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Temperature dependency of cell wall destruction of microalgae with liquid nitrogen pretreatment and hydraulic pressing

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ABSTRACT

Cell disruption of microalgae is often applied prior to oil extraction processes in order to breakdown the cell wall and to increase the release of intercellular products such as lipids for biodiesel production. However, microalgae require high-energy input to successfully break their cell walls. In this study, cell disruption of *Nannochloropsis oculata* cells was conducted using a manually operated hydraulic press. Cell disruption was implemented under a variable pressure range from 10 to 100 bars at 20 °C. Also, the temperature dependency of cell disruption was investigated over the range from 20 to 100 °C under two pressure conditions, 10 and 50 bars. In addition, the influence of cell disruption with liquid nitrogen (LN_2) pretreatment was studied. The average cell disruption was examined by means of microscopy and the results showed that higher cell disruption was achieved with the liquid nitrogen treated samples. At constant temperature, cell lysing was more effective with the additional LN_2 treatment. With pressures < 30 bars the differences were statistically significant. At a relatively low, applied pressure of 10 bars the LN_2 samples showed considerably higher disruption (~95% compared to ~51%) to those samples receiving identical pressure and temperature treatments. Interestingly, with the high pressure 50 bar treatment, approximately the same results with heat treatment and either with or without LN_2 were obtained. The relative cell destruction per applied MJ/kg was calculated for the different processes.

1. Introduction

Biofuel production from microalgae is one technology gaining importance and momentum due to its fit with current infrastructure, energy security and reduced dependency on fossil fuels, which significantly impact climate change. There are considerable environmental concerns from carbon emissions from conventional fuels whether from combustion engines [1] or power stations. High oil producing microalgae can be used to produce biodiesel [2]. Microalgae are plants which convert the energy from the sun into chemical energy which is generally stored as lipids [3]. These lipids are extracted from the microalgae and can be converted into biodiesel. This extraction process is generally expensive and reduces the cost competitive nature of biofuel production from microalgae. Microalgae, however, can yield 30–50% oil and their approximate oil production rate is estimated to be between 47,000 and 308,000 L/ha/year (5000–33,000 gal/acre/year) [4]. In order to increase the lipid yield, cell disruption treatment methods can be adopted [5], where there is some dependency on the method and oil yield for different species. Adopting the appropriate cell disruption method for the species being grown is a key to increasing the lipid extraction efficiency [6], otherwise extraction of oil from microalgal cells is a costlier procedure [7]. Cell disruption breaks the cell wall improving the release of intracellular material [8]. Cell disruption methods are classified into mechanical and non-mechanical methods. A non-exhaustive list of lysing processes include: water bath, ultrasonic, freeze drying (lyophilization), bead mills, rotor stator homogenizers, microwave, autoclaving or drying, gas decompression, hydrodynamic cavitation, micro-fluidization [9] high pressure homogenization [10], grinding, osmotic shock, enzymatic lysis [11], blender and laser treatment [12].

The optimal disruption process depends on a number of significant factors such as the rigidity of specific microalgae cell walls, the degree of lipid content, the cost effectiveness of the chosen method and the conversion from laboratory based to large scale processes [13]. Another factor is the state of the microalgae biomass sample (i.e. dry or wet). Osmotic shock [14] is a promising technique for wet algae lipid extraction, whereas microwave treatment can be applied to both dry and wet microalgae biomass samples [12]. Lee et al. [9] found that most microalgae strains are composed of cell walls with a high tensile strength and they therefore require high energy input for their disruption. Mechanical disruption is a physical method, which involves pressing [15] that is subjecting the microalgae biomass to high pressure, which ruptures the cell walls and in return releases the oil [16]. Mechanical cell breakage is due to shearing action above a threshold value [17] and helps in higher lipid extraction efficiency. Microalgae lipids consist of triglycerides, which can be converted into environmentally friendly biodiesel fuel by the process of transesterification [18].

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In the present case, the disruption of *Nannochloropsis oculata* is investigated as a function of applied pressure as delivered via a hydraulic press. Additionally, the cell destruction fraction dependency on pressure, heating and cooling to liquid nitrogen (LN_2) temperatures and the energy requirements for these processes were quantified.

2. Materials and methods

2.1. Strain and cultivation

N. oculata was cultivated at the University of Glasgow using a rectangular photobioreactor (PBR) tank with dimensions of 122 × 47 × 38 cm in a controlled environment. The temperature of the tank was maintained at 25 °C by means of four 25 W aquatic heaters (HT-825, JAD, China). Reef phyto Guillard F/2 nutrients were supplied at a ratio of 0.5 mL/L of culture water; the nutrients contain essential amounts of nitrogen and phosphorous trace element and required vitamins for microalgal growth. As microalgae are photosynthetic organisms, a 250 W sodium lamp source (Sun Master, Venture lighting, UK) provided 42 μmol m^2 s^{-1} at the surface of the tank. In addition, the tank was agitated with air from a pump (AC0308, HALCEA, UK). A salinity of 30 ppt for the photobioreactor was maintained by adding sea salt (Waitrose Limited, UK) and was measured by means of a salinity meter (HI-8733, HANNA Instruments, Italy).

