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Effect of ultrasonic frequency and power on the disruption of algal cells

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ABSTRACT

In this work the effect of ultrasonic waves on suspensions of *Chlamydomonas concordia* and *Dunaliella salina* have been investigated at frequencies of 20, 585, 864 and 1146 kHz and at different acoustic powers. Results showed that the reduction in algal numbers was dependent on both frequency and acoustic power. The order of efficiency of the ultrasonic disruption of *C. concordia* at different frequencies was 20 < 580 < 864 < 1146 kHz, and for *D. salina* was 20 < 580 ≅ 864 ⩽ 1146 kHz. It is clear that high-frequency sonication is more effective than conventional low-frequency sonication for the disruption of cells for both species. Results showed that suitable disruption frequencies for each algae were associated with the mechanical properties of the cell. The frequency dependence of the efficiency of algae disruption on the mechanical resonances of both the algae cell is discussed in terms of bubble oscillation in an ultrasonic field.

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1. Introduction

In recent years, algae have attracted attention as a renewable resource with the potential to be used as a substitute for non-renewable fossil fuels such as diesel oil [1–3]. The source of within the algae cells is in the form of lipids and a variety of different methods have been proposed for their extraction [4]. One of the more recent developments in extraction technology has been the use of ultrasound, so called "Ultrasound Assisted Extraction" or UAE [5] and this has been applied to the extraction of algal lipids [6]. Ultrasonic frequencies in the range of 20–100 kHz are commonly used for ultrasonic processing such as cleaning, homogenizing and cell disruption. Ultrasound within this range can be used to produce high acoustic pressure and acoustic cavitation which is referred to as "power ultrasound". Dynamic effects such as shear forces and shock waves can also be expected as a result of cavitation and these are used in chemical applications known as sonochemistry [7]. 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* [8–11]. *M. aeruginosa* contains gas vacuoles with a diameter of approximately 1 μm enclosed in a protein envelope. A theory has been proposed that these vacuoles can be effectively ruptured by high frequency sound because their size is approximately the same as the resonance radius of cavitation bubbles. We have investigated how the breakdown of algal cells depends on both the frequency of ultrasound used and the acoustic power generated in the algal suspension.

Algae species differ in terms of both their resistance to disruption and cell size. With this in mind we have chosen to examine two species *Chlamydomonas concordia* and *Dunaliella salina* which have cell sizes between 5 and 8 μm respectively. In terms of the dynamic mechanisms involved in the ultrasonic breakdown of the two species both the frequency and power of the ultrasound was varied. The shell model was used to calculate the mechanical resonant frequencies and vibration modes of the algae such models have been used previously for bacteria and viruses by treating them as spherical particles [12,13].

2. Materials and methods

2.1. C. concordia

The *C. concordia* subspecies used in these investigations is a non-motile spherical unicellular marine microalgae of size between 3 and 6 μm. It has a thin cell wall and contains lipid vesicles that are visible under a microscope. *C. concordia* master cultures were grown using *f*/2 growth media in sterile tissue culture flasks in a growth room at a constant temperature at 20 °C and a photoperiod of 12 h light and 12 h dark.

2.2. D. salina

D. salina is a halophile green microalga of ovoid shape with length 4–8 μm and diameter 1.5–3 μm found especially in sea salt

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fields. It is known for its anti-oxidant activity because of its ability to produce large amounts of carotenoids which are used in cosmetics and dietary supplements. Few organisms can survive under conditions such as highly saline salt evaporation ponds, making this alga a candidate for commercial biological production of biofuels. *D. salina* was cultured under the same sterile conditions as *C. concordia*.

2.3. High frequency sonication

Standard suspensions (400 ml) of *C. concordia* (absorbance of 1.0 at 680 nm) and *D. salina* (absorbance of 0.8 at 680 nm) were placed in a glass cylinder with a cooling jacket as shown in Fig. 1(a) and sonicated using a disk-type transducer capable of being driven at a range of individual frequencies (580, 864 and 1146 kHz) using a multifrequency high power system (Meindardt Ultrachalltechnik). Both standard suspensions for *C. concordia* and *D. salina* had cell concentrations of 10^7 cells/ml. The system was operated for up to 30 min using a cooling system (Julabo, FL 300) to maintain the temperature in the range of 15–20 °C. Experiments were undertaken at three acoustic power levels, low (~3 W), medium (~20 W) and high (~60 W). The precise acoustic power entering the system was determined using calorimetry prior to sonication. All experiments were performed in triplicate.

2.4. Low frequency sonication

Standard suspensions (400 ml) of *C. concordia* (absorbance of 1.0 at 680 nm) and *D. salina* (absorbance of 0.8 at 680 nm) were placed in a glass cylinder as outlined above using a bottom plate of stainless steel instead of the transducer shown in Fig. 1(b) to maintain the same experimental conditions as the high frequency sonication. Suspensions were sonicated for 30 min by directly inserting a 20 kHz probe (Vibra-cell, Sonics & Materials; acoustic power 32.3 W) using a cooling system to maintain temperature in the range of 15–20 °C. All experiments were carried out in triplicate.

