Energy Technology
Generation, Conversion, Storage, Distribution

Accepted Article

Title: Mild pyrolysis of manually pressed and liquid nitrogen treated de-lipid cake of microalgae for bioenergy utilisation

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To be cited as: *Energy Technol.* 10.1002/ente.201700805

Link to VoR: http://dx.doi.org/10.1002/ente.201700805

www.entechnol.de

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FULL PAPER WILEY-VCH

Mild pyrolysis of manually pressed and liquid nitrogen treated de-lipid cake of *Nannochloropsis oculata* for bioenergy utilisation

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Abstract: Due to the damaging impacts of continued use of fossil fuels, there is global interest in developing sustainable biofuel production to reduce society's dependency on carbon based energy resources. Microalgae cultivation can contribute to CO_2 fixation from the atmosphere, while simultaneously producing a source of lipids from the biomass for third generation biodiesel fuel production. The residual de-lipid cake left after lipid extraction can be treated with thermochemical techniques (such as mild pyrolysis) to produce solid biochar as an end product with a higher energy density and lower moisture content offering advantages for downstream processing or carbon sequestration. De-lipid cake was produced by solvent extraction from *Nannochloropsis oculata* that had been manually pressed and/or treated with liquid nitrogen (LN_2). The de-lipid cake was thermally treated at 200 or 300°C under partial vacuum in an oxygen free atmosphere. The solid biochar produced had a reduced moisture content (MC) resulting in a mass reduction of 25 and 66 wt. % of de-lipid cake without LN_2 and treatment at 200 and 300 °C respectively, while with LN_2 treated cake the mass reduction was 23 and 67 wt.% at 200 and 300 °C. The higher heating value of the control sample (without any manual pressing or LN_2 treatment) was 23.35 MJ kg^{-1}, while for the control sample it was enhanced to 26.82 and 30.56 MJ/kg with treatment at 200 and 300 °C respectively. With LN_2 treated samples with pressing the HHV was 21.98 MJ kg^{-1} for control sample as compared to 25.90 and 28.72 MJ kg^{-1} at 200 and 300 °C respectively, where the lower values were observed because of the lipid removal. The measured gas pressure developed, likely due to the production of CO_2 and CH_4 as major gases, was 0.19 and 0.53 bars without LN_2 treatment samples, while it was 0.13 and 0.58 bars with LN_2. The torrefaction process (mild pyrolysis) energy analysis showed that the ER (energy ratio) without LN_2 treatment sample with 0.485 at 200 °C was the highest and the lowest 0.407 energy ratio was found with LN_2 treated sample at the higher treatment temperature (300 °C).

Introduction

With the urgency to find environmentally friendly, reduced carbon fuels, microalgae have considerable benefits, allowing immediate benefits for carbon sequestration, water treatment and removal of nutrients and even production of high value products. Attempts to harness biofuels from microalgae however have been limited by several commercial bottlenecks and potential yields at scale have not been realised. Potential lipid (fats) yields are between 30-50% by dry weight [1] with favourable properties for conversion to biodiesel [2]. It has been estimated that with higher lipid productivity yields per unit area, roughly 20,000-80,000 L $acre^{-1}year^{-1}$ could be produced, which is 7-31 times higher than from conventional vegetable seed crops [3]. The transportation sector accounts for 21% of the CO_2 emissions from fossil fuels, causing climate change and global warming [4]. Whilst there is some likely uptake in electrical vehicles there remain serious problems in decarbonizing the aviation and shipping industries, primarily because of underdeveloped battery technology. Microalgae absorb CO_2 from the atmosphere through photosynthesis; thus helping in CO_2 sequestration, whilst not displacing land for cultivation of food crops [5]. There is growing recognition that microalgae are among the most productive biological systems for capturing carbon from the atmosphere [6] and generating biomass as a source of sustainable biofuel production [7] for ships, airplanes and high value products e.g. pharmaceutical and nutraceutical [8].

