**Temperature Effect on Hydrothermal Liquefaction of *Nannochloropsis*
gaditana and *Chlorella* sp.**

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Abstract

Temperature effect on hydrothermal liquefaction (HTL) of *Nannochloropsis gaditana* and *Chlorella* sp. was investigated with 10% biomass loading at HTL temperatures of 180-330°C, and reaction time of 30 minutes. Maximum yields of 47.5% for *Nannochloropsis* sp. and 32.5 % biocrude oil yields for *Chlorella* sp. were obtained at 300°C. The higher heating values of biocrude oils produced in this work ranged between 34 and 39 MJ/kg. 79% of energy in the *Nannochloropsis* sp. was recovered at 300°C and 62% of energy recovery from *Chlorella* sp. was achieved at 200°C. Valuable nutrients ($\text{NH}_3\text{-N}$ and PO_4^{3-}) produced during the HTL process were quantified from the aqueous phase for both strains of biomass. The aqueous phase samples obtained at all temperatures were also analyzed for amino acids and carbohydrates. The suitable temperatures for extraction of lipids, amino acids and carbohydrates have been identified. Sequential HTL experiments conducted have shown the prospect of recovering nutrients and other valuable byproducts along with biocrude oil. The experimental results and analysis indicate that sustainable biofuel production requires the development of strain based strategies for the hydrothermal liquefaction process.

Key words: Hydrothermal liquefaction, wet algae, sequential extraction and liquefaction, energy recovery, nutrients, by-products

1. Introduction

Algae have received much attention as a source of renewable energy source since 1970s due to depleting fossil fuel sources, unstable global energy markets and environmental issues. The previous generations of biofuels used food crops as feedstock, may not be sustainable and could impose new socio-economic problems in many developing and under developed countries [1]. The algae have highest photosynthesis efficiency compared to other energy plants [2], which grows much faster and produces more biomass. Algae could also be cultivated in wastewater [3, 4] to recover valuable nutrients and to clean various kinds of waste water. Algae are being used as a source of various bio-products like proteins, ω -3 fatty acids, and carbohydrates along with lipids as a feedstock for biofuels [5, 6].

In spite of having many advantages over the first two generations of biofuels, commercialization of algal biofuels has not yet been achieved due to a few technical and energy drawbacks. Cultivation and oil extraction of algal biomass have been identified as hurdles for large scale algal biofuel production. Recently, many researchers identified hydrothermal liquefaction as a possible method to extract biocrude oils from algal biomass. Hydrothermal liquefaction's major advantage is the elimination of the drying step completely, which provides several options to extract different compounds/products from algal biomass and whole algae liquefaction [7]. Based on the processing conditions, these processes can be divided as hydrothermal carbonization (150-250°C & less than 20 bar) [8], hydrothermal extraction and liquefaction (200-350°C & 40-200 bar), and hydrothermal gasification (above 350°C and 200 bar) [9, 10]. Water's dielectric constant decreases drastically after 200°C with an increase in temperature under pressure giving it greater solvating properties. Density also decreases slowly up to the critical pressure point and then drops sharply after the critical pressure. At this point,

the water medium attains gas like densities and liquid like solvent properties. The tendency of providing H^+ or OH^- ions, the ion product (K_w) of water, is another important property in performing acid or base catalyzed reactions without any external catalyst depending on temperature and pressure of the water [7, 9, 11].

Some researchers utilized these properties for selective extraction of desired compounds and for various applications at different processing conditions. Kim et al [12] extracted nutraceutical compounds from citrus pomaces and Ibanez et al., performed extraction of antioxidant compounds from rosemary plants [13]. The selective extraction of lipids from *Nannochloropsis salina* wet algal biomass using the subcritical water extraction method was demonstrated by Reddy et al. The optimum conditions (220°C and 20 min) for extraction of oil/lipids were determined and under these conditions valuable polyunsaturated fatty acids were preserved in algal oil [14]. Some other researchers performed whole algae liquefaction at higher temperatures than the previous work to produce more biocrude oil instead of lipids. Hydrothermal liquefaction of algal biomass was demonstrated by Vardon et al., [15] with *Scenedesmus* (raw and defatted), and *Spirulina* strains of biomass; by Reddy et al., with *Chlorella sorokiniana* and *Dunaliella tertiolecta* [16] at 300°C. Valdez et al., studied the hydrothermal liquefaction of *Nannochloropsis* sp. from 250°C – 400°C and analyzed each product fraction after liquefaction along with performing energy studies [17]. Influence of external catalyst in the HTL process have shown little or no effect on biocrude oil yield. Among several acid and base catalysts, only Na_2CO_3 was able to produce more biocrude yields than the normal HTL media [18, 19]. A life cycle analysis on HTL of algal biomass reported that better energy return on investment (EROI) and less emissions could be achieved, when compared to cellulosic ethanol production [20]. The bio jet fuel produced from algal biomass cultivated in

waste water treatment plant could reduce the greenhouse gas emissions by 38-76% than conventional jet fuel [21]. Recent studies on HTL of algal biomass also showed that the nutrients supplied could be recovered in the water phase after reaction and could be used to cultivate algal biomass with necessary dilutions. Residual water recovered from HTL of *Chlorella vulgaris*, *Scenedesmus dimorphus*, *Spirulina platensis*, and *Chlorogloeopsis fritschii* was used for cultivation at different dilutions. Near equal or increased growth was observed with residual water of *Spirulina platensis* and *Chlorogloeopsis fritschii* species at 400 times dilution. For *Chlorella vulgaris*, the best growth was achieved with 100 times dilution [22]. Most of the HTL studies on algal biomass have concentrated only on biocrude oil production and performed only at a single or few temperatures. In order to identify a strategy for producing sustainable algal biofuels or algal biorefinery, in this work we studied hydrothermal liquefaction patterns of two species of algal biomass. *Nannochloropsis gaditana* and *Chlorella* sp. biomasses at 10% biomass loading were processed from 180°C to 330°C for a reaction time of 30 min.. A sequential HTL run was conducted with the bio-char produced at 225°C to demonstrate the separation of valuable compounds and lipids. Selective ranges of temperatures for the recovery of lipids, amino acids, carbohydrates and nutrients have been identified. Along with biocrude oil, bio-char and water soluble compounds were also quantified and analyzed after HTL processing. The nutrients presented in HTL residual water were also quantified. Based on the HHVs of biocrude oil and bio-char, the energy recovery calculations were performed.

