MANUAL PRESSING OF NANNOCHLOROPSIS OCULATA DRIED BIOMASS FOR ENHANCED LIPID EXTRACTION

Mehmood Ali[1], Ian Watson[2]

ABSTRACT

Microalgae offer significant potential to produce high value products and biofuels, whilst simultaneously being used to bio-remediate water or capture carbon dioxide (CO_2). Microalgal cell disruption processes are often necessary to increase lipid extraction from microalgae before conventional solvent extraction processes are used to isolate lipids. The extracted lipids can be processed to produce biofuels. The combinations of hydraulic pressing with liquid nitrogen (LN_2) treatment were applied to samples of dried Nannochloropsis oculata in the presented study to enhance the cellular destruction and lipid yields. The results indicated higher lipid extraction with LN_2 treatment (0.159 g/g dry algae) compared to the LN_2 untreated samples (0.070 g/g dry algae). The corresponding cell disruptions were found to be seventy-eight and fifty percent, respectively, at the same 10 bar (145 psi) pressure level. The control sample (without any treatment) lipid yield was 0.006 g/g dry algae, while the lipid yield varied between 0.192-0.213 g/g dry algae with LN_2 treated biomass with pressure loadings of 70-100 bar (1015-1450 psi) and with a corresponding cell disruption of 93-98 percent. The presence of palmitate, oleate and linoleate found in the fatty acid methyl ester composition of the extracted lipids, shows a favourable profile to produce biodiesel.

Keywords: hydraulic pressing; biodiesel; lipid extraction; microalgae; liquid nitrogen; dried biomass; fossil fuels.

1. INTRODUCTION

Carbon neutral energy resources such as biofuel from microalgae [1] ostensibly appear as a viable alternative fuel source [2] for abatement of atmospheric environmental degradation due to carbon emissions with anthropogenic consumption of fossil fuels. Microalgae have higher biomass productivity (7-10 times [3]) as compared to vegetable seed crops and are identified as promising feed stock to produce carbon neutral biodiesel [4]. Microalgae oil has the potential to enable large scale biodiesel production without competing for arable land or biodiversity and natural landscapes [5]. Oil extraction is an important unit operation in biodiesel production and the most common techniques for oil extraction are mechanical expeller presses, organic solvent extraction and supercritical fluid extraction methods. Mechanical pressing extracts 70-75 percent algal oil by weight, while the addition of organic solvent increases the oil extraction yield to ninety-five percent from the microalgal biomass [6].

[1] Assistant Professor, Department of Environmental Engineering, NED University of Engineering and Technology, Pakistan, Ph. +92-(0)21-99261261x2366, Fax: +92(0)21-99261255, Email: mehmood@neduet.edu.pk.

[2] Professor, System, Power and Energy Research Division, School of Engineering, University of Glasgow, UK, Ph. +44-(0) 141-3305258, Fax: +44(0) 141-3302359, Email: ian.watson@glasgow.ac.uk.

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Mehmood Ali is an Associate Professor in the Department of Environmental Engineering at NED University of Engineering and Technology, Pakistan where he received his Bachelors and Masters in 1995 and 2002, respectively. He received his PhD in Mechanical Engineering from University of Glasgow, UK in 2016. His research interest includes biofuel production from vegetable oil seeds and microalgae, with fuel characterisation and emission testing/engine performance.

Ian Watson is a Reader in Applied Energy at University of Glasgow, UK where he received his PhD in 1993 in laser engineering. He is investigating and developing technologies to remove microalgae processing bottlenecks from scaling photobioreactors for efficient growth to dewatering strategies and efficient extraction processes. He is developing real time control of gasification systems to increase performance with feedstock variance and (in particular) developing robust tar detection systems.