Once, the microalgae have been grown, they can be extracted from the water and then processed to separate the lipids, which can subsequently be converted into biodiesel (methyl ester) by transesterification (chemical reaction with methyl or ethyl alcohol and with a base or acid catalyst) [9]. The residual, de-lipid cake has potential value e.g. the cake may contain nutrients and proteins and can be used as a feed or it can be used directly as a source of fuel but its higher heating value is relatively low 3000 kcal kg^{-1} (12.55 MJ kg^{-1}) as compared to the dried microalgae biomass i.e. 3500 kcal kg^{1} (14.64 MJ kg^{-1}) [10]. Previous studies have investigated thermochemical treatment of the de-lipid cake i.e. the residue remaining following extraction of the lipids from the microalgae. The de-lipid cake can account for ~ 75 % of the overall biomass, which for large scale cultivation represents a significant additional revenue stream. Torrefaction is a thermochemical treatment which can be applied to biomass, after which there is a subsequent increase in HHV, reduction in volume, moisture content and volatiles, which makes the fuel more useful for direct gasification or combustion for power generation applications [11]. Advantages of utilising torrefied feedstock include reduced emissions, in part because of the reduced volatiles, and reduced transportation costs of the torrefied feedstock due to the reduced volume because of the reduced moisture content, and the corresponding increase in HHV. Biomass, including microalgal sources, is one of the renewable sources of carbon that can be effectively converted into solid, liquid and gaseous biofuels through different thermochemical conversion technologies e.g. *via* pyrolysis, hydrothermal liquefaction, gasification, torrefaction, and direct combustion for heat and power production. Various studies have investigated slow or mild pyrolysis of a microalgal biomass yielding biochar, CH_4 and CO_2 gaseous products under operating conditions in the temperature range of 450–750°C, with a heating rate 50°Cs^{-1} at atmospheric pressure under reduced oxygen environments [12, 13]. Torrefaction treatment occurs over a relatively low temperature range (200-300 °C), and of course, in the absence of oxygen [12]; the process aims to increase the low energy density of biomass (by approximately 17-20%) by decomposing its reactive hemicellulose fraction [14]. At lower temperatures the torrefaction partially decomposes the biomass, releasing its moisture, low heat value volatile compounds and some non-condensable gases [15]. A typical biomass torrefaction process has main five thermal stages which can be described as follows [16]:

- Initial heating: the biomass is heated until the drying stage of the biomass is reached where moisture evaporation occurs with increasing temperature.

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- Pre-drying: starts at 100°C, where free water is evaporated from the biomass.
- Post-drying and intermediate heating: is the stage where the temperature of the biomass is increased to 200°C. Physically bound water is removed. During this stage some mass loss may occur as light fractions of hydrocarbons are also readily evaporated.
- Torrefaction: during this stage the actual process of torrefaction occurs, starting from about 200°C. Usually the temperature is increased to a higher value, generally not greater than 300 °C, and the material is held at this value for a fixed, residency time, and then allowed to cool.
- Cooling: the torrefaction process ends when the sample has cooled to below 200°C, eventually reaching ambient temperature.

The torrefaction temperature is defined as the maximum constant temperature reached during the torrefaction process. During this period most of the mass loss of the biomass occurs. The treatment efficacy is a function of temperature, residence time, heating rates and pressure and the optimal value depends on the desired outcome (i.e increasing HHV, moisture reduction) and will be heavily dependent on the biomass type and its properties. Of courses the torrifier chamber, rate of heating, heat losses and heat uniformity in the system may impact the system efficiency. Large scale torrefiers may need physical mixing to reduce temperature gradients in the system and provide a more uniform product. Previous studies showed that an increase in the torrefaction temperature and residence time of *Spirulina platensis* increased the net carbon content by evaporating the lower molecular weight hydrocarbons [17] and fixed carbon concentration, as well as the higher heating value (HHV) and hard grove grindability index (HGI) after torrefaction. The higher heating value (HHV) of a microalgae torrefied biomass at 300 °C for 30 min increased from 20.46 to 25.92 MJ kg^{-1}, and the upgraded biomass was consequently more suitable for partial replacement of coal employed in industrial power generation. In other words, the biochar is the torrefied end product, which is brittle in nature with a lower water content, higher heating value, due to its relatively higher carbon content, and a higher energy density, compared to the original feedstock [17]. Biochar is the porous, carbonaceous material produced by thermochemical treatment of organic materials in an oxygen-limited environment. In general, most biochar can be considered resistant to chemical and biological decomposition and therefore suitable for carbon (C) sequestration [18]. The biochar end product has a lower volume and reduced bulk density and it is hydrophobic in nature, thus it is more stable, offering longer storage periods whilst occupying less volume [19]. Another study investigated the pyrolysis behaviour of marine microalgae biomass, *Nannochloropsis gaditana*, at three different temperatures (400, 500, 600 °C). Experiments were conducted in the presence of N_2 with a flow rate of 50 cm^3/h using a 1-kg fixed-bed reactor. The effects of pyrolysis conditions such as temperature on product yields were studied [20].

