Removal of Microcystis aeruginosa using hydrodynamic cavitation: Performance and mechanisms

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1. Introduction

As a result of human activities, nutrients like nitrogen and phosphorus are continuously added to water bodies (Wang et al., 2013). The number of eutrophic lakes in China has increased rapidly over the last 30 years (Jin et al., 2005; Xu et al., 2013). Eutrophication causes seasonal growth of cyanobacteria such as Microcystis aeruginosa, which interferes with drinking water production (Bin Alam et al., 2001; Chow et al., 1999). Common problems, caused by both cyanobacterial growth and the water treatment this growth necessitates, include the risk of disinfection byproducts (DBP), high residual levels of coagulants, clogging of filters, undesirable taste and odor, and a high organic content in raw water (Zhang et al., 2006). Furthermore, production of hepatotoxin and
microcystin by M. aeruginosa are usually hazardous to human and animal health (Codd et al., 1999; Vasconcelos and Pereira, 2001). Therefore, it is necessary to find a reliable method for the inhibition of algal growth caused by eutrophication (Xu et al., 2006).

Chemical, mechanical, biological and ecological treatments of algae have been explored, among which enhanced coagulation and chemical treatment are the most commonly used methods. Enhanced coagulation involves high doses of coagulant, which are potentially harmful to the water ecology, and the removal of algal toxins by this process is not very effective (Wu et al., 2012b; Zhang et al., 2009). Chemicals, such as ClO₂, KMnO₄, ozone and copper sulfate, have been used to deal with algae for over 80 years (Albright, 1932; Wallace, 1939; Whiteley, 1933). However, most of these chemicals are harmful to the water ecology and may be associated with the generation of secondary pollution, such as the release of extracellular organic matter (EOM) and large amounts of algal sludge (Petrushevski et al., 1996; Wu et al., 2012b). Ultrasonic radiation, which causes acoustic cavitation, appears to be efficient for controlling cyanobacterial blooms (Ahn et al., 2003), but it is not economically applicable for large scale plants. Consequently, there is still a requirement for the investigation of new methods of water treatment.

Compared to acoustic cavitation, hydrodynamic cavitation is more energy efficient and better suited to large scale application (Gogate, 2007; Sivakumar and Pandit, 2002; Wu et al., 2013). Hydrodynamic cavitation (HC) is the formation, growth, and collapse of vapor cavities induced by a flow restricting device such as a pump, jet nozzle, propeller or orifice plate (Balasundaram and Harrison, 2006; Gogate and Pandit, 2001). As cavities collapse, a given bubble can reach temperatures as high as 5000K and pressures as high as 100 MPa (so-called “hot spots”). The drastic environmental change triggers high-speed micro-jets and free radical generation due to decomposing water vapor and noncondensable gases inside the bubbles (Suslick, 1988, 1990). The combination of these mechanical and chemical effects reduces Escherichia coli counts (Chand et al., 2007; Mezule et al., 2009) and removes zooplankton (Sawant et al., 2008) in aqueous suspensions. Nevertheless, little information on the removal of algae by HC is available in the literature (Gogate, 2011; Gogate and Kabadi, 2009; Nakano et al., 2001).

The aim of this study was to assess the effect of HC on algal removal and to elucidate the mechanism of any reduction observed. The experiments were carried out using M. aeruginosa as a model organism with a simple, laboratory scale hydrodynamic cavitation setup.

2. Materials and methods

2.1. Cultivation of algae

M. aeruginosa cells were purchased 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. The pH of the algal suspension was around 8.3—8.9. Algae were grown to exponential phase and then used for HC treatment experiments. As 680 nm is the maximal absorbance band of M. aeruginosa 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 3 x 10⁹ cells/L, corresponding to OD₆₈₀ = 0.25, which is close to the concentration in algal blooms (Lu et al., 2013).

2.2. Experimental setup

A schematic diagram of the experimental setup is shown in Fig. 1. The setup consists of a closed loop circuit including a tank, a centrifugal pump (20NPD04S, Nikuni pump Co., Japan), control valves, manometers, an air flow meter, and connecting pipes. All contacting materials are made of stainless steel. Two adjustable orifice valves were placed at the inlet (valve 1) and outlet (valve 2) of the pump, and two manometers were used to measure the inlet pressure and the outlet pressure. An air valve was used to control the air flow. Cavitation was induced by suction of air and water simultaneously and the extent of cavitation was controlled by the orifice valves. The temperature of the liquid in the tank was kept constant by a cooling circulator with heat exchange tubes.

