Short communication

The effect of ultrasound at 256 KHz on *Microcystis aeruginosa*, with and without gas vacuoles

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Abstract

The effect of ultrasound on the growth of *M. aeruginosa* confirmed to contain gas vacuoles and on a laboratory culture with no gas vacuoles was investigated. Both cultures were treated continuously for 9 d with an ultrasonic flow device. To evaluate the influence of ultrasound during the treatment, the chlorophyll-*a* concentration was measured daily. Furthermore, changes in culture characteristics, e.g., flotation and gas vesicle formation, were determined. The results showed that, in contrast to the control, both ultrasonic-treated cultures had a lower chlorophyll-*a* concentration and cell aggregates were disrupted. Transmission electron microscopy confirmed a collapse of gas vacuoles in the environmental culture, while the laboratory culture, which did not contain gas vacuoles, showed many membrane-damaged cells. It was concluded that ultrasonic treatment of *M. aeruginosa* caused the disruption of gas vacuoles and destruction of cell membranes.

Keywords: Ultrasound, cyanobacteria, *Microcystis aeruginosa*, chlorophyll, gas vacuoles

Introduction

Recent studies have shown that the application of ultrasound to a sample of cyanobacteria resulted in a reduction of chlorophyll-*a* concentration and cell number, due to the breaking up of cyanobacterial gas vacuoles, thus disabling the cyanobacteria and preventing their floating to the surface to absorb light-energy needed for photosynthesis (Zhang et al., 2006).

An increase in power input from 32 W to 80 W resulted in an increase in the release of microcystin; however, use of different ultrasound frequencies, of 20 kHz, 80 kHz and 150 kHz, showed limited impact on microcystin release. A lower energy consumption of 0.134 kWh was needed to remove 90% cells at 80 W, in comparison to 0.175 kWh at 32 W. A big disadvantage, despite the possible release of toxins, is the fast regeneration of cells after interruption of sonication. This occurrence is also influenced by frequency, power input and duration of treatment. It was shown that ultrasonic irradiation with 1.7 MHz for 5 min inhibits the growth of the cyanobacterium *Spirulina platensis* for 3 d, while a sonication with 20 kHz showed no constant effect (Hao et al., 2004).

Several mechanisms were discovered by which ultrasonic treatment affects the cells, of which the collapsing of gas vacuoles during cavitation was considered to be the main mechanism. The presence of gas vacuoles leads to a decrease in cell density, making the cell less dense than water. As a result the cell can migrate vertically and reach upper water layers with better light conditions, thus leading to a higher rate of photosynthesis (Oliver, 1981). Other mechanisms by which ultrasonic treatment affects cyanobacterial cells are the inhibition of cell division, damage to photosynthetic activities and cell lysis (Zhang et al., 2006).

The objective of this study was to investigate the effect of ultrasound on two different cultures of *Microcystis aeruginosa*, a laboratory culture lacking gas vacuoles and an environmental culture containing gas vacuoles.

Materials and methods

Cultivation of *Microcystis aeruginosa*

The laboratory culture of *M. aeruginosa* was obtained from the University of Pretoria culture collection and the environmental culture was collected in sterile Falcon tubes at the Hartbeespoort Dam (HB Dam), Pretoria, and immediately taken back to the laboratory to be cultured. Both cultures were cultivated in a 5 ℓ Erlenmeyer flask containing 3 ℓ of BG 11 culture medium in an incubator (continuous light of 2 000 lux provided by 18 W cool white fluorescent lamp, 24 ± 2°C). Before the start of the ultrasound experiment 500 mℓ of this culture was transferred into bigger plastic basins, each containing 35 ℓ BG 11, and cultivated for a further 4 d in a greenhouse with an average temperature of 26°C and environmental light conditions (sunrise: approximately 06:55, sunset: approximately 17:30). During these 4 days the medium was stirred twice a day, manually. Immediately before the onset of an experiment the chlorophyll-*a* concentration was measured and adjusted to be the same in both basins.

Ultrasonic treatment

For the ultrasonic treatment, an ultrasonic flow device was used (Fig. 1). The water was circulated through it at a constant flow rate of 15.4 ℓ/min. The frequency used was 256 KHz. For both cultures, a control culture was prepared. All basins were manually stirred twice a day to detach cells attached to surfaces.

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