magnitude that is large enough to rupture the gas vesicles with little mechanical power input. This approach makes an implicit assumption that the system's damping coefficient at its first resonance frequency is sufficiently low, so that little energy is required to collapse the structure. Unfortunately, little is known about the resonance frequency of gas vacuoles, except that it is in the MHz range, based on Miller's gas bubble resonance model (6), and no published work could be found about the damping coefficient of the first resonance frequency of gas vesicles.

The approach taken by all researchers referenced in this paper has been to attempt to collapse gas vesicles either by applying high pressure triggered by cavitation, possibly nucleated by the cells themselves, or by the resonant excitation of the gas vesicle structure. In most cases, the resonant excitation was also attempted by means of a cavitating pressure field. It should be noted that collapsing the gas vesicles is not sufficient to control bacteria since they can reform over time (7). However, sufficient light is required to support this reconstruction process. In a natural environment, judicious timing of the sonication process can help prevent the recovery of gas vesicles (8).

At frequencies well below gas vesicle resonance, ultrasound can create large pressure fluctuations that exceed the collapsing pressure threshold, and collapse may occur in a quasi-static manner as cavitation is not a requirement for this effect to take place. However, according to a review by Urick (9), cavitation will almost certainly occur at frequencies below 500 kHz, at the typical peak pressures of 4 to 9 atmospheres required to collapse the gas vesicles, noting that the cavitation threshold pressure increases with frequency.

4. ULTRASONIC PROCESSING

A recent review of sonication to control cyanobacteria is provided by Rajasekhar et al. (10), who noted the lack of a common method to report exposure in such a way that published experimental results can be easily compared. It is worth adding that all publications referenced in this article describe ultrasound exposure as an average over the entire fluid body, or as the sound intensity averaged over the surface of the transducer, while it is evident that individual cells will be exposed to vastly different conditions depending on their location in the sonication vessel. The literature, relating to ultrasound treatment of algae, describes the level of exposure in terms of duration, electrical power supplied to the transducer, acoustic energy injected into the medium, or average sound intensity or power density. However, in all cases the actual sound pressure in the volume is not reported. This is due to the fact that all sonication treatments are assumed to occur under cavitation, in which case, sound pressure measured with a hydrophone is of little use. The challenges associated with highly resolved spatial measurements in cavitating fields are well documented in a review by Hodnett and Zeqiri (11). Leighton (12) points out that cavitation-induced bubbles can dramatically alter the sound field in the sonicated medium, acting like an acoustic shield, or a focusing lens, which has the opposite effect on the energy distribution within the sonication vessel in front of the transducer. The shielding effect, also investigated experimentally and theoretically by Campos-Pozuelo et al. (13), was noted by Hao et al. (14) as a possible cause for saturation in bacterial inhibition once the sonication power increased beyond a certain level.

Unfortunately, little detail is provided regarding the general sonication arrangements, but most of them consist of one or more flat transducer(s) placed at the bottom surface of the sonication tank, or a horn transducer submerged in the fluid. This type of arrangement is likely to result in a highly non-uniform sound field, particularly in situations where there is no impedance matching layer between the hard ceramic or metal transducer and the medium, which would result in "hot spots" in the vicinity of the transducers and elsewhere. This point is eloquently illustrated in a review by Tudela et al. (15).

The limitation here is that, because of likely non-uniformities in the sound field, only a small region of the sonication volume, where sound pressure is above a certain threshold, could be active. In such a case, only the cells present in this effective region would be adequately affected by the ultrasound field. Further, it would take some time for all cells in the solution to pass through that region, by means of natural convection, streaming, or mixing. This would manifest itself as an increase of the effect with

sonication time, despite the fact that individual cells only require a small exposure time to be affected. In such a case, the required sonication duration would only be determined by the volume of the effective sonication region, the volume of solution being sonicated, and the rate of mixing.

1. ULTRASONIC TREATMENT OF ALGAE

Lehman and Jost (7) applied an unspecified ultrasound treatment to *Microcystis aeruginosa* in order to collapse gas vacuoles and observe and quantify the mechanism of their recovery. This initial work led a number of researchers to investigate the efficacy of ultrasound as a control measure for cyanobacteria, starting with Lee et al. (8), who found that both the gas vacuoles and photosynthetic activity of blue-green algae were affected in low frequency cavitating sound fields, and would not recover in low-light conditions as would occur at the bottom of a lake. This led them to develop a system combining flow circulation with ultrasonic treatment, and test a number of prototype systems in Lake Senba in Japan (16), where they associated some improvement in the water quality due to their device. Later, it was found that regular flushing of the lake was the dominant influence on water quality. The design presented in their paper subsequently appeared in a patent (17).