Prior to microalgae lipid extraction, several possible cell disruption pre-treatment methods can be employed to facilitate the extraction of intracellular material to enhance lipid yields with solvent extraction [7]. Cell disruption enhances the solvent's access to the stored lipids [8]. The cell wall toughness and impermeability to the solvent provides a barrier for lipid extraction. Mechanical and non-mechanical cell disruption methods are often used as an effective tool to enhance lipid extraction. Mechanical methods involve utilising compressive forces (with an expeller or a press) to break the cell wall due to shearing action [9]. Consequently, mechanical techniques are often preferred over non-mechanical ones for food derived products because they reduce contamination with organic solvents, here the de-lipid cake can safely be used as a fodder for animals or in cosmetic industries. Mechanical pressing helps to lower the operating costs through improved scale-up strategies [10]. The mechanical press generally requires dried algal biomass [11] and it entails the exertion of a compressive forces, which helps disrupt the algae cell walls [12] prior to oil extraction with solvents to enhance the extraction of the intracellular material [13, 14]. Microalgae are a potential feedstock to produce third generation biodiesel fuel but substantially more research is needed to improve its oil extraction yields [15], reduce operating costs and provide a commercial framework for production of such a relatively low value commodity. The production of biodiesel fuel from microalgae involves extracting the algal oils from the biomass as a major sub process that should be economically viable [16] and advantageous. The oil extraction energy input requirements were considered as a key factor in making biodiesel [17] and improvement in oil extraction techniques will have a direct impact on the sustainability of biodiesel production. This is mainly because oil extraction is a large part of the energy consumption of biodiesel production, which can jeopardize the overall energy balance of the processing system [18]. The combination of mechanical shearing action with conventional organic solvent extraction method is one of the best possible options to achieve higher algal oil yields with hydraulic pressing [11].

The present investigation was conducted to evaluate the performance of physical pre-treatment of hydraulic pressing applied to extract lipids from dried biomass of nannochloropsis oculata, for biofuels production. The extraction of the microalgal lipids were conducted with hydraulic pressing (10-100 bar (145-1450 psi)) both with and without liquid nitrogen (LN_2) pre-treated samples with a conventional organic solvent method. This builds on earlier work that investigated the lysis capability of liquid nitrogen, thermal treatment and high pressure [12].

2. MATERIALS AND METHODS

2.1 Cultivation and Harvesting

Nannochloropsis oculata was procured from a commercial source in the United Kingdom (UK) and cultivated in the School of Engineering at University of Glasgow, UK in a rectangular photo bioreactor (PBR) tank (200 ltr (53 gal)) in a controlled environment. The temperature of the tank was maintained at 25°C (77°F) by means of a 25 W aquatic heater. The microalgae culture was supplied nutrients at ratio of 1 ml (0.035 oz) nutrients per 1 ltr (0.26 gal) of culture water. As microalgae are photosynthetic organisms, an artificial light source (a sodium lamp 250W) was used with 42 µmol m^2/s (3444 lux), as measured with a light meter. In addition, the tank was supplied with air using an air pump and a salinity of 30 parts per trillion (ppt) was maintained with sea salt. Microalgae biomass was harvested with a centrifuge after 25 days of cultivation with a relative centrifugal force (RCF) of 2049G at a rotational speed of 4500 revolutions per minute (rpm). The microalgae culture was introduced into the centrifuge impeller with of a pump operating at a flow rate of 90 ltr/h (23.78 gal/h). After harvesting from the centrifuge, microalgae paste was spread over a rectangular metallic container and placed inside an incubator at 80°C (176°F) for 12 hrs for complete drying. The moisture content of the algal biomass was measured via the difference in its initial and final weights after drying as measured with an analytical balance. The dried microalgae layer was scraped off and the crisps were collected and crushed with a mallet for the experiments. The dried algal crisps had moisture content of 10.2 percent (w/w on dry basis). The dried algal crisps were found to have higher moisture content even after drying because original the moisture content of the wet algal paste collected was 76.77 percent.

2.2 Preparation of Samples for Lipid Extraction

Dried crushed samples of microalgae were used for lipid extraction without LN_2 treatment. For the liquid nitrogen (LN_2) pre-treated samples, the dried algal biomass was placed into a plastic container (123.15 cm^3 (7.5 in^3)) and fully covered with liquid nitrogen and allowed to evaporate before the pressing stage was conducted. It took approximately 3 min for the liquid nitrogen to evaporate completely.

