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Force and energy requirement for microalgal cell disruption: An atomic force microscope evaluation

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HIGHLIGHTS

- Cell disruption energy measured by an atomic force microscope.
- The indentation/disruption energy averaged 17.43 pJ per cell.
- This value is equivalent to 673 J kg^{-1} of the microalgal dry mass.
- Mechanical cell disruption requires energy that is higher by an order of 5.

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ABSTRACT

Cell disruption is an essential step in the release of cellular contents but mechanical cell disruption processes are highly energy intensive. This energy requirement may become a critical issue for the sustainability of low valued commodities such as microalgal biofuels derived from extracted lipids. By the use of an atomic force microscope (AFM), this study evaluated the force and energy required to indent and disrupt individual cells of the marine microalga, *Tetraselmis suecica*. It was found that the force and energy required for the indentation and disruption varies according to the location of the cell with the average being 17.43 pJ. This amount is the equivalent of 673 J kg^{-1} of the dry microalgal biomass. In comparison, the most energy efficient mechanical cell disruption process, hydrodynamic cavitation, has specific energy requirement that is approx. 5 orders of magnitude greater than that measured by AFM. The result clearly shows that existing mechanical cell disruption processes are highly energy inefficient and further research and innovation is required for sustainable microalgal biofuels production.

1. Introduction

1.1. Background

Lipids from microalgae have the potential to meet the global demand for biodiesel; furthermore, marine microalgae have the additional advantages of not competing with farm produce for arable land and fresh water. However, with the only known exception of *Botryococcus braunii*, the majority of the microalgal lipids are held intracellularly and separated from the surrounding media by cell membranes and/or cell walls. These structures form a barrier and the cells need to be disrupted for effective extraction of various cellular contents (Lee et al., 2010). A review of the scientific literature showed that microalgal cell disruption processes have energy requirements ranging from a low of 33 MJ kg^{-1} by hydrodynamic cavitation to a high of 529 MJ kg^{-1} by high pressure homogenizers. In comparison, the energy available by the combustion of the entire microalgal biomass is estimated to be about 29 MJ kg^{-1} (Lee et al., 2012). This net negative energy balance indicates current mechanical cell disruption processes are not sustainable for microalgal biofuels production. Some of the obvious questions are: is there any potential for further reductions in the disruption process energy or have the current disruption processes already reached the theoretical limit. To answer these questions, we need to find out the amount of energy required to disrupt the microalgal cells and compare it with the energy input during mechanical cell disruption processes.

Mechanical properties of various living tissues or cells such as tensile strength, adhesive force, visco-elasticity or Young's modulus have been studied (Rico et al., 2008), yet little research has been done on microalgae. Some of the studies performed on microalgae include: the determination of the tensile strength of cell walls of

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the microalga *Chlamydomonas eugametos* by compression/decompression (Carpita, 1985); evaluation of the mechanical resistive force of silicate shells from diatoms such as *Thalassiosira punctigera* by micro-needles (Hamm et al., 2003); theoretical estimation of the force and energy of disruption for a hypothetical microalgae (Lee et al., 2012). Apart from these, few measurements on mechanical properties of microalgae have been performed, and the actual force and energy required for algal cell indentation and disruption remain to be investigated.

1.2. Determination of the indentation force requirement

The relationship between the force F, applied by a rigid probe and the indentation depth, δ, on a deformable material can be determined either theoretically or experimentally. These two approaches are discussed below.

1.2.1. Theoretical

For a flat, homogeneous and deformable material, the force F, and the indentation depth, δ, can be estimated by the generalised equation (1):

$$F = \lambda \delta^{\beta} \tag{1}$$

Some values of λ and β are listed in Table 1 below (Bilodeau, 1992; Lin et al., 2007; Rico et al., 2008; Sneddon, 1965):

For microalgae, both Poisson's ratio and Young's modulus are unknown; the algal cell boundary is heterogeneous with tri-layered cell walls and membranes; cellular contents are viscous, heterogeneous and compartmentalised (Hori et al., 1982). Other factors to consider are the small sizes of the microalgae (in the order of a few μm) and the low indentation force required (in the order of a few μN); under such circumstances, the applied indentation forces may be influenced by the presence of other forces such as adhesive, salvation and/or electrostatic. For these reasons, a theoretical approach by using Eq. (1) will be too complex and direct experimental measurements may be preferable.