2. Experimental section

2.1. Materials

The *Nannochloropsis gaditana* (CCMP - 1775) culture used in this study was obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP).

The *Nannochloropsis* sp. was grown in the f/2 growth medium, modified to contain 5 mM NO_3^- and 0.287 mM PO_4^{3-} at a salinity of 20 g L^{-1} in a 4000 L outdoor Solix photo bioreactor. The algae was grown with 0.8% CO_2 enrichment and maintained at 25°C with ambient outdoor lighting. The *Chlorella* sp. (DOE 1412) culture used in this study was identified by J. Polle (Brooklyn University, N.Y). *Chlorella* sp. was grown in BG11 growth medium in a 4000 L outdoor Solix photobioreactor. The alga was grown with 0.8% CO_2 enrichment and at 25°C and ambient lighting conditions. The algal cultures were harvested using a high speed continuous centrifuge at 18,000 rpm and were stored at 4°C until used in the analysis and HTL experiments. The biochemical compositions of both strains of biomass are given in Table 1. The solvent used in this study was Dichloromethane (DCM) and of analytical grade (Pharmco-aaper).

2.2. Instruments

A PARR 4593 stainless steel bench top reactor accompanied by a 4843 controller unit manufactured by Parr Instrument Company (Moline, Illinois, USA) was used for conducting hydrothermal extraction and liquefaction experiments. A high pressure reactor manufactured by Supercritical fluid technologies, Inc; (Newark, Delaware, USA) was also used to perform the reaction at 330°C. Both reactors are equipped with pressure gauges to monitor pressure. A Hewlett Packard 5890 gas chromatograph with a 5972a mass selective detector equipped with a capillary column DB-23, 30m x .25mm diam. x .25 μm film was used for fatty acid methyl ester analysis of biocrude oil. The Leco Pegasus High Throughput Time of Flight Mass Spectrometer (HT TOFMS) with a CTC CombiPAL autosampler was used for qualitative analysis of biocrude oils. Compositional analysis of biocrude oils was performed with a hybrid linear ion trap 7 T FT-ICR mass spectrometer (LTQ FT, Thermo, San Jose, CA) equipped with an Advion Triversa NanoMate (Advion, Ithaca, NY). Thermo gravimetric analysis (TGA) of wet algal biomass was

performed using a Perkin Elmer Pyris 1 TGA (Perkin Elmer Inc., USA) instrument. The inorganic nutrients were measured using HACH DR6000 spectrophotometer (HACH, Colorado, USA).

2.3. FAME (GC-MS) and HTTOF-MS analysis of biocrude oils

Base catalyzed FAME (fatty acid methyl esters) analysis was performed by direct methylation of dry tissue of biomass and biocrude oils according to [14]. The acid catalyzed FAME analysis was performed with the same experimental procedure by using 0.4% H₂SO₄ and deionized water instead of NaOH and glacial acetic acid in base catalyzed FAME. In HTTOF MS, the biocrude oil samples were incubated for 5 minutes at 45°C, and samples were extracted for 5 minutes with a desorb time of 10 minutes for qualitative analysis. A ZB-5ms column (30m x .25mm I.D. x .25um film thickness) was used with helium as the carrier gas at a constant flow rate of 0.6 mL/minute. The inlet temperature was constant at 225°C and the transfer line was 275°C. The oven ramp started at 35°C and held for 4 minutes, then ramped at 5°C per minute to 110°C with no hold, then ramped at 7°C per minute to 280°C and held for 0.5 min for a total run time of 43.78 minutes.

2.4. Compositional analysis of biocrude oils by FT-ICR MS

Biocrude oil samples obtained from HTL runs were analyzed by direct infusion electrospray ionization Fourier Transform Ion Cyclotron Resonance mass spectrometry (ESI FT-ICR MS).. HTL bio-oil stock mixtures for both algal strains at each reaction temperature were prepared by dissolving the oil in 1:1 chloroform: methanol to a concentration of 1 mg/mL. Each stock mixture was further diluted to 0.5 mg/mL in an electrospray ionization solution of 1:2:4 chloroform:methanol:2-propanol containing either 0.1% formic acid or 0.1% ammonium hydroxide in positive- and negative-ion modes, respectively [23]. All solvents were high-

performance liquid chromatography (HPLC)-grade and purchased from Sigma-Aldrich (St. Louis, MO). The final diluted samples were centrifuged to remove any suspended particulate matter and FT-ICR mass spectrometry was performed for all oil samples as previously described [24]. Data was collected at a mass resolving power of $m/\Delta m_{50\%} = 400,000$ (m/z 400) and a total of 500 and 400 time-domain transients were co-added for each sample in positive- and negative-ion mode, respectively prior to fast Fourier transformation and frequency to m/z conversion.. Mass spectra were internally calibrated and elemental compositions were assigned to the observed ions in each mass spectrum. A variety of non-lipid constituent molecules of HTL biocrude oil were identified by their heteroatom class. Residual lipids in the oil were identified by matching the assigned elemental compositions to an in-house assembled lipid database derived from Lipid Maps (Nature, Lipidomics Gateway) as previously described[25].

