

Effects of biological and physical properties of microalgae on disruption induced by a low-frequency ultrasound

Zhipeng Duan¹ · Xiao Tan¹ · Jiujia Guo¹ · Christine Wairimu Kahehu¹ · Hanpei Yang¹ · Xueying Zheng¹ · Feng Zhu¹

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Abstract Ultrasonication has drawn an increasing attention as one of cell disruption methods for extracting cellular compounds or controlling algal blooms. However, the effects of biological and physical properties of microalgae on cell disruption were not well understood. In this work, cell disruption of six microalgae, namely, *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa*, *Microcystis aeruginosa* (three strains: PCC 7806, FACHB 469, and FACHB 1343), and *Synechococcus elongatus*, was compared mutually based on their characteristics induced by a low-frequency ultrasound (35 kHz, 0.043 W mL⁻¹). Results showed that the most sensitive strain was *C. reinhardtii* which has a hydroxyproline-rich-glycoproteins cell wall and a larger cell size (normally 10 μm in diameter). More than 80% of the cells of *C. reinhardtii* were ruptured after sonication for 5 min. In comparison, *C. pyrenoidosa*, a cellulose-rich-wall algal species with a medium size of 4–6 μm, and *M. aeruginosa* FACHB 1343, a peptidoglycan-wall species with a smaller average size of 2.3 μm, were highly resistant to ultrasound. Only 7.5 and 7.7% of cell disruption were achieved for *C. pyrenoidosa* and *M. aeruginosa* FACHB 1343, respectively, when they were sonicated for 60 min. Declumping effect was dominant in these strains. This suggested that cellulose-rich-wall algal species might be much more resistant than hydroxyproline-rich glycoproteins, and peptidoglycan-wall species to sonication. It also revealed that the larger cell size was more susceptible to sonication the cell would be. This

research provides useful insights into choosing the low-cost microalgae for extraction or controlling specific microalgal blooms in water systems using ultrasound.

Keywords Microalgae · Cell wall structure · Cell size · Ultrasonic disruption

Introduction

Microalgae have been researched for a large range of fine chemicals and bulk products since the 1960s (i.e., fat, β-carotene, polyunsaturated fatty acids, natural dyes, antioxidants, and high-value bioactive compounds, etc.) (Spolaore et al. 2006; Raja et al. 2008; Borowitzka 2013a). Meanwhile, interest in using microalgae for production of renewable energy has increased since the first period of oil crisis (Mata et al. 2010; Borowitzka 2013b). However, these components, including most of the high-value compounds, are generally circumscribed by cell membranes and/or cell walls, but the cell boundaries are usually quite dense and intact. The result is that these materials are not readily available for extraction from the whole cells (Lee et al. 2012). The acquisition efficiency of cellular inclusions could be improved by up to several times using disrupted cells (Lee et al. 2010; Keris-Sen et al. 2014; Yap et al. 2014). Therefore, cell disruption is identified as an effective pretreatment for extraction (Lee et al. 2015).

Recently, ultrasonication has received an increasing attention as one of the cell disruption methods or an effective assistance for extraction because of its advantages (Furuki et al. 2003; Wang et al. 2014). Firstly, it does not require an extra chemical addition as compared to chemical processes, and secondly, it does not require the process of downstream separation as compared to bead milling. For low-frequency

✉ Xiao Tan
cntanxiao@163.com

¹ Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes of Ministry of Education, College of Environment, Hohai University, No.1 Xikang Road, Nanjing 210098, China

ultrasound, the shear force and free radicals produced from cavitation-induce cell disruption (Hao et al. 2004; Rajasekhar et al. 2012b). More specifically, when ultrasound passes through a cell suspension, its rapid and intense compression/expansion cycles generate small bubbles via overcoming the liquid's tensile strength and then tearing it (Suslick 1989). These bubbles could result in transient cavitation when they undergo unsteady oscillations and eventually implode with localized, forced shear streams and “hot points”, which are points of extremely high temperature and high pressure (Lee et al. 2012). The transient cavitation can also trigger the production of free radical via decomposing water molecules (Luo et al. 2011).

Although ultrasound for cell disruption or controlling algal blooms has been extensively studied, most studies have focused on the optimization of ultrasound parameters, such as frequency and intensity (Tang et al. 2003; Ma et al. 2005; Joyce et al. 2010, 2014; Yamamoto et al. 2015; Kurokawa et al. 2016). The effect of biological and physical properties of microalgae on ultrasonic disruption is not yet well understood. Several reports have discovered that the susceptibility of cells to ultrasound varies with the species treated (Rajasekhar et al. 2012a). Tang et al. (2004) sonicated two cyanobacteria species, *Microcystis aeruginosa* (gas-vacuolated) and *Synechococcus* sp. (gas-vacuole negative), and found that only cells of *M. aeruginosa* were significantly damaged by ultrasound due to the presence of gas vacuoles in their cells. However, cells in these two cyanobacterial strains are also markedly distinct in terms of cell size, and the effect of cell size on ultrasonic damage was not discussed in their work. Similarly, Purcell et al. (2013) estimated the effectiveness of sonication on control of growth of four algal species which had a diversity of morphologies and were from three algal divisions. They revealed that *Aphanizomenon flos-aquae* (filamentous cyanobacterium) and *Melosira* sp. (chain-forming diatom) were much more susceptible to ultrasound than *M. aeruginosa* (unicellular cyanobacterium) and *Scenedesmus subspicatus* (colonial green alga). The difference in cell shape (filamentous/non-filamentous) was considered as one of the probable reasons for the different levels of susceptibility to sonication. In addition, Greenly and Tester (2015) suggested that cell size and wall composition might have a significant impact on cell lysis efficiency. However, it was difficult to find out which of these properties were the valid and working factors when numerous variables (different properties) were present synchronously in the treated strains. Moreover, explicit comparisons were not implemented between these different species in these documents above. Similar researches in bacteria were also ambiguous. Some reports showed that the larger and rod-shaped bacteria were more susceptible than smaller and coccus-shaped ones (Jacobs and Thornley 1954). Conversely, Cameron (2007) did not observe a direct influence of cell size and shape on ultrasonic

disruption. Therefore, more investigations in this topic are needed to be conducted and to further serve the commercial ultrasonic application in algal utilization.