2.3 Lipid Extraction

The lipid extraction from the samples with and without LN_2 treatment was conducted with hydraulic pressing to rupture the cells. Ethanol was subsequently used as a solvent for lipid extraction. Each sample of dry microalgae (1g (0.035 oz)) was weighed using an analytical balance and wrapped in aluminium foil 10×10 cm (4×4 in.) in size. It was later placed between the two plates of a manually operated hydraulic press to varying pressures (10-100 bar (145-1450 psi)) with increments of 10 bar (145 psi) for 1 min treatment duration. Every sample was placed at a specific location to provide consistent results. The dimensions of the hydraulic press plates were 30×30×4 cm (11×11×1.6 in.). The rate of applied pressure was measured as 0.58 and 2.21 MPa/s (84.12 and 320.53 psi/s) at 10 and 100 bar (145 and 1450 psi), respectively [12]. After each treatment, the sample was unfolded from the aluminium foil and was transferred to a beaker (100 mL (3.5 oz)), and analytical grade ethanol (as a solvent) was added in the ratio of 1:5 g/ml (8.34: 41.72 lb/gal) [19]. The sample was mixed with a magnetic stirrer for 1 hr. The sample was transferred to a centrifuge tube (50 mL (1.76 oz)) and was centrifuged at 3000 rpm (1449G) for 3 min. The supernatant layer obtained after centrifuging was transferred to another centrifuge tube and 1 mL (0.035 oz) of ethanol was added to remove any remaining lipids present in the algal cake and it was centrifuged again at 3000 rpm (1449G) for 1 min. The lipid solvent mixture was transferred to a pre-weighed beaker and the solvent was dried in an incubator at 80°C (176°F) (boiling point of ethanol) for 3 hrs and the weight of the extracted lipids were noted. The controlled sample was also treated in the same fashion for lipid extraction. For further comparison, the overall lipid content present in the microalgae sample was extracted using the conventional Bligh and Dyer method [20].

2.4 Percentage Cell Disruption

The cell disruption by hydraulic pressing with varying pressures was evaluated by determining the percentage cell disruption observed microscopically for each sample. Thirty images were captured at three different locations before and after each treatment and the number of intact cells were counted. The same protocol was followed as previously presented by McMillan et al. [7] and the results of this study were presented by Abbassi et al. [12].

2.5 Extracted Lipids Fatty Acids Analysis

The extracted lipid fatty acids methyl ester (FAME) composition from the samples with and without LN_2 treatment were measured in the School of Chemistry at University of Glasgow, UK using GC-FID equipped with a flame ionisation detector. A sample weighing 100 mg (0.0035 oz) was placed into a 20 mL (0.70 oz) reaction vial (with screw cap) and mixed with 5 mL (0.18 oz) hexane. 100 µltr (22 µgal) of 2 M potassium hydroxide in methanol was added (11.2 g in 100 ml (22 µgal)). The vial was sealed and shaken for 2 min and then centrifuged. The clear supernatant liquor (top layer) was collected and introduced into the auto-sampler for GC-analysis. The sample injection volume of 2 µltr (0.4 µgal) was introduced into a column at 200°C (392°F) while the detector temperature was maintained at 240°C (464°F). The GC machine column was 15 m (49 ft) high with capillary [P1]
tube internal diameter of 0.53 mm (0.02 in.). The GC machine was programmed to 60°C (140°F) for 2 min. The temperature was increased to 220°C/min (428°F) with a holding time of 10 min.

3. RESULTS AND DISCUSSIONS

3.1 Comparison of Lipid Extraction

The applied pressure loading on the microalgal biomass and its corresponding extracted lipid content are shown in **Figure 1**. The lipid extraction from the control sample with organic solvents (without any pressing) was found to be 0.006 g/g dry algae with five percent initial cell disruption [12], due to centrifugation for harvesting and drying in an incubator. However, the overall lipid present in the microalgae extracted by the Bligh and Dyer method [20] came out to be 0.168 g/g dry algae. The sample without LN_2 treatment at 10 bar (145 psi) loading pressure showed an increase in lipid extraction yield to 0.070 g/g of dry algae with fifty-one percent cell disruption [12]. In the present case, the hydraulic pressing helps in rupturing the microalgal cell wall and facilitates the organic

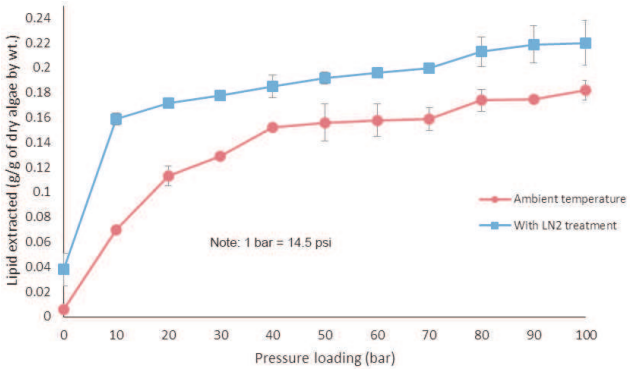

Figure 1. Lipid extracted versus applied pressure with LN_2 treatment and at ambient temperature. The lipid yield is presented as mean ± standard error (number of sample = 3).

