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Title

Influence of process conditions on pretreatment of microalgae for protein extraction and the production of biocrude during hydrothermal liquefaction of pretreated *Tetraselmis* sp.

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Abstract:

The direct conversion of microalgae to advanced biofuels with hydrothermal liquefaction (HTL) is an attractive option which has drawn attention in recent years. Meanwhile, the presence of undesired heteroatoms in the resultant biocrude, heat input and the process water has been a long-term concern. In this study, the pretreatment of microalgae biomass for protein extraction was conducted prior to hydrothermal liquefaction (HTL) for biocrude production. The impact of operating conditions on both the pretreatment and hydrothermal liquefaction steps was investigated. Following HTL using the pretreated microalgae with an initial solid content of 16%w/w for 30min at 310^{o}C, the biocrude yield was 65wt%, which was more than a 50% improvement in yield as compared to HTL of untreated algae at the same reaction conditions. To achieve a similar biocrude yield using the untreated algae required a much higher reaction temperature of 350^{o}C. Using recycled process water as reaction media led to a 25wt% higher biocrude yield. HTL of pretreated algae led to 32-46%

nitrogen reduction in resultant biocrude. The biocrude had a higher heating value (HHV) of 28MJ/kg to 34MJ/kg. A maximum of 15wt% protein extract was obtained during microalgae pretreatment at 150^{o}C, 20min. A similar energy input was required in biocrude production from untreated route and the combined pretreatment and HTL

Keywords: Biocrude; Hydrothermal liquefaction; Microalgae pre-treatment; process water.

1. Introduction

Hydrothermal liquefaction is a promising technology for the production of biofuel as it provides a direct pathway in the conversion of biomass to biocrude[1]. Hydrothermal liquefaction (HTL) is typically operated at subcritical temperature and pressure, and with hot compressed water as both reaction media and catalyst. This process is suitable for the conversion of low energy-density biomass feedstocks with higher moisture content, such as microalgae. Importantly, HTL avoids the high energy cost associated with drying feedstocks that is normally required in other conversion processes such as pyrolysis and transesterification. In addition, the resultant biocrude could potentially be used directly in existing crude oil refineries.

Recently, there has been increased study of the application of HTL for biocrude production from microalgae. These previous reports have investigated effects of temperature, residence time, solid concentration, catalyst, solvent, and microalgae strain parameters on the yield and composition of HTL products[2-9]. It is clear from the above work that biocrude production is feasible irrespective of differing biochemical composition of individual species and almost complete conversion can be achieved with reaction times in the order of minutes. However, the resultant biocrude typically contains undesirable heteroatoms, especially nitrogen. According to previous work, the nitrogen content of biocrude typically ranges between 5%w/w to 8%w/w, as compared to 0%w/w to 1.1%w/w for petroleum crude[10]. Also the sulphur and oxygen content are in the range 0.3%w/w to 3%w/w and 5%w/w to 28%w/w, respectively. The amount of the heteroatoms in the biocrude prevents its use directly in convectional petroleum crude refineries[1]. Thus, further research to reduce the nitrogen content of biocrude produce from microalgae is necessary.

Most of these previous reports agreed that the high nitrogen content in biocrude was due to decomposition of the microalgae protein fractions into simpler nitrogenous compounds (e.g. amides, amino and fatty acids) through series of decarboxylation and deamination reactions during HTL. Based on previous research investigations, three methods have been proposed to

deal with the issue of high nitrogen content in biocrude: the use of heterogeneous catalysts during liquefaction[11,12]; downstream catalytic upgrading; and the pre-treatment of microalgae prior to liquefaction[2,4].

The catalytic liquefaction of microalgae *Chlorella vulgaris and Nannochloropsis* sp. with Pt/Al_2O_3, Ni/ Al_2O_3, Ce/HZSM-5, and $Co/Mo/ Al_2O_3$ has been investigated[11-15] and was shown to improve the yield and energy density of the resultant biocrude and also lower its sulphur content. However, the nitrogen content of the biocrude was up to 6%w/w and is considered as too high compared to that of petroleum crude.

The subsequent catalytic hydrotreating of HTL biocrude could in principle be useful to reduce the nitrogen content of biocrude. A review of the scientific literature found that the catalytic hydrotreating step is a simple process, essentially aimed to improve biocrude quality without alteration of the boiling point. However, the nitrogen content of biocrude produced in this manner is still relatively high (4.5%w/w to 6%w/w) after catalytic hydrotreating of resultant biocrude[16-18].

Thus, while these previous reports have so far demonstrated that catalytic liquefaction and catalytic hydrotreating leads to increased biocrude yield and enhanced fuel properties, neither of these processes appear to have been successful in achieving substantial reductions in nitrogen content. Besides, the use of catalysts in HTL would potentially be expensive due to the high cost of the catalyst in addition to problems such as sintering, dissolution and instability within the hydrothermal environment[19,20]. Additionally, there could be increased energy consumption and loss of valuable products e.g. protein for pharmaceutical use[2]. Thus, the commercial application of catalyst for hydrotreating and in HTL to achieve drop-in biocrude could be uneconomical.

Another proposed route to address the challenge of biocrude with high nitrogen content is the pretreatment of microalgae for the extraction of protein prior to liquefaction. The extraction of protein from biomass before further processing of the solid residue to biocrude would not only reduce the nitrogen content in biocrude but also improve the economics of HTL since the extracted protein is a potential value co-product in the pharmaceutical industry. Limited information is available on the extraction of protein from microalgae prior to liquefaction. However, some previous studies have investigated the extraction of protein from other types

of biomass[21-23]. These reports made use of mild to harsh extraction techniques to recover highly soluble proteins with good techno-functional properties. As a result, protein denaturation was not a problem since the methods used were for analytical purposes and primarily aimed to achieve the production of enzymatic protein hydrolysates. It is clear from this previous work that, firstly, the amount of extracted protein and the properties of the by-products strongly depend on reaction conditions during pre-treatment, and secondly, that no information is available on applying HTL to the solid residue since there was there was no link to biocrude production. It is envisaged that HTL of the solid residue (pre-treated microalgae) obtained after extraction could be valuable for the production of biocrude potentially with lower nitrogen content since much of the nitrogen associated with the protein fraction is extracted during the pre-treatment step.

A review of the scientific literature shows that few studies have been conducted in the extraction of pharmaceutical grade products (e.g. protein, polysaccharides, and lipids) prior to biocrude production from microalgae. Biller *et al*.[24] investigated the extraction of lipids from *Nannochloropsis occulata* using microwave before further processing of the resultant pretreated microalgae biomass residue to biocrude. Chakraborty *et al*.[25] investigated sequential steps for hydrothermal liquefaction of *Chlorella sorokiniana* by extraction of polysaccharides with subsequent biocrude production from the recovered biomass residue. They concluded that biocrude production is feasible after microalgae pre-treatment. Also the production system does not need additional energy input as compared with conventional liquefaction at a lower reaction temperature. However, it was found that important quantity (about 40%) of residual organic carbon is loss in the wastewater stream following pretreatment. Recovering the organic carbon from the process water is necessary, as it will improve biocrude yield and potentially lower carbon footprint of HTL-microalgae biofuels systems. Also there is increased development in the production of co-products simultaneously with biocrude via hydrothermal liquefaction of microalgae biomass. The successful integration of this technique for production of chemicals with simultaneous production of the primary product biocrude could improve the viability of HTL microalgae biofuels.

The aim of this paper is to investigate the influence of thermal pretreatment of algae on HTL, the product yields, and biocrude characteristics. Also the effect of employing the recycled pretreatment process water as a reaction media on the HTL product yield and properties was examined.

2. Materials

The hypersaline microalga *Tetraselmis* sp. was used in the present study. The *Tetraselmis* sp. was grown and cultivated in an outdoor open raceway pond owned and operated by Muradel Pty Ltd in Karratha, Australia. Commercial grade carbon dioxide, nitrate and phosphate were employed as nutrients during biomass culturing period. The other essential mineral elements for cultivation were source from natural seawater. The *Tetraselmis* sp. biomass was harvested by centrifugation after electrofocculation. The detail of cultivation, growth and harvesting has been reported elsewhere[26]. This present study (pretreatment and HTL) was conducted at the Aban Infrastructure Pvt Ltd, Biotechnology Division Pilot Plant in Chennai, India. To convey the harvested *Tetraselmis* sp. biomass to Chennai, the biomass was freeze-dried (Labcono FreeZone) at a temperature of -48^{o}C and pressure and 0.133 mBar (absolute), respectively. The biochemical and elemental compositions of the microalgae are shown in Table 1.