The present investigation was initiated to fill the research gap between the performance of mild pyrolysis (low temperature up to 300°C) of the residual de-lipid cake of *Nannochloropsis oculata* after lipid extraction, to convert it into a more energy dense solid fuel for power generation and to conclude previous studies where the impact of liquid nitrogen and high pressure pre-treatment on lipid extraction was studied [21]; this work assessed the residual delipid cake. Previous studies from other authors on *Nannochloropsis oculata* only investigated high temperature pyrolysis and using different methods of cell lysis and lipid extraction. In the present work the cake was torrified or treated at low temperature in an oxygen free environment (mild pyrolysis to examine the impact of the treatment on its HHV. The extraction of the microalgal lipids were conducted with hydraulic pressing (10-100 bar) both with and without liquid nitrogen (LN_2) pre-treatment following conventional organic solvent extraction methods by Ali and Watson [21]. This builds on the authors' earlier work that investigated microalgal lysis with liquid nitrogen, thermal treatment and high pressure [1]. The properties of the residual de-lipid cake before and after torrefaction were investigated and compared. The *Energy Ratios* (ER) i.e. energy output over energy input for mild pyrolysis of the de-lipid cake at two different temperature conditions (200 ± 5 and 300 ± 5 °C) for a treatment time of 30 min were calculated to estimate the efficiency of the system and likely scalability.

Results and Discussion

Torrefaction of microalgal biomass

The torrefaction of the residual cake after lipid extraction was conducted in a non-oxidizing atmosphere (vacuum environment), in order to avoid oxidation and ignition [15]. The experimental results are depicted in **Table 1**, which shows that a mass reduction was observed in each case of pyrolysis with treatment temperatures of 200 and 300 °C, and both with and without LN_2 pre-treatment. The mass reduction was greater with the higher temperature treatment, in accordance with previous researchers [15, 17]; however, there was no observable difference found in the weight loss due to the LN_2 treatment (0.67 ± 0.01) g and without LN_2 treatment (0.66 ± 0.02) g . careful that was one of my comments! The weight loss of the biomass with respect to torrefaction temperature occurs due to the thermal degradation of its hemicellulose content [26]. A layer of condensation was formed on the inner surface of the pyrex tube during experimentation, indicating the formation of condensable products (liquid phase) which includes water (due to dehydration and reactions between the organic molecules) [15] and organic acids such as: acetic, lactic and formic. These organics are usually formed during torrefaction with devolatilization and carbonization of the hemicellulose fraction present in the biomass. Due to the production of gases, an increase in pressure was noticed after 10-20 min of treatment, after which equilibrium was established. It is assumed that the non-condensable products (i.e. gaseous phase) developed in the pyrex tube includes the: CO_2, CO and traces of CH_4 and H_2. The degradation of hemicellulose present in microalgae biomass with higher carboxyl content (-COOH) accounted for CO_2 production [28] and the presence of cellulose results in a higher CO yield, this is mainly attributed to the thermal cracking of carbonyl (C=O) and carboxyl (-COOH). In general, microalgae biomass does not contain lignin, which is required for structural support in larger plants but some exceptions exists e.g. in *Calliarthron cheilosporioide* lignin is found and also in some sea weeds, compounds resembling

lignin are found [27]. The cracking and deformation of lignin in plants is responsible for the release of H_2 and CH_4 in the reaction, which is difficult to decompose due to the presence of aromatic rings and methoxyl (-O-CH_3) groups [28] but in the present study, lignin free microalgae biomass offers advantages for downstream processing . Furthermore, the production of biohydrogen (H_2) is mainly dependent on the decomposition of polysaccharides, which are the polymers of carbohydrates molecules [29]. The pressure developed due to the liberation of gases recorded at 300 °C was greater than compared to 200 °C treatment with the experimental conditions as shown in **Table 1.** This is due to the higher amount of gases evolved due to the degradation of biomass at the higher temperature.

Table 1. The experimental results of the control and torrefaction of the residual de-lipid cake with and without LN_2 extraction (results presented as mean ± standard error of the mean, sample size n=3).