Ahn et al. (18) applied a 20 kHz ultrasound field by means of a commercial horn transducer for two minutes twice a day for six consecutive days. They found that sonication inhibited population growth for the duration of the sonication, but observed a quick recovery after sonication had ceased. They identified that the effect of sonication was optimal just after cell division, which occurred at the end of the day in the artificial light cycle, and found that adjusting the timing of sonication accordingly resulted in a significant effect on cell density. They recommended that *Microcystis aeruginosa* be sonicated just before sunset in field applications. Aside from any direct effect on the individual cells, they suggest that another way to impair *Microcystis*' ability to position themselves in a favourable environment, is to break up the colonies they form naturally to facilitate their vertical migration. This declumping effect is another well-known outcome of exposure to ultrasound.

Hao et al. (14) concluded that two control mechanisms were at play: cavitation at low frequencies, and cell resonance at higher frequencies. They estimated the optimal frequency to be in the order of 100 kHz. Tang et al. (19) subjected two types of cyanobacteria, with and without gas vacuoles, to the same sonication process, and found that only *Microcystis*, which contains gas vacuoles, was affected by ultrasound at 1.7 MHz, at an unspecified power density. They argued that this was because gas vesicle resonance triggered cavitation at the selected sonication intensity. They proposed that sonication affects cell viability by collapsing gas vesicles through resonant excitation or cavitation nucleated by the cells themselves, destruction of the photosynthetic apparatus, or cell lysis, either chemically, by the formation of free radical by-products from cavitation, or mechanically from the intense forces as cavitation bubbles collapse. Evidence of cavitation was supported by increased electrical conductivity of the culture water after cavitation, cell membrane lipid peroxidation, and increased cell membrane permeability—effects that were only observed on the gas-vacuolate cyanobacteria. It should be noted, that the authors' estimate of a gas vacuole resonance of 1.30–2.16 MHz seems low, considering that they approximated the gas vacuole size as 3–5 μm bubbles, a dimension that is characteristic of an entire *Microcystis aeruginosa* cell, and that the model does not account for honeycomb-like structure of the gas vacuole, or the rigid structures of the gas vesicles. Zhang et al. (21) found that 5 minutes of sonication at 25 kHz and 320 W/l (26.7 kWh/kl) was sufficient to markedly disrupt the culture's photosynthetic activity, as well as population growth.

In summary, ultrasonic cavitating fields have been reported to affect cyanobacteria populations through one or more of the following mechanisms or symptoms:

- Gas vesicle collapse and subsequent sedimentation,
- Destruction of multi-cellular organisms,
- Cavitation-induced mechanical damage at low frequencies, or chemical damage related to the production of free radicals in high frequency cavitation, and
- Photosynthetic activity reduction, where the structure of phycocyanin is damaged.

Rodriguez-Molares et al. (22) provide a useful summary table of published works and the reported mode of action.

Resonance is frequently cited as a primary mechanism of gas vesicle collapse, but the excitation frequencies are well below the expected resonance frequency of the gas vesicles, or even the gas vacuole. If the first resonance was to be excited, it would have to be through the low frequency tail of a very broad resonance peak, which is associated with a high damping coefficient. This would protect the structure from resonant destruction. Rather, it is likely that if resonance is at all involved in gas

vesicle collapse, it is one of the cavitation-induced higher harmonics of the fundamental excitation frequency that is responsible for resonant destruction. This would explain why cavitation is required, as it enables cell stimulations at frequencies much higher than the transducer excitation frequency. This is important to note in light of the fact that very little is known about the dynamic response of a single cyanobacteria cell, its resonance frequencies and associated damping coefficients.

The general consensus is that cyanobacterial control results from the effects of inertial cavitation, and that the mode of action can be mechanical, chemical or both. However, sonication conditions are seldom reported in a precise and unambiguous manner, which would make it at least very difficult for other researchers to attempt to independently reproduce the published results. This is further complicated by the fact that the inception of cavitation is dependent on controllable parameters such as sound intensity and ambient pressure, as well as a range of parameters that are impossible to control in a practical setting, such as dissolved gas, water purity, as well as self-induced cavitation, where inertial cavitation is nucleated by bubbles generated as part of previous pressure cycles (12).

Despite these difficulties, a significant number of patents relating to ultrasonic control of algae have been lodged, and a number of products using the technology are now commercially available.