In order to study how ultrasonic disruption of algae cells might be affected by some of their biological (species, cell wall structure, or composition) and physical (cell shape, cell size, and arrangement) properties, six microalgae strains were treated in this work, which included two green algae (*Chlamydomonas reinhardtii* FACHB 359 and *Chlorella pyrenoidosa* FACHB 5) and four cyanobacterial strains (*M. aeruginosa* PCC 7806, FACHB 469 and FACHB 1343; *Synechococcus elongatus* PCC 7942). These microorganisms were used due to a variety of their comparable properties. In addition, *M. aeruginosa* is blooming in eutrophic freshwater lakes (Tan et al. 2009). It thereby could be a cheap feed stock for hydrogen or bioethanol products after ultrasonic pretreatment. *Synechococcus* also has been observed ubiquitously in freshwater and seawater (Scanlan et al. 2009; Callieri 2010). The two green algae have been investigated broadly as promising feed stocks for health products or biofuels production (Görs et al. 2010; Bigelow et al. 2014).

Materials and methods

Algal cultures and cell suspensions preparation

Microcystis aeruginosa PCC 7806 and *Synechococcus elongatus* PCC 7942 were kindly provided by the Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences. *Chlorella pyrenoidosa* FACHB 5, *Chlamydomonas reinhardtii* FACHB 359, *M. aeruginosa* FACHB 469, and *Microcystis aeruginosa* FACHB 1343 were from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB), Chinese Academy of Sciences. Cyanobacterial strains and *C. pyrenoidosa* were grown in 250-mL Erlenmeyer flasks with the standard BG-11 medium, whereas *C. reinhardtii* was cultured in flasks with SE medium (Bold and Parker 1962). Cultures were grown in a sterile illumination incubator at 25 ± 0.5 °C under $30 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ light intensity with a light:dark cycle of 12 h:12 h. All algal cells were collected at stationary phase through centrifuging ($5000 \times g$ for 10 min at 4 °C), and then were re-suspended in fresh medium. Well-mixed algal suspensions of *M. aeruginosa*, *C. pyrenoidosa*, and *C. reinhardtii* were prepared with similar cell concentrations at $1.25\text{--}1.33 \times 10^7$, 1.23×10^7 , and 1.29×10^7 cells mL^{-1} , respectively. The cell concentration was determined via cell counting under a light microscope using a hemocytometer. In comparison, the suspension of *S. elongatus* was prepared with an optical density of 1.0 at 680 nm (Ma et al. 2005). This measurement was not done using the microscope because of the limited accuracy to acquire the absolute cell number of such small cells.

Ultrasonic equipment

In this work, an ultrasonic apparatus (bath-type) was used, which was equipped with a disk-type transducer (35 kHz; DAS Corp., Hangzhou China) (Fig. 1). The transducer was fixed centrally at the bottom of the cylindrical stainless steel tank (with a maximal capacity of 200 mL). The tank was surrounded by a cooling jacket made of a glass to keep the temperature of the cell suspension below 25 °C. The calorimetric method was used to measure the accurate acoustic power which passed through the system (Mason et al. 1992). Briefly, in order to determine the stable power output of this device, 150 mL of distilled water was sonicated in the tank for 15 min with the cooling system not in operation, and the temperature was recorded every 5 min using a digital thermometer. The top of the device's sample tank was covered using a lid during the measurement of the power output in order to minimize the heat loss. This measurement was carried out in triplicate and the data then were averaged. The acoustic power was computed using the equation below:

$$I = \left(\frac{dT}{dt} C_p M \right) / V \quad (1)$$

where, I is the ultrasonic intensity (W mL^{-1}); T is the temperature of treated liquid ($^{\circ}\text{C}$) and t is processing time (seconds); C_p indicates the heat capacity of water at 25 °C ($\text{J kg}^{-1} \text{ } ^{\circ}\text{C}^{-1}$); M is the mass of water (kg); and V is the volume of treated liquid (mL). After the measurement and calculation, it was confirmed that I was 0.043 W mL^{-1} in this system.

Experimental design

For *M. aeruginosa* and *C. pyrenoidosa*, the 150 mL of suspensions prepared above were sonicated for 60 min in the