2.1 Methods

2.1.1. Pre-treatment

The microalgae biomass pretreatment and liquefaction steps were performed batch-wise in a custom made 1L high-pressure Inconel reactor (100mm × 160mm). Microalgae pre-treatment was performed isothermally at temperatures in the range 130^{o}C to 200^{o}C for a fixed reaction time of 20min with 16%w/w solid content. A microalgae slurry was prepared by adding 60g of freeze-dried *Tetraselmis* sp. (moisture content = 7wt%) to 300mL distilled water. Then the reactor was sealed and the slurry heated with an electrical band ceramic heater to the desired set-point temperature while stirring at 300rpm using an inbuilt specialized two bladed magnetic drive impeller type device made of Inconel. At the end of desired reaction time, the reactor was then cooled to room temperature. Depending on the predefined temperature, it took about 30min and 90min to attain set-point temperature and cooling to room temperature, respectively. After cooling, the reaction mixture was transferred to a separating funnel followed by filtration to recover the pretreated algae as the solid phase. The solid phase was oven dried at 40^{o}C, the resultant mass, labelled as “pretreated algae”, was determined using Eq. (1).

The filtrate was mixed with ethanol in the ratio 1:4 and vigorously agitated for about 5min. The mixed mixture was allowed to sit for 10-12 hours and then decanted to separate the upper and lower phases. A soluble protein extract is recovered from the lower phase using ethanol extraction following previous method[27]. The lower phase was again mixed with ethanol

(0.5vol/vol) and vigorously agitated for about 5min. The remixed mixture was allowed to settle and the lower phase was decanted. This step was then repeated up to four times, or until the lower phase became clear in colour. The lower phase from the final ethanol extraction step was then oven dried at 50-70^{o}C. The mass yield of the resultant solid, labelled as "Protein Extract", was determined also using Eq. (1). The upper phases (ethanol wastewater mixture) remaining after each ethanol extraction stage (above) are combined. Ethanol is recovered from this mixture using vacuum distillation at ~80^{o}C. A fraction of the wastewater was oven dried at 100^{o}C. The mass yield of the wastewater was estimated by relating the resultant dried fraction to the mass of feed.

$$Y_i = \frac{M_i}{M_f} \times 100\% \quad (1)$$

where Y_i is the yield of the *ith* fraction, *i* is the pretreated algae, protein extract or process water, M is mass, and *f* is original feed.

2.1.2. Hydrothermal liquefaction

For hydrothermal liquefaction, the recovered pretreated algae were diluted to ~16%w/w solid concentration with deionised water or the recycled process water. HTL experiments were performed at 310 and 350^{o}C, for either 5 or 30min. We followed the HTL operational and separation procedures as described in previous work[6]. In each HTL experiment, duplicate runs were performed and average yield reported. The list of separate treatments with experimental variables used is presented in Table 2.

The gravimetric yields (wt%) of biocrude, residual char and aqueous phase (precipitate) were determined by relating the weight of the product to the initial mass of microalgae loaded to the reactor. The biocrude yield was estimated on ash free dry basis. The gas phase yield was determined by difference using the calculated yields of the remaining fractions.

2.2. Analysis

Dried fractions of pretreated algae, process water, protein extract, residual char, aqueous phase and the liquid biocrude were analysed for elemental composition (carbon, hydrogen, nitrogen, and sulphur) according to ASTM D-5291 method using a VarioEL III Elemental Analyser System GmbH. The oxygen content was determined by difference. The elemental

composition data was used to calculate the higher heating value (HHV) using the unified correlation (Eq. 2) proposed by Chinnawala and Parikh[28].

$$HHV\ (MJ/kg) = 0.3491C + 1.1783H + 0.1005S - 0.1034O - 0.0151N - 0.0211A \quad (2)$$

where C H N S O and A represents the mass of carbon, hydrogen, nitrogen, oxygen, sulphur and ash, on a dry weight basis.

The dried fractions were also analysed for their functional groups and structure with Fourier Transform Infrared Spectroscopy (FT-IR) (Nicolet 6700, Thermo scientific) according to the method explained by Duan and Savage[12]. The FT-IR spectra were assigned with reference to scientific literature, for example[16,29-31].

The micrographs of initial microalgae biomass and the product fractions (in powder form) were inspected with Philips XL 30 FEG scanning electron microscopy (SEM). This allows a high-resolution of samples, providing more details that are undetected using a convectional optical microscopy. A fraction of the powder samples were coated with platinum after being placed in a sticky and aluminium stab, before being placed in the SEM. The metallic compositions of dried fraction were analyzed by Inductively Couple Plasma-Mass Spectrometry (ICP-MS), Agilent 7500 series. About 5g of dried sample were prepared by wet digestion in nitric acid, followed by diluting into different folds with reverse osmosis water.

The chemical composition of the biocrude was determined using an Agilent 6890N series GC-MS coupled with a HP5-MS column (length: 30m, internal diameter: 0.25mm, and film thickness: 0.25μm). The GC-MS analyses were performed according to the method described by Jena *et al.*[7]. The biocrude sample size was 1μL, prepared by diluting to 2.5%v/v with acetone. The injector temperature was 200^{o}C, oven temperature 50^{o}C to 250^{o}C at 10^{o}C/min, carrier gas was HP helium, flow rate: 1mL/min, GC interface temperature: 250^{o}C, mass spectrometer temperature: 250^{o}C, scan range: 50 to 600amu, detector: photon multiplier, ionisation method: electron impact ionisation. The chemical composition of the biocrude was identified with the mass spectral library of National Institute of Standards and Technology's 1998 version (NIST 98).

The carbon and nitrogen balance in the pretreated algae, biocrude, residual char and aqueous phase was calculated by using the mass balances across the product. The carbon and nitrogen

balance in the gas phase plus losses was obtained by mass difference using the estimated recovery of remaining elements[32].

The percentage energy recovery (ER%) in individual product fractions from initial feedstock were calculated using Eq. (3). The energy recovery does not include the external energy input for heating the reactor.

$$ER = \frac{M_i \times HHV_i}{M_f \times HHV_f} \times 100\% \quad (3)$$

where M is mass (g), HHV is the higher heating value (MJ/kg), i represent biocrude, residual char or aqueous phase and f the initial algae feed. Assuming the initial microalgae biomass slurry loaded into the reactor was 80%w/w with 20%w/w dried microalgae. The reactor being insulated, the heat capacity of dry microalgae biomass was assumed to be half of that of water[4,33]. The heat input (Eq. 4) required to produce a unit of biocrude from untreated algae, by heating from room temperature to predefined temperature with no vaporization was estimated using the enthalpies of saturated liquids (h_f). Similarly, the heat input for HTL of pretreated algae (E_{LP}) was determined, but after substituting M_{dw} and M_{ua} with M_{pw} and M_{pa}, respectively.

$$E_L \text{ (MJ/kg)} = \frac{\Delta h_f \times M_{dw} + 0.5 \times \Delta h_f \times M_{ua}}{M_b} \quad (4)$$

where Δh_f is the change in enthalpies of water at T_1 base temperature, assumed to be ~28^{o}C and T_2 predefined temperature. M_{dw}, M_{ua}, M_{pw}, M_{pa}, and M_b are the mass (kg) of deionised water, untreated algae, process water, pretreated algae and biocrude, respectively.

Assuming heat loss to environment and container is negligible, the heat input for vacuum distillation (E_{VD}) of ethanol from the mixture of ethanol and water was determined using Eq. (5).

$$E_{VD} = \frac{((M_e \times C_{pe} \times \Delta T) + (M_e \times HVAP_e \times \Delta T) + (M_w \times C_{pw} \times \Delta T) + (M_{wd} \times HVAP_w \times \Delta T))}{M_m} \quad (5)$$

where M_e, M_w is the mass (kg) of ethanol and water respectively, M_{wd} is the mass (Kg) of water in the distilled liquid (mass of water distilled alongside the ethanol), M_m the mass of (kg) ethanol-water mixture, ΔT is the temperature difference, C_{pe} and C_{pw} is the specific heat capacity of ethanol (2.3KJ/kg/k) and water (4.2KJ/kg/k), respectively, $HVAP_e$ the latent heat of vaporization for ethanol (841KJ/kg), and $HVAP_w$ for water (2260MJ/kg). In principle, an ethanol-water solution forms an azetrope at 78.2^{o}C at standard atmospheric pressure[34]. The boiling point of ethanol is 78.4^{o}C, and 100^{o}C for water but the azetrope boils at 78.2^{o}C,

which is lower than that of either ethanol or water[35]. Also the distilled liquid from a mixture of ethanol-water contains 95.6% ethanol and 4.4% water. For example, assuming the total amount of ethanol-water mixture for vacuum distillation was 5kg, containing 80% ethanol (4kg) and 20% water (1kg). Then T_1 = 28°C (room temperature), T_2 = 78.2°C (azetrope boiling point), ΔT = 50.2°C. 4.4% water of 4kg ethanol = 0.176kg = M_{wd}. The amount of water remaining = 1kg – 0.176kg = 0.824kg, then the total mixture evaporated = 4kg ethanol + 0.824kg water.

