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Effect of sonication frequency on the disruption of algae

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ABSTRACT

In this study, the efficiency of ultrasonic disruption of *Chaetoceros gracilis*, *Chaetoceros calcitrans*, and *Nannochloropsis* sp. was investigated by applying ultrasonic waves of 0.02, 0.4, 1.0, 2.2, 3.3, and 4.3 MHz to algal suspensions. The results showed that reduction in the number of algae was frequency dependent and that the highest efficiency was achieved at 2.2, 3.3, and 4.3 MHz for *C. gracilis*, *C. calcitrans*, and *Nannochloropsis* sp., respectively. A review of the literature suggested that cavitation, rather than direct effects of ultrasonication, are required for ultrasonic algae disruption, and that chemical effects are likely not the main mechanism for algal cell disruption. The mechanical resonance frequencies estimated by a shell model, taking into account elastic properties, demonstrated that suitable disruption frequencies for each alga were associated with the cell's mechanical properties. Taken together, we consider here that physical effects of ultrasonication were responsible for algae disruption.

1. Introduction

Algal cells provide a readily available source of lipids for the biofuel industry [1–3] and a variety of different lipid extraction methods have been proposed [4]. One of the more recent developments in extraction technology has been the use of ultrasound, and at present, there have been reports of inactivation via ultrasound for many microorganisms [5–7]. Inactivation of microorganisms by ultrasound was first reported in the 1920s [8], and the specific mechanism began to be reported in the 1960s [9]. The inactivation mechanism differs with different parameters, such as temperature, ultrasonic frequency, and acoustic power, and between microorganisms; the inactivation mechanisms for ultrasonic inactivation of *Escherichia coli* [10], *Listeria monocytogenes* [11] and *Alicyclobacillus acidiphilus* [12] have been reported. Ultrasound has been found to affect *Microcystis aeruginosa* at several tens to several hundreds of kilohertz [13–16]. However, in another study, both ultrasonic frequency and power were reported to be necessary for ultrasonic inactivation in *Chlamydomonas concordia* and *Dunaliella salina* [17]. Yet despite these reports, there remain many unanswered questions about the mechanisms of algal disruption by ultrasound.

Ultrasonic waves exert several different effects, grouped broadly into two types. The first type comprises direct effects without cavitation at very low power, such as radiation force and acoustic streaming [18]. Bioeffects caused by acoustic streaming have been confirmed to occur in cell cytoplasm [19]. The second type comprises cavitation effects, which consist of both chemical and physical effects, for example, free radical reactions, shock waves, shear stress, and microjet [20]. Although chemical effects have been reported as a major cause of inactivation [21], inactivation mechanisms due to shock waves [22] and shear stress of microstreaming [23] have also been suggested. Against this background, we investigate the effects of 0.02, 0.4, 1.0, 2.2, 3.3, and 4.3 MHz ultrasound on suspensions of *Chaetoceros gracilis*, *C. calcitrans*, and *Nannochloropsis* sp. to clarify the mechanism by which ultrasound causes disruption in algae.

2. Materials and methods

2.1. Algae

C gracilis, *C. calcitrans*, and *Nannochloropsis* sp. were selected for this study. *C. gracilis* and *C. calcitrans,* which are diatoms, contain chlorophyll a and c, and *Nannochloropsis* sp. belonging to Eustigmatophyceae, contains only chlorophyll a. These algae are widely available; *C. gracilis* and *C. calcitrans* were purchased from Yanmar

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Co., Ltd. and *Nannochloropsis* sp. was purchased from ISC Co., Ltd. All algae were cultured and concentrated under appropriate conditions recommended by the manufacture, and were transported at a controlled temperature. Initial concentrations of *C. gracilis* and *C. calcitrans* were 1.1×10^8 cells/mL, and that of *Nannochloropsis* sp. was 1.1×10^{10} cells/mL. All experiments were performed within 48 h of the arrival of the algal suspension to ensure that the condition of the algae did not change. *C. gracilis*, *C. calcitrans*, and *Nannochloropsis* sp. were measured by using a nanoparticle size analyzer (SALD-7500 nano; Shimadzu Co.) and the mean particle sizes were 2.5, 2.4, and 1.3 μm, respectively. The Young's moduli were 91, 142, and 29 MPa, respectively, measured with a scanning probe microscope (Shimadzu Co., SPM-9700).

2.2. Ultrasonic treatment

Algal suspensions (100 mL) were placed in a stainless steel cylinder and sonicated by using an ultrasonic processor (Sonics & Materials, Inc., VC750) at 0.02 MHz (Fig. 1(a)) and then by a PZT ceramic disk-type transducer (Fuji Ceramics Co.) at 0.4, 1.0, 2.2, 3.3, and 4.3 MHz (Fig. 1(b)) for 10 min. Algae samples were taken at 0, 2, 4, 6, 8 and 10 min of sonication. The suspension temperature was kept at 15 °C using a water circulator (Cool Line CL301; Yamato Scientific Co., Ltd.). In all suspensions, the initial cell number was 10^7 cells/mL. To ensure reproducibility, all experiments were carried out in triplicate. The acoustic power P was 10 W and was measured by calorimetry [24]

$$P = mC_P(\Delta T/\Delta t) \quad (1)$$

where C_P is the specific heat capacity of water, m is the mass of water, Δ_T is the increase in the temperature of the sonicated water, and Δ_t is ultrasonic irradiation time.