2.5. Amino acids quantification and carbohydrate profiling

Amino acids were separated from the aqueous HTL fraction by strong cation exchange on a Supel Select SCX SPE 30 mg/1.0 mL SPE Tube (Supelco, Bellefonte, PA). The SPE columns were conditioned with two volumes of 0.1N HCl. A 2.0 mL aliquot of the aqueous fraction was acidified (to 0.1N HCl) by the addition of 34 μ L of 6.0 N HCl and then passed through the SPE Column. The column was washed with two volumes of 0.1 N HCl and the bound amino acids were eluted with two volumes of NH_4OH . A total of 200 μ L of 2.5 mM norleucine solution was added as an internal standard to the eluted extracts before they were completely dried in glass vials by a speedvac (Eppendorf, Hauppauge, NY). The dried residue was resuspended in 100 μ L of pyridine and subsequently derivatized by the addition of 100 μ L N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA; Sigma, St. Louis, MO) and incubated at 110°C for 1 hour. The resulting silylated derivatives were chromatographed on a

Varian 3800 GC coupled to a Saturn 2000R scanning from 40–450 m/z with a 12 min filament delay. Samples were injected by splitless injection, and the MS temperatures were set as follows: inlet and transfer line were held at 250°C, trap 150°C, manifold 35°C. Separation was achieved with a temperature program of 80°C for 2 min, then ramped at 4 °C min⁻¹ to 250°C on a 30 m ZB-5 column 0.25 mm ID, 0.25 µm film thickness (Phenomenex, Torrance, CA) at a constant flow of 1.0 mL min⁻¹. Internal standard quantification was performed by relative response factors of Norleucine to amino acid mix cat # A6407 (Sigma, St. Louis, MO).

The carbohydrate profiling of the aqueous HTL fraction was adapted from the method described by Broeckling et al., 2004 using 0.2 mL of evaporated aqueous residue[26]. All samples were analyzed using a Varian 3800 GC coupled to a Saturn 2000R scanning from 45–550 m/z with a 10 min filament delay. Samples were injected at a 20:1 split ratio, and the MS temperatures were set as follows: inlet and transfer line were held at 250°C, trap 150°C, manifold 35°C. Separation was achieved with a temperature program of 70°C for 1 min, then ramped at 3°C min⁻¹ to 330°C on a 30 m ZB-5 column 0.25 mm ID, 0.25 µm film thickness (Phenomenex, Torrance, Ca) at a constant flow of 1.0 mL min⁻¹. The resulting peaks were identified by matching to the NIST mass spectral database.

2.6. Experimental procedures

Biomass with 10% biomass loading (w/v) was prepared by adding the proper amount of supernatant water collected after harvesting. 50 mL of this slurry was fed to the reactor, and nitrogen was purged three times to dispose of any air in the reactor. 200 psi of initial pressure was maintained to control the rapid boiling of water. Subsequently, the reactor was heated according to the experimental plan by using the controller unit and held for 30 min, and the pressure was recorded at target temperature. The recorded pressures at each experimental run

211 were presented in supplementary information Table S5. The reactor was cooled down to room
 212 temperature by means of an external fan before adding 30 mL of Dichloromethane (DCM) to
 213 separate biocrude oil from the product mixture. The DCM mixture was transferred to a
 214 separation funnel through a filter paper and allowed to stand for 15 minutes for phase separation.
 215 The collected bio-char was separated, dried and quantified for the material and energy
 216 calculations. The denser DCM phase, which includes biocrude oil, settled at the bottom and the
 217 residual water comprised the less dense upper phase. HTL product was recovered from the
 218 separated DCM phase by a rotary evaporator to yield biocrude oil (B.C.O) at 65°C under vacuum
 219 (94-96% recovery). Weight of the biocrude oil was noted carefully, and each experimental run
 220 was performed three times. The weight of water soluble compounds (W.S.C) was also measured
 221 gravimetrically. Biocrude oil samples and residual water samples were stored at -5°C for further
 222 analysis. Yields of biocrude oil, bio-char (B.Ch), and water soluble compounds were calculated
 223 based on the following equations. The remaining mass fraction was considered as gaseous
 224 products.

$$\text{Biocrude oil yield (\%)} = \frac{\text{weight of biocrude oil}}{\text{dry weight of biomass}} * 100$$

$$\text{Bio – char yield(\%)} = \frac{\text{weight of bio – char}}{\text{dry weight of biomass}} * 100$$

$$\text{Yield of W. S. C (\%)} = \frac{\text{weight of W. S. C}}{\text{dry weight of biomass}} * 100$$

225 The high heating values (H.H.V) of biocrude oil, and bio-char were determined with a micro
 226 bomb calorimeter. These determined H.H.V numbers were used to calculate energy recovery
 227 calculations. Energy recovery and consumption ratios were calculated based on the following
 228 equations on dry weight basis.

Energy recovery % (ER)

$$= \frac{(\text{Wt. of B. C. O} * \text{HHV of B. C. O}) + (\text{Wt. of B. Ch} * \text{HHV of B. Ch})}{(\text{Wt. of algal biomass} * \text{HHV of algal biomass})} * 100$$

$$\text{Energy consumption ratio (ECR)} = \frac{\text{Energy consumed for HTL process}}{\text{Energy available in biocrude oil}}$$

$$\text{Energy available in biocrude oil} = \text{Wt. of biocrude oil} * \text{HHV of biocrude oil}$$

The amount of energy consumed in the HTL process was calculated using 1.31 kJ/kg.K as specific heat for algal biomass [27]. The energy recovery from HTL process was taken as 50% though heat recovery and the ECR was calculated with and without heat recovery.

3. Results and discussion

3.1. Influence of temperature on HTL products

Major product fractions identified during this work were biocrude oil, water soluble compounds (W.S.C), bio-char or solid residue and gaseous product (calculated by difference with losses during experiments). Yields of the above mentioned products obtained from hydrothermal processing of both *Nannochloropsis* sp. and *Chlorella* sp. are presented in Figure 1 on the dry weight basis. Increase in temperature improved the biocrude oil yield for both strains of biomass, but followed a different pattern. In the case of *Nannochloropsis* sp., yield of biocrude oil was increased from 16.85 % at 180°C and stabilized at 25% between 225°C and 250°C. A slight increase of biocrude oil yield was observed at 275°C than the previous temperature, but at 300°C drastic increase in yield was achieved recording 47.5%. Further increase in temperature resulted in reduction of biocrude oil yield due to higher gasification. Larger amounts of gases were formed above 275°C, where 31% of the biomass was converted to gaseous product at 330°C due to rapid gasification.