2.5. Analytical methods

Algae samples were taken after periods of 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 min of sonication and the number of algae cells was enumerated using haemocytometry. Cell counting was performed in triplicate and the average was taken. The reduction in algae cell numbers after sonication for n minutes (CR_{nmin}) was calculated using the following equation from the number of cells counted at n min CN_{nmin} and the original number of cells i.e. at 0 min CN_{0min}:

$$CR_{n\ min}\ [\%] = (CN_{n\ min} - CN_{0\ min})/CN_{0\ min} \times 100 \quad (1)$$

C. concordia and *D. salina* suspensions both exhibited strong absorbance at around 430 and 680 nm. A second method for analyzing the condition of algae cells during sonication involved a closed flow loop system through a spectrophotometer. This method enabled measurements of the absorbance of the algae samples at 430 and 680 nm using very short time intervals.

3. Results and discussion

3.1. Ultrasonic treatment of C. concordia

Fig. 2 shows the absorbance spectra of *C. concordia* suspensions before sonication (0 min) and after 30 min sonication at 580 kHz at an acoustic power of 60.1 W with the wavelength plotted as the abscissa and the absorbance plotted as the ordinate. The absorbance spectrum at 0 min exhibited no discernible peaks in the measured wavelength range of 300–800 nm. After 30 min sonication, two new peaks appeared at specific wavelengths of around 430 and 680 nm. This means that chlorophyll was released from the algae during sonication due to collapse of the algae cells (see chap. 2 in Ref. [10]). The spectrum after sonication exhibits a tendency to rise to a level greater than the control suspension (0 min) at short wavelengths and drop at longer wavelengths. The absorbance spectra contains information not only on light absorption, but also on light scattering from the algae cells and fragments in the suspension. Mie scattering occurs from fragments of algae in the suspension during sonication, with the absorbance spectrum increasing as cell disruption progresses. The sonication fragments become smaller than the wavelength and Rayleigh scattering occurs as this process continues, indicating that strong wavelength dependence on scattering power is dominant at longer wavelengths. The chlorophyll peak value at around 680 nm does not directly indicate the algae cell disruption rate. The peak value at around 430 nm however can be correlated with algae cell reduction.

3.1.1. Sonication at different acoustic powers

The results for the time change in cell reduction and absorbance during sonication at the three different frequencies are shown in Fig. 3:

- At 580 kHz applied at three acoustic powers of 3.0, 18.5 and 60.1 W are shown in Fig. 3(a) at intensities of 0.13, 0.82 and 2.67 W/cm^2, respectively. Cell reduction was calculated from the aforementioned Eq. (1). Absorbance at 430 nm was adopted

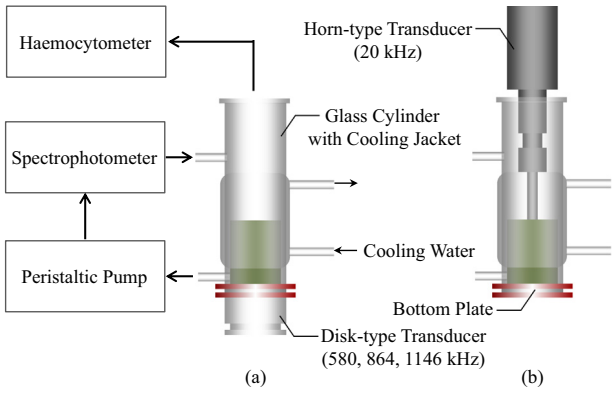

Fig. 1. Experimental apparatus for sonication at (a) 580, 864, 1146 kHz and (b) 20 kHz.

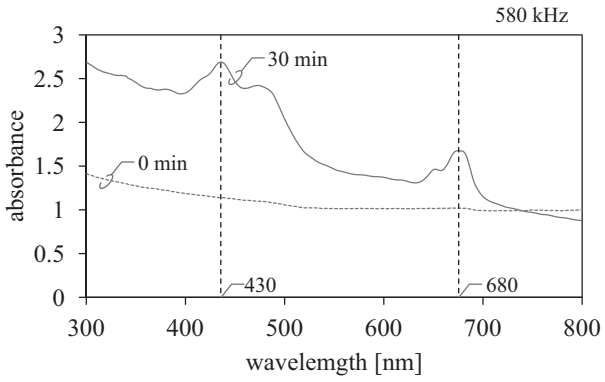

Fig. 2. Absorbance spectra of *Chlamydomonas concordia* suspensions before sonication (0 min) and after 30 min sonication at 580 kHz at an acoustic power of 60.1 W.

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