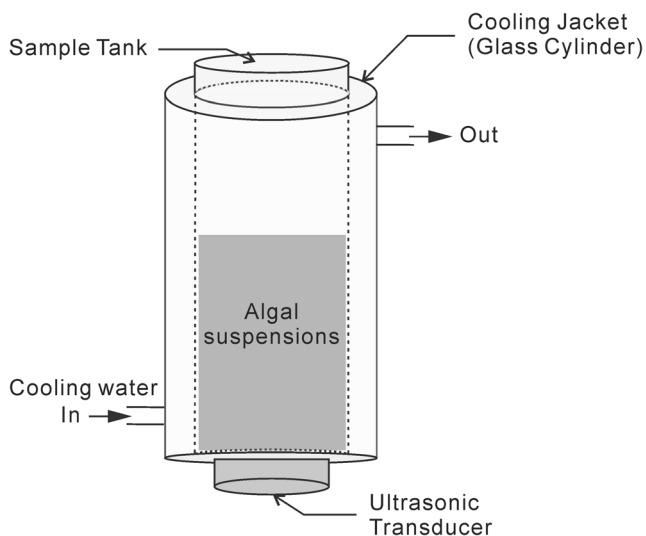


Fig. 1 A diagram of the ultrasonic apparatus

ultrasonic apparatus. In the course of treatment, 2 mL of the suspension was sampled at 0, 5, 10, 15, 20, 30, 40, 50, and 60 min, respectively, for cell counting and microscopic image analysis. Before sampling, the suspension was stirred to ensure better sampling. Three other 150-mL suspensions were treated for 0, 30, and 60 min, respectively, under the same ultrasonic conditions to assess the changes of the particle size distribution (PSD). In the case of *C. reinhardtii*, due to its high susceptibility to ultrasound, 150 mL of suspension was treated and analyzed as described above, but samples were taken at 0, 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, and 60 min, respectively. Other three suspensions of *C. reinhardtii* were treated for 0, 15, and 30 min, respectively, for the purpose of PSD analysis. Suspension of the *S. elongatus* was treated identically to the *M. aeruginosa* and the *C. pyrenoidosa*, but its cell reduction was measured by the decrease of OD₆₈₀ of the suspension using a spectrophotometer. All experiments were conducted in triplicate.

Microscopic analysis and quantifying cell disruption

Morphological characteristics of these microalgae were observed while images were taken at different levels of disruption using a light imaging microscope (Carl Zeiss, Germany) which was equipped with a camera lens (AxioCam ICc 3) and connected with a computer. These microscopic images were analyzed through a software; AxioVision 4.8.2.0 (Carl Zeiss). The cell size of these strains except for *S. elongatus* was analyzed using the software. For *S. elongatus*, cell size was determined by PSD analysis and further referenced in literature.

Cell counting used to calculate cell disruption was conducted using a hemocytometer under an optical microscope (Olympus CX31, Japan). More than six replicate cell counts were made on each sample, and the average was computed. The cell disruption, CD_t (%), was calculated using the following equation:

$$CD_t = (C_0 - C_t) / C_0 \times 100\% \quad (2)$$

where, C_0 is the initial cell concentration, and C_t is the cell concentration after sonication for t min (For *S. elongatus*, C indicates the value of OD₆₈₀).

PSD analysis

A Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK) was used to measure the particle size distribution of these algae. The dispersant was Milli-Q water with a refractive index of 1.33. A refractive index of 1.45 was used for the green algae (Spiden et al. 2013b) and 1.40 for the cyanobacterial strains (Li et al. 2014). A light absorption index of 0.1 was used for all the strains (Li et al. 2014). It should be noted that the Mastersizer 2000 software obtains the particle size value

using a prerequisite assumption that the algal cells are spherical.

Statistical analysis

Significant differences in cell disruption among algae strains were compared through the Wilcoxon signed-rank test (* indicates $P < 0.05$, ** indicates $P < 0.01$). Pearson correlation analysis was conducted to describe the correlation between cell size and cell disruption ($P < 0.05$ was considered as significant). All statistical analyses were completed using SPSS 19.0. The values in this paper are expressed as mean \pm standard deviation (SD).

Results

Some biological and physical properties of the treated microalgae

In this study, the major aim is to investigate the effect of several biological (species, major structures of the cell wall) and physical features (shape, cell size, and arrangement) of these microalgae on cell disruption induced by ultrasound. These features obtained by light microscopic analysis and literature review were displayed and in Table 1.

Cells of *C. reinhardtii* are spherical and unicellular with two flagella, whose diameters are approximately 10 μm (Table 1). They can also form large aggregates or colonies (up to 100 μm in size, Fig. 3a). *Chlorella pyrenoidosa* has a diameter which is about a half of the *C. reinhardtii*. Some aggregates or colonies were also observed in the suspension (Fig. 3c).

Four cyanobacterial strains of the genera of *Microcystis* and *Synechococcus* were employed in this case. This is

because their characteristics were pronouncedly different from the green algae. Actually, they are Gram-negative microorganisms. Furthermore, they possessed a range of comparable properties. For example, the *M. aeruginosa* strains (PCC 7806, FACHB 469 and FACHB 1343) shared a similar coccoid shape (Figs. 2a, b and 3b) and biological features, but they were significantly different in cell size (ranging from 2.3 to 5.3 μm in diameter) (Table 1). In their axenic cultures, stains of PCC 7806 and FACHB 469 mainly presented as single cells with a few small aggregates or colonies, whereas large aggregates were observed in FACHB 1343 suspensions (Figs. 2a, b and 3b). In comparison, cells in the strain of *S. elongatus* PCC 7942 shared rod-like shapes with the smallest cell size (Table 1). They were able to form microcolonies or aggregates of 5–100 cells (Fig. 2c).

Ultrasonic disruption on algal cells

Disruption efficiency varied among algal species (Fig. 4). More than 80% of the cells in *C. reinhardtii* were ruptured after sonication for 5 min. When the suspension was treated for 15 min, most of the cells were no longer intact as observed in the micrographs (Fig. 3b). And a great number of collapsed cells and cell debris were captured. Moreover, there was no visible lumpy biomass when exposure time increased to 30 min (Fig. 3c).