Reaction temperature is the major influencing factor that affects the production of biocrude oil and its properties, and varies the polarity of the water [7, 28]. A similar pattern of biocrude oil yield was observed from the *Chlorella* sp., but the amount of biocrude oil produced was low over the range of all temperatures when compared to *Nannochloropsis* biomass. This difference can primarily be attributed to the lower lipid content of the biomass. Despite having lower lipids, a maximum of 32.54% of yield was achieved at 300°C and a further increase in temperature slightly reduced the yield. Interestingly, more gaseous product was observed from 180°C and above for *Chlorella* sp. biomass. 15.5% of gaseous product was observed at 180°C and reached a maximum of 52.24% at 330°C. In the case of both strains more biocrude oil was generated due to an increase in temperature, where the ionic product ($K_w = [H^+][OH^-]$) of water increases. Higher ionic product of water provides more H^+ and OH^- ions, which drives the hydrothermal cleavage of biochemical compounds present in the biomass into smaller compounds. Hydrothermal liquefaction of *Nannochloropsis salina* and *Spirulina platensis* reported much higher biocrude production than their original lipid content. 47% of biocrude oil yields for *Nannochloropsis* sp. at 350°C and 38% for *Spirulina* sp. at 310°C were reported [29]. Depending upon reaction conditions, condensation and repolymerization reactions influence the formation of biocrude oil [7, 30].

As shown in Figure 1, water soluble compounds (W.S.C) were observed to form beginning from lower temperatures with *Nannochloropsis* sp. and *Chlorella* sp.. In the case of *Nannochloropsis* sp., the amount of W.S.C was increased up to 250°C, where a maximum of 27% W.S.C were observed. Further increase in temperature caused repolymerization of these water soluble compounds, which might have been converted to either biocrude oil or gaseous products. In the case of *Chlorella* sp., W.S.C's were increased up to 225°C, where a maximum of

24.06% were recovered. Further increase in temperature caused repolymerization or condensation of these compounds into biocrude oil or gaseous phase. Their weight fraction was drastically reduced at higher temperatures. The yield of W.S.Cs reached 3.85% for *Chlorella* sp. and 10.50% for *Nannochloropsis* sp. at 330°C. High amounts of ash content present in the biomass may also have contributed to this high W.S.C. in the case of *Nannochloropsis* sp. Water soluble compounds produced at lower temperatures from proteins and carbohydrate compounds were repolymerized at higher temperature, which also contributed to the increase in yield of biocrude oil and this trend was observed with both strains of biomass. A similar pattern was previously reported, where conversion of water soluble compounds into bio-oil at higher temperatures was observed [31, 32].

The subsequent important product is bio-char, which decreased from 62% at 180°C to 16.5% at 330°C for *Nannochloropsis* sp.. At 300 and 330°C, drastic reductions in mass of bio-char were observed due to disintegration of algae cells and conversion of metabolites into biocrude oil and gaseous products. Trends of bio-char yield of *Chlorella* sp., were also similar to the former strains trends, and major weight reductions occurred between 200-250°C. Yield of bio-char is reduced from 55.2% to 13.35% at 330°C. In this study, we identified bio-char as one of the crucial products which improves the overall energetics of the process and provides options for other applications. Along with changes in weight fractions of bio-char, its heating values were also varied. This indicates the extraction and liquefaction of biochemical compounds present in the algal biomass. Yu et al., have reported that bio-char or solid residue yield was decreased with an increase in temperature. A rapid reduction in bio-char was observed between 0 to 30 min. and compared to a slow decrease from 30 to 120 min., as most of the compounds converted into bio-oil or into other phases [33].

3.2. Analysis of biocrude oils

The biocrude oils were analyzed thoroughly for their chemical, thermal properties and calorific value over the range of all temperatures. The primary constituents of the biocrude oil at a lower temperature were lipids and their contribution to the yield of biocrude oil was different in both strains of biomass. In the case of *Nannochloropsis* sp., the amount of lipids presented in the biocrude oil increased with temperature up to 300°C. But a greater variation in their chemical form was observed, as a majority of the lipids were converted into free fatty acids (FFA) from 225°C and upwards. This was due to hydrolysis of lipids above 200°C, and formation of FFAs is shown in Figure 2. Up to 275°C, half or more than 50% of the biocrude oil was formed by lipids. At 300°C, 43.5% of the biocrude oil was from lipids and it was ~84% of the total lipid content of the biomass. At lower temperatures membrane constituents like phytol, 2-phytene, minor quantities of alkanes, nitrogen containing amides and many other minute compounds were observed in the remaining portion of biocrude oil. The high throughput mass spectrometer detected as low as 180 compounds at lowest temperature to 1300 compounds at highest temperature. At higher temperatures the larger compounds like proteins, lipids, and cellulosic compounds defragmented to form smaller compounds. Due to wide variety of possible reactions (hydrolysis, dehydration, decarboxylation, deamination, repolymerization, isomerization etc.,) in the hydrothermal liquefaction process, different classes of N, O, and S containing compounds were formed at increasing temperatures. These compounds were observed in both FT-ICR MS and HT TOF MS analyses.

Above 225°C, most of the membrane associated compounds started degrading and disappeared completely at higher temperatures. As temperatures increased above 250°C, the contribution of lipids to biocrude oils decreased primarily due to contribution from other

biochemical compounds like proteins and carbohydrates. This phenomenon was also confirmed by the FT-ICR MS analysis.

. For the positive-ion mode, distribution of non-lipid compounds ($N_{1-3} O_{1-3}$) in the biocrude oils and their variation in the abundance with reaction temperature are very similar for both algal strains. Primary lipids observed for *N. gaditana* low-temperature ($< 250^{\circ}\text{C}$) oil in the positive-ion ESI FT-ICR MS are acylglycerols. Major compounds observed in the negative ion mode include free fatty acids regardless of reaction temperature. A more detailed description of the compositional analysis of the biocrude oils has been reported by Sudasinghe et al.[24].