5. PATENTS AND PRODUCTS FOR ULTRASOUND TREATMENT

Numerous patents have been lodged in relation to the use of ultrasound to control microscopic life in water. For the benefit of conciseness, the focus here is on patents that describe ultrasonic treatment, leaving aside the sheer volume of patents referring to ultrasound as part of more general implementation using other technologies such as flocculation, ultraviolet or circulation, merely making a generic reference to a sonication device. Patents that refer generally to ultrasonic treatment but do not provide an indication of exposure levels and frequency ranges were also disregarded.

Despite the lack of evidence that the sonication/circulation device of Nakano, Lee and Matsamura (16) (8) has a significant effect, relative to the impact of regular flushing of Lake Semba, a patent application for the design they used in their experiment (23) was submitted. However, no commercial product relating to this patent could be found. Similarly, Ahn and his co-workers lodged three patents in relation to their research. Their first patent (24) describes an ultrasonic transducer attached to a solar powered floating platform, where the transducer is submerged at a depth between 1 and 2 m, and emits 0.1 to 2 kW of acoustic power between 15 and 100 kHz towards the surface, one to three times a day. This patent also calls for a pump injecting active oxygen, which is a known biocide, into the same layer of water. Their second patent (25) relates to the selective treatment of bacteria based on the time of the day and the circadian cycle of the target organism, in order to maximise treatment efficiency. Their third patent (26) describes a pump assisted system, where the water intake is positioned in a region of high-concentration, and discharged after sonication in a “specific direction”. Two years later, a related group of inventors were awarded a patent (27) that combines ultrasonication with a range of other treatments. This submission was prompted by the inventors’ own conclusion that their previously “*patented technologies were not effective in inhibiting algal growth in the field. Since ultrasonication covers a narrow area, several ultrasonic transducers are needed to treat a wide body of water.*” They claim to have demonstrated the technology over a five month trial in a 12,000 m³ pond, and that their success was promoted by the synergistic relationship between the treatments involved.

Another patent attributed to Bahk (28) describes an array of tuned ultrasonic transducers where a control system measures the acoustic energy reflected by the medium, to determine algae concentration and the optimal sonication frequency, assumed to be that at which the reflected energy is the highest. They claim that energy is only used at the “correct” frequency and when the presence of algae is detected by echolocation, thereby optimising energy usage. It is difficult to see how this technology could be used in a realistic environment where acoustic reflection from a suspension of microscopic cells is to be detected in an extremely complex natural environment. Perhaps it is not surprising that no commercial product was found that uses this proposed method.

The patent awarded to Lee (29) describes a set of submerged ultrasonic transducers attached to a floating platform, arranged in such a way as to radiate energy in all horizontal directions at frequencies between 30 and 200 kHz. Using transducers rated at 20–60 W, the patent claims that the device has an effective range of 10 to 600 m. The system, designed to control filamentous algae, is powered by solar panels. This patent is worth noting as it provides a close description of the fundamental features of the commercial products that are now available. Subsequent patents in this direction use the same arrangements, and novelty is claimed on the addition of energy sources, water quality measurement, or wireless communication.

Li was granted a patent (30) for a flow-through sonication vessel designed to create a sound field dominated by standing waves, where the cells nucleate cavitation as described by (19), which results in the collapse of gas vesicles.

Li, Jin, Duan and co-workers submitted a number of patents using ultrasound between 20 and 150 kHz, where four 100–200 W transducers are used to cover all directions in the horizontal plane within a range of 120–150m. The patents describe a solar and/or wind powered floating platform supporting a submerged transducer that is controlled via wireless connection. The single patent that has been granted (31) and is currently valid, describes an ultrasonic transducer mounted on the hull of a boat that is powered by a battery charged by a wind turbine and a solar array. Here again, the patented novelty is in the implementation of ultrasound treatment rather than the treatment process.

Huang and co-workers have been granted two Chinese patents (32), (33) for a solar powered platform equipped with wireless communication devices that monitors the water quality and reports it to an on-shore central processing unit, which then selects a sonication programme and transmits it back to the platform. The inventors claim that the master/slave arrangement allows for precise monitoring and control of the algae population. With a rotating transducer rated at 20 W or less and a frequency range of 20–150 kHz, they claim an effective treatment range of 50 to 100 m. Building on this, Yousef (34) was granted a patent describing a similar arrangement, augmented by a database containing the characteristics of the ultrasound signals to be used to control various types of algae, a self-cleaning set of transducers able to cover 360 degrees at the resonance frequency of the target cells, and a measurement of the absorption coefficient on sound transmission between slave platforms to estimate local cell concentrations. The main claims of this patent are for energy efficiency, flexible choice of treatment programs over multiple frequencies, and maintenance-free operation.

