



The effect of hydrodynamic cavitation on *Microcystis aeruginosa*: Physical and chemical factors



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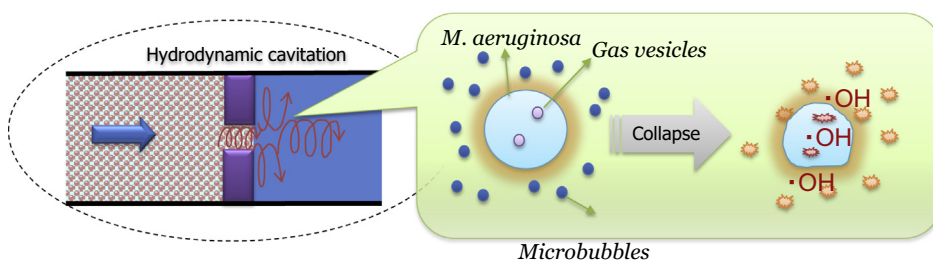
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HIGHLIGHTS

- HC has higher cavitation efficiency in algal growth inhibition than acoustic cavitation.
- Gas vacuoles within algae enhance the growth limiting effects of HC treatment.
- HC treatment causes lipid peroxidation of algal membranes.
- Limited effect of shear stress on algal removal.
- HC has significant potential as a novel treatment for algal bloom control.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 22 January 2015

Received in revised form 13 April 2015

Accepted 10 May 2015

Available online 27 May 2015

Keywords:

Algae
Hydrodynamic cavitation
Gas vacuole
Lipid peroxidation
Free radical

ABSTRACT

The various effects of hydrodynamic cavitation (HC) on algal growth inhibition were investigated. The gas-vacuolate species *Microcystis aeruginosa* responded differently to the gas-vacuole-negative alga *Chlorella* sp. When *M. aeruginosa* was subjected to HC, both its cell density and photosynthetic activity were subsequently reduced by nearly 90% after three days culture. However, the cell density of *Chlorella* sp. was reduced by only 63%, and its final photosynthetic activity was unaffected. Electron microscopy confirmed that HC had a minimal impact on algal cells that lack gas vacuoles. Shear stress during recirculation only modestly inhibited the growth of *M. aeruginosa*. The relative malondialdehyde (MDA) content, a quantitative indicator of lipid peroxidation, increased significantly during HC treatment, indicating the production of free radicals. Accordingly, the addition of H₂O₂ to the HC process promoted the production of free radicals, which also improved algal reduction. A comparison of the outcomes and energy efficiency of HC and ultrasonic cavitation indicated that HC gives the best performance: under 10 min cavitation treatment, the algal removal rate of HC could reach 88% while that of sonication was only 39%.

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1. Introduction

Surface water is the main water source for public and private water supplies in China (Zhang et al., 2009). However, the discharge of excessive nitrogen-containing and phosphate-containing nutrients into the natural environment has led to

increased blue-green algal (i.e. cyanobacterial) blooms in water bodies since the 1950s (Wu et al., 2012). Surface cyanobacterial blooms in eutrophic lakes and rivers have been recognized as a serious environmental problem (Smith et al., 1999). Algal blooms lead to oxygen depletion, phycotoxin production and high water turbidity (Wang et al., 2013). These factors can inhibit the growth of fish, aquatic plants and aquatic microfauna, eventually causing the aquatic ecosystem to fail, resulting in harm to human health (Klemas, 2012).

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To overcome this form of pollution, many control strategies have been used to inhibit cyanobacterial blooms, including chemical, mechanical, biological and ecological treatments, among which the use of chemicals is currently the most common. However, chemical treatment may not only bring another kind of pollution, but also result in a burst of microcystins into the water body after killing the algae (Lam et al., 1995).

Cavitation is the combination of the formation, growth and subsequent collapse of microbubbles or cavities occurring over an extremely small interval of time (milliseconds), which releases a large amount of energy at the location of transformation (Gogate, 2011). Very high energy densities (energy released per unit volume) are obtained locally, resulting in high pressures (in the range of 100–5000 bar) and temperatures (in the range of 1000–10,000 K) (Suslick, 1990). Moreover, free radicals are generated in the process due to the dissociation of vapors trapped in the cavitating bubbles, which results either in intensification of chemical reactions in the vicinity or in alteration of their reaction mechanisms (Gogate and Kabadi, 2009). If the cavitation is caused by the passage of high frequency sound waves, then it is called acoustic cavitation (ultrasonication), whereas if it is caused by pressure variations in the flowing liquid due to a change in the geometry of the flowing system, it is called hydrodynamic cavitation (HC) (Jyoti and Pandit, 2001). Power ultrasound is harmful to the structure and functional state of organisms (Rott, 1998) and has been applied in algal bloom control, but it is not economically viable for large scale plants.

Compared to acoustic cavitation, HC is more energy efficient and better suited to large scale applications (Gogate, 2007; Sivakumar and Pandit, 2002; Wu et al., 2012). However, until now studies were limited to analyses on water disinfection by disruption of *Escherichia coli* (Chand et al., 2007; Mezule et al., 2009) and zooplankton (Sawant et al., 2008), and little information is available in the literature on the removal of algae by HC (Gogate, 2011; Gogate and Kabadi, 2009; Nakano et al., 2001).