Unlike the biocrude oil produced from *Nannochloropsis* sp., biocrude oil produced from *Chlorella* sp., has shown different chemical properties and this difference can be attributed to their respective biochemical composition. A maximum of 43% of the lipids was observed at 200°C in biocrude, which was the highest contribution of lipids to biocrude over the range of temperatures. Hydrolysis was observed right from 180°C and reached highest at 200°C , where 76% of the lipids extracted were in the form of FFA. Interestingly, as shown in Figure 2 these FFA compounds started degrading above 200°C . The membrane alcohols like phytol, isophytol, and other minor compounds were detected below 225°C , and most of these compounds were degraded above 225°C and formed different compounds similar to the previous case. The difference in hydrolysis of lipids in both these strains could be due to a higher proportion of unsaturated fatty acids present in *Chlorella* sp., which are very sensitive to high temperatures. In both cases of biomass 65-70% of the neutral lipids were extracted at 225°C into the biocrude oil. Positive-ion ESI FT-ICR MS analysis indicates that low temperature ($< 225^{\circ}\text{C}$) *Chlorella* sp. biocrude oil is primarily comprised of betaine lipids. Similar to *N. gaditana*, acidic compounds

observed for *Chlorella* sp. oil in the negative ion mode are dominated by free fatty acids for all reaction temperatures..

In both cases of biomass many valuable compounds have been extracted into biocrude oil, providing a possible commercial application of hydrothermal extraction. Biocrude oil produced from *Nannochloropsis* sp., at lower temperatures (225°C) preserved valuable polyunsaturated fatty acids. They are Arachidonic acid (C20:4n6 – 4% of lipids), Eicosapentaenoic acid (C20: 5n3-3% lipids), and membrane alcohols like phytol (3% based on peak area-not quantified). *Chlorella* sp., has more membrane compounds than the previous strain, they are isophytol (16.28%), and phytol (7.5%) based on peak area (not quantified) at 200°C, which also have commercial value. Compound identity is confirmed by fragment ion mass spectra provided by electron impaction ionization. These compounds are also thermo labile, which were degraded from 225°C and above.

As shown in Figure S1, the TGA curves of biocrude oils shows increasing presence of volatile compounds with increasing HTL processing temperatures. The biocrude oils produced from *Nannochloropsis* sp., have showed this phenomenon. The biocrude oils produced with *Chlorella* sp., have minor differences in their TGA curves, and this is due to less lipids or contribution of protein based compounds than *Nannochloropsis* sp. The biocrude oil produced at the maximum yield point (300°C) has showed the same thermal behavior. When compared to distillation curve of Ural crude oil [34], the distillation curves of the biocrude oils produced at 300°C are very near to distillation curve of diesel (shown in Figure S2). This shows that the biocrude oils produced from algal biomass could be used as feedstock to produce transportation fuels with necessary denitrification and deoxygenation prior to the refining.

3.3 Analysis of water soluble compounds

Water soluble compounds are valuable products after biocrude oils, as they consists valuable nutrients, byproducts like amino acids and carbohydrates. The residual water was analyzed in two phases, one for quantification of nutrients and the other for water soluble organic compounds.

HTL water samples were analyzed for ammoniacal nitrogen ($\text{NH}_3\text{-N}$) and Phosphate (PO_4^{3-}) according to Selvaratnam et al. [35]. In both cases of biomass, the ammoniacal nitrogen recovery was detected at different concentrations at each temperature. In both cases, the total nitrogen (TN) increased slightly at lower temperatures up to 250°C and stabilized at higher temperatures. Total nitrogen quantities varied greatly for both strains due to their biochemical composition. The highest total nitrogen detected was 5808 ppm at 250°C for *Chlorella* sp., and 1761 ppm for *Nannochloropsis* sp. at 225°C . For both strains of biomass, the amount of $\text{NH}_3\text{-N}$ increased with an increase in processing temperature and was found to be highest at 300°C . The highest being 820 ppm with *Nannochloropsis* sp. and 2475 ppm of $\text{NH}_3\text{-N}$ with *chlorella* biomass were obtained (Figure 3a). Proteins present in the biomass decompose into water soluble amino acids and ammonia [17], and the concentration of these compounds varied with processing temperature. Ammonium was formed by deamination of amino acids, which are produced due to hydrolysis of proteins [22]. The difference in TN and $\text{NH}_3\text{-N}$ for both cases of biomass at the same temperature was due to variation in biochemical composition and less protein content in *Nannochloropsis* sp. A larger contribution of proteins to the biocrude oil with the *chlorella* biomass is observed, also contributed to more TN and $\text{NH}_3\text{-N}$ in water phase. Another valuable nutrient quantified was phosphate (PO_4^{3-}). As shown in Figure 3b, in both cases of biomass the amount of phosphate increased with increase in temperature up to 200°C

then decreased as temperature increased above 200°C. The highest phosphate concentration of 280 ppm with *Nannochloropsis* sp. and 26 ppm with *Chlorella* sp. at 200°C were found. Anyhow for the recycling of these nutrients, analysis of organic compounds also needed to be conducted as phenolic compounds present in the water may inhibit the growth of algal biomass. After appropriate dilutions of the water fraction, it was used successfully to grow algal biomass [22].

HTL aqueous phase was analyzed as a source of recyclable soluble organic compounds. The amino acids are one of the valuable compounds being produced due to hydrolysis of protein at lower temperatures. In Table S1 and S2, most abundant and quantified amino acids are presented. The highest concentrations of amino acids at 1.9 and 1.92 nmol/mL were observed for *Nannochloropsis* sp. and *Chlorella* sp. respectively at 200°C. The mol% of simple amino acid glycine was increased with ascending processing temperatures as the remaining amino acids were degraded continuously with the increasing temperatures. This degradation of amino acids with increasing temperatures may have contributed to the increase of NH₃-N at higher temperatures. The deamination and decarboxylation reactions cause the degradation of amino acids forming pyrolyzed compounds and gaseous compounds at higher temperatures [36]. These results also suggest the need to track peptides which are intermediate compounds between proteins and amino acids, which will provide more understanding of protein behavior in the complex HTL reaction medium.