Commercially available ultrasound treatment devices match the embodiment described in Lee's Korean patent (29), with one or several ultrasonic transducers attached under a float, pointing in a horizontal direction and attached to a remote, ground based control/power supply system. Although a comprehensive list is difficult to establish and beyond the scope of this paper, these devices generally have a power rating of less than 100W, and a claimed range of the order of several hundred metres. When explanations are provided, it is claimed that these systems are tuned to frequencies targeting specific algae and that the mode of operation is to collapse the gas vesicles. Based on the published works reviewed above, this implies that operating frequencies are in the MHz range where the attenuation is high, and possibly significantly higher in a natural environment, and the beamwidth of the representative transducer is narrow, therefore covering a limited volume in the water body of interest.

No peer-reviewed articles could be found to demonstrate the effectiveness of these products in a field application under controlled conditions. However, there is anecdotal evidence in the form of marketing literature, with “before and after” photographs. Algal blooms are strongly seasonal events, and it is difficult to assess the effectiveness of ultrasonic treatment from a field trial lasting several weeks or months, as the trial might have coincided with the end of the bloom season.

2. FIELD APPLICATION

The vast majority of the reported work was carried out in a laboratory setting, where a transducer was either submerged in a small volume of solution, or attached to the vessel containing it. In all cases, all cells in the solution were within a few wavelengths of the sound source in the sub-MHz range. The confined volume also facilitates the establishment of a very intense sound field, due to multiple reflections at the boundaries of the fluid volume. However, the situation in an open environment, such as a lake, is very different. Sonication devices are generally submerged close to the surface, which can in the first instance be approximated as a reflector through an imposed pressure boundary condition, but any other surface is likely to be much further away or much less reflective, such as a bottom surface covered in pebbles, mud, or vegetation. In that sense, the field of application for these sonication devices can be approximated as a semi-anechoic environment, in stark contrast with the flasks of one litre or less used in laboratory tests.

Sound intensity at any distance from a given transducer is limited by beam spread as well as sound absorption in the propagation medium, which increases with frequency. Sound absorption in distilled water is approximately 0.02 dB/m and 0.2 dB/m at 100 kHz and 1MHz, respectively (9). In order to achieve an effective treatment range of 100 m, it is therefore necessary to operate at a frequency no higher than a few hundred kHz, which is well away from the estimated gas vacuole resonance frequency of the target organisms, estimated to be of the order of several megahertz. A practical design

will have to reflect the choice between a low-intensity field at high frequency confined to a small sonication volume to directly target gas vacuole resonance, or a low frequency, high intensity field that can cover a larger volume where gas vacuoles are collapsed in a near-static manner, or at resonance through a harmonic of the excitation frequency brought about by the cavitation process.

To develop some understanding of this physical constraint, a representative transducer was modelled as a circular piston having a diameter of 65 mm, and assuming a simple free piston model in an anechoic environment as a crude approximation of a large lake, neglecting reflections off the free surface, the far-field sound pressure was calculated using the free piston formulation (35). Considering a transducer emitting 100 W of acoustic power and neglecting any effect of cavitation, the volume in which the sound pressure exceeded 1 atm was calculated as a function of frequency. This estimate of the effective sonication volume is shown in Figure 2(a).