2.3. Analytical methods

Two analytical methods were used. The first method was to use the rate of algal cell disruption, calculated from the number of cells enumerated by hemocytometry. Cell counting was performed in triplicate and averaged. The reduction in algal cell numbers after sonication for n minutes (Δ_t) was calculated using the following equation:

$$CR_{n\,\mathrm{min}}[\%] = (CR_{0\,\mathrm{min}} - CR_{n\,\mathrm{min}})/CR_{0\,\mathrm{min}} \times 100 \quad (2)$$

where $CR_{n\,\mathrm{min}}$ is the number of cells counted at n min and $CR_{0\,\mathrm{min}}$ is the original number of cells at 0 min. Because chlorophyll a, which is present in the algae, has a peak optical absorbance at 680 nm, the second method for analyzing the condition of algal cells was to measure the peak height (PH) obtained by subtracting absorbance at 630 nm ($Abs_{630\mathrm{nm}}$) from absorbance at 680 nm ($Abs_{630\mathrm{nm}}$)

$$PH = Abs_{680\mathrm{nm}} - Abs_{630\mathrm{nm}} \quad (3)$$

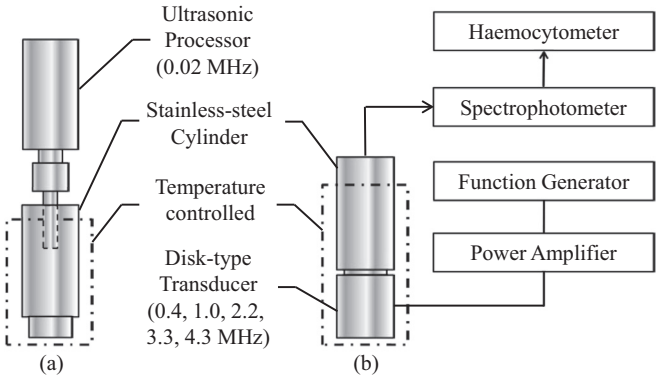

Fig. 1. Experimental apparatus for sonication at 0.02 (a), and 0.4, 1.0, 2.2, 3.3 and 4.3 MHz (b).

3. Results

3.1. Ultrasonic treatment of C. gracilis

Fig. 2 shows the absorbance spectra of *C. gracilis* suspensions before sonication (0 min) and after 10 min sonication at 2.3 MHz, with the wavelength plotted on the abscissa and the absorbance plotted on the ordinate. Upon ultrasonication, absorbance at longer wavelengths decreased, but the peak height at 680 nm increased. This decreased absorbance was possibly a result of the influence of Rayleigh scattering due to the algal cells, and the increased peak height was possibly caused by chlorophyll released from individual algae [15]. Therefore, we used peak height in this experiment.

Figs. 3 and 4 show the changes in cell reduction and peak height over time during sonication at the six frequencies of 0.02, 0.4, 1.0, 2.2, 3.3, and 4.3 MHz, at an acoustic power of 10 W. Cell reduction

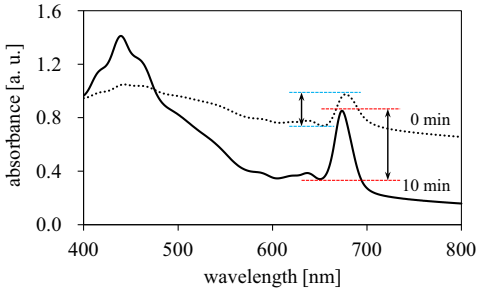

Fig. 2. Absorbance spectra of *Chaetoceros gracilis* suspensions before sonication (0 min) and after 10 min sonication at 2.2 MHz.

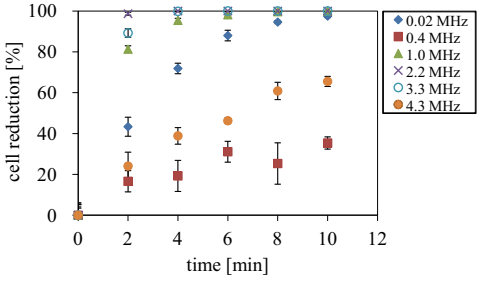

Fig. 3. Cell reduction of *C. gracilis* over time during sonication at an acoustic power of 10 W at 0.02, 0.4, 1.0, 2.2, 3.3, and 4.3 MHz.

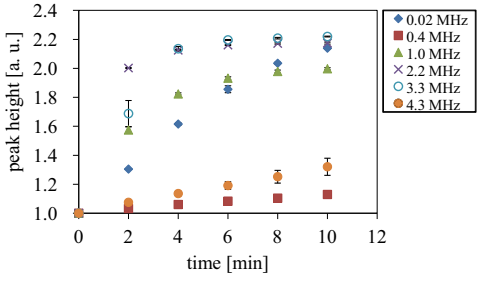

Fig. 4. Peak height of absorbance of *C. gracilis* over time during sonication at an acoustic power of 10 W at 0.02, 0.4, 1.0, 2.2, 3.3, and 4.3 MHz.