For the algal removal experiments, a suspension of algae (5L) was introduced into the tank and circulated by the pump at a flow rate of 6—14 L min⁻¹ with the change of pump discharge pressure. After switching on the pump, Valve 1 was immediately adjusted to maintain the pump intake pressure at ~0.03 MPa. Valve 2 was then throttled to obtain the required pump discharge pressure. Experiments were carried out at 0.2—0.5 MPa pump discharge pressures. Air flow rates were varied from 0 to 1 L min⁻¹ by controlling the air valve. When air was introduced into the pump, many microbubbles were generated, which gave the water a milky appearance.

Samples of 150 mL were taken periodically after initiating HC treatment; control samples were taken before treatment. All samples were grown continuously in the incubator for 3 days and algal density was monitored over this period.

2.3. Analytical methods

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

![Fig. 1 – Bench scale device for creating hydrodynamic cavitation.](image-url)
where OD_{680,1} is the algal density of treated samples that had been cultured for time t (h), while OD_{680,0} is the initial algal density.

Chlorophyll-a content and photosynthetic activity were determined with a PHYTO-PAM phytoplankton analyzer (Heinz Walz, German). 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).

Extracellular organic matter (EOM) deriving from algae was analyzed according to the following procedure: (1) the algal suspension was centrifuged at 10,000 rpm for 10 min; (2) supernatants were filtered through 0.45 μm cellulose acetate membranes; the organic matter in the filtrate represented EOM (Li et al., 2012); (3) dissolved organic carbon (DOC) was used as a measure of EOM (Sugiyama et al., 2005) and was quantified with a TOC analyzer (TOX-VCPH, Shimazu, Japan).

Free radicals generated by HC were detected using the method of Liao (Liao et al., 1996). A scanning electron microscope (SEM515, Royal Dutch Philips Electronics Ltd., Holland) was used to observe the morphology of algal cells during HC treatment. The zeta potentials of algal suspensions were obtained using a Malvern ZetaSizer 2000 (Malvern, UK). All treated samples were measured shortly after the cavitation treatment (within 1 h).

All experiments were carried out three times and the standard deviations for different batches were normally <12%.

3. Results

3.1. Effect of hydrodynamic cavitation on growth of algae

Fig. 2 shows the changes in optical density of the cell suspension and in its chlorophyll-a content following HC treatment for different times. The pump discharge pressure was 0.4 MPa and the air flow rate was 0.5 L min^{-1}. The density of the untreated sample of algae (OD_{680}) gradually increased from 0.25 to 0.38 over 3 days as shown in Fig. 2a. In contrast, the density of all treated samples was reduced to different degrees during 72 h of culture. For algae treated for ≤5 min, the OD_{680} decreased almost linearly to as low as 0.04 after 72 h culture, amounting to a reduction of 84%. We observed that treated samples turned from green to yellow and some algae settled to the bottom. When the treatment period was less than 5 min, the algal suspension showed a different trend. In the first 36 h of culture following HC, the decrease in density was comparable to that of other treated groups, but then density began to increase again. The final optical density in these regrowing cultures after 72 h was about 0.18.

The change in chlorophyll-a content resembled that of cell density (which was reasonable, since the concentration of chlorophyll-a was in proportion to cell number). The chlorophyll-a content of untreated algae increased significantly from 171 μg L^{-1} to 285 μg L^{-1} over the 72 h growth period, while all treated samples showed a rapid decrease (Fig. 2b). However, in contrast to what was observed for cell density, the chlorophyll-a content in samples treated for <5 min did not show any sign of recovery after 36 h of culture. These results demonstrate a significant effect of HC on algal growth.

Cavitation treatment time was an important parameter that determined the reduction efficiency. Fig. 2 shows that longer cavitation times resulted in more efficient reduction in algal growth. Specifically, algae treated for less than 5 min exhibited a degree of regrowth. Given that longer treatment times require more energy and therefore incur higher operational costs, the optimum treatment time would appear to be 5–10 min in the present configuration.