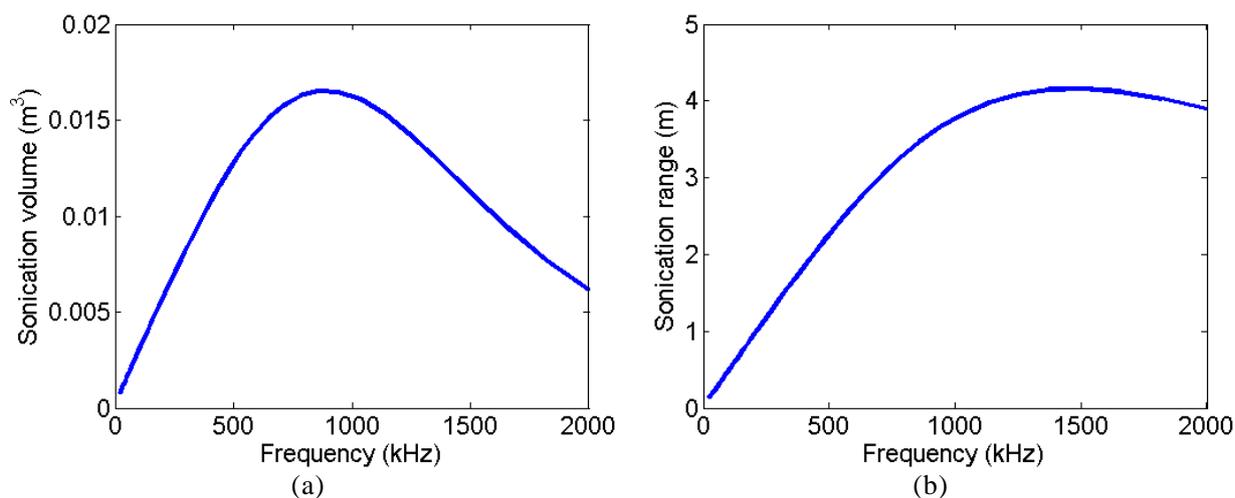


Figure 2 – Sonication volume(a) and range(b) calculated in a free field as a function of frequency for a 100 W, 65 mm transducer. The sonication volume is arbitrarily determined as the volume in which the sound pressure amplitude exceeds 1 atm.

In the case of this hypothetical 100 W transducer, the sonication volume varies between less than 1 l at low frequencies, peaks slightly above 17 l around 900 kHz, and decreases again at higher frequencies due to increasing sound absorption in water. This sonication volume is determined by the source directivity as well as the range, defined as the distance in the axis of the transducer where the pressure amplitude exceeds 1 atm, which is plotted in Figure 2(b). This threshold of 1 atm is well below the value of 3–7 atm determined by Holland and Walsby (5) as the external cell pressure required to collapse the gas vesicles, but the purpose of this calculation is to provide a best case scenario of what can be achieved by a simple ultrasonic transducer immersed in a large reservoir. The 1 atm threshold indicates where the effect on cyanobacteria may be promoted by weak cavitation effects. Again, in an attempt to depict a best case scenario, the attenuation of the far-field sound field by any cavitation that may occur near the transducer has been neglected, and the sound absorption model used was that described by Ulrick in distilled water (9).

From this simple model, and in light of the cell inhibition mechanisms described in the scientific literature published on the control of cyanobacteria by ultrasound, it is difficult to imagine how the sound field generated by a typical commercially available ultrasonic device can have a significant effect on a cells' internal structure at a distance of several hundred metres from the transducer. Of course, in much smaller enclosures of sufficiently long reverberation time, where the sound field builds up from multiple reflections of the walls, it becomes much easier to reach the sound intensity levels required to treat the target organisms.

6. CONCLUSIONS

The vast majority of the published research into the control of cyanobacteria with ultrasound is focused on high-intensity sound fields, where cavitation is the primary cause of a number of symptoms affecting the cells. Some researchers raised the possibility that mechanical resonance of the cells or gas vacuoles could facilitate the process, but noted that this was only possible at frequencies that were well above typical sonochemistry processes. The general consensus is that the extreme pressure events caused by inertial cavitation, and to a lesser degree, its chemical by-products, are the primary agents inhibiting the cells. As a result, all conclusively successful treatments have been carried out in small vessels where high intensity sound fields can be maintained. For practical field applications, numerous patent applications have been lodged, most of them suggesting that ultrasonication be used along with another process such as filtration or chemical dosing. Of the patents that focus primarily on ultrasonic treatment, the core claims are centred on minimising energy usage by selecting the appropriate frequency, duration and time of sonication, matched to the organism of interest. The main distinction between the patents is in the implementation details such as the use of solar panels or wireless communication. However, the optimised frequency and time parameters are not listed in the patent documents and appear to remain trade secrets. This situation may be a reflection of the scientific work carried out to date from which it is difficult to build a definitive picture of minimum sonication requirements to control any given organism.

Despite this, a number of ultrasonic devices, broadly similar in appearance and functionality, are now commercialised, supported by claims that optimised and algae-specific treatment programmes developed in-house are the key differentiator against competing products, and constitute the trade secret to collapsing gas vesicles at low frequency, low power and long range. Unfortunately, scientific validation of the efficacy of these systems is yet to materialise in the form of solid experiments published in peer-reviewed articles, which, hopefully, will replace the numerous testimonies and anecdotal evidence accompanied by “before and after” photographs that can be found in the marketing literature.

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