For ethanol:

Heat input for temperature rise = $M_e \times C_{pe} \times \Delta T$ = 4kg × 2.3KJ/kg/k × 50.2°C = 461.84KJ

Heat input for vaporization = $M_e \times HVAP_e \times \Delta T$ = 4kg × 841KJ/kg/k = 3364KJ

Similarly for water:

Heat input for temperature rise = $M_w \times C_{pw} \times \Delta T$ = 1kg × 4.2KJ/kg/k × 50.2°C = 210.84KJ

Heat input for vaporization = $M_{wd} \times HVAP_w \times \Delta T$ = 0.176kg × 2260KJ/kg/k = 397.76KJ

Total amount of heat input = 461.84KJ + 3364KJ + 210.84KJ + 397.76KJ = 4434.44KJ/kg

Therefore, the E_{VD} required to evaporate 5kg of ethanol-water solution = 4434.44KJ/5kg = 0.8868MJ/kg.

The energy consumption ratio (ECR) for processing untreated algae was calculated with Eq (6a), while that for the combined process including pretreatment (E_P), vacuum distillation of ethanol (E_{VD}) and liquefaction of pretreated algae (E_{LP}) was calculated with the Eq. (6b).

$$ECR = \frac{E_L}{E_{OUT}} \quad (6a)$$

$$ECR = \frac{E_{LP} + E_P + E_{VD}}{E_{OUT}} \quad (6b)$$

where E_L , $E_{LP,}$ E_P, E_{VD} is the amount of heat input for liquefaction of untreated algae, liquefaction of pretreated algae, pretreatment and vacuum distillation, respectively, E_{OUT} energy produced from the biocrude (MJ/kg biocrude). An ECR greater than 1 suggest that the process consumes more energy than it produces while a ratio <1 means that a net energy producer[36]. If equal to unity indicates that same amount of heat is used for liquefaction as is produced from the biocrude[4].

3. Result and Discussion

3.1. Algae Pre-treatment

The mass yields of pretreated algae, protein extract and process water following pretreatment at 130, 150, 170 and 200^{o}C are shown in Figure 1. The yield of pretreated algae varies in the range 54-65wt% and decreases with increasing pretreatment temperature. The combined yield of the remaining phases (proteins extract and process water) varies in the range 28wt% to 39wt%. The yield of protein extract varies in the range 4wt% to 15wt%, with the maximum yield observed for a pretreatment temperature of 150^{o}C. The decrease in the pretreated algae is mostly due to the increase breakdown of the algae cells into water soluble products, which also led to an increase in process water. Also the lower protein extract at 130^{o}C could be that the pretreatment temperature was not high enough to break down the algae cell, while the decreasing extract after 150^{o}C could be due to formation of new products. The slight increase in the process water with an increase in pretreatment temperature could be mostly due to the increase in the algae cell intracellular metabolites such as lipids that could not precipitate along the protein extract phase. It should be noted that a portion of the initial mass of microalgae remains unaccounted for following pretreatment. This mass loss could be due to the production of gases (such as CO_2) as a result of decarboxylation reactions during pretreatment, and is labelled as the gas phase in Figure 1. This amount is in the range 6wt% to 10wt% and is independent of the pretreatment temperature. This could relate to the original moisture content of the feed or loss of volatiles, which was more pronounced at pretreatment temperature of 200^{o}C.

The carbon recovery and nitrogen recovery in the pretreated algae is presented in Figure 2. It was found that there was general reduction in each of the element with an increase in the pretreatment temperature. The amount of carbon and nitrogen obtained after pretreatment were 59-95% and 54-88%, respectively. This suggests that up to 41% carbon and 46% nitrogen is fractionated in other product fractions such as protein extract and process water. At the optimum pretreatment temperature (based on maximum yield of protein extract and recovered pretreated algae) about 85% of the carbon was recovered while the nitrogen content in the pretreated algae was reduced by 33%. The pretreatment of microalgae biomass resulted in structural changes to the microalgae biomass, as shown in Figure 3. These modifications led to the fractionation of carbon and nitrogen in protein extracts, process water and gas phase. These structural changes could improve the quality of the pretreated algae as a feedstock for biocrude production via HTL. Based on the data in Figure 2 these changes were

higher at the pretreatment temperature of 200^{o}C, but led to undesired low carbon recovery in the pretreated algae. This suggests that operating at 200^{o}C is unsuitable to pretreat microalgae biomass.

The micrographs of untreated and pretreated algae are presented in Figure 3. The micrographs of the pretreated algae for different pretreatment conditions appear similar; hence, only one is presented and compared with that for the untreated algae. As shown in Figure 3 the untreated algae cells appear to be highly clustered, however, the cells seem disrupted after pre-treatment. This cell disruption reaffirmed the fractionation of microalgae components during pretreatment, thus a substantial impact on recovered products. It is also noted that the microalgae is not a monoculture as Figure. 3b revealed the presence of diatoms.

The FT-IR spectra of the untreated and pretreated algae biomass is illustrated in Figure 4. As shown in Figure 4, there were no substantial changes in the peak and wave-numbers of the untreated and pretreated algae. One of the revelations is the wider transmittance in the peak 1200cm^{-1} to 800cm^{-1}, which represent the C-O bonds, O-H phenoxy structures, aliphatic ester, sulphonic acid and aromatic substituted banzenes. These compounds such as phenoxy are undesired nitrogenated (N) compounds because they lead to higher nitrogen content in biocrude following liquefaction. This finding suggests that the undesired N-compounds could have been reduced during algae pretreatment. Also the similar peak and wave-numbers suggests that the pretreated algae still contain similar functional groups to that of the untreated algae. It could be inferred that the functional group of the pretreated algae is unaffected following pretreatment. For the process water, there were changes in peak 2900 cm^{-1} to 2800cm^{-1}, 1550cm^{-1} to 550 cm^{-1} compared to either untreated or pretreated algae. These changes could be due to new products formed, as a result of the interactions among the algae components during pretreatment. Moreover, the similarity in the process water wavenumbers to that of the algae (untreated or pretreated) suggests that the obtained process water could relatively still contain important dissolved organic compounds such as hydrocarbons. Therefore employing the process water as HTL reaction media could enhance biocrude yield.

The data from the elemental and ICP-MS analysis of the process water is presented in Table 3. The ICP-MS data revealed presence of dissolved trace elements of vary concentration. The

elemental analysis of the process water show the presence of residual organic carbon up to 32%, which is believed to have been decomposed from the initial microalgae biomass during pretreatment. It is therefore envisaged that recycling the process water as HTL reaction media will influence product yields and distribution, particularly biocrude yield. The reuse of the process water to mix/dilute the pretreated algae is to take advantage of the added benefits of the dissolved organics, thus a potential means to improve the carbon efficiency of HTL-microalgae-biocrude. Also it will avoid employing freshwater for liquefaction of pretreated algae, thus important to water conservation. Although recycling the process water to algae cultivation pond will be useful in cultivating additional microalgae biomass as suggested by previous report[24]. But it will be of more benefit to be initially employed as hydrothermal media, recovering loss residual organic carbon. The essential nutrient for cultivation will be eventually recovered in the final aqueous phase. In addition, employing the process water is a potential means to reduce the organic and toxicity that has been identified to adversely inhibit microalgae growth during cultivation[37,38].

From observation, the colour of the protein extract was whitish. An increase in pretreatment temperature led to changes in colour for the pretreated algae and the process water. The pretreated algae colour slightly changed from green to dark green with an increase in the pretreatment temperature. At 200^{o}C, a darker green colour was observed with pretreated algae, which could be mostly due to hydrophobic peptides or Maillard reaction. For the filtrate colour, if was light green, which was similar to that observed when untreated algae was mixed deionised water and stirred for 20min at room temperature. This suggests that the temperature below 130^{o}C has no substantial effect on disruption of the algae molecule. The filtrate colour became light yellow, light brown and ember at 150^{o}C, 170^{o}C and 200^{o}C, respectively.

After complete cycle, about 85% of the employed ethanol was recovered following distillation, which can be reused after purification. The remaining fraction could have been lost as vapour during distillation. In order to avoid error, fresh ethanol was used for each experiment.

The previous section has clearly demonstrated the feasibility to extract protein from microalgae prior to liquefaction; effects of the pre-treatment conditions in the composition and structural changes of product yields and properties compared to the untreated algae. The

effect of reaction conditions on HTL products yield and properties, particularly biocrude during liquefaction of the pretreated algae is discussed in the next section.

3.2. HTL Product Yields

The yield in biocrude, residual char, aqueous and gas phases during processing of pretreated algae is presented in Figure 5. Due to the recycled process water having residual carbon led to positive effect on biocrude yields. As shown in Figure 5a biocrude yield from pretreated algae with the process water were generally higher than that from pretreated algae with deionised water. The HTL of pretreated algae with recycled process water led to additional 25wt% biocrude yield at 350^{o}C. Also at this condition, the residual char, aqueous and gas phase yields were lower with 10wt%, and 2.5wt% and 38wt%, respectively, than that obtained without recycled process water (i.e. using deionised water). It is therefore imperative to recycle the process water for liquefaction as it favours more biocrude yields.