However, the high efficiency of cell disruption observed in *C. reinhardtii* was not observed in *M. aeruginosa* and *C. pyrenoidosa*. In the case of *M. aeruginosa*, cell reduction increased in a linear model as a function of sonication time ($R^2 = 0.97$, $P < 0.01$, $n = 9$; Fig. 4). After treatment for 30 min, 39.9% of *Microcystis* cells were ruptured. The maximal cell disruption at the end of the treatment was 69.0%. Cells in the suspension had complete and clear cell edges at the beginning, but they turned out to be ambiguous and crippled after

Table 1 Several biological and physical properties of the studied microalgae in this case

Properties	Green algae (eukaryote)		Cyanobacteria (prokaryote)			
	<i>C. reinhardtii</i> FACHB 359	<i>C. pyrenoidosa</i> FACHB 5	<i>M. aeruginosa</i> PCC 7806	<i>M. aeruginosa</i> FACHB 469	<i>M. aeruginosa</i> FACHB 1343	<i>S. elongatus</i> PCC 7942
Shape	Spherical	Spherical	Coccoid	Coccoid	Coccoid	Rod-like
Average single cell size (μm) ^a	10 (5–13)	4.4 (3.4–6.1)	5.3 (4.4–6)	4.7 (4.3–5.1)	2.3 (1.8–2.6)	1.1 (0.6–1.6)
Arrangement	Aggregate or single	Single or aggregate	Single, pairs, or small aggregate	Single, pairs, or small aggregate	Single, pairs, or large aggregate	Single or micro-colony
Major structures of cell wall ^b	Hydroxyproline-rich glycoproteins; crystalline layer	Cellulose-rich polysaccharides	Gram-negative; peptidoglycan layer; periplasmic space; extracellular mucilage layer; serrated external layer (S-layer); or hair-like fibers			

^a The values were acquired from microscopic analysis except for *S. elongatus* PCC 7942 which was from PSD analysis, and the minimum and maximum diameters of spherical cell or long axes of rod-like cell are shown in brackets.

^b These information were obtained from literature review (Domozych 2011).

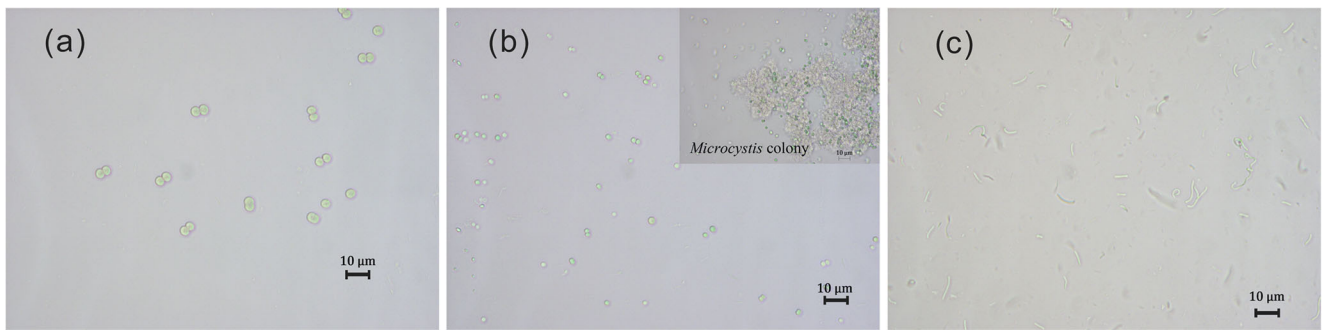


Fig. 2 The morphological characteristics of cyanobacterial cultures used in this study. **a** *M. aeruginosa* FACHB 469, **b** *M. aeruginosa* FACHB 1343 (the embedded picture showed its aggregated form), **c** *S. elongatus* PCC 7942 (microcolonies can be seen in *short-line shape*)

sonication (Fig. 3d–f). More fragmented cells were observed with increased exposure time.

Chlorella pyrenoidosa was highly resistant to sonication. Despite the fact that the suspension was treated for 60 min, the cell rupture was only found to be 7.5% (Fig. 4). Evidence from micrographs was well in agreement with the result of cell counting. The declumping effect rather than the rupturing effect would be dominant in the suspension because no visible fragmented cells were observed (Fig. 3h, i).

In this study, PSDs of microalgae with different disruption extents were presented in Fig. 5. The PSD of *C. reinhardtii* was mono-modal before sonication, then bimodal after exposure for 15 min with a new distribution of small sizes between 3.8–10 µm (accounted for 20.1% of the total mass of particles). On the other hand, D_{90} , D_{50} , and D_{10} of the suspension reduced significantly ($P < 0.05$), indicating that not only were aggregates of this microorganism dispersed, but also cells were fragmented in the course of sonication. These effects were reinforced in the continuing process. The D_{90} decreased