solvent migration deep into the lipid containing algal cellular matrix [21], which resulted in higher lipid extraction. This is basically due to the uniaxial compression developed between the two plates of the hydraulic press, creating a mechanical solid shearing action on the microalgae biomass. The microalgae cells are almost perfectly symmetrically spherical in shape and filled with an incompressible fluid. The pressure loading on the cells is axially perpendicular to the hydraulic press plates. The rupturing of cells depends on its uniaxial compressive strength (which is the strength of a material to withstand axially directed forces); its fracture behaviour depends on its stress-strain curves [22] and the presence of water content which hinders the development of adequate shear to promote cell wall rupture [21]. The present experimental work showed that the low moisture content of the dry microalgae biomass (ten percent (w/w on dry basis)) helped in creating sufficient shear forces to rupture the cells, enabling higher extraction of lipids. Due to the applied pressure loading on the cell walls, the volume of the spherical cells increases with a corresponding increase in surface area. This stretches its cell membrane and increases its internal pressure. The cell wall ruptures as its membrane is stretched due to the tensile forces developed due to pressing which causes deformation in the surface of the cell wall.

The modulus of elasticity (E) for cell walls was generally high enough so that substantial deformation before bursting is unlikely. The cell wall rupture depends on the breaking pressure, cell radius and its wall thickness. In the present case, the spherical cells were under equatorial wall stress (δE) developed which is opposite to the applied pressure over the area of an equatorial plane through the sphere. Mathematically, it can be expressed by Eq. (1) [23]

$$\delta = P\, r/2\, t \tag{1}$$

where δ is the equatorial wall stress; r is the radius of the cell; P is the hydrostatic pressure; and t is the thickness of the cell wall

The internal turgor pressure in the cell resists the bursting of the cell wall with applied compressive breaking pressure. The applied pressure above turgor pressure breaks the cell wall and the intracellular material spills out. This was experienced in the present case where the lipid extraction from the samples without LN_2 treatment showed an increasing linear trend ranging 0.070-0.182 g/g dry algae with a change in pressure loading from 10-100 bar (145-1450 psi) pressure, with a corresponding increase in cell disruption between 51-98 percent [12].

The LN_2 treated samples showed a sharp increase in the lipid extraction compared to those pressed without LN_2. The controlled LN_2 treated sample without any pressing showed 0.038 g/g dry algae with forty-two percent cell disruption, while at 10 bar (145 psi) pressure it leaped to 0.159 g/g of dry algae with seventy-eight percent cell disruption. This is due to the sudden change in the temperature of the biomass with LN_2 treatment. An extremely cold fluid (LN_2 boiling point is -196°C (-321°F)) would instantly vaporise the raw material after treatment which causes deformation of the cell walls. Moreover, it could also be hypothesised that such sudden cooling down of the material results in enormous compressive forces applied on the cell walls. This leads to cracks on the wall surface and overall weakening/deterioration of the wall structure which could partly explain the reason for a more efficient lipid extraction. The evaporation of LN_2 at atmospheric pressure took approximately 3 min for each sample. The microalgae biomass during its decompression (thawing) resulted in cracks in the cell walls of all undamaged cells [23]. Between 70-100 bar (1015-1450 psi) pressure and with LN_2 treatment, there was a consistent pattern of lipid extraction with minimum and maximum values ranging 0.192-0.213 g/g of dry algae and the cell disruption was found to be between 93-98 percent. LN_2 treatment has been found to be an effective method of causing cell disruption with no effect on the downstream lipid extraction [24]. The low temperature LN_2 treatment also prevented lipids oxidation, thus improving its fatty acid composition.

It is worth mentioning here that higher cell disruption was achieved with LN_2 treatment and pressing biomass (> ninety percent cell disruption) while similar lipid extraction yields were found at 70-100 bar (1015-1450 psi). It is concluded that 70 bar (1015 psi) pressure is sufficient to extract the maximum yield from the biomass with LN_2 treatment which saves the energy consumption of the hydraulic press.

