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Short Communication

Algal cell disruption using microbubbles to localize ultrasonic energy

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HIGHLIGHTS

- Ultrasound with pre-loaded microbubbles was used to enhance algal cell disruption.
- This process requires less than one-fourth the energy of current disruption methods.
- Disruption scales with ultrasound pressure and microbubble concentration.
- Separating bubble formation and growth increases efficiency by localizing energy.
- This process can potentially synergize with dissolved air flotation cell harvesting.

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ABSTRACT

Microbubbles were added to an algal solution with the goal of improving cell disruption efficiency and the net energy balance for algal biofuel production. Experimental results showed that disruption increases with increasing peak rarefaction ultrasound pressure over the range studied: 1.90 to 3.07 MPa. Additionally, ultrasound cell disruption increased by up to 58% by adding microbubbles, with peak disruption occurring in the range of 10^8 microbubbles/ml. The localization of energy in space and time provided by the bubbles improve efficiency: energy requirements for such a process were estimated to be one-fourth of the available heat of combustion of algal biomass and one-fifth of currently used cell disruption methods. This increase in energy efficiency could make microbubble enhanced ultrasound viable for bioenergy applications and is expected to integrate well with current cell harvesting methods based upon dissolved air flotation.

1. Introduction

Lipid extraction is a key step in algal biofuel production, and disrupting the cell prior to extraction has been shown to improve the recovery of lipids by up to a factor of four (Lee et al., 2012). Several disruption methods are used in labs or full-scale processes, but the energy for disruption of these techniques is typically higher than the energy of combustion of algal biomass (Lee et al., 2013). A survey of disruption techniques suggests energy requirements between 3.3×10^7 J/kg of dry biomass for hydrodynamic cavitation up to 5.3×10^8 J/kg for high-pressure homogenizers (HPH) (Lee et al., 2012). Unfortunately, the heat of combustion of algal biomass is only 2.7×10^7 J/kg (Lee et al., 2012), so current cell disruption techniques typically result in a negative net energy balance in biofuel applications.

However, there is no expectation that the energy is applied efficiently in these devices. For example, when algae are disrupted with an atomic force microscopy (AFM) tip, the specific energy of disruption is only 6.73×10^2 J/kg, approximately 10^5 times smaller than the current state-of-the-art hydrodynamic cavitation (Lee et al., 2013). Theoretical estimates based upon individual cell properties also suggest significantly lower specific disruption energy than current processes. One estimate based on the tensile strength of the cell walls suggests a cell disruption energy of 2.26×10^2 J/kg dry biomass (Lee et al., 2012). A similar estimate based upon anticipated bonding energy in the cell walls is 3.32×10^2 J/kg (Lee et al., 2012). The energy for cell disruption using the critical tension to rupture a lipid membrane suggests only 1.3×10^{-1} J/kg of cell biomass (Krehbiel, 2014).

There are several aspects of current sonication techniques that could potentially increase the energy efficiency substantially.

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Ultrasonic disruption operates with a continuous low-frequency (about 10 kHz) wave (Lee et al., 2010), which is designed to increase the probability of microbubble generation during a low pressure portion of the wave (Bendicho and Lavilla, 2000). The fluid motion due to the bubble and the shock wave accompanying the strong collapse of a microbubble disrupt the cells with shear, heat, or free radical formation (Lee et al., 2012). The dissipation associated with these mechanisms is evidenced by the need to remove heat from sonicators (Balasundaram and Pandit, 2001). A low probability of strong interaction with nearby cells decreases efficiency (Lee et al., 2013).

Significant flexibility would be afforded by separating the bubble loading from the insonification. In this case, the frequency and bubble sizes could be set near resonance to amplify its efficacy. Similarly, the probability of cell-bubble interactions could be specified by changing the concentration of pre-loaded microbubbles. The bubbles have the potential to localize the ultrasonic energy in space, near the algal cells. Their presence, however, also facilitates localization in time. Since the bubble response will be rapid with properly tuned ultrasound, it can then be applied in pulses rather than continuously. This application will further reduce energy required because the ultrasound can be off most of the time. Furthermore, the energy of bubble formation in sonication processes is inefficient (Lee et al., 2012), but bubbles could be generated during a separate stage much more efficiently. For example, the energy for shelled microbubble generation is 6.17×10^6 J/kg (Krehbiel, 2014).

Ultrasound contrast agents (UCAs) provide the regularity needed to assess the potential of this pre-loading approach. These microbubbles were initially designed to enhance diagnostic ultrasound by increasing the acoustic impedance mismatch. They are made of thin protein or lipid shells with typical diameters between 1 and 10 μm (Stride and Saffari, 2003). It has been established in biomedical applications that when these microbubbles collapse, due to strong acoustic forcing, the resulting pressure gradients and microjets disrupt cellular membranes (Stride and Saffari, 2003). It has been demonstrated that they can be used to transiently disrupt cell membranes so that molecules, such as drug molecules, can be transported into cells (Stride and Saffari, 2003). Here, their potential for total disruption of algal cells is investigated.