Along with amino acid analysis, exploratory runs were conducted to detect the presence of carbohydrates and other compounds. This analysis revealed the presence of large amounts of sugars (including mono, di and polysaccharides), sugar alcohols, glycerol and nitrogen containing compounds. A ratio of the abundance of the compounds at the higher temperature and lowest temperature (180°C) indicates that each compound exhibits independent behavior with

increasing temperature (Shown in Table S3 and S4). Both strains of biomass have only a few common and far more different compounds due to their biochemical composition. But interestingly both strains of biomass have compounds like sugars (glucose, melezitose, galactose), sugar alcohols (mannitol, galactitol, sorbitol) and many unidentified carbohydrates. In both cases highest concentrations of these carbohydrates were found at 250°C and below (Figure 4). As discussed in the biocrude oil analysis section, due to hydrolysis of lipids abundance of glycerol started increasing with increasing temperature up to 300°C and started degrading above 300°C. The above mentioned sugar alcohols have commercial pharmaceutical applications and needed to be quantified. The original GC-MS spectra have identified 180-200 compounds, but with very low abundance. For quantification of the highly abundant and valuable compounds separate quantification methods have to be used for the individual class of compounds.

3.4. Higher heating values (HHV) and energy recovery

The Higher heating values (HHV) of biocrude oils and bio-char were determined with a micro-bomb calorimeter. Results of the HHV of biocrude oils and bio-char are presented in Table 2. Higher values of HHV were observed for biocrude oils produced at lower temperatures. In the case of *Nannochloropsis* sp., HHVs of biocrude oils started decreasing at 250°C and continued decreasing as temperature increased, reaching a minimum at 300°C. This reducing behavior could be attributed to the presence of more nitrogenous compounds with increase in temperature above 250°C as observed in FT-ICR, HT TOFMS analysis. Further increase in temperature increased the energy content again, due to deoxygenation of biocrude oils. Similar observation was reported by Valdez et al., where reduction in oxygen content improved the HHV of the biocrude oil [17]. A similar trend of HHV variation was observed with *Chlorella* sp., where higher HHVs were observed when compared to biocrude oils produced at high

temperature. When compared to HHVs of biocrude oils produced with *Nannochloropsis* sp., the HHVs of biocrude oils produced with *Chlorella* sp. were slightly lower due to the presence of nitrogenous compounds from the 180°C. As temperature increased above 275°C, the HHV of the biocrude oils was increased due to deoxygenation at high temperatures. Alba et al., also have observed similar behavior of increase in nitrogen and decrease in oxygen content in biocrude oils, which influences the HHV of the biocrude oils [32]. The bio-char recovered after liquefaction also has considerable energy value associated with it. In both cases of biomass, at lower temperatures the HHVs for these char samples have energy content between 18-20 MJ/Kg. In the case of *Chlorella* sp., above 250°C lower HHVs were observed as most of the energy dense compounds were converted into other product fractions. Similar observations were recorded with bio-char samples obtained above 275°C with *Nannochloropsis* sp. The energy recovery from the biomass was calculated based on the HHVs measured.

Energy recovery (%) was calculated based on HHVs of biocrude oil, bio-char and feed biomass. The highest energy recovery of 78% was achieved with *Nannochloropsis* sp. at 300°C. Above 70% of energy recovery was attained at all temperatures except at 330°C due to gasification of biomass, reduced contribution of biocrude oil and bio-char. In the case of *Chlorella* sp., only 61.3% of the energy was recovered at 200°C and the energy recovery was decreased with increase in temperature. Reasons for lower energy recoveries for *Chlorella* biomass were lower lipid based energy, and more gasification. The ECR numbers shows that most of the experimental runs are energy positive except very few even only with biocrude oil as the product. When heat recovery is employed the energy consumption is much reduced making HTL as an attractive option for biofuel production. The lowest energy consuming temperature or

ECR (0.41 for *Nannochloropsis* sp., and 0.54 for *Chlorella* sp.) is observed at 300°C for both strains of biomass due to highest production of biocrude oil at that temperature.

3.5. Sequential HTL (SE-HTL) and byproducts

To investigate the possibility of an algal biorefinery, we have conducted sequential HTL runs with bio-char of *Nannochloropsis* sp. obtained at 225°C. Triplicate experiments at 300°C with D.I water as media were conducted. The results showed a slight decrease in cumulative biocrude yield (25.6 (225°C) + 16.52 (SE HTL - 300°C) = 42.12 %. The HHV of the biocrude oil was found to be 39.14 ± 0.66 MJ/Kg. This decrease in yield of biocrude oil was due to separation of water soluble compounds at 225°C. Because of the separation of nitrogen in the water phase at lower temperatures may have resulted in improved HHV of the biocrude [18]. These experimental results indicate the possibility of separating nutrients, valuable water soluble organic compounds, preservation of temperature sensitive compounds at lower temperatures, and conversion of remaining biomass into biocrude oil. Similarly, the bio-char obtained from *Chlorella* sp. processed at 200°C contains high amounts of crude protein. This high protein content makes it a good animal feed source or the recoverable protein can be extracted for commercial human food applications as per demand. The bio-char can also be fired along with coal in power generation because of its higher calorific values. The gasification of the bio-char could be used to make syngas, which can be further converted into valuable industrial chemical products [37]. Other possible applications for bio-char are water purification [38] and soil remediation [39]. It has been reported that the production of biogas through anaerobic digestion of lipid extracted algae after lipid extraction could reduce the energy consumption from 56.9 MJ/kg to 46.0 MJ/kg to produce 1 kg biodiesel [27]. The utilization of this char for other energy byproducts development produces more energy and provides more alternatives for the algal

biofuels sustainability. But, the final path of processing strategies and product identification could only be possible after extensive life cycle and techno economic assessments for individual strains of biomass.

4. Conclusions

Hydrothermal extraction and liquefaction experiments were conducted with two strains of algal biomass having different biochemical composition. The experimental results indicate that biocrude oil could be produced from proteins and carbohydrates along with lipid content present in the biomass. The highest biocrude oil yield of 47.5% was observed at 300°C for *Nannochloropsis* sp. and 32.5% for *Chlorella* sp. at 275°C. The HHV of biocrude oil ranged between 34-39 MJ/Kg. The bio-char also has 8-21 MJ/Kg of HHV depending upon the processing temperature. The analysis of the water fraction shows the recovery of valuable nutrients like $\text{NH}_3\text{-N}$ and PO_4^{3-} , which reduces nutrient costs in cultivation. The water soluble compounds like amino acids, sugars, sugar alcohols, and glycerol provide possible profitable byproducts. The SE-HTL experiments show the possibility of an algal biorefinery with hydrothermal extraction and liquefaction methods to produce fuels, valuable nutrients for humans and algae as byproducts.