Sample type	Treatment temperature [°C]	Treatment time [min]	Mass reduction[g]	Bulk density [g cm^{-3}] (on dry basis)	HHV [MJ kg^{-1}]	Gas pressure [bar]
Control (No LN_2)	-	-	-	0.54 ± 0.07	23.35 ± 0.63	-
Pyrolysis without LN_2	200	30	0.25 ± 0.01	0.47 ± 0.01	26.82 ± 0.49	0.19 ± 0.04
Pyrolysis without LN2	300	30	0.66 ± 0.02	0.35 ± 0.01	30.56 ± 0.94	0.53 ± 0.04
Control with LN_2	-	-	-	0.44 ± 0.01	21.98 ±0.25	-
Pyrolysis with LN_2	200	30	0.23 ± 0.04	0.41 ± 0.01	25.90 ± 0.31	0.13 ± 0.04
Pyrolysis with LN_2	300	30	0.67 ± 0.01	0.32 ± 0.01	28.72 ± 0.35	0.58 ± 0.01

The higher heating values (HHV) of the torrefied product both with and without LN_2 increased with increasing temperature as compared to the control sample (23.35 MJ kg^{-1}). Without LN_2 treatment the HHV values were 26.82 and 30.56 MJ/kg at 200 and 300 °C respectively. With LN_2 treatment, an increase in HHV between the control sample (21.98 MJ kg^{-1}) and LN_2 treated samples at 200 °C (25.90 MJ kg^{-1}) and 300 °C (28.72 MJ kg^{-1}) were observed. It is seen that the with LN_2 treatment, there is a slight decrease in the HHV for the same temperature treatment, which is indicative of a higher lipid removal from the samples with the LN_2 treatment due to the removal of hydrocarbons [21]. Overall, the results demonstrate a reduction in the oxygen content in the torrefied end product, showing the amount of C-O bonds were exhausted in the formation of CO_2 and CO. While the C-C bonds were higher in number, due to the removal of hydrogen and oxygen in the formation of water and low molecular weight volatile organics in the reaction [21]. The higher HHV is indicative of higher carbon content in the torrefied biomass. The HHV increases with increasing temperature [17] and of course makes the energy density higher as compared to the control sample.

The elemental analysis showed an increase in the carbon, hydrogen and nitrogen content after thermal treatment for both the LN_2 treated and untreated samples (see **Table** 2). This is due to the formation of water, which is the main product [28] generated by drying at low temperatures and by de-hydration reactions between organic molecules at high temperature [15, 17]. In addition, the fibrous component structure function is to maintain the position of the nucleus in the cell cycle. The structure of the biomass was destroyed because of decomposition of alkanes, aldehydes, ketones and carboxylic acid, alcohols and other macro molecules [27] and, furthermore, torrefaction improves the grindability properties of the torrefied biomass as a fuel feedstock e.g. combustion or gasification [30]. The torrefied biomass becomes hydrophobic in nature and does not absorb moisture, improving storage characteristics. The transportation cost for torrefied biomass is lower compared to untreated biomass because of its reduced volume and higher HHV.

Table 2. Elemental analysis of the residual de-lipid cake before and after torrefaction, results are depicted as an average of two results.

Sample type	C [% wt.]	H [% wt.]	N [% wt.]
Control without LN_2	42.31	6.35	3.78
200 °C without LN_2	47.97	6.76	4.31
300 °C without LN_2	54.82	6.58	4.86
Control with LN_2	46.92	7.14	4.21
200 °C with LN_2	50.42	7.00	4.44
300 °C with LN_2	58.24	6.82	5.18

Energy analysis of torrified biomass

The energy required to create the torrefied biomass from both LN_2 treated and untreated samples were calculated and the results can be seen in **Table 3**. By considering the ratio of the input energy and the available energy from the samples HHV, the ER values were determined. The highest value was 0.485 without LN_2 treatment at 200 °C which reduced to 0.435 at 300 °C. The ERs with LN_2 treatment were 0.469 and 0.407 at 200 and 300 °C respectively. It can be concluded that the torrefaction of de-lipid algal residual cake with and without LN_2 at 200 °C was more energy efficient compared to the higher temperature treatment (300 °C). This is primarily due to the higher amount of energy required to achieve and maintain 300°C. The ER are low due to the processing energy cost and can be improved operating at scale by using solar thermal or waste heat for torrefaction, reducing these costs entirely. Clearly the LN_2 treated samples had a lower value with their corresponding sample without LN_2. This is because of the removal of the extra lipids with the LN_2 [21] see **Table 1**.