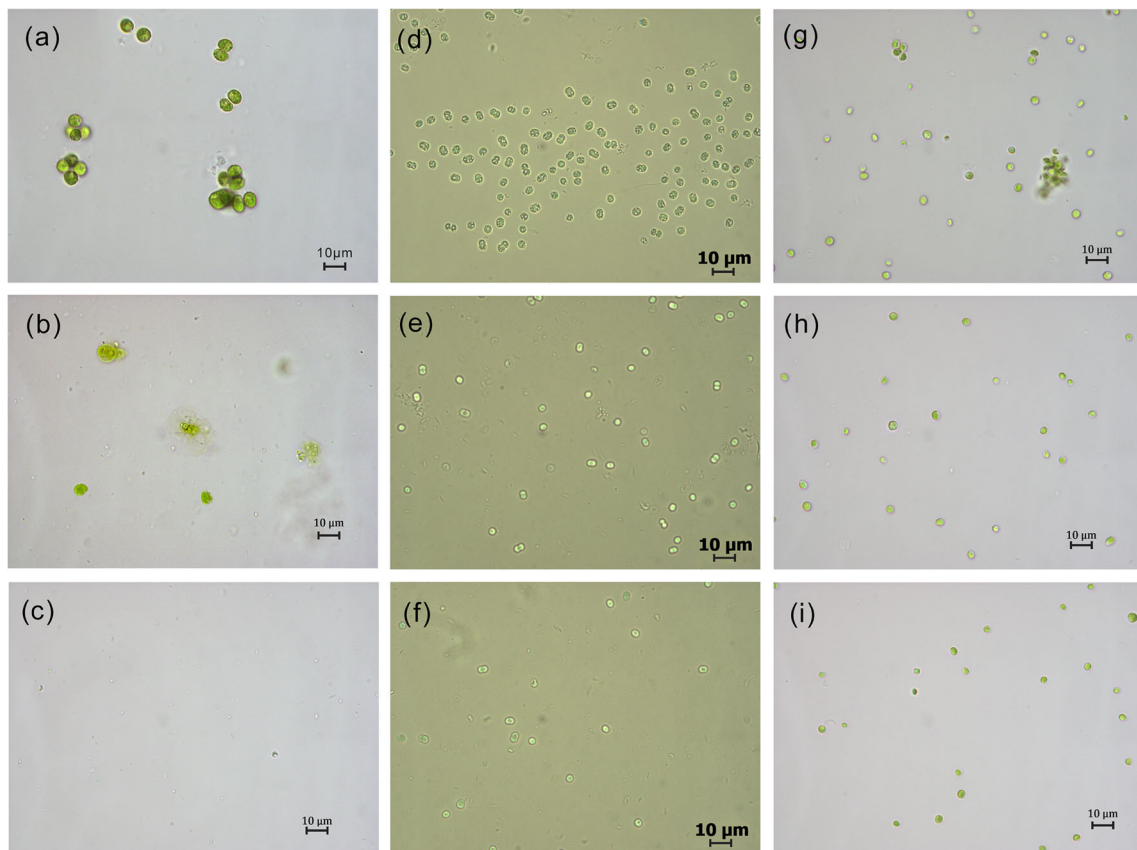


Fig. 3 Micrographs of algal cells with various ruptured extents. *C. reinhardtii* (control (a), sonication for 15 min (b), sonication for 30 min (c)); *M. aeruginosa* PCC 7806 (control (d), sonication for 30 min (e), sonication for 60 min (f)); and *C. pyrenoidosa* (control (g), sonication for 30 min (h); sonication for 60 min (i))

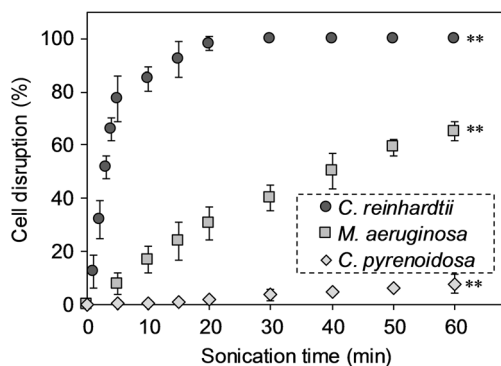


Fig. 4 Ultrasonic cell disruption of different microalgae as a function of sonication time. *M. aeruginosa* meant *M. aeruginosa* PCC 7806 in this figure. Error bars indicated the standard deviation (SD). Significance level is shown (** $P < 0.01$)

to 1.3 μm when the suspension was treated for 30 min, indicating that almost all the cells were disrupted (Fig. 5a). The result of PSD analysis was highly consistent with those of cell counting and microscopy (Figs. 3a–c, and 4).

The PSD of *M. aeruginosa* PCC 7806 suspension had a bimodal distribution, indicating that there were aggregates (up to 17.4–363.1 μm , accounting for 25% of the total) at the beginning. During the first half of the exposure period, declumping effect coupled with cell disruption (39.9% of disruption was achieved according to the cell counting) was considered as the main reason for the significant decrease in D_{90} and D_{10} (Fig. 5b). The rupturing effect was predominant within the next 30 min, since the D_{90} reduced to 4.5 μm from 6.3 μm which is nearly the diameter of a single cell (Table 1, Fig. 5b). In the case of *C. pyrenoidosa*, in contrast, declumping effect was predominant during the whole period of treatment. The PSD and its parameters (D_{90} , D_{50} , and D_{10}) of the *C. pyrenoidosa* suspension insignificantly changed when the suspension was sonicated from 30 to 60 min (Fig. 5c).

Effect of cell size and shape on disruption

To investigate the effect of cell size and shape on ultrasonic disruption, three *M. aeruginosa* stains (PCC 7806, FACHB 469 and FACHB 1343) with various diameters and a strain of *S. elongatus* (PCC 7942) were sonicated under the same conditions. Several of their characteristics are displayed in Table 1. Fig. 6 is a plot of the ultrasonic disruption of these microorganisms as a function of sonication time. The order of disruption efficiency was PCC 7806 > FACHB 469 > PCC 7942 > FACHB 1343, which was in line with the decreasing tendency of cell size except for PCC 7942 (Fig. 6). The cells of PCC 7942 are not only smaller in size but different in shape (rod-like) compared to the other strains (Table 1).