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496 **References**

497

- 498 [1] Demirbas A, Fatih Demirbas M. Importance of algae oil as a source of biodiesel. *Energy Convers*
499 *Manage.* 2011;52:163-70.
- 500 [2] Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, et al. Second Generation
501 Biofuels: High-Efficiency Microalgae for Biodiesel Production. *Bioenergy Research.* 2008;1:20-43.
- 502 [3] Wang M, Kuo-Dahab WC, Dolan S, Park C. Kinetics of nutrient removal and expression of extracellular
503 polymeric substances of the microalgae, *Chlorella* sp. and *Micractinium* sp., in wastewater treatment.
504 *Bioresource Technology.* 2014;154:131-7.
- 505 [4] Rawat I, Kumar RR, Mutanda T, Bux F. Dual role of microalgae: Phycoremediation of domestic
506 wastewater and biomass production for sustainable biofuels production. *Appl Energy.* 2011;88:3411-24.
- 507 [5] Wijffels RH, Barbosa MJ, Eppink MHM. Microalgae for the production of bulk chemicals and biofuels.
508 *Biofuels Bioproducts & Biorefining-Biofpr.* 2010;4:287-95.
- 509 [6] Demirbas MF. Biofuels from algae for sustainable development. *Appl Energy.* 2011;88:3473-80.
- 510 [7] Savage PE. A perspective on catalysis in sub- and supercritical water. *J Supercritical Fluids.*
511 2009;47:407-14.
- 512 [8] Heilmann SM, Davis HT, Jader LR, Lefebvre PA, Sadowsky MJ, Schendel FJ, et al. Hydrothermal
513 carbonization of microalgae. *Biomass & Bioenergy.* 2010;34:875-82.
- 514 [9] Peterson AA, Vogel F, Lachance RP, Froeling M, Antal MJ, Jr., Tester JW. Thermochemical biofuel
515 production in hydrothermal media: A review of sub- and supercritical water technologies. *Energy &*
516 *Environmental Science.* 2008;1:32-65.
- 517 [10] Moeller M, Nilges P, Harnisch F, Schroeder U. Subcritical Water as Reaction Environment:
518 Fundamentals of Hydrothermal Biomass Transformation. *Chemsuschem.* 2011;4:566-79.
- 519 [11] Akiya N, Savage PE. Roles of water for chemical reactions in high-temperature water. *Chemical*
520 *Reviews.* 2002;102:2725-50.
- 521 [12] Kim J-W, Nagaoka T, Ishida Y, Hasegawa T, Kitagawa K, Lee S-C. Subcritical Water Extraction of
522 Nutraceutical Compounds from Citrus Pomaces. *Separation Science and Technology.* 2009;44:2598-608.
- 523 [13] Ibanez E, Kubatova A, Senorans FJ, Cavero S, Reglero G, Hawthorne SB. Subcritical water extraction
524 of antioxidant compounds from rosemary plants. *Journal of Agricultural and Food Chemistry.*
525 2003;51:375-82.
- 526 [14] Reddy HK, Muppaneni T, Sun Y, Li Y, Ponnusamy S, Patil PD, et al. Subcritical water extraction of
527 lipids from wet algae for biodiesel production. *Fuel.* 2014;133:73-81.
- 528 [15] Vardon DR, Sharma BK, Blazina GV, Rajagopalan K, Strathmann TJ. Thermochemical conversion of
529 raw and defatted algal biomass via hydrothermal liquefaction and slow pyrolysis. *Bioresource*
530 *Technology.* 2012;109:178-87.
- 531 [16] Reddy HK, Muppaneni T, Rastegary J, Shirazi SA, Ghassemi A, Deng SG. ASI: Hydrothermal Extraction
532 and Characterization of Bio-Crude Oils from Wet *Chlorella* Sorokiniana and *Dunaliella Tertiolecta*.
533 *Environ Prog Sustain.* 2013;32:910-5.
- 534 [17] Valdez PJ, Nelson MC, Wang HY, Lin XN, Savage PE. Hydrothermal liquefaction of *Nannochloropsis*
535 sp.: Systematic study of process variables and analysis of the product fractions. *Biomass and Bioenergy.*
536 2012;46:317-31.
- 537 [18] Biller P, Ross AB. Potential yields and properties of oil from the hydrothermal liquefaction of
538 microalgae with different biochemical content. *Bioresource Technology.* 2011;102.
- 539 [19] Jena U, Das KC, Kastner JR. Comparison of the effects of Na₂CO₃, Ca₃(PO₄)₂, and NiO catalysts on
540 the thermochemical liquefaction of microalga *Spirulina platensis*. *Appl Energy.* 2012;98:368-75.