Figure 2 shows the relationship between the ratio of extracted lipid yield and percent cell disruption with respect to pressure loading. It is seen in **Figure 2** that the lipid yield was approximately proportional to the cell disruption in both cases (with and without LN_2 treatment) with respect to the applied pressure. Nevertheless, the ratio of lipid yield and percent cell disruption with LN_2 treatment was higher as compared to the case of no LN_2 treatment (ambient temperature sample). This was due to the reason that LN_2 penetrates into the microalgae biomass, freezes the moisture and (during thawing at room temperature) cracks the cell walls which facilitates lipid extraction. Overall, the lipid yield and percent cell disruption ratios were found to have a positive trend with applied pressure (**Figure 2**); a sharp increase was noted in **Figure 2** for both samples from the 0-20 bar (0-290 psi) before the values raised less steeply for both conditions.

3.2 Fatty Acid Methyl Esters Analysis

The extracted lipids from the untreated and the LN_2 treated samples showed similar FAME compositions as can be seen in **Table 1**. The fatty acids with carbon chain (C16-C18) displayed a favourable fatty acid profile for biodiesel [24]. In addition, the lipids with a high content of unsaturated fatty acids (such as C18:1 with less than four double bonds) is highly desirable for biodiesel production with its favourable cold flow properties during the winter [3]. The FAME composition of the extracted lipids showed a higher quantity of palmitate (C16:0), oleate (C18:1) and linoleate (C18:2) for both untreated and the LN_2 treated pressed samples compared to the control sample. In particular, the oils with higher quantities of these fatty acids have been reported to have a reasonable balance of fuel properties. The properties of biodiesel [25] such as ignition quality, combustion heat, cold filter plugging point (CFPP), oxidative stability, viscosity and lubricity are determined by the structure of its component fatty esters. The presence of higher oleate content increases the oxidative stability, allowing longer storage and decreasing CFPP for use in cold regions [26]. Overall, the properties of extracted lipids were found to be suitable for biodiesel production.

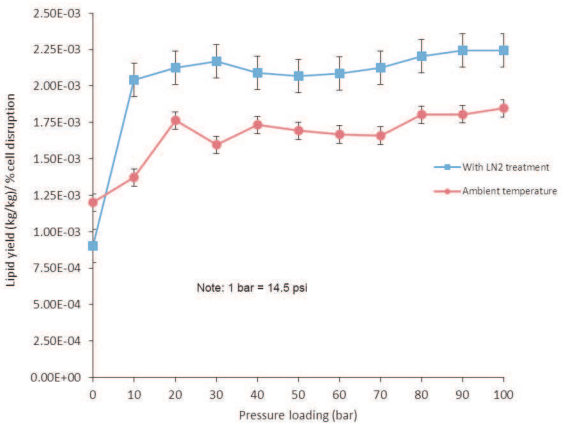

Figure 2. Lipid extracted yield/percent cell disruption versus pressure loading with LN_2 treatment at ambient temperature. The lipid yield is presented as mean ± standard error (number of sample = 3).

Table 1. Fatty acid methly ester composition

FAME	Without any treatment (FAME mg/100 mg (0.004 oz) sample)	LN_2 treated (FAME mg/100 mg (0.004 oz) sample)
Palmitate (C16:0)	9.61 ± 0.63	9.78 ± 0.07
Palmitoleate (C16:1)	0.71 ± 0.04	1.03 ± 0.02
Stearate (C18:0)	0.37 ± 0.01	0.58 ± 0.01
Oleate (C18:1)	9.33 ± 0.12	9.87 ± 0.24
Linoleate (C18:2)	14.23 ± 0.23	14.95 ± 0.34

Note: results are presented as mean ± standard error of the mean; number of sample = 3

4. CONCLUSION

Lipid extraction was conducted on dry microalgae biomass samples both with and without LN_2 treatment. The effect of the percent cell disruption on the lipid yield was investigated by using hydraulic pressing with a conventional solvent extraction method. Additionally the samples were subjected to pressure loading using hydraulic pressing. The lipids extracted from the dry microalgae biomass with LN_2 pre-treatment showed a higher lipid yield with low pressure loadings as compared to the sample without LN_2; this correlates with the cell disruption efficiency. The highest extracted lipid without LN_2 treated sample came out to be 0.182 g/g dry algae at 100 bar (1450 psi) pressure loading and a corresponding ninety-eight percent cell disruption. The lipid yields with LN_2 treatment was found to be between 0.192-0.213 g/g dry algae from 70-100 bar (1450 psi) with 93-98 percent cell disruption. The FAME composition showed the presence of palmitate, oleate and linoleate in greater amounts in both samples compared to the control one, suggesting favourable properties for biodiesel production.

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