The PSD of treated cyanobacterial strains with different levels of rupture were drawn in Fig. 7. The dynamic trend of

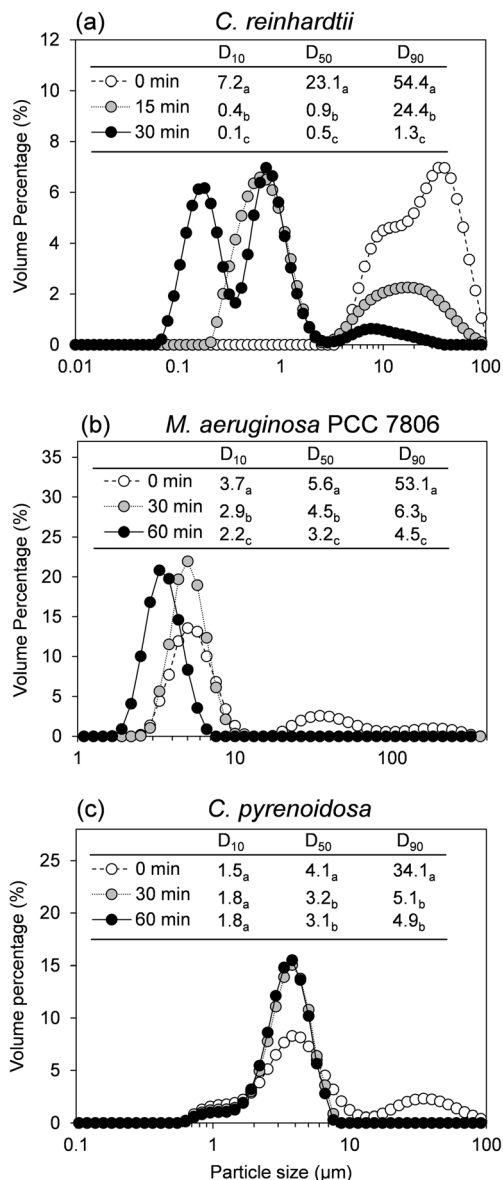


Fig. 5 Effect of ultrasonic treatment on the particle size distributions in different microalgae suspensions. D_x is particle size (μm) indicating that $x\%$ of the total mass of particles were smaller. Therefore, D_{10} , D_{50} , and D_{90} represent minimum, median, and maximum particle sizes in suspensions, respectively. Significant differences are marked with different letters ($P < 0.05$)

particle size in the suspension of FACHB 469 was similar to that of PCC 7806 described above (Figs. 5b, and 7a). Declumping effect and cell disruption were detected in this suspension during treatment. However, according to PSD analysis, only declumping effect was determined in suspensions of FACHB 1343 and PCC 7942, which was proved by their almost exact initial values of D_{10} (Fig. 7b–c). In other words, there were no additional smaller particles in suspensions of FACHB 1343 and PCC 7942 after sonication. In addition, PSD of PCC 7942 suspension displayed a bimodal

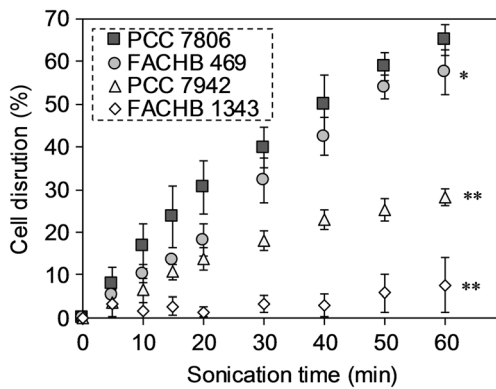


Fig. 6 Ultrasonic disruption of several cyanobacterial algae with a variety of cell sizes as a function of sonication time. Error bar indicated the standard deviation (SD). Significance level is shown after comparison with PCC 7806 (* $P < 0.05$, ** $P < 0.01$)

distribution with two peaks at 1.0 and 0.18 μm , which could represent the long and short axes of the rod-like cell, respectively.

Discussion

Six algal strains were sonicated to investigate the effect of biological and physical properties of algae on ultrasonic disruption. Cell disruption or damage was estimated by four techniques: cell counting, microscopic analysis, PSD of suspensions, and spectrophotometry (the last was used only to quantify cell disruption of *S. elongatus* PCC 7942). In previous studies, cell counting was used extensively to examine cell damage induced by ultrasound (Ahn et al. 2003; Wu et al. 2012) and even was considered as the only reliable method for quantitative comparisons of all microalgae (Spiden et al. 2013b). As a visualisation tool, microscopic images are also usually used to analyze microorganisms under a variety of treatments (Hao et al. 2004; Gao et al. 2014b). In addition, particle sizes of *Microcystis* colonies and microorganisms have been determined by using PSD analysis (Spiden et al. 2013a; Gao et al. 2014a). However, Spiden et al. (2013b) reported that the PSD is derived from light scattering data using the assumption of spherical particles, and it needs to be interpreted and used with caution. In this study, all morphologies of the tested microalgal cells were spherical or nearly spherical except for *S. elongatus* PCC 7942 (Figs. 2 and 3 a, d and g). Furthermore, the result of PSD was well in line with those of microscopic analysis and cell counting (see in “Ultrasonic disruption on algal cells” and “Effect of cell size and shape on disruption” for details). This suggested that results acquired from this work could be reliable.

