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Frequency Dependence of the Ultrasonic Disruption of Algae
藻類の超音波破壊における周波数依存性

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1. Introduction

Algal cells provide a readily available source of lipids for the biofuel industry and a variety of different methods have been proposed for their extraction.[1] One of the more recent developments in extraction technology has been the use of ultrasound.[2-4] Ultrasonic irradiation in the several tens of kilohertz to several hundreds of kilohertz range has also been found to be effective for the deactivation of the planktonic blue-green alga Microcystis aeruginosa.[5–9] In this work the effect of ultrasonic waves on suspensions of *Chaetoceros gracilis*, *Chaetoceros calcitrans* and *Nannochloropsis* sp. have been investigated at frequencies of 20 kHz, 400 kHz, 1.0 MHz, 2.2 MHz, 3.3 MHz and 4.3 MHz at acoustic power of 10 W. Results showed that the reduction in algal numbers was dependent on frequency. It is clear that high-frequency sonication is more effective than conventional low-frequency sonication for the disruption of cells for all species.

2. Materials and methods

Chaetoceros gracilis, *Chaetoceros calcitrans* (Yammar Co.) and *Nannochloropsis* sp. (I.S.C Co.) used in these investigations are spherical marine algae and their mean radiuses are 2.5, 2.4 and 1.3 μm, respectively. These were measured by the nano particle analyzer (SALD-7500nano, Shimadzu Co.). Figure 1 shows the experimental apparatus for sonication. Standard suspensions (100 ml) of algae (10^7 cells/ml) were placed in a stainless steel cylinder with a cooling jacket to keep the temperature in the range of 13 - 15°C and sonicated using a disk-type PZT ceramics transducers at frequencies of 400 kHz, 1.0 MHz, 2.2 MHz, 3.3 MHz and 4.3 MHz. Suspensions were sonicated by directly inserting a 20 kHz probe (Vibra-cell, Sonics & Materials) using a bottom plate of stainless steel instead of the transducer to maintain the same experimental conditions as the high frequency sonication. Experiments were undertaken at the acoustic power of 10 W. The precise acoustic power entering the system was determined using calorimetry prior to sonication. All experiments were carried out in triplicate. Algae samples were taken after periods of 0, 2, 4, 6, 8, 10 min of sonication and the number of algae cells was enumerated using haemocytometry. Cell counting was performed in triplicate and the average taken. Algae suspensions exhibited strong absorbance at around 680 nm. A second method for analyzing the condition of algae cells during sonication involved a closed flow loop system through a spectrophotometer.

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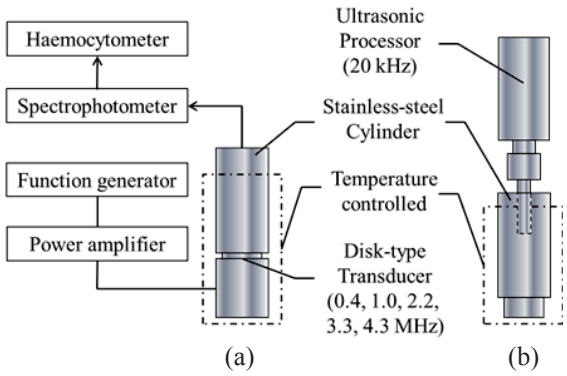
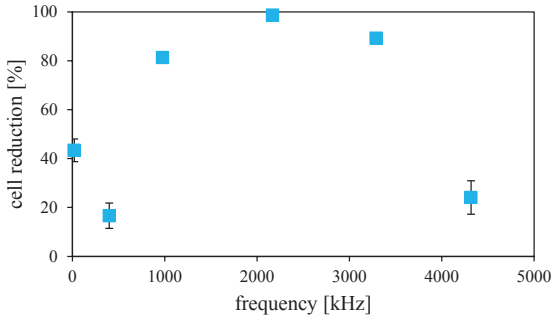


Fig. 1 Experimental apparatus for sonication at 400 kHz, 1.0 MHz, 2.2 MHz, 3.3 MHz and 4.3 MHz (a), and 20 kHz (b).

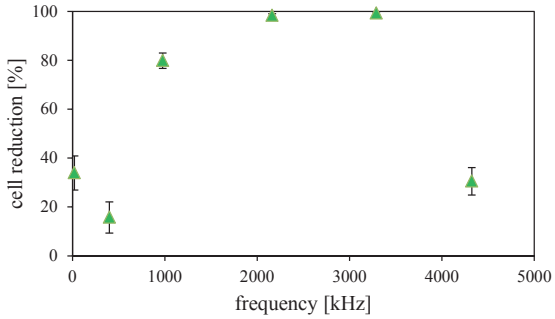
3. Results and discussion

Figure 2 shows frequency dependence of cell reduction of algae suspensions by sonication for 2 min at the acoustic power of 10 W. The ultrasonic disruption of *Chaetoceros gracilis* at different frequencies was 400 kHz < 4.3 MHz < 20 kHz < 1.0 MHz < 3.3 MHz < 2.2 MHz, for *Chaetoceros calcitrans* was 400 kHz < 4.3 MHz < 20 kHz < 1.0 MHz < 2.2 MHz < 3.3 MHz, and *Nannochloropsis* sp. was 20 kHz ≈ 400 kHz ≈ 1.0 MHz < 2.2 MHz < 3.3 MHz < 4.3 MHz. The wavelength in water of the highest frequency ultrasonic wave used in our experiments, 4.4 MHz, is estimated to be 350 μm. This is 270 times greater than the radius of the *Nannochloropsis* sp. cell of 1.3 μm. The direct effect of the ultrasonic wave on algae at the frequency of mechanical resonance is thus negligible, and the acoustic impedance of algae is

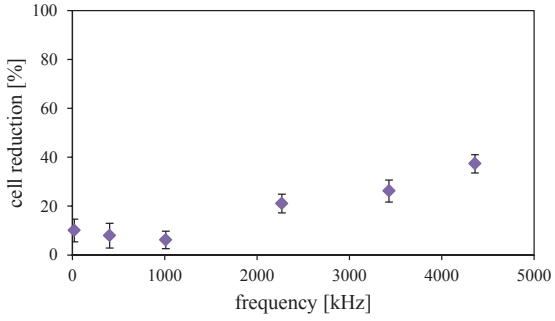
very close to that of water. However, a small stable bubble oscillating near an algae cell may be a source of excitation for shape vibrations. The resonance radius of a linearly oscillating bubble in an ultrasonic field at frequency can be determined from Minnaert's formula.[10-13] Calculated resonance radiuses of the bubble in water are 1.9, 1.3 and 1.1μm at frequencies of 2.2, 3.3 and 4.3 MHz, respectively. On the other hand the mechanical resonance frequencies of some bacteria can be calculated from their elastic shell properties and are in the 10^5 - 10^6 Hz range depending on the size and elasticity of the cells. The results demonstrate that suitable disruption frequencies for each alga were associated with the cell's mechanical properties.



(a) *Chaetoceros gracilis*



(b) *Chaetoceros calcitrans*



(c) *Nannochloropsis* sp.

Fig. 2 Ultrasonic treatment (2min, 10 W) of 100 ml algae solutions at different frequencies.

Acknowledgment

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