



## Project Update

# Ultrasound for the control of cyanobacteria

### Context

Cyanobacterial blooms are frequently disruptive and recurrent in natural and managed waterbodies. Options that are used to inhibit blooms in a water storage include nutrient control, destratification/aeration and algacides. Cyanobacterial blooms have traditionally been treated with the algacide copper sulphate, a biocide that whilst very effective, is expensive, kills non-target species, and results in contaminated water treatment residuals. Alternative non-chemical treatments are an important future sustainable goal for the industry to reduce both environmental impact and costs of chemical treatment.

The use of ultrasound as an alternative treatment has been the subject of research since the 1970s and treatment units are now commercially available. However, the efficacy of ultrasound treatment has been questioned by some utilities. Furthermore, there is no real technical understanding of the interaction between ultrasound and cyanobacteria and how it impacts on cyanobacterial cells.

The first part of this project involved 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. The project builds upon this knowledge to better understand the science underlying the impact of ultrasound on algal eco-physiology and help develop a working prototype.

### Ultrasound induced sedimentation: A non-chemical alternative for the treatment of cyanobacteria

*Microcystis*, *Anabaena* and *Cylindrospermopsis* are the major problem cyanobacteria for reservoir managers. They have a significant competitive advantage over other algae due to

buoyancy regulation involving a natural balance between gas vesicle synthesis and carbohydrate accumulation and dissipation, which allows these cyanobacteria to migrate vertically in the water column and access nutrients and light. The growth of many nuisance cyanobacteria in colonies accelerates this migration. This balance can be upset by artificially collapsing the gas vesicles, causing cyanobacteria

to sink to the bottom of the water column, reducing their access to light and ultimately causing their death.

A number of previous studies have shown that cyanobacterial gas vesicles can be collapsed mechanically by static pressure, shockwave or ultrasound. However, these studies have also shown that the cyanobacteria reform their gas vesicles. No detailed investigations on the correlation of vesicle reformation and buoyancy have been conducted. The challenge for this project was to determine how ultrasound could be used to cause permanent damage to vesicles and cell buoyancy while not causing cell lysis which can have the unwanted consequence of taste and odour compounds and toxin release.



*Microcystis* buoyancy (photos this page courtesy M Burch SA Water, AWQC)

### The effects of ultrasound treatment

Previously published laboratory experiments have tested the impact of a range of ultrasound frequencies (20kHz-1.7 MHz) and various power levels on vesicle collapse and cell function inhibition.

Two factors appear to dictate the impact of ultrasound on cyanobacterial blooms:

**Cell resonance**, entails very high frequencies (several MHz) and operates at short range due to high attenuation in water. There is limited evidence of its effectiveness, and no direct measurements are available. Also, this mechanism is more likely to cause cell lysis releasing toxins into the water body.

**Cavitation**, which is the formation of vapour cavities in a liquid, requires very high energy, is easiest to achieve at low frequencies and has a long acoustic range. There is a large body of scientific evidence for effectiveness of cavitation to collapse gas vesicles.

Cavitation, which results in localised high pressure, temperatures and free radical formation, can also cause damage to cyanobacteria (other than vesicle collapse) by:

- Damage to the phycocyanin structure, reducing photosynthetic activity,
- Decrease in cell concentration,
- Colony de-clumping, promoting natural control through decrease in flotation and increased grazing pressure, and
- Destruction of multi-cellular organisms

Researchers have been successful in controlling cyanobacteria using ultrasound in laboratory-based studies, however, the limitations of these experiments are that they have used high power over a long duration, while still generating a non-uniform sound field.

### Limitations of ultrasound propagation in water

The sound pressure level at any distance from a transducer is limited by beam spread, sound absorption in the water, and turbidity. For example, in distilled water the sound pressure level from a transducer radiating in all directions will decrease due to beam spreading at a rate of 6 dB (halving of pressure amplitude) per doubling of distance, and sound is absorbed at a rate of approximately 0.003 dB/m and 0.3 dB/m at 100 kHz and 1 MHz respectively. The typical operating environment for an ultrasound unit in a large waterbody with variable water turbidity will also further attenuate ultrasound as it travels through the water. Because of the physical limitations listed above, when a transducer is placed in an open body of water, the sound pressure level of ultrasound decreases with increasing distance. Also, the duration of sonication applied to cells must take into consideration the time for cells in the solution to pass through the ultrasound region, by means of natural convection, streaming or mixing.

### ARC Linkage Project

The recent ARC Linkage project investigated the application of ultrasound to cyanobacteria by adopting a systematic approach (frequency, amplitude, duration) using specially designed in-house sonication vessels.

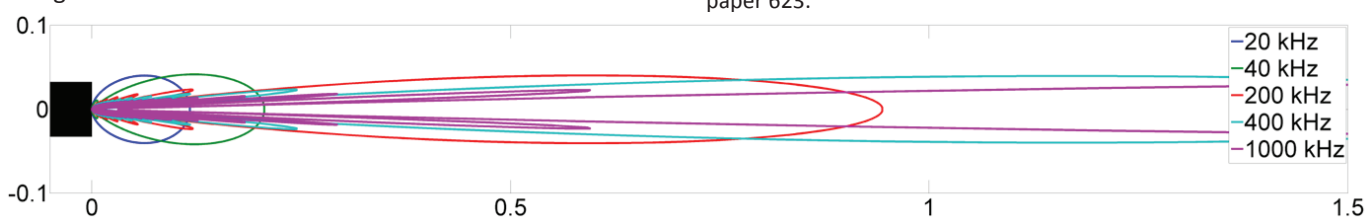


Diagram showing the beam pattern of ultrasound at various frequencies from presentation by D. Leclercq, WaterRA Research Symposium July 2014

Through their research the project team:

- Showed that ultrasound can be used to collapse gas vesicles in cyanobacteria, causing them to sink.
- Developed a relationship between ultrasound acoustic power and duration that causes a given percentage of vesicles to collapse.
- Determined that higher ultrasound power/duration levels (but still below levels that cause cell rupture) can impact metabolic and photosynthetic activity and so reduce the ability for cell recovery.
- Developed a prototype ultrasound device, tested it using laboratory cultures and environmental samples, and showed that it causes the collapse of gas vesicles.

### Conclusions

The effect of ultrasound on cyanobacteria has been demonstrated in the laboratory to include permanent removal of cell buoyancy using relatively low energy levels. Work with higher energy levels and greater exposure time has impacted on photosynthetic/metabolic activity and growth - without cell disruption and lysis.

Right now there is no direct path to field application. Current results have been achieved with a nutrient-rich culture in relatively small volumes in containers where the ultrasound levels have been measured. However, results of this project are very promising and suggest that by using the correct balance between power and exposure time it could be possible to create an economically viable option for the control of cyanobacteria at large scale. The next step is to take the laboratory-based findings and the prototype unit into the field to test and further develop the use of ultrasound to control cyanobacterial blooms. (ARC Linkage Project 100200366 & WaterRA project 1031)

### References

- Rodriguez-Molares A, Dickson S, Hobson P, Howard CQ, Zander AC, Burch M (2014) Quantification of the ultrasound induced sedimentation of *Microcystis aeruginosa*. *Ultrasonics Sonochemistry*, 21 (4):1299-1304.
- Rodriguez-Molares A, Howard CQ, Zander AC (2014) Determination of biomass concentration by measurement of ultrasonic attenuation. *Applied Acoustics*, 81:26-30.
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