Table 3. Energy consumption analysis of torrefaction treatment

Sample type	Treatment temperature [°C]	Total input energy [MJkg^{-1}]	Total output energy [MJkg^{-1}]	Energy Ratio (ER)
Without LN_2	200	55.20	26.82	0.485
With LN_2	200	55.20	25.90	0.469
Without LN_2	300	70.55	30.56	0.435
With LN_2	300	70.55	28.72	0.407

Microscopic investigation of torrefied biomass

Fig. 1 shows that the microscopic investigation for different samples. The control sample with no heating (a) looked brighter in colour. The torrefied biomass without LN_2 at (b) 200 °C and (c) 300 °C were black in colour as compared to their control samples, primarily due to thermal degradation of hemicellulose and dehydration. The control sample (no heat) with LN_2 (d) turned dark in colour due to severe freezing effects. The torrefied end product with LN_2 at (e) 200 °C and (f) 300 °C were converted into a dark black colour, with the combined effects of liquid nitrogen and thermal treatment. Additionally, cracks were visible on the sample surfaces and they were highly reflective under the optical microscope, these were observed on other samples. A change in colour and surface morphology was observed from yellowish to light brown and then to black with respect to the increase in treatment temperature. The surface morphology showed cracks appearing on the surface due to shrinking effects with the removal of moisture and other low molecular weight compounds. This is attributed to the evaporation of water and reduction in carbon and hydrogen content from the biomass with respect to increase in temperature (from 200 to 300 °C for 30 min) [24].

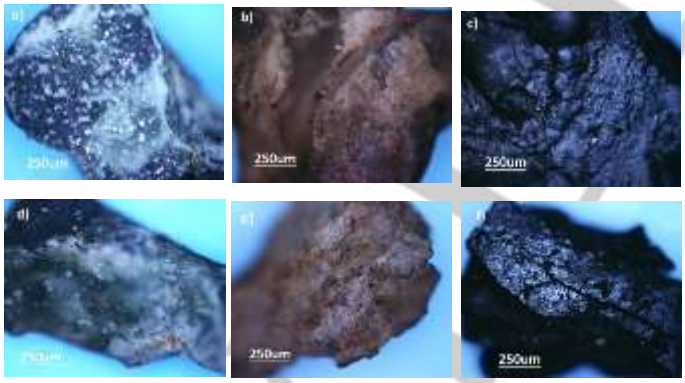

Figure 1. Torrefied biomass microscopic images (a) control sample without LN_2 treatment, (b) without LN_2 at 200 °C (c) without LN_2 at 300 °C, (d) control sample with LN_2 treatment, (e) with LN_2 at 200 °C and (f) With LN_2 at 300 °C.

Conclusions

Lipid extraction was conducted on dry microalgae biomass samples both with and without LN_2 treatment by subjecting the samples to pressure loading via hydraulic pressing. Mild pyrolysis of the residual de-lipid cake resulted in an increase in the HHV and a decrease in the bulk density at both treatment temperatures (200 and 300 °C) as compared to the control samples (without any treatment). The mass reduction in the biomass also increased with temperature due to the increased formation of gases and condensation of evaporated organics in the aqueous phase. The torrefaction of the residual cake causes the biomass to become more porous and shrink, which results in lower bulk density. The decrease in moisture content of the biomass resulted in mass reduction and an increase in the higher heating value of the torrefied samples. It was also observed that the gas pressure was higher at 300 °C as compared to treatment at 200 °C; this is likely due to the production of CO_2 and CH_4 in the pyrex tube (reactor). The torrefaction energy analysis showed that the ER was highest (0.485) with the lowest operating temperature (200°C) and lowest (0.407) with LN_2 treated sample at 300 °C. This was primarily due the extra energy requirements for heating to 300°C and maintaining this temperature. It should also be noted that extracted lipids increased with LN_2 treatment which reduced the HHV of the residual cake, which was the basis of this analysis. This work shows the potential energy cost implications of using LN_2 treatment for enhanced lipid extraction and its impact on the properties of the downstream residual cake both with and without torrefaction. It is seen that the low temperature torrefaction is preferable in terms of process energy feasibility unless waste heat or solar energy can be used for the torrefaction process. A higher treatment temperature is desirable to increase the HHV and reduce bulk volume.