However, in the case of *S. elongatus* PCC 7942, the result of PSD was inconsistent with that of cell disruption (Figs. 6 and 7c). It should be noted that its cell disruption was calculated from the reduction of OD_{680} measured by

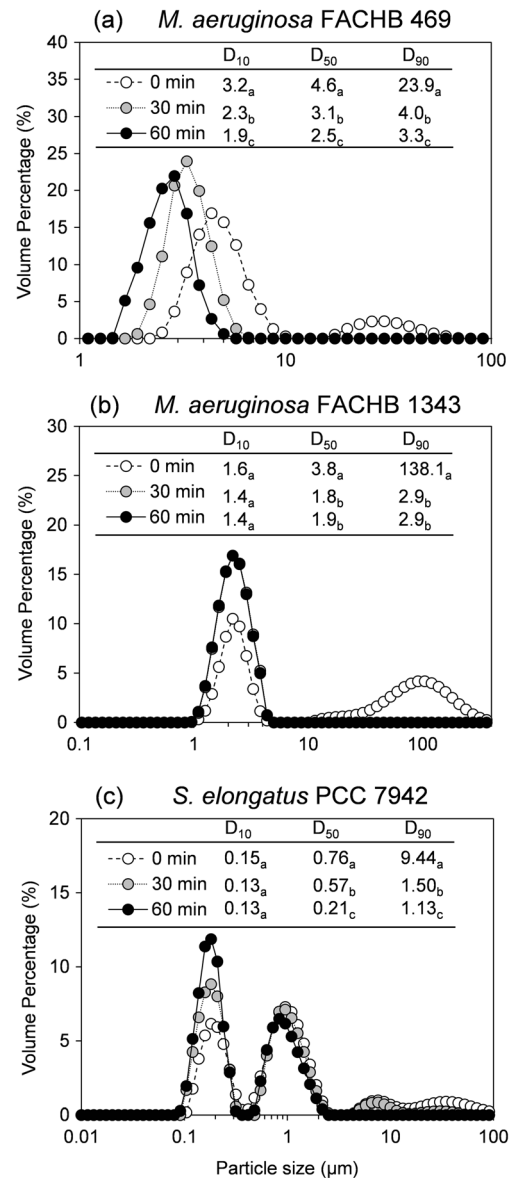


Fig. 7 Effect of ultrasonic treatment on the particle size distributions in several cyanobacterial algae. D_x is defined identically as in Fig. 5. Significant differences are marked with different letters ($P < 0.05$)

spectrophotometry, since it was difficult to distinguish such small intact cell or tiny cell debris under a light microscope. Although many previous researchers have used this technique to quantify cell disruption, a discovery made by us (unpublished) showed that it was not ideal for all microalgae species due to the optical density’s complexity, which consists of light scattering, absorption, and refraction or reflection. Yamamoto et al. (2015) also reported that OD_{680} of treated suspension did not directly indicate the algae cell disruption because it inter-related with the chlorophyll content. In addition, the PSD of *S. elongatus* PCC 7942 should be further confirmed by other more advanced techniques such as TEM due to the inconsistency of its rod-like shape and the assumption of the Mastersizer 2000 mentioned above. Therefore, due to the

complexity and uncertainty of the results of *S. elongatus* PCC 7942, the effect of its properties on ultrasonic cell disruption is not stated in the following discussion.

Effect of cell wall structures or compositions on ultrasonic disruption The cell wall is the dominant barrier protecting microalgae from being damaged by external pressures. Structure or composition of the wall and its thickness are recognized as the two major factors that impact its strength. Generally the thicker the wall thickness is, the more the resistance of the cell wall could be to pressures (Joyce et al. 2014). Although the wall thickness of the strains tested in this work was not measured, it was estimated to account for approximately 5% of a cell size (Kurokawa et al. 2016). Accordingly, the cells of the *C. reinhardtii* should have the thickest wall and the highest resistance to sonication because of their larger cell size among all the treated algae (Table 1). The greatest disruption efficiency, however, was gained from the *C. reinhardtii* (Fig. 4). The thickest wall therefore fails to cause the greatest resistance to sonication if that evaluation on wall thickness is correct. Nevertheless, the effect of wall thickness on cell disruption merits further investigation.

Cell wall structures or compositions of microalgae used in this work have been reviewed briefly in Table 1. The two green algae, *C. reinhardtii* and *C. pyrenoidosa*, possess different cell walls in terms of structure or composition. Hydroxyproline-rich glycoproteins and crystalline layers compose the major structure or composition of the *C. reinhardtii* wall, but there is no cellulose or other polysaccharides (Voigt 1988; Adair and Snell 1990; Domozych 2011). For instance, 17–30 glycoproteins were found in their wall complexes, arranged into interlocking fibrillar and granular elements. Crystalline layers that provide strength also made up their walls. On the other hand, *C. pyrenoidosa* generally consists of cellulose arranged in fibrils and a polysaccharide-rich matrix sheath (Northcote et al. 1958). Results of cell disruption and PSD analysis showed that *C. reinhardtii* was much more sensitive than *C. pyrenoidosa* to sonication (Figs. 4 and 5a, c). Specifically, the proportion of intact cells in the *C. reinhardtii* suspension decreased by 80% after sonication only for 5 min. Its D_{90} was smaller than 1.3 μm when the suspension was treated for 30 min. In comparison, even though the suspension of *C. pyrenoidosa* was sonicated for 60 min, the cell disruption was found to be only 7.5% (Fig. 4). These results suggested that cellulose-rich wall could be stronger than hydroxyproline-rich-glycoprotein wall in the ultrasonic field. A similar experiment conducted by Purcell et al. (2013), suggested that *Melosira* sp. (a diatom) has a higher vulnerability to the acoustic field than *S. subspicatus* (a green alga); probably due to differences in their wall structure. However, it should be noted that significant difference in cell sizes, ranging from 3.4 to 13 μm in diameter, between two green algal strains treated in this work was observed (Fig. 3a, g), which

could be also responsible for the various disruption efficiencies.