- [20] Liu X, Saydah B, Eranki P, Colosi LM, Greg Mitchell B, Rhodes J, et al. Pilot-scale data provide enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via hydrothermal liquefaction. *Bioresource Technology*. 2013;148:163-71.
- [21] Fortier MOP, Roberts GW, Stagg-Williams SM, Sturm BSM. Life cycle assessment of bio-jet fuel from hydrothermal liquefaction of microalgae. *Appl Energy*. 2014;122:73-82.
- [22] Biller P, Ross AB, Skill SC, Lea-Langton A, Balasundaram B, Hall C, et al. Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process. *Algal Research*. 2012;1:70-6.
- [23] Sudasinghe N, Dungan B, Lammers P, Albrecht K, Elliott D, Hallen R, et al. High resolution FT-ICR mass spectral analysis of bio-oil and residual water soluble organics produced by hydrothermal liquefaction of the marine microalga *Nannochloropsis salina*. *Fuel*. 2014;119:47-56.
- [24] Sudasinghe N, Reddy H, Csakan N, Deng S, Lammers P, Schaub T. Temperature-Dependent Lipid Conversion and Nonlipid Composition of Microalgal Hydrothermal Liquefaction Oils Monitored by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *BioEnergy Research*. 2015:1-11.
- [25] Holguin FO, Schaub T. Characterization of microalgal lipid feedstock by direct-infusion FT-ICR mass spectrometry. *Algal Research-Biomass Biofuels and Bioproducts*. 2013;2:43-50.
- [26] Broeckling CD, Huhman DV, Farag MA, Smith JT, May GD, Mendes P, et al. Metabolic profiling of *Medicago truncatula* cell cultures reveals the effects of biotic and abiotic elicitors on metabolism. *J Exp Bot*. 2005;56:323-36.
- [27] Ponnusamy S, Reddy HK, Muppaneni T, Downes CM, Deng S. Life cycle assessment of biodiesel production from algal bio-crude oils extracted under subcritical water conditions. *Bioresource Technology* (under review). 2014.
- [28] Akhtar J, Amin NAS. A review on process conditions for optimum bio-oil yield in hydrothermal liquefaction of biomass. *Renewable & Sustainable Energy Reviews*. 2011;15:1615-24.
- [29] Toor SS, Reddy H, Deng S, Hoffmann J, Spangsmark D, Madsen LB, et al. Hydrothermal liquefaction of *Spirulina* and *Nannochloropsis salina* under subcritical and supercritical water conditions. *Bioresource Technology*. 2013;131:413-9.
- [30] Toor SS, Rosendahl L, Rudolf A. Hydrothermal liquefaction of biomass: A review of subcritical water technologies. *Energy*. 2011;36:2328-42.
- [31] Zhou D, Zhang L, Zhang S, Fu H, Chen J. Hydrothermal Liquefaction of Macroalgae *Enteromorpha prolifera* to Bio-oil. *Energy & Fuels*. 2010;24:4054-61.
- [32] Alba LG, Torri C, Samori C, van der Spek J, Fabbri D, Kersten SRA, et al. Hydrothermal Treatment (HIT) of Microalgae: Evaluation of the Process As Conversion Method in an Algae Biorefinery Concept. *Energy & Fuels*. 2012;26:642-57.
- [33] Yu G, Zhang Y, Schideman L, Funk TL, Wang Z. Hydrothermal liquefaction of low lipid content microalgae into bio-crude oil. *Transactions of the ASABE*. 2011;54:239-46.
- [34] Nedelchev A, Stratiev D, Ivanov A, Stoilov G. BOILING POINT DISTRIBUTION OF CRUDE OILS BASED ON TBP AND ASTM D-86 DISTILLATION DATA. *Petroleum & Coal*. 2011;53.
- [35] Selvaratnam T, Pegallapati AK, Montelya F, Rodriguez G, Nirmalakhandan N, Van Voorhies W, et al. Evaluation of a thermo-tolerant acidophilic alga, *Galdieria sulphuraria*, for nutrient removal from urban wastewaters. *Bioresource Technology*. 2014;156:395-9.
- [36] Moscoso JLG, Obeid W, Kumar S, Hatcher PG. Flash Hydrolysis of Microalgae (*Scenedesmus* sp.) for Protein Extraction and Production of Biofuels Intermediates. *The Journal of Supercritical Fluids*. 2013;82:183-90.
- [37] Brewer CE, Schmidt-Rohr K, Satrio JA, Brown RC. Characterization of Biochar from Fast Pyrolysis and Gasification Systems. *Environmental Progress & Sustainable Energy*. 2009;28:386-96.
- [38] Sandau E, Sandau P, Pulz O. Heavy metal sorption by microalgae. *Acta Biotechnologica*. 1996;16:227-35.

589 [39] Libra JA. Hydrothermal carbonization of biomass residuals: a comparative review of the chemistry,
590 processes and applications of wet and dry pyrolysis. Biofuels. 2011;2:71-106.

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623 Figure 4: Water soluble organic compounds a) *Nannochloropsis sp.*, and b) *Chlorella sp.*

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626 Table 1. Biochemical properties of algal biomass

	<i>Nannochloropsis</i> sp.	<i>Chlorella</i> sp.
Water content (%)	79.6	74
Ash content (%)	7.0	2.50
Lipid content (%)	21.76	10.7
Crude protein (%)	14.26	44.62
Crude Carbohydrate (%)	56.97	42.88
HHV (MJ/kg)	25.0	24.3
pH of supernatant water	7.0	7.3

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630 Table 2. Higher heating values and energy calculations

	Temperature (°C)	180	200	225	250	275	300	330
<i>Nannochloropsis</i> sp.	Biocrude oil							
	Avg.	37.92	39.19	38.06	36.76	36.83	34.60	37.37
	S.D	1.07	0.92	0.31	1.33	0.88	0.00	1.03
	Bio-char							
	Avg.	19.21	18.50	18.55	21.17	17.97	13.65	12.54
	S.D	0.69	0.62	0.75	1.13	1.41	0.36	2.87
	Energy recovery (%)	71.99	73.18	74.14	76.36	72.20	78.39	65.25
	ECR (w/o heat recovery)	1.15	0.98	0.93	1.08	1.09	0.73	0.93
	ECR (with heat recovery)	0.68	0.57	0.53	0.61	0.61	0.41	0.51
<i>Chlorella</i> sp.	Biocrude oil							
	Avg.	36.45	37.30	37.38	36.13	34.37	37.74	36.78
	S.D	1.01	0.44	0.66	0.44	0.85	0.17	0.49
	Bio-char							
	Avg.	19.82	21.23	20.73	12.69	13.63	8.62	12.05
	S.D	1.70	1.33	1.66	1.13	0.62	1.25	2.34
	Energy recovery (%)	59.3	61.3	57.5	53.7	55.7	56.8	53.9
	ECR (w/o heat recovery)	2.23	1.77	1.23	1.00	0.99	0.98	1.17
	ECR (with heat recovery)	1.32	1.03	0.70	0.57	0.55	0.54	0.65

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Graphical abstract