Experimental Section

Cultivation and harvesting

Nannochloropsis oculata was procured from a commercial source (Reefphyto, UK) and cultivated in the School of Engineering, University of Glasgow in a rectangular photobioreactor (PBR) tank (200 L) in a controlled environment. The temperature of the tank was maintained at 25 °C by means of a 25 W aquatic heater (HT-825, JAD, China). The microalgae culture was supplied with nutrients (Reef phyto Guillard F/2) at ratio of 1 mL of nutrients per 1 L of culture. As microalgae are photosynthetic organisms, an artificial light source, a sodium lamp 250W (Sun Master, Venture lighting, UK), was used with 42 µmol m^2 s^{-1}, as measured with a light meter (LX-1108, Lutron, UK). In addition, the tank was aerated using an air pump (AC0308, HALCEA, UK) and a salinity of 30ppt was maintained with sea salt (Waitrose Ltd, UK). Microalgae biomass was harvested with a centrifuge (Extreme Raw Power Centrifuge WV0 Designs, USA) after 25 days of cultivation with a centrifugal force (RCF) of 2049 G at a rotational speed of 4500 rpm. The microalgae culture was introduced into the centrifuge impeller with of a pump (7524-05 Master Flex, Cole Parmer Inc., USA) operating at a flow rate of 90 L/h. After harvesting from the centrifuge, microalgae paste was spread over a rectangular metallic container and placed inside an incubator (PIN-120, Carbolite, UK) at 80°C for 12 hours for complete drying. The moisture content of the

algal biomass was calculated after measuring the difference in the sample's initial and final weights after drying, as measured with an analytical balance (AS120, Ohaus, USA). 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% (w/w on dry basis) which was considerably less than the wet algal paste which had a moisture content of 76.77%.

Lipid extraction from samples with and without LN_2 treated microalgae biomass using hydraulic press

Control lipid samples were extracted from the dried, crushed samples of microalgae without LN_2 treatment; while, for the liquid nitrogen (LN_2) pre-treated samples, the dried algal biomass was placed into a plastic container (123.15 cm^3) and fully covered with liquid nitrogen (BOC Industrial Gases, UK) and allowed to evaporate before the pressing stage was conducted. It took approximately 3 min for the liquid nitrogen to evaporate completely.

The lipid from the control and LN treated samples were extracted with hydraulic pressing to rupture the cells [21] followed by ethanol extraction. Each sample of dry microalgae (1g) was weighed using an analytical balance and wrapped in aluminium foil, 10 x 10 cm (Tesco, UK). The wrapped sample was then placed between two plates of a manually operated hydraulic press (S10316/95, Mackey Bowley International Ltd, England) to varying pressures (10-100 bar) with increments of 10 bar for 1 min treatment duration. This followed the authors previous protocols where the impact of the LN_2 and pressure on the lysing fraction was investigated [21]. Every sample was placed at the same specific location to provide consistent results. The dimensions of the hydraulic press plates were 30 x 30 x 4 cm. The rate of applied pressure was measured as 0.58 and 2.21 MPa s^{-1} at 10 and 100 bar respectively [1]. After each treatment, the sample was unfolded from the aluminium foil and was transferred to a beaker (100 mL) and analytical grade ethanol (Fisher Scientific, UK) was added as a solvent in the ratio of 1:5 g ml^{-1} [22]. The sample was mixed with a magnetic stirrer (CB-302 Stuart, Bibby Scientific Ltd, UK) for 1 hr. Then the sample was transferred to a centrifuge tube (50 mL) and was centrifuged (CFH-240-010A, Biofuge Primo, Fisher Scientific, UK) at 3000 rpm (1449G) for 3 min. The supernatant layer obtained after centrifuging was transferred to another centrifuge tube and 1 mL of ethanol was added to remove any remaining lipids present in the algal cake and it was centrifuged again at 3000 rpm (1449 G) 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 (boiling point of ethanol) for 3 hrs and the weight of the extracted lipids were noted. The control sample was treated in an identical 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 [23], and was found to be 0.168 g/g of dry algae. While the LN_2 treated and without LN_2 treated algal biomass sample, lipid yields were found to be 0.159 and 0.070 g g^{-1} of dry algae respectively [21].

Thermochemical treatment (torrefaction) of de-lipid residual cake of microalgae biomass

2g samples of the residual microalgal cake treated with and without LN_2 were subjected to thermal torrefaction treatment in a temperature controlled, batch, single zone tube furnace (MTF 12/25/250, Carbolite, England), in a non-oxidizing atmosphere, for a treatment time of 30 min. The samples were introduced in an aluminium rectangular container with a surface area 14.4 cm^2, which were fed inside the pyrex tube (20mm dia. and 770 mm length) and evacuated to 0.5 bar with a rotary peristaltic pump (501, Watson-Marlow Ltd, UK) using the same experimental setup for torrefaction of the de-oiled cake of flaxseed [25]. The samples were treated at two temperatures 200 ± 5 and 300 ± 5 °C and the rise in pressure was noted for the evolved gases (the gas composition was not analysed). After the treatment, the sample mass reduction (g), higher heating value (MJ kg^{-1}) and bulk density (g cm^{-3}) were measured. The bulk density (g cm^{-3}) was calculated with an analytical balance (AS120, Ohaus Corp., USA) and a pre-weighed plastic container with a $1cm^3$ capacity.