The efficacy of bubble action in disrupting cells, whether in biomedical or algal applications, is expected to depend upon the intensity of the ultrasound, the character and dynamics of the bubble, and the relative proximity of the cells to the bubble, though the relative importance of these factors is unclear. The overall objective of this study is to examine the potential of pre-loading with microbubbles to significantly advance the efficiency of ultrasonic algae disruption. Ultrasound contrast agents are used to provide a controlled microbubble geometry and pre-loaded concentration.

2. Methods

2.1. Algae

Chlamydomonas rheinhardtii colonies were cultivated on Petri dishes and then transferred to 250 ml Erlenmeyer flasks with 150 ml TAP medium. They were subsequently grown on a shaker table with continuous lighting (110 ± 30 μmol photons m^{-2} s^{-1}) at 24 °C. Tests were initiated during exponential growth phase, and a cell count (with hemocytometer) showed 8.9×10^6 cells/ml. Total suspended solids were 0.76 mg/ml.

2.2. Microbubbles

The contrast agent used for these experiments was Definity™ (Lantheus Medical Imaging, N. Billerica, MA, USA). These are lipid-shelled microbubbles with a diameter in the range of 1.1–3.3 μm with a reported mean of 1.98 μm (King and O'Brien, 2011). The experimentally-measured resonant frequency is between 4.0 and 4.5 MHz for 2 μm diameter Definity™ microbubbles (Sun et al., 2005).

2.3. Ultrasound setup

The experimental configuration involved flowing the algal solution through a clear vinyl tube with inner diameter 1.6 mm and wall thickness 0.79 mm. A section of the tube in a water bath was insonified by a 0.9 MHz transducer with f-number of 2 (Valpey Fisher, Hopkinton, MA). This frequency provided a beamwidth that covered the cross section of the tube: the −6 dB beamwidth of the pulse is 4.6 mm (Cobbald, 2007). The alignment of the transducer relative to the tube was determined by transmitting a pulse to an air-filled tube; the point of peak amplitude reflection in pulse-echo mode was selected.

Ten-cycle tone bursts were generated at a pulse repetition frequency of 1000 Hz using a pulse-receiver system (RITEC RAM5000, Warwick, RI) providing a duty cycle of 1.1%. Solutions with microbubble concentrations (C_b) between 0 and 15×10^7 UCAs/ml were prepared by pipetting the appropriate volume of a stock solution of 10^{10} UCAs/ml into a 20 ml algae-filled syringe. Uniform distribution of the UCAs in the syringe was ensured by gently rolling the syringe vertically and horizontally for 30 s prior to each test. The solution was visually well-mixed and the rise times based on Stokes drag of the microbubbles (16 min) far exceeded the testing time. Peak acoustic rarefaction pressures (P_r) of 0, 1.90, 2.38, 2.83, and 3.07 MPa were measured with a polyvinylidene fluoride hydrophone following established procedures (Raum and O'Brien, 1997).

For each test, 3.0 ml of the algae and microbubble solution was pumped through the tube at a rate of 40 ml/hr (0.67 ml/min) with a syringe pump, though only the final 1.5 ml were collected to ensure that only samples treated with the selected test conditions were collected. For each test condition, four 180 μl samples were analyzed in a microplate reader, and each test condition was repeated three to ten times.

2.4. SYTOX fluorescence diagnostic

To quantify cell viability, SYTOX green fluorescent probe was used (Molecular Probes Inc. Eugene, OR, USA) because it has been shown to correlate well with disruption and extractable lipids (Roth et al., 1997; Sheng et al., 2011). To do this, the 5 mM solution was diluted to 10 μM with deionized water, and 20 μl were mixed with 180 μl of treated algal solution in each well of a 96-well plate. This produced a final 1.0 μM SYTOX concentration in accordance with recommendations for eukaryotes (Life Technologies, 2006). The well plate and cover were placed in a microplate reader (Infinite 200 series, Tecan Group Ltd. Männedorf, Switzerland) and the samples were shaken for 10 s with a 1 mm orbital amplitude and then excited with 488 nm light. Fluorescent emission was measured at 534 nm with gain set at 80%. This measurement was repeated for 30 min. Maximum fluorescence values during the 30 min are reported.

To correlate SYTOX fluorescence with disruption of *C. rheinhardtii*, the algal cells were made to be permeable with 70% isopropyl alcohol as proposed by Roth et al. (1997) with the modification that the sterile dilute medium was replaced with deionized water. The disrupted cells were then combined with untreated cells, and three separate calibration samples with 0%, 20%, 40%, 60%, 80%, and 100% of disrupted cells were analyzed. The measured relationship between fraction disrupted (Δd_f) and