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Measurement of acoustic properties of microalgae and implications for the performance of ultrasonic harvesting systems

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ABSTRACT

Microalgae are a promising feedstock for biofuel production, but difficulties associated with harvesting suspended cultures contribute to the high costs of algal feedstock production. Ultrasonic harvesting has been identified as a potential low-cost technique, but limited data are available on the response of microalgae cells in the presence of an acoustic field. The acoustic radiation force acting on a cell depends upon cell size and the acoustic contrast factor (ACF) of the cell in the media. The ACF depends upon the density and compressibility of the cell and the media. Cell size and ACF were measured for *Microchloropsis gaditana, Nannochloropsis oculata, Phaeodactylum tricornutum,* and *Chlamydomonas reinhardtii.* The average ACFs, which were determined by measuring the densities and sound velocities of suspensions containing varying concentrations of cells in growth media, were 0.04 (range = 0.03–0.05) for *M. gaditana,* 0.02 (range = 0.01–0.04) for *N. oculata,* 0.05 (range = 0.04–0.07) for *P. tricornutum,* and 0.05 (range = 0.049–0.053) for *C. reinhardtii.* The ratio of the acoustic radiation force to the drag force would be highest for *C. reinhardtii* cells due to their larger effective radius (5.6 μm compared to 1.9–2.7 μm for the other species). The effective ACF of *C. reinhardtii* was also evaluated by recording the motion of cells in the presence of an acoustic field, using particle tracking velocimetry, and then modeling the recorded motion using COMSOL Multiphysics software. The result (ACF = 0.04) demonstrated agreement with the density/sound velocity meter method. Experiments with starch null sta6 mutant *C. reinhardtii* cells demonstrated that the effective ACF can transition from positive to zero and eventually become negative as microalgae cells accumulate lipids. The dynamic nature of the ACF represents an opportunity and a challenge for acoustic harvesting of algal cells.

1. Introduction

Biofuels have the potential to reduce lifecycle greenhouse gas emissions associated with the use of liquid transportation fuels, reduce dependence on imported petroleum, and serve as a renewable alternative to finite fossil fuel supplies [1,2]. Microalgae represent a promising biofuel feedstock because they grow rapidly, can be cultivated year-round on non-arable land, require less area for cultivation per gallon of fuel produced than conventional land-based biofuel feedstocks, and can integrate with various waste streams [3–6]. Microalgae have been grown in wastewater and brackish water (i.e., non-potable water) and can utilize CO_2 emitted from stationary sources [4]. Microalgae can be cultivated to produce a high lipid content (typically 20–50% by dry mass) [7] and can be converted into liquid biofuels through a variety of technologies [3]. Algae biofuels have potential to qualify as advanced biofuels under the Renewable Fuel Standard, meaning that they are capable of reducing greenhouse gas emissions by at least 50% compared to petroleum-derived fuels [8–10]. Meeting the

Renewable Fuel Standard requires the integration of innovative processes across the algal production value chain, including the harvesting stage.

Major challenges associated with production of algae biofuels include the costs and energy requirements associated with cultivation, harvesting, and conversion of the algal biomass [11]. Biofuels produced from algae at commercial scale have been estimated to cost between $1.65 and $33.16 per gallon (in 2014 dollars) [12]. Costs associated with harvesting, which often involves flocculation, centrifugation, and/or filtration [13–15], have been estimated to contribute to 15% of the final fuel cost [16].

Ultrasonic harvesting has been investigated as a low-cost method that can dramatically reduce energy consumption compared to traditional harvesting through a centrifuge [15,17,18]. Ultrasonic harvesting also has the advantages of operating continuously, requiring no moving parts (which could help minimize maintenance requirements), and not damaging the cells [17]. In an ultrasonic harvesting process, an algal culture is exposed to an acoustic field and cells agglomerate at the

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nodes or antinodes of the resulting standing wave. When the agglomerated cells obtain a critical mean diameter, the gravitational force on the agglomerates becomes larger than the drag force imposed by the flowing media [15,17]. Several ultrasonic harvesting systems for microalgae have been described in the literature. One is a batch-continuous system in which the acoustic field causes cells to agglomerate and settle in the bottom of a chamber. The concentrated media is then drained from the chamber periodically [19]. Hincapié Gómez and Marchese [18] describe an ultrasonically-enhanced inclined plate settler that operates continuously.

Ultrasonic harvesting has been described as the only technology capable of meeting the National Alliance for Advanced Biofuels and Bioproducts (NAABB) goal of reducing operating costs associated with harvesting to less than $0.013 per gallon of gasoline equivalent [15]. However, this goal can only be achieved if the algal cells are sufficiently responsive to the acoustophoretic force [15]. The responsiveness of the cells is dependent on the physical properties of the microalgae and the media in which the cells are suspended.