Energy analysis of torrefaction (mild pyrolysis) process

The energy analysis of torrefing the microalgal de-lipid cake for both cases (with and without LN_2 pre-treatment) was conducted by multiplying the power consumption of the equipment used with its operating time and volume fraction. The volume fraction was calculated by [25];

Volume fraction = Sample of volume/ Equipment volume used (1)

The power consumption of the rotary peristaltic pump (44W) was measured with a power plug-in meter (2000MU, Maplin Electronics Ltd., UK) and multiplied with its operating time (15 sec) and volume fraction which was considered as unity (~ 1). Then the energy consumption was divided by the mass of the sample ($2x10^{-3}$kg) to provide the energy consumption in MJ kg^{-1}. The furnace electrical power consumption was measured with a power plug-in meter and multiplied by its operating time to reach the desired temperature plus the time required for the furnace to run to maintain that temperature (~ 2 min) during 30 min holding time, as presented in equation:

Time to reach required temperature = (T_2-T_1)/ Heating rate of the furnace (2)

Where T_1 and T_2 are the room temperature and the required torrefaction temperature in °C respectively. The heating rate of the furnace was measured as 1.500 ± 0.033 °C sec^{-1} (mean ± standard deviation, n=3).

This was multiplied with the volume fraction, in this case considered as unity, and divided by the mass of the sample. The total energy input was calculated by summing up the energy consumption of the pump and the furnace, while the total energy output is the higher heating value (HHV) of the torrefied biomass (MJ kg^{-1}). The *Energy Ratio* (ER) was calculated by dividing the total energy output / total energy input. No heat losses were measured during this study, but it is suggested to include the heat loses from the furnace in future investigations.

Microscopy of torrefied biomass

Microscopy of the control samples and torrefied biomass (both with and without LN_2 treatment) was conducted to investigate any surface, structural and topographical changes developed due to the thermal treatment. A standard Nomarsky optical microscope (Eclipse ME 600, Nikon, Japan) with an X10 objective lens, was used for this analysis.

Elemental analysis of control and torrefied biomass

The elemental composition (C, H and N) of the residual biomass samples before and after the torrefaction treatment was measured at the School of Chemistry, University of Glasgow, UK with an analytical elemental analyser (CE-440, Exeter Analytical, UK).

Measurement of higher heating values (HHV) of the residual cake and the torrefied biomass

Each 1g sample was introduced into an oxygen bomb calorimeter (Model: 1341, Parr Instrument Company, USA). ASTM D2015 standard method was followed to measure the higher heating value (calorific values) of the extracted lipids, the control and the torrefied residual cake biomass samples (with and without LN_2 treatment).

Acknowledgements

The authors are thankful to the School of Chemistry, University of Glasgow, UK for their help in measuring elemental analysis of the torrefied biomass. The PhD research scholar Mehmood Ali was funded by NED University of Engineering and Technology, Karachi Pakistan for his PhD studies under the faculty development programme.

Keywords: Hydraulic pressing • Torrefaction • Mild pyrolysis • Microalgae • Liquid nitrogen (LN_2) • *Nannochloropsis oculata*