3.2. Effect of hydrodynamic cavitation on photosynthetic activity

A widely used fluorescence parameter, F_0/F_m, is a measure of the maximum photochemical efficiency of PSII (maximal PSII quantum yield) (Wang et al., 2010), which represents photosynthetic activity. Table 1 illustrates the change in F_0/F_m of M. aeruginosa after hydrodynamic cavitation treatment. The pump discharge pressure was 0.4 MPa and the air flow rate was 0.5 L min^{-1}. The treatment time was varied from 1 to 60 min, while the untreated sample served as the control. Photosynthetic activity given by F_0/F_m decreased slightly
immediately after treatment, from 0.33 to 0.24. Then, after two days culture, photosynthetic activity in the treated algae significantly weakened by over 90%, with some samples (treatment time $\geq$ 10 min) having undetectable levels of activity. By the second and third day, none of the algal samples treated by HC showed photosynthetic activity. In contrast, photosynthetic activity of the control sample increased about 20% during the culture period.

### 3.3. Effect of pump discharge pressure on algae growth

Algal samples were treated at various pressures for 10 min with an air flow rate of 0.5 L min$^{-1}$, and then grown continuously for 72 h. The algal reduction rate following HC treatment is presented in Fig. 3. It was found that a critical pump pressure existed for generation of significant intensity cavitation to bring about desired effect of algae removal (Amin et al., 2010; Gogate, 2011). When the pump pressure was greater than 0.2 MPa, growth of the treated algae was dramatically inhibited, regardless of the specific pressure used (up to 0.5 MPa). The greatest removal rate (>90%) was achieved at a pump pressure of 0.4 MPa. At a pump pressure of 0.2 MPa, however, algal reduction declined after 36 h, which indicated a degree of growth recovery.

### 3.4. Effect of air flow rate on algal growth

HC was carried out at different air flow rates to investigate the effect of microbubbles on inhibition of algal growth. Treatment time was 10 min and the pump pressure was 0.4 MPa. The results are presented in Fig. 4. Algae reduction after 72 h culture was 78% when the air intake was shut off. It increased to 90% when air was introduced into the pump at a flow rate of 1.0 L min$^{-1}$, but the trends of algal reduction rate under different air flow rates were very similar. Therefore, the generation of air microbubbles during HC appears to enhance its inhibitory effect on algal growth, although the effect was slight.

### 3.5. Change of cellular morphology after hydrodynamic cavitation treatment

To directly verify the effect of HC treatment on $M$. aeruginosa, a scanning electron microscope (SEM) was used to observe changes in the morphology of the algae. Fig. 5a shows the untreated algae with intact cytoderm and epicyte, and with viscous material adhering to the cell surface. After HC, the algal cells exhibited different degrees of damage (Fig. 5b–d). After 10 min treatment, some cells were no longer spherical and seemed to have collapsed (Fig. 5b). After 30 min HC, ruptures were apparent on the surface of almost all cells. Furthermore, the algae accumulated in large clumps (Fig. 5c). This phenomenon was observed in all treated samples, but never occurred in the control sample. Fig. 5d shows the cell morphology after treatment for 60 min, when cell rupture became much more serious: the cell has disintegrated, with loss of intracellular material.

### 3.6. Effect of hydrodynamic cavitation on EOM concentration

Aqueous EOM concentration changes after HC treatment are illustrated in Fig. 6. The initial $M$. aeruginosa suspension had an EOM concentration of 3.06 mg L$^{-1}$. When the algal suspension was treated for <10 min, there were no distinct changes in EOM concentration, which remained around 3.1 mg L$^{-1}$.

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**Table 1 – Effect of HC treatment on chlorophyll fluorescence ($F_0/F_m$).**

<table>
<thead>
<tr>
<th>Culture time (d)</th>
<th>Control</th>
<th>1 min</th>
<th>3 min</th>
<th>5 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>0.33</td>
<td>0.31</td>
<td>0.3</td>
<td>0.28</td>
<td>0.27</td>
<td>0.26</td>
<td>0.24</td>
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<tr>
<td>1</td>
<td>0.44</td>
<td>0.08</td>
<td>0.05</td>
<td>0.03</td>
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<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>0.36</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>3</td>
<td>0.40</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA: not analyzed.

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**Fig. 3** – Effect of pump discharge pressure on subsequent algal growth.

**Fig. 4** – Effect of air flow rate during HC on algal growth.
However, after HC for 30 min, EOM concentration clearly increased to 3.53 mg L$^{-1}$, and then after 60 min, it rose further to 3.87 mg L$^{-1}$.