In order to further investigate the effect of cell wall structure or composition on acoustic rupture, three strains (*C. pyrenoidosa*, *M. aeruginosa* PCC 7806, and *M. aeruginosa* FACHB 469) which shared a similar cell shape (coccolid-like) and cell size (4.4–5.3 μm in diameter), but were diverse in wall structure, were treated by the same ultrasound. Strains of *M. aeruginosa* are Gram-negative microorganisms and possess peptidoglycan and lipopolysaccharide wall (Tang et al. 2004). Results showed that *C. pyrenoidosa* was also more resistant than *M. aeruginosa*. The two *M. aeruginosa* strains achieved a similar cell disruption and a similar PSD model (Figs. 4; 5b, c; and 7a). Rajasekhar et al. (2012a) reported a similar finding that under the same sonication conditions, the order of decreasing growth inhibition of the two algae was: *M. aeruginosa* > *Chlorella* sp.. Therefore, a cellulose-rich wall might be highly more indestructible than a peptidoglycan-rich wall in the acoustic field. However, one still should be cautious to note such a conclusion because their internal cell structures could also be a reason to explain these different levels of susceptibility. For instance, gas vesicles in cyanobacterial cells (*M. aeruginosa*, *Aphanizomenon flos-aquae*, and *Anabaena flos-aquae*, etc.) resulted in high susceptibility of these species to ultrasound (Purcell 2009). Nevertheless, the cell wall structure or composition combined with the internal cell structure could be an important factor to explain the various levels of susceptibility of these algae to sonication.

Effect of cell size on ultrasonic disruption The effect of cell size on disruption was investigated with three *M. aeruginosa* strains (PCC 7806, FACHB 469, and FACHB 1343) after eliminating interferences from other characteristics as far as possible. They had similar biological characteristics and cell shapes, but were significantly different in cell sizes and cell arrangements (Table 1, Figs. 2a, b and 3d). Despite larger aggregates or colonies observed in the strain FACHB 1343, declumping effect was completed without remarkable cell disruption in the first half of the sonicated period (Fig. 6), and there was no detectable cell debris in the suspension after treatment for 60 min (Fig. 7b), indicating ultrasonic disruption was insignificantly affected by the cell arrangement. Conversely, intense rupturing effect and declumping effect were shown synchronously in the suspensions of PCC 7806 and FACHB 469 during the first half of the period, and many smaller particles rather than intact single cells were detected in these suspensions after sonication for 60 min (Figs. 5a, 6, and 7b). These results demonstrated that the order of disruption efficiency was directly related to the cell size, which was PCC 7806 > FACHB 469 > FACHB 1343 (Table 1 and Fig. 6). Furthermore, even if numerous biological features were included (meaning that all of these algae here were taken into

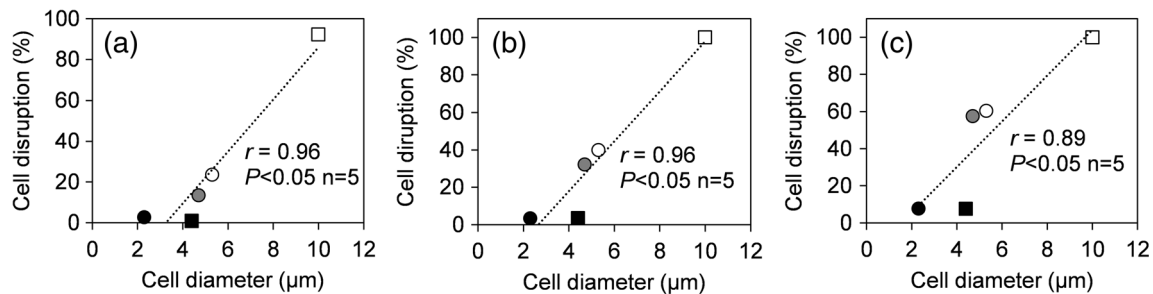


Fig. 8 The relationship of cell size and ultrasonic disruption efficiency. **a**, **b**, and **c** indicate the suspensions which were sonicated for 15, 30, and 60 min, respectively. Symbols are *white square C. reinhardtii*; *black*

square C. pyrenoidosa; *white circle M. aeruginosa* PCC 7806; *gray circle M. aeruginosa* FACHB 469; *black circle M. aeruginosa* FACHB 1343

account except for PCC 7942 due to the reasons mentioned above), cell disruption was still positive correlated to cell size ($r > 0.89$, $P < 0.05$) (Fig. 8). This is in agreement with the report conducted by Greenly and Tester (2015), who demonstrated that *Nannochloropsis oculata* (CCMP 525), a 2- μm marine green alga, was more indestructible than the other bigger microalgae.

In conclusion, six algal strains with a variety of biological and physical properties were sonicated, and cell disruption efficiency varied with the microalgae treated. *Chlamydomonas reinhardtii* was the most susceptible strain to ultrasound followed by *M. aeruginosa* PCC 7806, *M. aeruginosa* FACHB 469, and *S. elongatus* PCC 7942. *C. pyrenoidosa* and *M. aeruginosa* FACHB 1343 were not easily ruptured, and declumping effect, instead of cell disruption was the main result of sonication. Cell wall structures or compositions and cell sizes significantly affected the level of susceptibility to sonication. Cellulose-rich-wall algal species might be a lot more resistant than hydroxyproline-rich-glycoproteins, and peptidoglycan cell-walled species in the acoustic field. In addition, there was a positive correlation between ultrasonic cell disruption and cell size ($P < 0.05$, $n = 5$), that is the larger the cell size is, the more susceptible it would be to sonication.

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