However, employing the recycled process water with pretreated algae led to lower biocrude yields compared to that from HTL of untreated microalgae biomass at 350^{o}C, shown in Figure 5b. The biocrude yields from the untreated algae were generally higher with 10-16wt%, lower with about 6wt% in gas phase. Similar yields in residual char and aqueous phases were obtained at same condition. The slight variations in product yields from among pretreated algae (shown in Figure 5b) were simply the manifestation of amount of pretreated algae during pre-treatment (Figure 1). This finding suggests that: processing of pretreated algae at high temperatures (350^{o}C) simply favours gasification; and an intact cell may require more energy than an already hydrolysed algae cell. The difference in biocrude yields obtained from the pretreated and untreated algae at 350^{o}C was in agreement with previous reports. For example, a 10wt% less biocrude yield was reported by Zou *et al*.[39] after HTL of pretreated *Dunaliella tertiolecta* compared to processing of untreated *D. tertiolecta*[40]. Vardon *et al*.[41] reported 31wt% biocrude yield from defatted *Scenedesmus* sp. compared to 45wt% obtained from non-lipid extracted *Scenedesmus* sp. These studies were mostly conducted at high reaction temperature (360^{o}C), hence, the lower yields in biocrude from pretreated microalgae compared to untreated algae.

At low reaction temperature (310^{o}C), there was no substantial difference in biocrude yield from liquefaction of pretreated algae compared with that from untreated algae processed at much higher temperature. Processing of pretreated algae at 310^{o}C, led to ~65wt% biocrude

yield similar to 68wt% obtained from untreated microalgae at 350°C. The difference in biocrude yield was only about 3wt%, which reaffirms that higher reaction temperature may not be suitable for HTL of pretreated algae. Moreover, the biocrude yield from pretreated algae at 310°C (150WRL) was 23.6wt% higher than that obtained from untreated algae at same condition (310NPL). Lower aqueous phase of 15.8wt% and 10.1wt% in gas phase with similar residual char yields was obtained at same condition.

In comparison with previous research investigation, the biocrude yield obtained in this study was within range compared to yields from catalysed, un-catalysed HTL and microalgae pretreatment prior to liquefaction. At optimum condition, the biocrude yields obtained in this study was relatively higher than the 50wt% biocrude yield from *Chlorella. pyrenoidsa* at 300°C, 20min using HZSM-5 catalyst[14], higher than 46wt% derived from *Chlorella pyrenoidosa* at 280°C, 120min using heterogeneous catalyst (Pd/Al_2O_3)[15], higher than 57wt% from *Nannochloropsis* sp. at 350°C, 60min with heterogeneous catalyst (Pd/C, Pt/C, $NiSiO_2$, $Co/Mo/\gamma\text{-}Al_2O_3$, Zeolite)[12] but lower than 82wt% from *Chlorella* sp. at 220°C, 90min[40]. The biocrude yield was also found higher than ~24wt% to 49.5wt% from *Nannochloropsis oc*., *Chlorogloeopsis fritschii* and *Pseudochoricystis ellipsoidea* obtained at 300°C, 15min[24] and also higher than 30wt% to 45wt% from *Chlorella sorokiniana* and *Scenedesmus* sp. at 240°C to 300°C in previous reports[12,43] following pretreatment of microalgae.

Furthermore, the residual char obtained from HTL of pretreated and untreated algae were fairly constant. Although, lowest residual char (3.3wt%) was obtained from pretreated algae without recycled process water at 350°C (Figure 5a) which contain some inorganics such as salt (Table 3). At 310°C, the aqueous phases from liquefaction of pretreated algae were 3.3wt% to 13.7wt% lower compared to 16wt% to 22.7wt% for untreated algae at same reaction condition. At higher reaction temperature (350°C), the yields in gas phase (10wt% to 22wt%) from HTL of pretreated algae was generally higher than those from untreated algae. However, at lower process temperature, the gas phase yield from pretreated algae was ~12wt% lower than the 22wt% from untreated algae. This finding again reaffirms that liquefaction of pretreated algae at high temperature appear to favour gasification, thus much lower reaction temperature is required for the processing of pretreated algae.

This present study has shown that high yields in biocrude could still be produced from pretreated algae, but more favourable at a lower temperature. Application of the recycled

process water as reaction media appear to increase biocrude yields, suggesting that higher concentration of organic carbon in the process enhances biocrude yields. If the pretreated algae are processed at high temperature as normally required for conventional liquefaction, would lead to ~10wt% to 16wt% reduced biocrude yields. It is therefore concluded that biocrude yields similar to that obtained from untreated algae at high operating temperature (350^{o}C) can be produced at much lower temperature (310^{o}C) from pretreated algae. This is encouraging as producing maximum biocrude at lower reaction temperature will economically favour the envisaged HTL commercialization. Decreasing liquefaction reaction temperature from 350^{o}C to 300^{o}C will reduced the energy consumption by 22% and only by 3wt% the yield of biocrude[11]. In addition Akhtar and Amin[44] reported that high operating temperature is usually unsuitable for the production of biocrude in terms of operational cost and biocrude yield. Moreover, the cultivation and harvesting of microalgae biomass for biofuels production is relatively still too expensive[3,45,46]. Therefore producing valuable co-products alongside the primary product biocrude at lower temperature and the use of waste products could improve the life cycle assessment of HTL microalgae biofuels.

3.3. Biocrude analysis

A dark biocrude was produced from both pretreated and untreated algae, but a lighter; more volatile and less viscous biocrude was obtained from the former. This could suggest the elimination of some of the fatty acids and nitrogenous compounds or the formation of new compounds in the biocrude. There was no much difference in the biocrude HHV produced from both routes. The higher heating values were 32.1MJ/kg to 34MJ/kg and 28MJ/kg to 32.3MJ/kg with H/C ratio of 1.03-1.41 and 1.3-1.57 for biocrude obtained from untreated algae biomass and pretreated algae, respectively.

3.3.1. Elemental composition of biocrude

The elemental composition of biocrude produced at different operating condition is shown in Figure 6. There were some slight variation in carbon and nitrogen content of the biocrudes. The carbon content was 66%w/w to 70%w/w and 68%w/w to 72%w/w for biocrude from pretreated and untreated algae, respectively. It was found that the biocrude carbon and nitrogen content was lower in experiments involving non application of recycled process water (Figure 6a) at 350^{o}C. At high process temperature (350^{o}C) (Figure 6b), the nitrogen content was generally lower in biocrudes obtained from pretreated algae than untreated algae. But there were no substantial difference in nitrogen content at lower reaction temperature

(310^{o}C). This was expected and thus signified the importance of the pretreatment step prior to liquefaction. During pretreatment, hydrolysis of the microalgae led to reduction in protein fraction which was recovered as protein extract (Figure 1). At the initial hydrolysis of microalgae, it is inferred that some protein fraction can decomposed to N-compounds such as amines, amino acid[4,24,27] via decarboxylation and deamintaion reactions[47], leading to lower nitrogen content in biocrude (Figure 6). This finding confirms with our previous report[6] that low nitrogen content biocrude could be produced from liquefaction of pretreated algae. However, the level of nitrogen in resultant biocrude is unsuitable to be directly applied as transportation fuel. For transportation fuel, further upgrading of the biocrude is required or blended with petroleum crude. Also, it was also found that the nitrogen content in biocrude obtained at lower reaction temperature were relatively lower compared to those obtained at high process temperature The increase in nitrogen content at higher reaction temperature could be due to an increase in biocrude yield from the increase decomposition of protein fractions. But a trade-off of increase nitrogen content in biocrude. This find is in agreement with previous reports reporting increase in nitrogen content. For example, Alba *et al.*[2] that reported an increase in nitrogen content of 0.4%w/w to 6.5%w/w at 175^{o}C to 450^{o}C during the liquefaction of *Desmodesmus* sp. Jazwari *et al*.[48] reported increase in nitrogen content from 2.6%w/w to 7.7%w/w with an increase in process temperature of 250^{o}C to 350^{o}C.