2.2. Harvesting and drying

After the culture was sufficiently dense (approximately 2.5 × 10^9 cells/mL), microalgal biomass paste was collected with a centrifuge (Extreme Raw Power Centrifuge WVO Designs, USA) with a rotational speed of 4500 rpm (2094 g). Microalgae were fed into the centrifuge with a pump (7524-05 Master Flex, Cole Parmer Inc., USA) at a flow rate of 90 L/h. After harvesting, the microalgae paste was spread over a rectangular metallic container and placed inside an incubator (PIN-120, Carbolite, UK) at 80 °C for 12 h for drying. The dried microalgae layer (average thickness of 1 mm) was scraped off and the microalgae chips were collected for the experiments and weighed using an analytical balance (AS120, Ohaus, USA) into 0.1 g samples. The moisture content of the sample after drying at 80 °C was 10.2% (w/w on a dry basis).

2.3. Cell disruption with a hydraulic press

A manually operated hydraulic press (S10316/95, Mackey Bowley International Ltd., England) was used to apply different pressures to the dried microalgal samples. The press is provided with an electrical heating element on both surfaces, to vary the temperature, and a water cooling circulation system to avoid any damage to the equipment from overheating. The size of the hydraulic press plates was 30 ×30 × 4 cm.

2.3.1. Cell disruption of controlled microalgae dried biomass under variable pressure

The dried microalgae samples were treated to a pressure range from atmospheric (control) to 100 bars in increments of 10 bars (1 MPa). For all of the experiments the ambient temperature was 21 ± 1 °C. At 10 bars the rate of pressure application was ~0.58 MPa/s, while at 100 bars it was ~2.21 MPa/s. Each sample was wrapped in baking paper (10 × 10 cm) (Tesco, UK) with a thickness of 0.2 mm; this prevented the samples sticking to the surfaces of the hydraulic press and allowed collection of the compressed samples. Before disruption treatment, the initial microalgae samples were examined under a microscope to determine cell disturbance due to the centrifugation or the drying process and to provide a baseline to quantify the pressure and temperature effects. The samples were placed between the two surfaces of the hydraulic press and the pressure exerted to the desired value, where it was maintained for 1 min for each sample. Identical procedures were followed for all of the samples. The microalgae disruption was examined microscopically (Orthoplan, Ernst Leitz Wetzlar GmbH, Germany). The cell disruption was calculated using the same protocol as previously described by McMillan et al. [12].

2.3.2. Cell disruption of liquid nitrogen treated dried microalgae biomass under variable pressure

Cell disruption was evaluated under variable pressure from 10 to 100 bars (1–10 MPa) using the hydraulic press after the 0.1 g samples were pretreated using LN_2 (BOC Gases Ltd., UK) for 3 min. This treatment time was based on the time for complete evaporation of the LN_2 in the sample container (volume = 123.15 cm^3). All of the experiments were conducted at the same ambient temperature to compare the results obtained from the control samples and pretreated microalgae. Each sample was placed in a plastic container and covered with liquid nitrogen for approximately 3 min. Then the same method was adopted for pressing the microalgae samples with hydraulic press as described in Section 2.3.1.

2.3.3. Cell disruption of dried microalgae biomass under variable temperature

The disruption treatment was applied to the dried microalgae samples under variable temperature using the hydraulic press. Variable temperatures of 20, 40, 60, 80 and 100 °C were applied at two pressure levels of 10 and 50 bars (1 and 5 MPa respectively). Along with heating the pressure was applied for 1 min for each sample. All the samples were treated in an identical fashion. Cell disruption treatment was repeated three times for each sample. In each case the same weight of 0.1 g of microalgal samples was used. The temperature was stabilized over a 15 min period to ensure that the temperature of the surfaces was stable. Identical procedures were used for all of the samples.

2.3.4. Cell disruption of liquid nitrogen treated microalgae under variable temperature

The dried samples were exposed to liquid nitrogen pre-treatment for 3 min and then were pressed under variable temperatures of 20, 40, 60, 80 and 100 °C at two pressure levels of 10 and 50 bars (1 and 5 MPa respectively). The same protocol of pressing the samples was followed as described in Section 2.3.3.

2.3.5. Quantifying cell disruption

The microscope (Orthoplan, Ernst Leitz Wetzlar GmbH, Germany) with 25× objective lens was used for the analysis. In order to examine the dry microalgae samples under the microscope, the sample was diluted with distilled water to avoid contamination and transformed into a liquid form. A weight of 0.01 g of dry microalgal sample was measured by the balance and mixed with 1000 μL of distilled water using a pipette (Finn pipette, Thermo-Scientific, UK). A volume of 30 μL was pipetted and placed on the microscopic slide (26 × 76 mm, Delta Lab, Spain) and covered with a 16 mm diameter round glass cover slip (Chance Propper Ltd., England). 10 images were captured for each sample at three different locations (n = 30) to increase the precision of the average disruption of cells. The results of the cell disruption analysis are represented as the mean ± standard error of the mean (SEM). Microscopic analysis was conducted before and after the treatment for each sample.

The cell disruption induced with the hydraulic press treatments was evaluated by determining the percentage disruption and the number of intact cells microscopically, before and after each treatment. The centrifugation of algal culture to collect biomass and drying of the wet algae paste also have an effect on the cell disruption. Consequently, the initial disruption was calculated from [12],

$$C_0\% = \left(\frac{C_0}{C_0 + I_0}\right) \times 100 \quad (1)$$

Where C_0 is the number of disrupted cells for the control and I_0 is the number of initial cells. After treatment the efficacy of the treatment was