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Short Communication

Ultrasonic cavitation for disruption of microalgae

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HIGHLIGHTS

- We study ultrasonic cell disruption as a processing step that fractionates microalgae.
- A range of species types with different sizes and cell wall compositions are treated.
- The initial seconds of sonication cause the most significant cell disruption.
- Higher concentrations of *Isochrysis* disintegrate only marginally slower.
- Lessening of cell disruption is attributed by acoustic measurements to attenuation.

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ABSTRACT

Challenges with mid-stream fractionation steps in proposed microalgae biofuel pathways arise from the typically dilute cell density in growth media, micron scale cell sizes, and often durable cell walls. For microalgae to be a sustainable source of biofuels and co-products, efficient fractionation by some method will be necessary. This study evaluates ultrasonic cell disruption as a processing step that fractionates microalgae. A range of species types with different sizes and cell wall compositions were treated. The initial seconds of sonication offered the most significant disruption, even for the more durable *Nannochloropsis* cells. Following this initial period, diminishing effectiveness was attributed, by acoustic measurements, to attenuation of the ultrasound in the ensuing cloud of cavitating bubbles. At longer exposure times, differences between species were more pronounced. Processing higher concentrations of *Isochrysis* slowed cell disintegration only marginally, making the expenditure of energy more worthwhile.

1. Introduction

Our global society has a compelling interest in establishing a more sustainable energy infrastructure for the long term. In the transportation sector, a particular need exists for high energy density liquid fuels in the jet and diesel range. Renewable fuel use for transportation is still small, and fuel ethanol makes the only important contribution. Bio-oil production from microalgae shows significant potential as a pathway that does not compete with conventional agriculture in terms of land use. This process has been shown to require a comparatively smaller amount of even non-arable land compared to conventional terrestrial crops, and can utilize carbon dioxide from industrial sources often in brackish or salt water (Clarens et al., 2010; Davis et al., 2011).

This work with cavitation induced by high intensity, low frequency ultrasound is motivated by the potential to alleviate the key challenges in the mid-stream steps of harvesting and fractionation of microalgae. Cavitation is characterized by the violent collapse of bubbles in an alternating pressure field. The cavitation of bubbles in the aqueous algal suspension produces severe, localized, short-lived temperature and pressure increases as well as microstreaming effects and shock waves that rupture algal cells. Acoustic cavitation is employed for its chemical and physical effects in a wide range of applications, including sonochemistry, mixing, and cleaning applications. The central mechanism responsible for the desired effects is the drastic convergence of a collapsing bubble under the influence of a sound field.

The destructive effects of ultrasound on cells were documented early in the last century (Harvey and Loomis, 1929; Hugo, 1954), and correctly attributed to acoustic cavitation (Hughes and Nyborg, 1962), not a direct effect of the ultrasound itself. Giordano and coworkers (1976) conducted experiments with low power (3 W/cm^2), high frequency (1 MHz) ultrasound. This was

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transmitted from the walls of a cylindrical container for long times, exceeding a half hour, for disruption of the unicellular microalga *Scenedesmus quadricauda*. They measured cavitation intensity by monitoring the power of the subharmonic frequency (De Santis, 1967), and showed that the extent of microalgae cell breakage directly followed cavitation intensity not acoustic power. When the water was degassed, cavitation – which relies on pre-existing bubble nuclei under ordinary ultrasonic power – was prevented. This inhibition continued up to the maximum power available.

A few recent studies, motivated by the potential for biofuel processing, present work done in the utilization of ultrasonic cavitation for microalgae cell disruption. Gerde and coworkers (2012) evaluated cell disruption in 10 mL aliquots of two species, heterotrophic *Schizochytrium limacinum* and photosynthetic *Chlamydomonas reinhardtii*, with 20 kHz high power ultrasound. Disruption was gauged by measurement of released chlorophyll and carotenoids for *C. reinhardtii*, and by Nile red lipid fluorescence (of an extracted ethanol/water supernatant) with *S. limacinum*. They reported that, with both types of algae, when 800 J was applied to the 10 mL suspensions, the release of intracellular components was maximized. *C. reinhardtii* was used at concentrations from 0.15–1.4 wt%. They opted not to do microscopic cell counting due to its time intensiveness. A bilinear fit of cell disruption vs. energy was proposed, consisting of two linearly fitted sections where – after some threshold value was reached – the second line's slope is greatly decreased, and it is implied that further sonication is not beneficial. They called for studies with other species, which vary in cell wall strength and size.