1. A. Abbassi, M. Ali, I.A. Watson, *Algal Research* 2014, 5, 190-194.
2. J. Plácido, S. Capareda, *Bioresources and Bioprocessing* 2016, **3**(1), 1-12.
3. A. Demirbas, M.F. Demirbas, *Energy Conversion and Management* 2011, **52**(1), 163-170.
4. K. Ullah, M. Ahmad,Sofia, V.K. Sharma,P. Lu P, A. Harvey, M. Zafar, S. Sultana, C.N. Anyanwu, *Progress in Natural Science: Materials International* 2014, **24**(4), 329-339.
5. J.R. McMillan, I.A. Watson, M. Ali, W. Jaafar, *Applied Energy* 2013, 103, 128-134.
6. R. Sayre, *Bio Science* 2010, 60(9), 722-727.
7. K. Worasaung, Y. Yi-Hung, H. Chih-Hung, T. Chung-Sung, *Aerosol and Air Quality Research* 2015, **15**, 712-742.
8. *Algae as a Source of Pharmaceuticals & Nutraceuticals*. 2017; Available from: http://www.oilgae.com/blog/2010/09/algae-as-a-source-of-pharmaceuticals-nutraceuticals.html.
9. B. Naveena, P. Armshaw, J. T. Pembroke, *Biotechnology for Biofuels* 2015, **8**(1), 1-13.
10. M.S. Ghayal, M.T. Pandya, *Energy Procedia* 2013; 32 (Supplement C): 242-250.
11. H.H. Khoo, C.Y. Koh, M.S. Shaik, P.N. Sharratt. *Bioresource Technology* 2013; **143**(0): 298-307.
12. K. Gopalakrishnan, S. Sutha, W.H. Chen, Q.V. Bach, S.H. Kim, A. E. Atabani, J.S. Chang, *Green Chemistry* 2017, **19,** 44-67.
13. M. Francavilla, P. Kamaterou, S. Intini, M. Monteleone, A. Zabaniotou, Algal Research 2015; **11**: 184-193.
14. M.J. Prins, K.J. Ptasinski, F.J.J.G. Janssen, *Energy* 2006, **31**(15), 3458-3470.
15. J.S. Tumuluru, S. Sokhansanj, T. Christopher, J. Wright, R. Hess , R.D. Boardman, *A Review on Biomass Torrefaction Process and Product Properties*. in *Symposium on Thermochemical Conversion*. 2011. Oklahoma State University.
16. M.J.C van der Stelt, H. Gerhauser, J.H.A. Kiel, K.J. Ptasinski, *Biomass and Bioenergy* 2011; **35**(9): 3748-3762.
17. K.T. Wu, C.J. Tsai, C.S. Chen, H.W. Chen, *Applied Energy* 2012, **100,** 52-57.
18. C. Kyle, M. Ondrej, P. Saran, *GCB Bioenergy* 2013, 5**,** 122-131.
19. D. Thrän, J. Witt, K. Schaubach, J. Kiel, M. Carbo, J. Maier, C. Ndibe, J. Koppejan, E. Alakangas, S. Majer, F. Schipfer, *Biomass and Bioenergy* 2016, **89**, 184-200.
20. M. Adamczyk, · M. Sajdak, *Waste and Biomass Valorization* 2017, 1-15. DOI 10.1007/s12649-017-9996-8.
21. M. Ali, I.A. Watson, *NED University Journal of Research - Applied Sciences* 2017, **XIV** (4), 105-111.
22. B.Y. Wiyarno, R.M Yunus, M. Mel, *Journal of Applied Sciences* 2011, **11**, 3607-3612.
23. E. G. Bligh, W.J. Dyer, C*anadian Journal of Biochemistry and Physiology* 1959, **37**(8), 911-917.
24. M. Ali, I.A. Watson, *Energy Technology* 2016, **4**(8), 980-989.
25. M. Ali, I.A. Watson, *International Journal of Energy Research* 2014, 38(5), 614-625.
26. W.H. Chen, W.Y. Cheng, K.M. Lu, Y.P. Huang, *Applied Energy* 2011, 88 (11), 3636-3644.
27. C.M. Welker, V.K. Balasubramanian, P. Carloalberto, K.M. Rai, S.D. Bolt, V. Mendu *Energies* 2015, 8, 7654-7676.
28. H. Yang, R. Yan, H. Chen, D.H. Lee, C. Zheng, *Fuel* 2007, **86**(12), 1781-1788.
29. G.D. Saratale, R.G. Saratale, J.S. Chang, *Biohydrogen*. 2013: Elsevier.
30. Q. Chen, J. Zhou, B. Liu, Q. Mei, Z. Luo, *Chinese Science Bulletin* 2011, **56**(14), 1449-1456.

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Layout 1:

FULL PAPER

The mild pyrolysis of the residual cake of *Nannochloropsis oculata* after lipid extraction by hydraulicc pressing and LN_2 treatment was conducted in a non-oxidizing atmosphere. The results showed an increase in HHV and a decrease in bulk density at both treatment temperatures (200 and 300 ^oC) as compared to the control samples (without any treatment). Gas pressure was developed due to the formation of CH4 and CO2 gases in the reactor. The Energy ratio was highest (0.485) with low temperature treatment and lowest (0.405) with higher temperature treatment,this can be increased if the energy for torrefaction can be supplied by waste heat or solar thermal energy.

*M.Ali *, I.A. Watson*

Mild pyrolysis of manually pressed and liquid nitrogen treated de-lipid cake of microalgae for bioenergy utilisation

Layout 2:

FULL PAPER

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