3.3.2. FT-IR characterization of biocrude

The FT-IR spectra resultant biocrude is illustrated in Figure 7. Only one each of the FT-IR spectra for biocrude from pretreated and untreated algae is presented. As presented in Figure 7, the FT-IR spectral of both biocrude obtained from pretreated algae and untreated algae appear similar. This suggesting biocrude from pretreated algae has similar functional groups to that of untreated algae. However, the composition of the biocrude from pretreated and untreated algae may differ due to the difference in transmittance of the biocrudes. The use of GC-MS may provide more detail, which will be discussed later. One of the main features in the FT-IR spectra of both biocrudes is the absence of major peaks in 3500cm^{-1} to 3000cm^{-1} wavenumbers, initially exhibited by the spectra of initial and pretreated algae (Figure 3). This major peak absence shows scission of protein derivatives such as amine[29]. Both the FT-IR spectra of biocrude from pre-treated and untreated algae display sharp peaks between 3000cm^{-1} to 2800cm^{-1}, suggesting higher content of C-H stretching vibration of methylene group[49]. The band initially present at 2926.25cm^{-1} in the biocrude from initial microalgae shifted towards a lower wavenumber of 2920.32cm^{-1} in biocrude produced from pretreated

algae, which suggests the formation of new compound[50]. Similarly, the shift in peak 1700cm^{-1} to 1600cm^{-1} representing C=O group stretching vibration in carboxylic acids. The peak originally present at 1701.32cm^{-1} in untreated algae biocrude shifted to 1666.49cm^{-1} with much lower intensity. The biocrude produced from pretreated algae show a proportionately lower transmittance in this wavenumbers. The peak located at 1451.19cm^{-1} to 1258.05cm^{-1} shows the presence of CH_2 and CH_3C-O bending vibrations, and possibly with some aromatic rings[30,31]. The weak peaks between 1163.06cm^{-1} to 966.75cm^{1} strongly suggests C-O bonds, O-H in phenoxy structures, aliphatic esters and sulphonic acid. The peaks detected at 887.60cm^{-1} to 694cm^{-1} wavenumbers shows the presence of aromatic substituted benzenes[31]. Since both biocrude show relatively similar peaks, it reaffirms that the biocrude from both route still had similar functional groups as determined by the FT-IR. Therefore, pretreatment of microalgae lead to no negative influence, but rather to enhance the quality of resultant biocrude.

In comparison, previous reports[16,47,51] reported differences in intensities of FT-IR spectra among the produced and catalytically upgraded biocrudes. This was attributed to improved quality/properties such as reduced carboxylic acids, nitrogen content in upgraded biocrude. Similar phenomenon was observed in the present study, thus, it reaffirmed that microalga pretreatment led to improved quality biocrude. Nevertheless, the resultant biocrude still contains some heteroatoms, suggesting that pretreatment route does not totally remove heteroatoms such as nitrogen in the biocrude. Hence, further upgrading of the biocrude is required before direct utilization as transportation fuels.

3.3.3. GC-MS analysis of biocrude.

The data obtained from the GC-MS analysis of the biocrudes are presented in Table 4. As shown in Table 4, the GC-MS results revealed presence of complex compounds, mainly containing long-chain fatty acids, alcohols, nitrogenated compounds and some alkane hydrocarbons. The N-containing compounds such as pyrrole, indole, phenol, egtazic, are typical protein derivatives obtained through series of decarboxylation and deamination reactions[4,47] during liquefaction. However, these N-compounds were below detection level in the biocrude derived from pretreated algae. This suggests that the protein that leads to the formation of these compounds could have been removed during the pretreatment step. The biocrude produced from untreated algae contained heavier molecular weight compounds unlike that from pretreated algae. The constituents of the biocrude derived from pretreated

algae were simpler compared to untreated algae biocrude but still high in long-chain fatty acids. The presence of long-chain fatty acids could due to the reactions of fatty acids and ammonia released from the pretreated algae. Nevertheless, the long-chain fatty acids can be catalytically converted to hydrocarbons. In summary, it could be inferred that the biocrude obtained from pretreated algae was of better quality due to the reduced amount of the N-compounds.

3.4 Nitrogen recovery in biocrude, solid residue, aqueous and gas phases

The fate of nitrogen and its recovery in HTL products fractions is important as it significantly affects product quality[32,52,53]. For example, it is desirable for biocrude to be low in nitrogen in order to reduce NO_x emission during combustion[46,54]. The nitrogen recovery (NR) in biocrude, residual char, aqueous and gas phases from liquefaction of Recovered Algae and untreated microalgae is presented in Figure 8. As shown in Figure 8, there was general lower nitrogen distribution in biocrude obtained from pretreated algae than untreated algae. The NR were 15.4% to 33.7% and 28.6% to 49.6% in biocrude derived from pretreated and untreated algae, respectively. This suggests that 32% to 46% of nitrogen was reduced in biocrudes derived from pretreated algae.

The nitrogen recovery (NR) in the residual char was 3.6% to 7.2% and 3.8% to 6.2% from HTL of pretreated algae and untreated algae, respectively. The NR in aqueous phase varies in 3.8% to 22.4% for pretreated algae and 12.2% to 21.2% for untreated algae. This finding confirmed with earlier suggestion of still recovering nutrient such as nitrogen in the aqueous phase if the recycled process water is employed as a reaction media. The recovery of nitrogen in the aqueous phase is important as it facilitate nutrient recycling[2,8] for cultivation of additional microalgae biomass[37,38]. The NR in the gas phases from HTL of pretreated and untreated algae was 40.3% to 76.7% and ~53%, respectively, similar to 20% to 70% at 200^{o}C to 450^{o}C, 60min reported by Alba *et al.*[2].

3.5 Energy Analysis

The heat input and output applied for the pre-treatment and liquefaction of pretreated and untreated algae is presented in Table 5. It was found that the pretreatment step required an energy input of 2.95MJ/kg to 3.7MJ/kg. At optimum conditions, the heat-input for HTL of untreated algae was 10.28MJ/kg at 350^{o}C and 13.92MJ/kg for pretreated algae at 310^{o}C. The heat input was found to be similar to 14MJ/kg obtained for liquefaction of *Spirulina* sp.[54].

The differences in energy values were predominantly due to the variation in biocrude yield and higher heating value. It was found that the combined heat load of 13.81MJ/kg for microalgae pre-treatment at 150^{o}C and liquefaction at 310^{o}C was similar (13.92MJ/kg) to that required for only HTL of untreated algae at same reaction condition.

Considering total heat-input for both downstream productions of microalgae biomass and in HTL of biomass, shows the possibility of net energy production. As shown in Table 5, the energy consumption ratios of 0.3 to 0.66 suggests that processing pretreated and untreated algae could produce net renewable fuels. The ECR values were in range to those reported by Sawayama *et al*.[9], but were considerably lower than those in some previous reports[4,7]. The ECR difference is predominantly due to varying biocrude yields, heating values and process temperatures. It also suggests that a favourable ECR at lower temperature is recommended based on energy consumption. In conclusion, pretreatment at 150^{o}C with subsequent liquefaction of pretreated algae at 310^{o}C with recycled process water appear more suitable for a biorefinery concept. Operating at 350^{o}C, 5min was found as the optimum condition based on biocrude yield and HHV for convectional liquefaction of untreated algae.

Furthermore, the ER in biocrude from pretreated algae at 310^{o}C (150WRL) was 54.9% and higher than 37.3% derived from untreated algae at the same reaction condition. However, it was ~ 10% less compared to that obtained from untreated algae at 350^{o}C (350NP). The ER difference was mainly due to the slight <3wt% less biocrude yield. However, the ECR could still be improved if higher biocrude yields are obtained at lower liquefaction temperature. The chemical energy recovered in the biocrude was found to be similar to that of previous investigations reporting ER[2,4,5,55]. Alba *et al*.[2] reported 11% to 75% ER during HTL of *Desmodesmus* sp. at 175^{o}C to 450^{o}C, 5and 60min. Biller and Ross[4] operating at 350^{O}C, 60min reported an ER of 50.7% for *Spirulina* sp., 51.6% for *Porphyridium* sp., 54.2% for *Chlorella* sp. and 66% for *Nannochloropsis* sp. Brown *et al*.[5] obtained 55-90% ER from *Nannochloropsis* sp. at 200^{o}C to 500^{o}C, 60min while 56-90% ER was reported by Jena *et al*.[54] for S*pirulina* sp. at 350^{o}C, 60min. This suggests that the pretreatment of microalgae had no negative effect on the energy recovery in biocrude rather a potential means to improve product yields, properties and the viability of HTL microalgae biofuels

The ER and HHV distributed to the residual char and aqueous phases following HTL of pretreated and untreated algae is presented in Figure 9. As shown in Figure 9, the ER and

HHV of residual char and aqueous phases obtained from liquefaction of pretreated algae were generally lower compared to that from untreated algae. The lower ER and HHV data could be mostly due to the energy loss during the initial pretreatment process. In summary, the total amount of energy recovered in biocrude, residual char and aqueous phase was less than 100%, the remaining is believed to have been fractionated to the gas phase. It was found that the combined ER for biocrude, residual char and aqueous phases obtained from pretreated algae was lower than that from untreated algae. The trade-off here is the improved quality biocrude that will require less amount of catalyst (and hence cost) during refining to transportation fuels and the revenue from the extracts. The energy balance can still be improved with a continuous reactor. Though no study has compared biocrude yields from continuous and batch reactors, it is generally believed that the former is more efficient mostly due to heat exchange systems, far less heating and cooling periods leading to reduce energy consumption and higher biocrude yields[48,56], although the pumping of feedstock still remains a challenge.