Recently, Bigelow et al. (2014) probed the performance of ultrasonic cavitation at a much higher frequency of 1.1 MHz applied with a spherically focused transducer acting on 1 mL of cell suspension. The spherically focused transducer generates cavitation at a distance its vibrating surface, where the sound waves converge. This is a fundamentally different configuration than the ultrasonic horn, which produces cavitation in the media near the horn tip as well as beyond it. The specific system employed by the authors diminished tangibly in effectiveness past 2.5 wt% due to reduced mixing in the increasingly viscous suspension, though the authors comment (without showing results) that a simple flow system was constructed to provide mixing in the volume being treated across a range of cell concentrations from 0.1–5 wt%. This better mixing in the flow system enabled processing which was equally effective for the range of concentrations. The authors concluded that release of protein and chlorophyll, as well as lipid extractability, were complete after 15 s of sonication.

Wang et al. (2014) also assessed high-frequency focused ultrasound at 3.2 MHz, 40 W in reference to a lower 20 kHz frequency probe setup operating at 100 W. Particle counting and two fluorescence density measurements were used to evaluate the effect of ultrasound. A volume of 30 mL of approximately 0.03 wt% suspensions were treated for two different species of microalgae. Initial declumping and subsequent breakage of the 10–20 μm *Scenedesmus dimorphus* cells caused the particle count with a flow cytometer to increase. An appreciable decrease of 28% for the particle count of 2–4 μm *Nannochloropsis oculata* did not occur until 5 min of treatment time with the low frequency ultrasound, when the cells broke into smaller fragments than the detectable particle size in the flow cytometer. Wide variation in cell and fragment particle size and uncertainties related to the sensitivity of the counting device for small particles make the reported data less useful for evaluating morphological changes in cells as a function of treatment time. The authors also demonstrate that, for both species, a final combined treatment at high and then low frequency for 1 min each caused a more significant change in lipid detection compared to 2 min at either single frequency. They claim that this improvement cannot be attributed to total energy input because the dual treatment employed less energy than the low frequency one.

Keris-Sen et al. (2014) studied the effect of 30 kHz ultrasound on mixed microalgal cultures known to be primarily *Scenedesmus* sp., *Chloroccum* sp., and related species which were cultivated in a standard medium (BG11) and secondary effluent wastewater. With a reported power of 50 W (likely the nominal value, not the actual power applied), volumes from 100 to 500 mL were sonicated for 5–60 min. Without specifying the exact volume and sonication time used, the authors demonstrate that the highest material release is attained at 0.4 kW h/L energy intensity. The article provides useful experimental data on improved lipid extraction yields with combinations of solvents and ultrasound. However, the uncertainty regarding the sonication time, a lack of error reporting of replicate measurements when concluding that an energy intensity of 0.4 kW h/L is optimal, and the apparently very large energy expenditures for dilute cells in suspension leave room for further clarification in subsequent studies.

2. Experimental methods

2.1. Cell suspension preparation

Suspensions of five strains of microalgae were prepared for sonication experiments. A 10 μm freshwater green algae, *C. reinhardtii*, both wild type and a mutant strain (4349) with deficient cell wall formation were grown in TAP media prepared according to the formula of Gorman and Levine (1965). A 5 μm marine diatom with a siliceous cell wall, *Thalassiosira pseudonana*, was also cultured in Aquil seawater medium prepared according to a standard recipe and method (Morel et al., 1979) with slight modifications (Price et al., 1989). The growth of both of these species was monitored by absorbance measurements. At the beginning of the stationary phase (when growth slowed), the suspension was harvested and used within an hour. Two other species, *Isochrysis galbana* (CCMP1324), a 5 μm marine Golden/Brown species lacking a cell wall, and *N. oculata* (CCMP525), a 2 μm marine green algae with a robust cell wall, were supplied by Reed Mariculture Inc. (Campbell, CA, USA) in unfrozen concentrated slurries of 19.5 wt% and 32 wt%, respectively, and used in a few days. These concentrated slurries were resuspended in tap water (pH 7.2 with no added salts) to 0.5%, nearer to growth concentrations. Additionally, a series of higher concentrations were run with *Isochrysis* up to 7.5%.

2.2. Experiments with an ultrasonic horn

A Branson Ultrasonics Corporation 450 digital ultrasonic processor (Danbury, CT, USA) operating at 20 kHz was employed. A Sonics & Materials, Inc. ultrasonic horn (Newtown, CT, USA) with a 3/8 inch diameter tip was inserted 3.5 cm into a 45 mL cell suspension in a VWR Brand 50 mL polypropylene centrifuge tube. Just before each individual treatment, a cell suspension was retrieved from a large, well-mixed volume that was stirred for an hour prior to the experiments.

Once inserted, the distance from the tip of the horn to the bottom of the tube was 5.5 cm. The highest amplitude setting was employed for all experiments. Given the maximum converter amplitude of 25 μm with the horn's amplification factor of 7.5, the maximum amplitude (peak-to-peak displacement) of vibration is almost 190 μm. At this maximum amplitude, the typical average power drawn was approximately 114 W (an average of 2.5 W/mL of suspension). This power was calculated from the total energy drawn during an experiment (recorded digitally by the sonifier), divided by the time of operation. Considering the area of the horn tip, this translates to 160 W/cm^2. The cell suspension was not