We have previously shown that HC is effective in controlling growth of the alga *Microcystis aeruginosa* (Li et al., 2014), but the mechanism of action – for example, whether there is a chemical effect involving the generation of free radicals – remains unclear. The energy efficiency of HC treatment as an algal control strategy also needs to be evaluated. In this study, we systematically analyze the mechanism of algal inhibition by HC treatment, and compare the efficiency of algal removal by HC and acoustic cavitation.

2. Materials and methods

2.1. Test materials and culture conditions

The unicellular gas-vacuolate cyanobacterium *M. aeruginosa* and the gas-vacuole negative algae *Chlorella* sp. were obtained from the Chinese Academy of Sciences and cultivated at 25 °C in axenic BG-11 medium. Illumination intensity was 2000 lux with a light–dark cycle of 12 h: 12 h. Algae were grown to exponential phase and then used for experiments. As 680 nm is the maximal absorbance band of *M. aeruginosa* and *Chlorella* sp. cell suspensions (Liang et al., 2005), the cell concentration was measured by spectrophotometer (UV-765, Shanghai Science Instrument Company Limited, China) at this wavelength. The concentration of algae used in this study was about $2\text{--}3 \times 10^9$ cells/L, corresponding to $\text{OD}_{680} = 0.15\text{--}0.25$, which is close to the concentration in algal blooms (Lu et al., 2013).

2.2. Experimental setup

The hydrodynamic cavitation setup consisted of a closed loop circuit including a tank, a centrifugal pump (power: 0.27 kW),

control valves, manometers, an air-flow meter and connecting pipes as shown elsewhere (Li et al., 2015, 2014). All contacting materials are made of stainless steel. Cavitation was induced by suction of air and water simultaneously and the extent of cavitation was controlled by the orifice valves. For the algal removal experiments, a suspension of algae (5 L) was introduced into the tank and circulated by the pump at a flow rate of 6–14 L min⁻¹ by changing pump discharge pressure. After switching on the pump, a valve was immediately adjusted to maintain the pump intake pressure at -0.03 MPa. Experiments in this study were carried out at a pump discharge pressure of 0.2–0.5 MPa with an air-flow rate of 0.5 L min⁻¹. The velocity in orifice was from 0.55 m/s to 1.35 m/s and the cavitation number varied from 1.32 to 0.55. Many microbubbles were generated and circulated during the treatment process. The temperature of the liquid in the tank was kept constant at 25 °C.

The ultrasonic apparatus used in this study was an ultrasonic bath designed by our laboratory and has a resonance frequency of 40 kHz and the power of 40 W. For each sonication experiment, an algal sample (5 L) was placed in a 5 L beaker and exposed to a continuous 40 kHz ultrasound signal at 0.04 W/mL, while the temperature was kept at 25 °C. The sonication parameters used in this study are close to those used in the majority of studies on the use of sonication to control algal blooms (frequency: 20 kHz–1.7 MHz, power: 0.02–0.32/mL) (Rajasekhar et al., 2012b).

In both hydrodynamic cavitation and ultrasonic experiments, algal samples of 150 ml were taken periodically after initiating HC or ultrasonic agitation; control samples were taken before treatment. All samples were grown continuously in the incubator for 3 days.

The H₂O₂ solution was prepared by dissolving 30% H₂O₂ (Sigma Chemical Co.) in distilled water at a concentration of 0.1 mmol/L. Fresh solutions were used. In the free radical oxidation experiment, various doses of H₂O₂ were added into the HC setup at the beginning of the cavitation treatment. The production of free radicals in the setup, as well as those only generated by H₂O₂, was measured. After 10 min HC treatment (24 circulations), oxidizing reactions were allowed to continue for 2 h, after which 4 ml of the suspension was taken from 1 cm below the surface and the optical density was measured. A sample only treated with H₂O₂ was used as a control to enable comparison of the effects of free radicals produced in HC on algal removal.

2.3. Analytical methods

The algal reduction efficiency (*R*) was calculated according to following equation:

$$R = \left(\frac{\text{OD}_{680,t} - \text{OD}_{680,0}}{\text{OD}_{680,0}} \right) \times 100\% \quad (1)$$

where $\text{OD}_{680,t}$ is the algal density of treated samples that had been cultured for time *t* (h), while $\text{OD}_{680,0}$ is the initial algal density.

Photosynthetic activity was determined with a PHYTO-PAM phytoplankton analyzer (Heinz Walz, Germany). Samples were dark-adapted for 30 min before determination of the minimal (*F*₀) and maximal (*F*_{*m*}) fluorescence yield, corresponding to open and closed PSII reaction centers, respectively (Matsubara and Chow, 2004).

Free radicals generated by HC were detected using the method of Liao et al. (1996). Methylene blue reacts with free radicals and can be detected by absorbance at 660 nm. Therefore, experiments were conducted using methylene blue as an indicator to evaluate the influence of various factors on ·OH concentration.

Lipid peroxidation was measured based on the thiobarbituric acid reactive substances (TBARS) method (Halliwell, 1999). Algal

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