Ultrasonics Sonochemistry 21 (2014) 1299–1304

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Quantification of the ultrasound induced sedimentation of *Microcystis aeruginosa*

Alfonso Rodriguez-Molares [a,*], Sandy Dickson [b,c], Peter Hobson [b], Carl Howard [a], Anthony Zander [a], Mike Burch [b,c]

[a] School of Mechanical Engineering, University of Adelaide, Australia
[b] Australian Water Quality Centre, SA Water Corporation, Australia
[c] School of Earth and Environmental Sciences, University of Adelaide, Australia

ARTICLE INFO

Article history:
Received 8 November 2013
Received in revised form 12 January 2014
Accepted 20 January 2014
Available online 27 February 2014

Keywords:
Cyanobacteria
Sedimentation
Gas vesicle
Collapse
Buoyancy

ABSTRACT

It has been known for more than 40 years that vacuolate organisms can be induced to sediment with ultrasound. However, robust indicators are still needed to compare the efficacy of different treatments. A repeatable index is proposed that makes it possible to quantify the ultrasonic induced sedimentation. The procedure is used to monitor the long term sedimentation of *Microcystis aeruginosa* after sonication. Results reveal that the sedimentation process continues after gas vesicles have fully recovered, although at a slower rate.

1. Introduction

Microcystis aeruginosa is a phototropic bacterium that grows in nutrient-rich, slowly moving water. This species of cyanobacteria produces the potent hepatotoxin microcystin, that can be released into the water when its cell wall breaks. Ingestion of water containing microcystin is known to cause animal and human poisoning [1]. Several treatment methods are available to control cyanobacterial populations, but economic and environmental factors are still motivating the search for alternatives. In the last 12 years, ultrasonic irradiation has been intensively studied as a potential pollutant-free method to control cyanobacterial blooms.

M. aeruginosa cells contain gas filled pockets called gas vesicles. It has been shown that gas vesicles can be collapsed by applying ultrasound [2]. Consequently, the cells lose buoyancy and move to lower levels in the water column [3], having less exposure to sunlight and, potentially, less ability to photosynthesise. The collapse of gas vesicles induced by ultrasound has been observed by electron microscopy [2–4] and quantified by flow cytometric measurements [5]. However, the study of the subsequent sedimentation has attracted very little attention. To date, most of the reported data consists of qualitative observations.

Previous studies on the effects of ultrasound have often focused on gross general impacts upon cell activity, such as cell growth or photosynthetic activity. But by doing so the study of ultrasound induced effects may have been biased toward the region of high acoustic power. The gas vesicles of *M. aeruginosa* can be collapsed with relatively low acoustic intensities, while high power ultrasonic fields are required to cause significant damage to cell division and the photosynthetic mechanisms.

Investigations involving the specific analysis of threshold effects and mechanisms of subtle changes to gas vesicles may lead to the development of a more energy efficient control without the risk of gross cell damage and metabolite release. The collapse of gas vesicles and loss of buoyancy would allow the cells to sink without being lysed.

In this paper we show that the sedimentation induced by ultrasound is a much slower process than previously assumed. Inconsistencies in quantifying and reporting that sedimentation allow results to be easily mistaken as an overall reduction in cell concentration. We believe that the experimental techniques used to quantify this phenomenon so far are subject to high variability due to the sampling and culturing techniques. In consequence, we propose an experimental method to quantify the ultrasound induced sedimentation with greater accuracy. The technique was applied

* Corresponding author. Tel.: +47 728 28098.
E-mail address: alfonso.rodriguezmolares@adelaide.edu.au (A. Rodriguez-Molares).

http://dx.doi.org/10.1016/j.ultsonch.2014.01.027
1350-4177/

to systematically measure the time span of the sedimentation process under standard illumination conditions and found that it continues after the gas vesicles have fully recovered, however at lower velocities.

This result reinforce the original proposition by Lee et al. [3] that the application of ultrasound to induce sedimentation could be applied as a cost-effective method for controlling cyanobacterial populations.

2. Prior work

Lehman and Jost [2] were the first to use ultrasound to collapse the gas vesicles that regulate the buoyancy of *M. aeruginosa*. However, at that time, they did not consider ultrasound as a practical method to control cyanobacterial blooms. It was not until 2001 that Lee et al. [3] proposed that ultrasonic irradiation could be used to induce the sedimentation of cyanobacteria, and showed that sedimentation was followed by a reduction in the photosynthetic activity of the cells.

Since the work by Lee et al. [3], many researchers have investigated the effect of ultrasound on cyanobacteria and a number of different effects have been shown (see Table 1). Ultrasound induced sedimentation is qualitatively observed in Refs. [3,4,6], in the form of photographs. An assessment of the published experimental procedures used in this field indicates that scarce quantitative data is available, as some authors report having measured cell concentration at the top of containers after allowing the sonicated sample to settle [7–9]. Variation in both sampling techniques and the calculation of cell regrowth makes it difficult to distinguish whether the reported results describe the sedimentation process or an actual reduction in cell concentration of the whole sample.

The absence of reliable information on the settling time and the sampling depth leads to non-reproducible outcomes, but even with this information the concentration gradient with depth makes the quantitative measurements extremely sensitive to the depth at which the sample is taken. These small variations in depth give rise to very different concentration values, and hence measurements subjected to a large amount of uncertainty.

The collapse of gas vesicles is accepted as the primary cause for the loss of buoyancy of vacuolate organisms [10]. Vesicle collapse has an immediate effect on cyanobacterial suspensions, which visibly lose their optical characteristics due to the disappearance of

Table 1
Review of the effects of ultrasound on cyanobacteria as reported in literature. TEM: transmission electron microscopy, SEM: scanning electron microscopy, OD: optical density, Chl-a: chlorophyll-a.

Observed effect	Assessment method	Reference
Loss of buoyancy	Photography	Lee et al. [3], Jachlewski et al. [4], Mahvi and Dehghano [6]
Gas vesicle collapse	TEM	Lee et al. [3]
	SEM	Lehman and Jost [2]
	Flow cytometry	Lee et al. [5]
Growth inhibition	Cell counting	Lee et al. [3], Tang et al. [12], Ahn et al. [13],
	OD_{684}	Zhang et al. [9, 8, 14]
	OD_{560}	Hao et al. [15, 16]
Immediate reduction in cell concentration	Cell counting	Wu et al. [17]
	OD_{680}	Wu et al. [17], Joyce et al. [18]
	OD_{684}	Rajasekhar et al. [7], Zhang et al. [9], Ma et al. [19]
Reduction of cell integrity	Spectrofluoro	Wu et al. [17]
	Electrolyte measurement	Tang et al. [12]
	Differential Interference Microscopy	Hao et al. [15, 16]
	TEM	Jachlewski et al. [4]
Reduction of cell viability	Flow cytometer	Wu et al. [17]
Reduction of photosynthetic activity	Chl-a concentration	Lee et al. [3], Jachlewski et al. [4], Zhang et al. [9, 14], Ahn et al. [13], Hao et al. [15]
	Fluorescense spectroscopy	Hao et al. [15]
Toxin release	Microcystins concentration	Lee et al [3], Zhang et al. [9, 14], Ma et al. [19]

See above-mentioned references for further information.