1.2.2. Experimental measurements

A range of techniques are available for the measurement of cell mechanical properties, for example: micropipette aspiration (Evans, 1983), compression and decompression (Carpita, 1985), dynamic light scattering (Janmey et al., 1994), optical tweezers (Ashkin, 1997), micro-compression (Mashmoushy et al., 1998), cytoindenter (Shin and Athanasiou, 1999), magnetic twisting cytometry (MTC) (Fabry et al., 1999) and atomic force microscopy (AFM) (Radmacher, 2002). Micropipette aspiration and fluid shear flow measure the response of the entire cell to the applied mechanical stress; MTC measures mechanical properties based on the collective responses from a group of cells; AFM indentation and optical tweezers evaluate cell mechanical properties in localised areas on individual cells. Comparing with these measurement techniques, AFM has the advantages of being capable of both imaging the cells with a resolution in the order of low tens of nanometers and measuring the mechanical strength in the nN to N scale. In addition, the majority of large scale mechanical cell disruption processes such as high pressure homogenisation are carried out in liquid media; the ability for the AFM to operate in liquid (Radmacher, 2002) allows force measurements to be made under similar conditions.

Table 1
Values for λ and β, where: E is Young's modulus and v is Poisson's ratio.

Probe tip geometry	λ	β	Reference
Sphere of radius, R	$\frac{4E\sqrt{R}}{3(1-v^2)}$	3/2	Rico et al., (2005)
Cone of semi-included angle, θ	$\frac{2E\tan\theta}{\pi(1-v^2)}$	2	Sneddon, (1965)
Flat-ended cylinder of radius, a	$\frac{2Ea}{(1-v^2)}$	1	Sneddon, (1965)
Four-sided regular pyramid of semi-included angle, θ	$\frac{0.7453E\tan\theta}{(1-v^2)}$	2	Bilodeau, (1992)

1.3. AFM indentation and types of cavitation collapse

The collapse of cavitations can create extremely localised high temperatures of a few thousand degrees Kelvin and high pressures of tens of MPa (Franc and Michel, 2005; Prentice et al., 2005). The shock waves and shear accompanied with such collapse are the common disruption mechanisms for many mechanical cell disruption processes (Lee et al., 2012). There are two main types of cavitation collapse, namely: spherical and central water jet, they are described below:

i. Spherical – When a cavitation is located at the bulk of the liquid and is not under the influence of any solid surface, the collapse is spherically symmetrical and the cavitation boundary travels uniformly towards the centre of the sphere (Shah et al., 1999). During this type of cavitation collapse, cell walls will be burst outward by the supersonic collapse of the cavitations (Balasundaram and Harrison, 2006).
ii. Central water jet – When a cavitation is located near a solid boundary and the collapse is restricted on the side adjacent to the boundary, a central water jet is formed on the opposite side of the cavitation with the jet indenting the solid boundary (Prentice et al., 2005). During this type of collapse, cell membranes will be pushed inwards by the localised impinging water jet (Xu et al., 2006).

Under a controlled environment, Prentice et al. (2005) reported the number of spherical to central water jet collapse occurred in the ratio of 3:1, and indentations made by AFM probes during force measurements correspond to the latter.

1.4. Objectives

This study aims to determine the energy required to disrupt individual microalgal cells by the use of AFM. The results, in J cell^{-1}, will be converted to specific disruption energy in J kg^{-1} of the dry mass. This specific energy will be compared with that from mechanical cell disruption processes to determine if there is any potential for the reduction in the disruption energy requirement.

2. Methods

2.1. Microalgae

Tetraselmis suecica was used for this cell disruption study; this microalga has a theca of carbohydrate scales as an outer cell barrier. The starting culture was obtained from The Australian National Algae Culture Collection, Hobart, Tasmania. The microalga was grown in modified *f/2* medium at 22 °C on a shake table in a culture tube. Modified *f/2* medium was prepared by the addition to 1 L of filtered (pore size 0.22 μm) sea water using the following chemicals (Guillard and Ryther, 1962): $NaNO_3$, 75 mg; $Na_2HPO_43H_2O$, 5 mg; Na_2EDTA, 4.36 mg; $FeCl_36H_2O$, 3.15 mg; $CuSO_45H_2O$, 10 μg; $ZnSO_47H_2O$, 22 μg; $CoCl_26H_2O$, 10 μg; $MnCl_24H_2O$, 18 μg; $NaMoO_45H_2O$, 6.3 μg; Cyanocobalamin, 1 ng; Biotin, 1 ng and Thiamine, 2 ng. The medium was first autoclaved and vitamins were added after cooling. The pH was adjusted to about pH 8.2 by the addition of sodium bicarbonate prior to the introduction of the culture. The contents were later introduced to a 250 mL Erlenmeyer