Although, an improved quality biocrude was obtained from pretreated algae, an important long-term concern is the high heat input for algae cultivation and harvesting, which in most cases were more than that required for the combined process route (Table 5). For a viable and sustainable production of biofuels and chemicals from algae, developing advanced technology that requires less amount of heat input for downstream processing (cultivation and harvesting) of algae is necessary. In addition, the extractability of valuable pharmaceutical grade chemicals from the original biocrude prior to refining to transportation fuels may be interesting.

4. Conclusion

This study has demonstrated the feasibility of producing biocrude low in nitrogen content from liquefaction of pre-treated algae. The pre-treatment of algae led to the lowest nitrogen content in resultant biocrude. The pretreated algae do not require high reaction temperature to achieve high yield in biocrude as normally applied in liquefaction of untreated algae. Recycling of process water from the pre-treatment step generally improved the biocrude yield. Though, the pre-treatment process did not totally eliminate the nitrogen content in the resultant biocrude, it significantly reduced 32% to 46% nitrogen content. This suggests that further reduction of protein fraction during pretreatment could reduce more nitrogen content

in the biocrude. Therefore further optimization studies might be necessary, but caution needs to be adhered as this could have negative effect with expected result.

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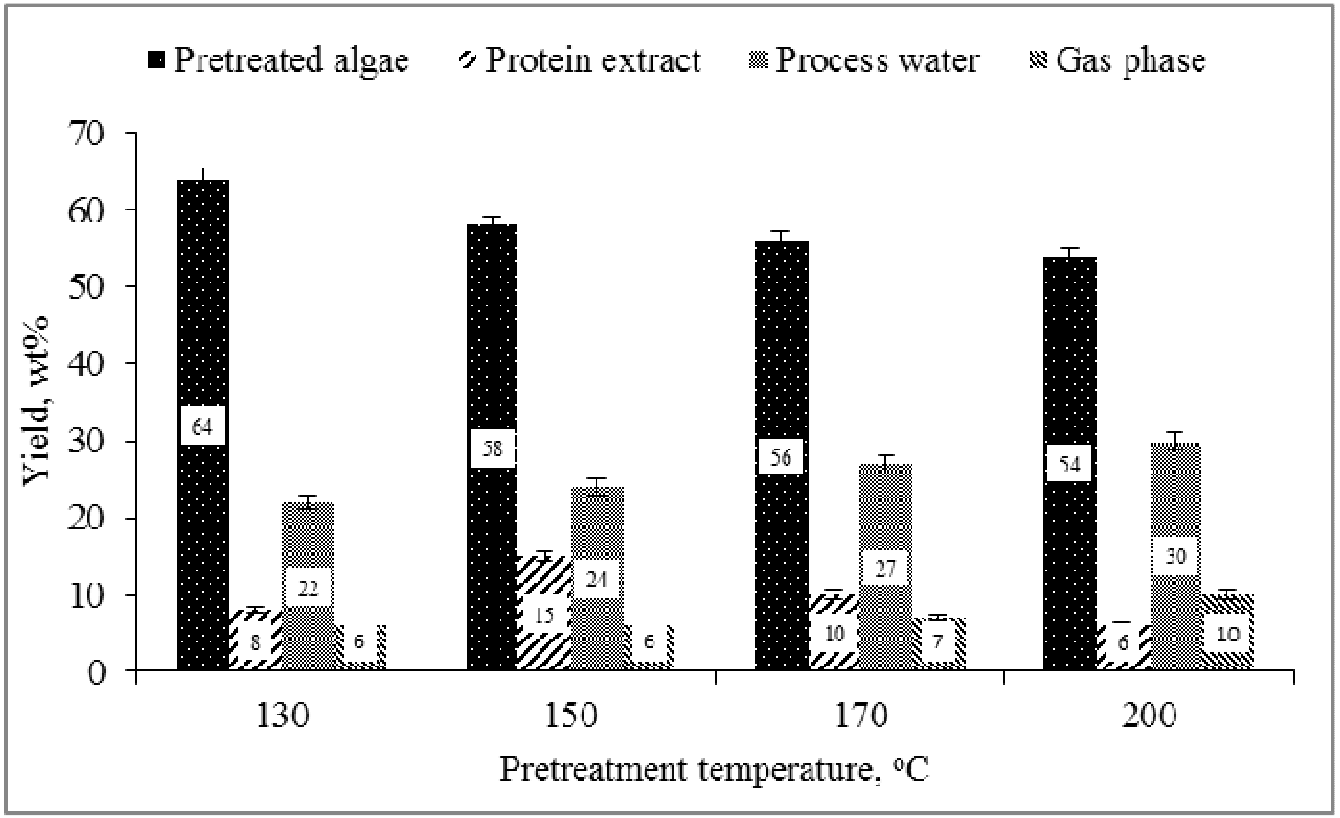

Figure 1: Mass yield of pretreated algae, protein extract, process water and gas phase following pre-treatment of *Tetraselmis* sp. microalgal.

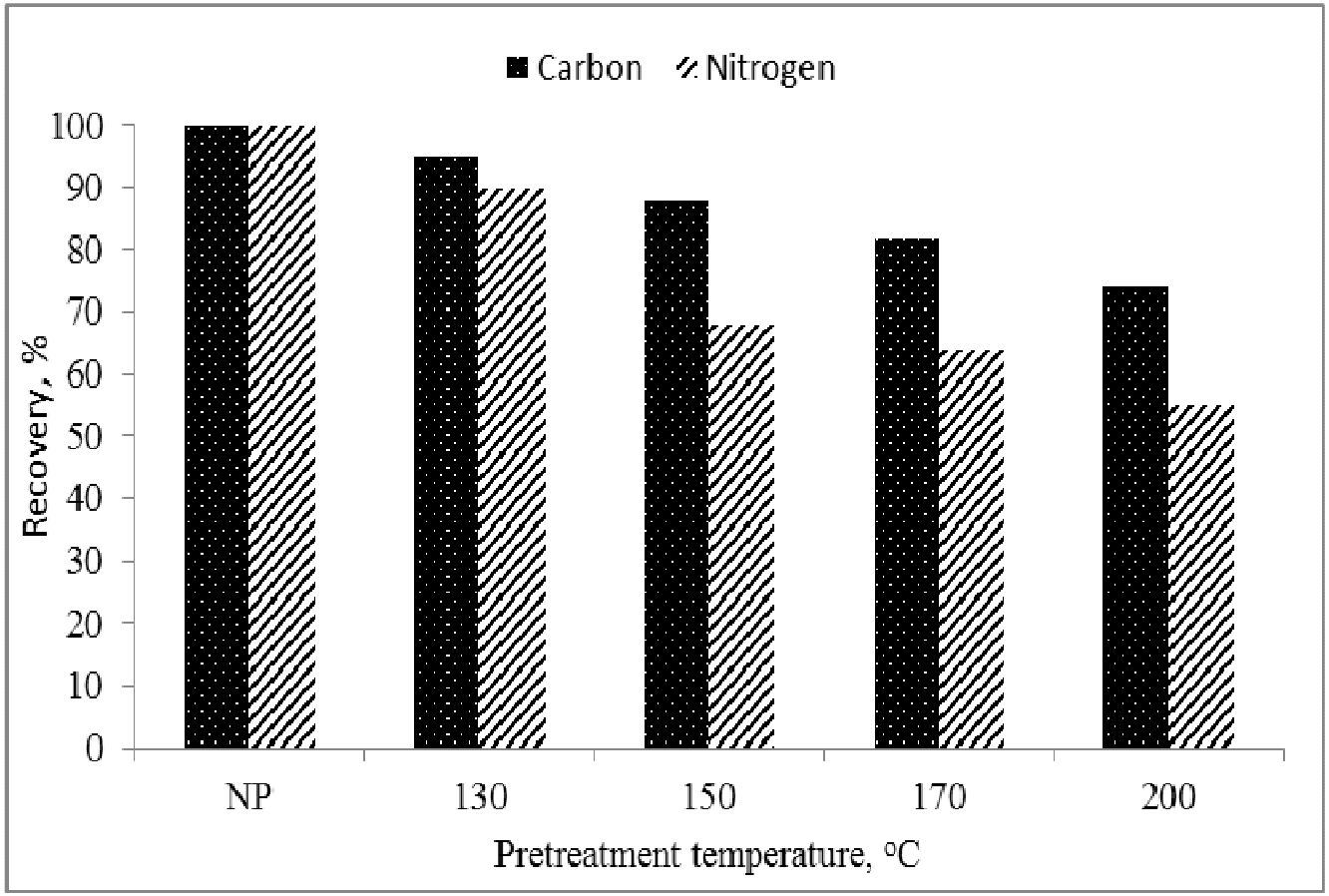

Figure 2: Comparison of carbon and nitrogen recovery in pretreated algae and no pretreatment (NP)