### 3.7. Free radical generation in the hydrodynamic cavitation process

The production of free radicals by HC, whose intensity can be assessed by quantifying 'OH generation (Li et al., 2009; Takahashi et al., 2007), can destroy algae by inhibiting photosynthesis, damaging membranes and lipid peroxidation (Ahn et al., 2003; Tang et al., 2003; Wu et al., 2012a). Therefore, experiments were conducted using methylene blue as an indicator to evaluate the influence of various factors on 'OH concentration. Results are listed in Table 2. Free radical generation increased by 2.3 times when the duration of HC treatment was prolonged from 1 min to 10 min, but the rate of increase reduced after 10 min. A significant difference in 'OH generation was detected at different pump discharge pressures. The highest level of 'OH production was achieved at a pressure of 0.4 MPa, and was almost 1.6 times that produced at 0.2 MPa. An increase in 'OH concentration with air flow rate was observed.

A positive correlation was observed between free radical concentration and algal reduction efficiency (Fig. S1 in the supporting information). When 'OH concentration was less than 1 μmol L$^{-1}$, growth inhibition was not effective, and regrowth occurred. With increasing free radical concentrations, the algal reduction efficiency gradually increased and reached high levels, i.e. over 95%.

### 3.8. Change in zeta potential of $M$. aeruginosa during hydrodynamic cavitation

The change in the zeta potential of the algae after treatment by hydrodynamic cavitation is illustrated in Fig. 7. Before cavitation, $M$. aeruginosa cells in water were electrically charged with a zeta potential of −33.9 mV. This value...
decreased markedly to −23.0 mV after 1 min treatment, and then gradually decreased to −19.6 mV over 1 h.

Zeta potential is a measure of the surface charge (Henderson et al., 2008) of algal cells. EOM adsorbed on the surface of algal cells has a crucial effect on the charge of the algae–water interface. EOM has an anionic character (Zhang et al., 2013), which makes algal cells negatively charged in water, thereby enhancing their stability. HC strips EOM off the surface of the algae by mechanical forces, as confirmed by the smooth cell surface observed after treatment (Fig. 5c), giving rise to a decline in absolute zeta potential values. As the electrical charge on the surface of the algae is reduced, the cells lose their stability, forming aggregates that settle out of suspension. This aggregation effect presumably contributes to algal removal efficiency (Roh et al., 2008).

### 4. Discussion

The mechanism of algal reduction resulting from HC is thought to be similar to that induced by ultrasound. Algae contain gas vesicles that control cell buoyancy in water (Reynolds et al., 1987). It was shown previously that the major adverse effect of acoustic cavitation on cyanobacteria could be attributed to the disruption of gas vesicles (Henderson et al., 2008; Nakano et al., 2001; Oliver, 1994). It is likely that a similar mechanism contributes to algal reduction by HC: when a suspension of algae was circulated through the HC system, large pressure differentials (0.3–0.5 MPa) were generated, leading to the collapse of gas vesicles in *M. aeruginosa* cells (Henderson et al., 2008). As a result, the algae settle out of suspension and accumulate at the bottom of the water column. This was confirmed by our SEM observations (Fig. 5), which demonstrated the dramatic changes in algal cell shape produced by HC. A clear depression could be seen in the cell surface, which was very likely due to the collapse of gas vesicles.

A second contributory factor to algal reduction by HC is the change in zeta potential, which decreased sharply immediately after treatment (Fig. 7). Consequently, the repulsive force between the algal cells diminished and they showed a greater tendency to aggregate, as seen by SEM in Fig. 5c. Thus, the mean particle volume (MPV) of the algae increased, further enhancing their settleability.

HC treatment of *M. aeruginosa* may also damage its photosynthetic apparatus, which is affected by various stress conditions (Lee et al., 2001), including high temperature (Gamon and Pearcy, 1989). Measurement of photosynthetic activity (Table 1) confirmed this possibility. Damage to PSII reaction centers not only results in photoinhibition, but also prevents carbon fixation (Renger et al., 1989) and lowers growth rate (Li et al., 2012). In this study, we found that the reduction in photosynthetic activity following HC was more rapid than the decrease in cell density (Fig. 1). Such damage to the photosynthetic apparatus would be expected to inhibit algae proliferation effectively.