The acoustic radiation force acting on a small, spherical particle suspended in a fluid and exposed to a one-dimensional standing acoustic wave is proportional to the particle/fluid acoustic contrast factor (ACF) and the radius of the particle cubed [20]:

$$F_{rad} = 4\pi\Phi k a^3 E_{ac}\sin(2kz) \quad (1)$$

where k is the wavenumber (m^{-1}), a is the radius of the particle (m), E_{ac} is the acoustic energy density ($J \cdot m^{-3}$), z is the position of the particle along the axis of the standing wave (m), and Φ is the acoustic contrast factor:

$$\Phi = \frac{1}{3}\left[\frac{5(\rho_p/\rho_m) - 2}{2(\rho_p/\rho_m) + 1} - \frac{\kappa_p}{\kappa_m}\right] \quad (2)$$

where ρ_p is the density ($kg \cdot m^{-3}$) of the particle (an algal cell in this case), ρ_m is the density ($kg \cdot m^{-3}$) of the fluid (growth media in this case), and κ_p and κ_m are the compressibility of the particle and fluid, respectively, (Pa^{-1}). Compressibility is related to density and speed of sound as shown in Eq. (3):

$$\kappa = \frac{1}{\rho c^2} \quad (3)$$

where c is the speed of sound ($m \cdot s^{-1}$).

Eqs. (1) and (2) are valid when the particle radius is small compared to the sound wavelength and when the particle and fluid densities are on the same order of magnitude. When the ACF is positive, the acoustic radiation force pushes the particle toward the node of the standing wave. When the ACF is negative, the acoustic radiation force pushes the particle toward the antinode of the standing wave [20,21].

For microalgae cells, the sign on the ACF could theoretically be positive or negative depending on the cell composition. Microalgae cells are composed primarily of carbohydrates, proteins, and lipids. As shown in Table 1, carbohydrates and proteins have positive ACFs, whereas lipids have a negative ACF. As microalgae cells accumulate lipids, which is desirable for conversion to biofuels, the ACF of the cells could approach zero. Cells with an ACF of zero would render acoustic harvesting ineffective.

In addition to the ACF, the magnitude of the acoustic radiation force is strongly dependent on cell size. This size dependence presents another potential challenge, since many of the microalgae species targeted for biofuels production have small radii ($< 10\,\mu m$) [15]. When an algal cell is exposed to an acoustic field, the acoustic radiation force is opposed by the drag force:

$$F_D = C_D A \frac{\rho_m V^2}{2} \quad (4)$$

where C_D is the drag coefficient, A is the cross sectional area of the cell (m^2), and V is the velocity of the cell ($m \cdot s^{-1}$).

Assuming a spherical cell, the coefficient of drag is given by Eq. (5) when $Re \lesssim 1$, and the resulting drag force is given by Eq. (6):

$$C_D = \frac{24}{Re} \quad (5)$$

$$F_D = 6\pi\mu_m Va \quad (6)$$

where μ_m is the dynamic viscosity of the media ($kg \cdot m^{-1}\,s^{-1}$). The ratio of the acoustic radiation force to the drag force is therefore proportional to Φa^2.

No information is available on the manner in which the acoustic response of microalgae cells varies with strain, growth conditions, and growth stage. Accordingly, the objectives of the present study were to: (1) quantify the density, speed of sound, and compressibility of different microalgae species; (2) evaluate the acoustic contrast factor of those species; (3) quantify the acoustic radiation force acting on microalgae cells in the presence of a standing acoustic wave; and (4) assess how the response of microalgae cells changed with growth conditions and stage in the growth process. The Discussion Section focuses on the impact of a dynamic ACF on the implementation of an acoustic harvesting system.

2. Materials and methods

In this study, the acoustic contrast factors for four species of microalgae (*Microchloropsis gaditana*, *Nannochloropsis oculata*, *Phaeodactylum tricornutum*, and *Chlamydomonas reinhardtii*) were evaluated by measuring the density and speed of sound of suspensions of microalgae cells in growth media. In addition, the trajectories of polyamide particles and *Chlamydomonas reinhardtii* cells exposed to an acoustic field were measured using particle tracking velocimetry (PTV) and modeled using COMSOL Multiphysics to determine the acoustic energy density (E_{ac}) for the system and the effective ACF of the cells in the media. The PTV system was then used to illustrate the change in ACF that can occur as microalgae accumulate lipids under nitrogen-deprived growth conditions.

2.1. Microalgae cultures and cultivation

Microchloropsis gaditana (CCMP 527), *Nannochloropsis oculata* (CCMP 525) and *Phaeodactylum tricornutum* (CCMP 630) were obtained from the National Center for Marine Algae and Microbiota. The

Table 1
Density, speed of sound, compressibility, and acoustic contrast factor for various substances, including the saline growth media used in the present study. Properties of substances without citations were measured by the authors. Properties of substances with citations are from the literature.

Substance	Density, ρ ($kg \cdot m^{-3}$)	Speed of sound, c ($m \cdot s^{-1}$)	Compressibility, κ (Pa^{-1})	Acoustic contrast factor in media, Φ ($-$)
Water @ 20 °C	998	1483	4.56×10^{-10}	–
Saline growth media	1013	1504	4.37×10^{-10}	–
Carbohydrates (corn starch) [22]	1620	2810	7.82×10^{-11}	0.42
Protein (Myoglobin) [23]	1370	3473	6.05×10^{-11}	0.38
Lipid @ 20 °C (soybean oil)	919	1470	5.03×10^{-10}	-0.08
Microalgae (*Chlorella vulgaris*) [24]	1100	1540	3.83×10^{-10}	0.07
Polyamide seeding particles [25]	1030	2200	2.01×10^{-10}	0.19