a b

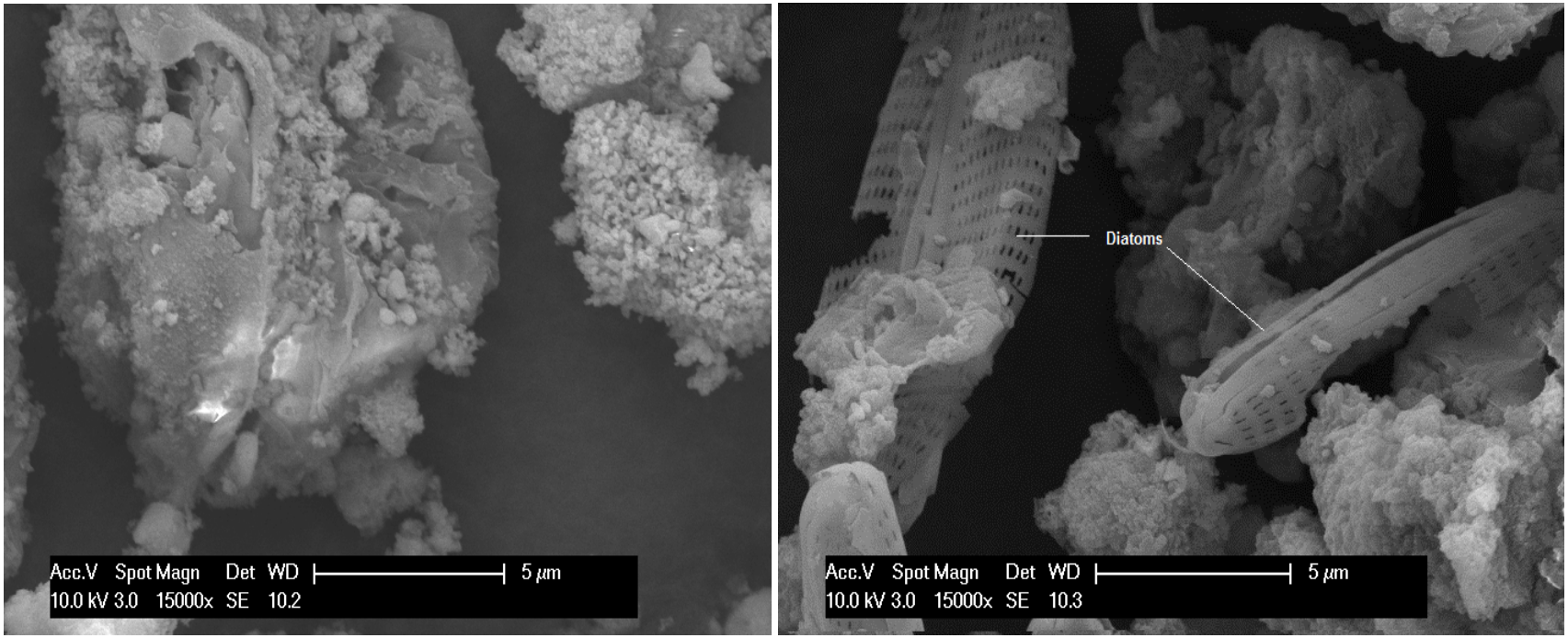

Figure 3: SEM micrographs of (a) untreated algae and (b) pretreated algae following pretreatment at 150^{o}C

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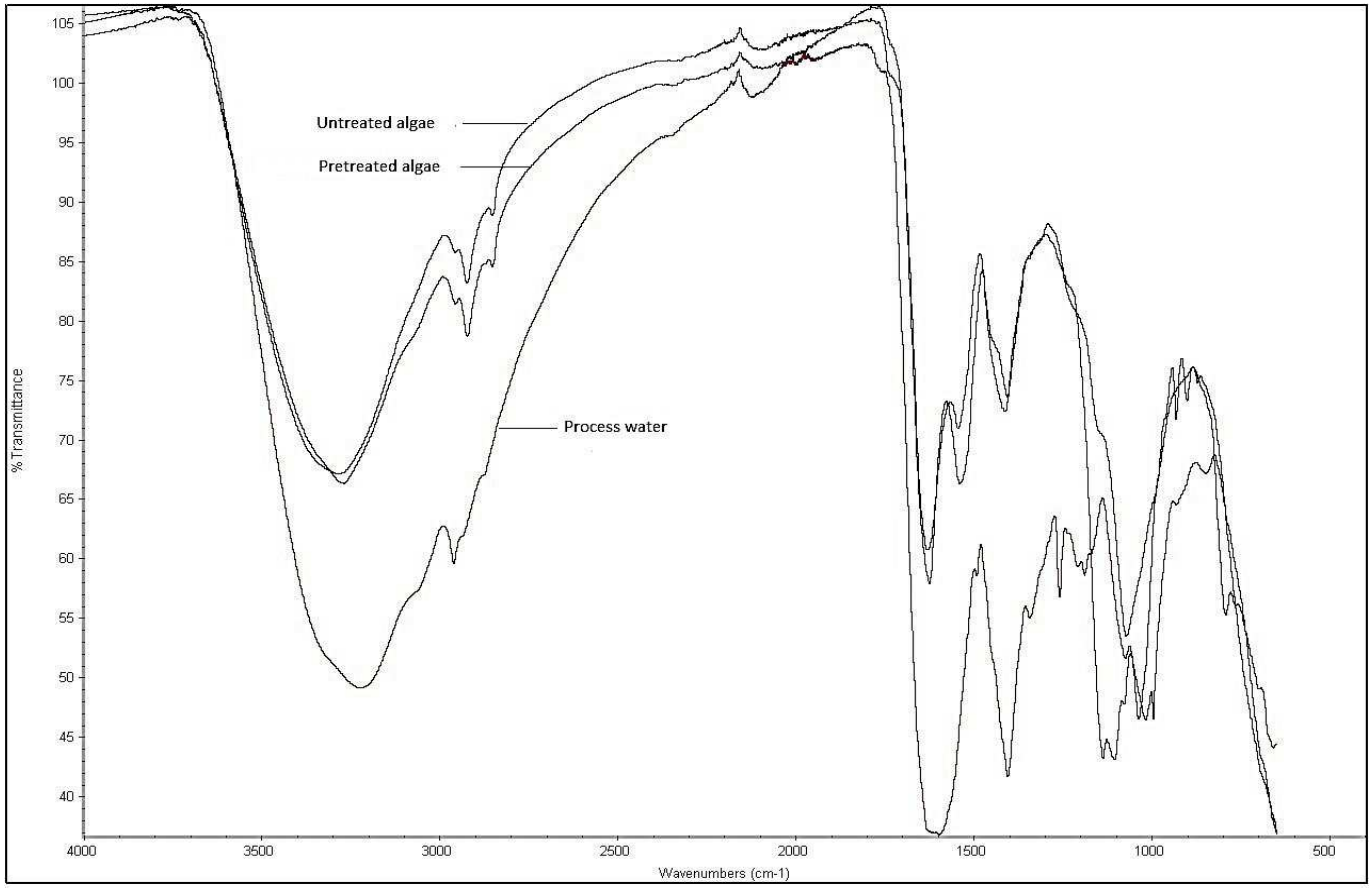
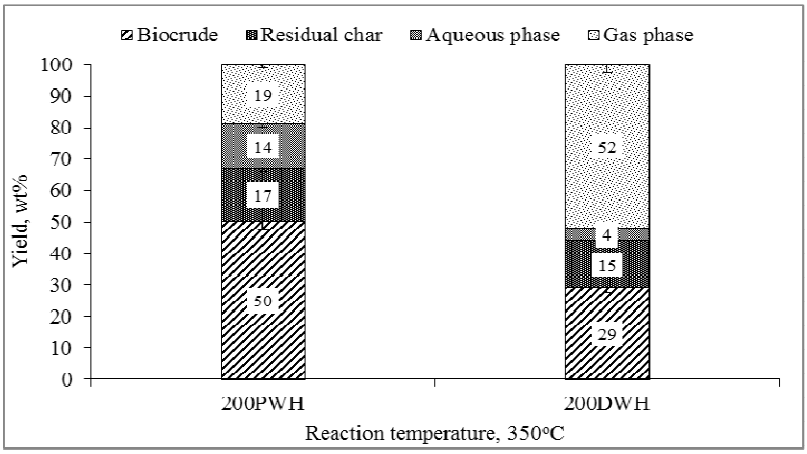


Figure4: Comparison of FT-IR of untreated algae with pretreated algae and process water.