In addition to the physical damage experienced by *M. aeruginosa* cells, chemical damage due to the generation of free radicals was also a possible inhibitory effect of HC. Free radicals are known to attack cellular constituents and functions by various mechanisms, such as lipid peroxidation (Rajasekhar et al., 2012). Most studies on sonication have

<table>
<thead>
<tr>
<th>Pump discharge pressure (MPa)</th>
<th>Air flow rate (L min⁻¹)</th>
<th>Cavitation time (min)</th>
<th>'OH generation (µMol L⁻¹)</th>
<th>Algal reduction after 3 days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>0.5</td>
<td>1</td>
<td>0.67 ± 0.03</td>
<td>25.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0.89 ± 0.04</td>
<td>35.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>1.26 ± 0.02</td>
<td>77.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>1.55 ± 0.03</td>
<td>88.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>1.68 ± 0.04</td>
<td>92.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>1.79 ± 0.03</td>
<td>93.8</td>
</tr>
<tr>
<td>0.2</td>
<td>0.5</td>
<td>10</td>
<td>0.97 ± 0.04</td>
<td>25.6</td>
</tr>
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<td>0.3</td>
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</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
<td>0.83 ± 0.05</td>
<td>83.3</td>
</tr>
<tr>
<td>0.4</td>
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<td>10</td>
<td>1.60 ± 0.04</td>
<td>78.9</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td></td>
<td>1.46 ± 0.01</td>
<td>83.4</td>
</tr>
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<td></td>
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<td>1.55 ± 0.03</td>
<td>88.1</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td></td>
<td>2.03 ± 0.06</td>
<td>91.2</td>
</tr>
</tbody>
</table>

Fig. 7 – Change in zeta potential of *M. aeruginosa* over a 1 h cavitation treatment period (0.4 MPa, 0.5 L min⁻¹).
shown that chemical damage due to free radicals is insignificant (Ahn et al., 2003; Kudo et al., 2009), but there has been insufficient data on HC until our demonstration of free radical generation by HC in this paper (Table 2). The cavitation conditions, i.e. pump discharge pressure, treatment period and air flow rate, had a marked influence on the concentration of free radicals (Chakinala et al., 2009). Algal reduction was improved with the increase of free radical concentration. To further evaluate the effect of free radicals, we next investigated the HC process in the presence of carbonate, which is a known scavenger of OH radicals. However, addition of 5 mmol L\(^{-1}\) sodium carbonate did not cause any effect on algal reduction efficiency. These observations indicate that free radicals produced by HC are not a significant factor in growth inhibition, at least at the concentrations generated in our experiments. However, an impact of free radicals cannot be completely ruled out: algae could perhaps withstand HC treatment under comparatively mild conditions, but become damaged and nonviable at higher cavitation intensities. In this regard, free radical concentrations might be useful as an indirect indicator of the intensity of HC treatment: the greater the intensity of HC, the more free radicals are generated after cavitation events.

The simultaneous physical and chemical effects of HC on the EOM of M. aeruginosa suspensions are also of interest. Rupture of algal cells by intense physical stress will cause leakage of organic material, leading to an increase in EOM concentration; at the same time, the free radicals produced by HC may cause decomposition of the molecular constituents of EOM. The combination of these two opposing effects may account for the observation that EOM concentration remained at an almost constant level for up to 10 min of treatment. Beyond this time point, however, the physical damage to the algae became increasingly severe, such that some cells began to disintegrate (Fig. 5c and d). In turn, this probably caused the release of intracellular organic matter (IOM), which contributed to the further increase in DOC. Once the amount of IOM released exceeds the amount oxidized by free radicals, we would expect the total concentration of EOM to increase, which is what was observed. Therefore, it may be appropriate to restrict cavitation time to 5–10 min with an experimental setup of the type used in this study, so that algal cells remain relatively intact.

5. Conclusion

Hydrodynamic cavitation was effective in controlling M. aeruginosa growth. In addition to disrupting gas vesicles and causing rapid settling of algae, cavitation was also found to damage the photosynthetic apparatus. The optimal cavitation time was 5–10 min with a pump pressure of 0.4 MPa. Appropriate choice of treatment time should allow the relative integrity of algal cells to be maintained and thus avoid secretion of a large amount of organic material. HC was also shown to increase the zeta potential of algal cells, resulting in cell aggregation. Furthermore, HC should not cause secondary pollution, and therefore represents a sustainable technology for algal removal in eutrophic water bodies.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2014.05.052.

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