a



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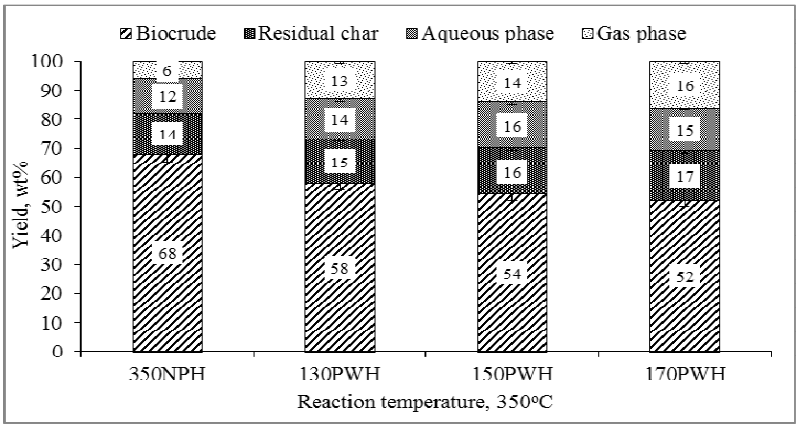

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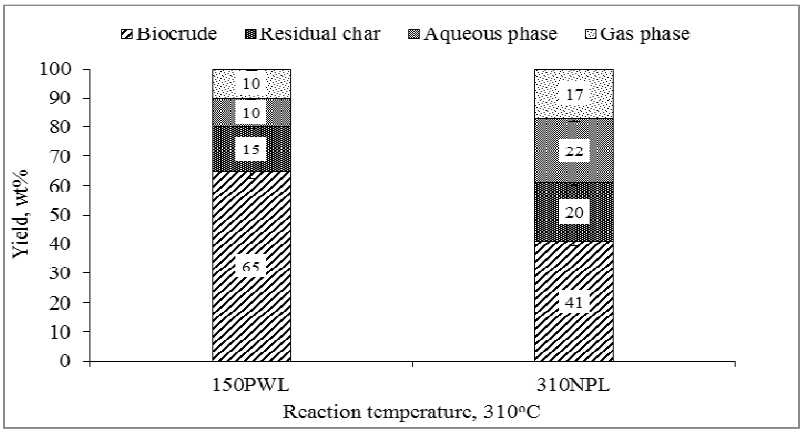


Figure 5: Mass yield of biocrude, residual char, aqueous and gas phases yield following hydrothermal liquefaction pretreated and untreated microalgae (a) HTL of pretreated algae with and without recycled process water. (b) Comparison of HTL product from untreated algae with and untreated algae at higher temperature (350°C) (c) comparison of HTL of pre-treated and untreated algae at lower temperature (310°C).

200PWH: Algae pretreated at 200°C, followed with HTL of pretreated algae with process water (PW) at high temperature (H) (350°C).

200DWH: Algae pretreated at 200°C, followed with HTL of pretreated algae with deionised water (DW) at high temperature (H) (350°C)

350NPH: HTL of untreated algae (no pretreatment (NP)) at high temperature (H) (350°C)

130PWH: Algae pretreated at 130°C then HTL of pretreated algae with process water (PW) at high temperature (H) (350°C)

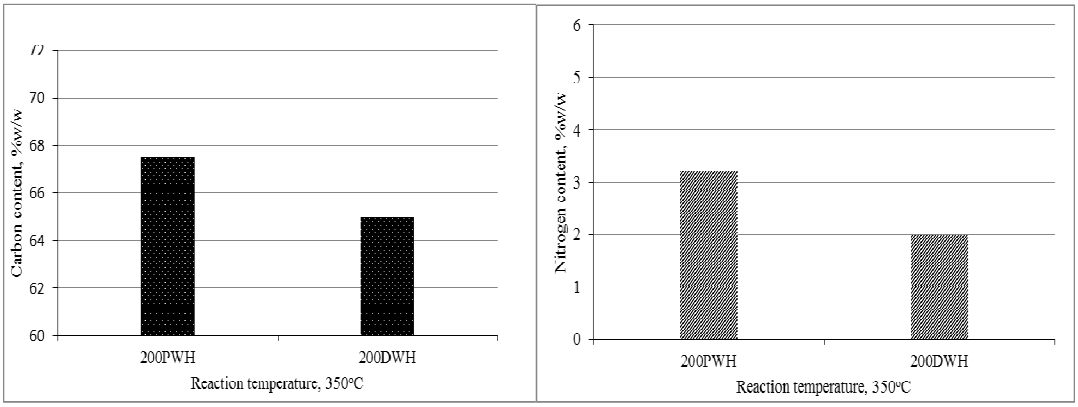
150PWH: Algae pretreated at 150°C then HTL of pretreated algae with process water (PW) at high temperature (H) (350°C)

170PWH: Algae pretreated at 170°C then HTL of pretreated algae with process water (PW) at high temperature (H) (350°C)

150PWL: Algae pretreated at 150°C followed by HTL of pretreated algae with process water (PW) at low temperature (L) (310°C)

310NPL: HTL of untreated algae (no pretreatment (NP)) at low temperature (L) (310°C).

a



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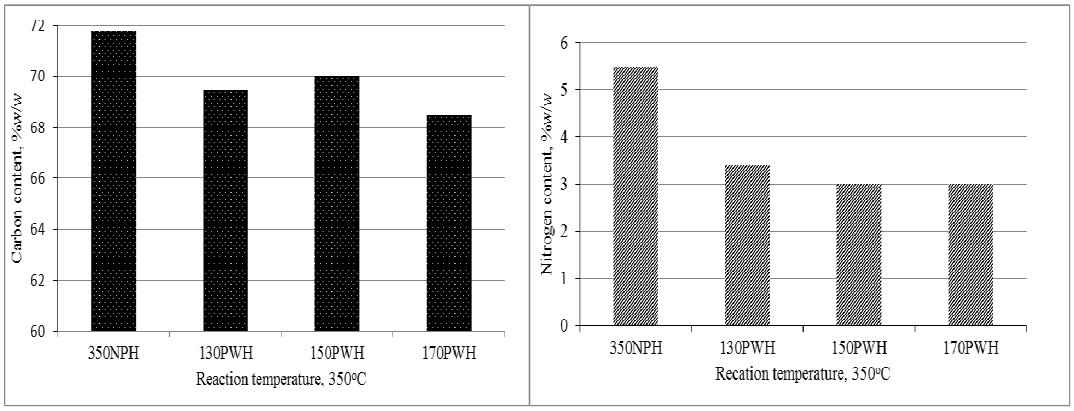

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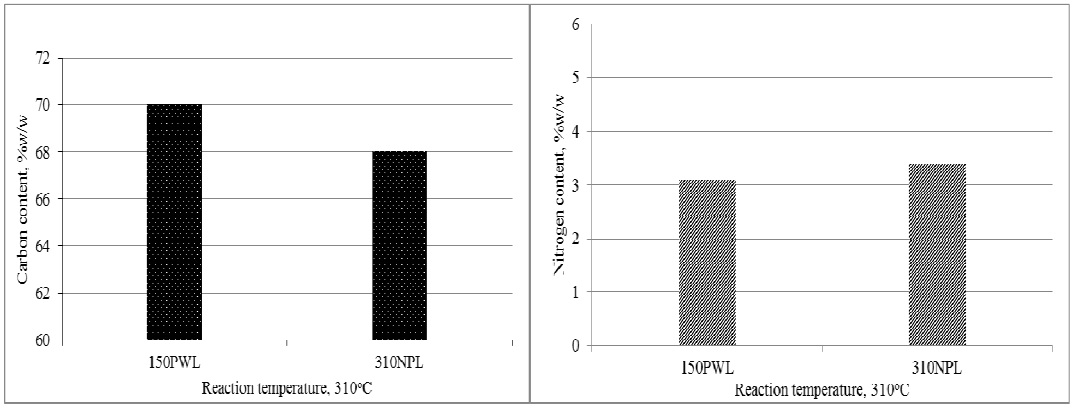

Figure 6: Elemental composition of biocrude from pretreated and untreated algae at different reaction condition.

200PWH: Algae pretreated at 200°C, followed with HTL of pretreated algae with process water (PW) at high temperature (H) (350°C)

200DWH: Algae pretreated at 200°C, followed with HTL of pretreated algae with deionised water (DW) at high temperature (H) (350°C)

350NPH: HTL of untreated algae (no pretreatment) (NP)) at high temperature (H) (350°C)

130PWH: Algae pretreated at 130°C then HTL of pretreated algae with process water (PW) at high temperature (H) (350°C)

150PWH: Algae pretreated at 150°C then HTL of pretreated algae with process water (PW) at high temperature (H) (350°C)

170PWH: Algae pretreated at 170°C then HTL of pretreated algae with process water (PW) at high temperature (H) (350°C)

150PWL: Algae pretreated at 150°C followed by HTL of pretreated algae with process water (PW) at low temperature (L) (310°C)

310NPL: HTL of untreated algae (no pretreatment (NP)) at low temperature (L). (310°C)

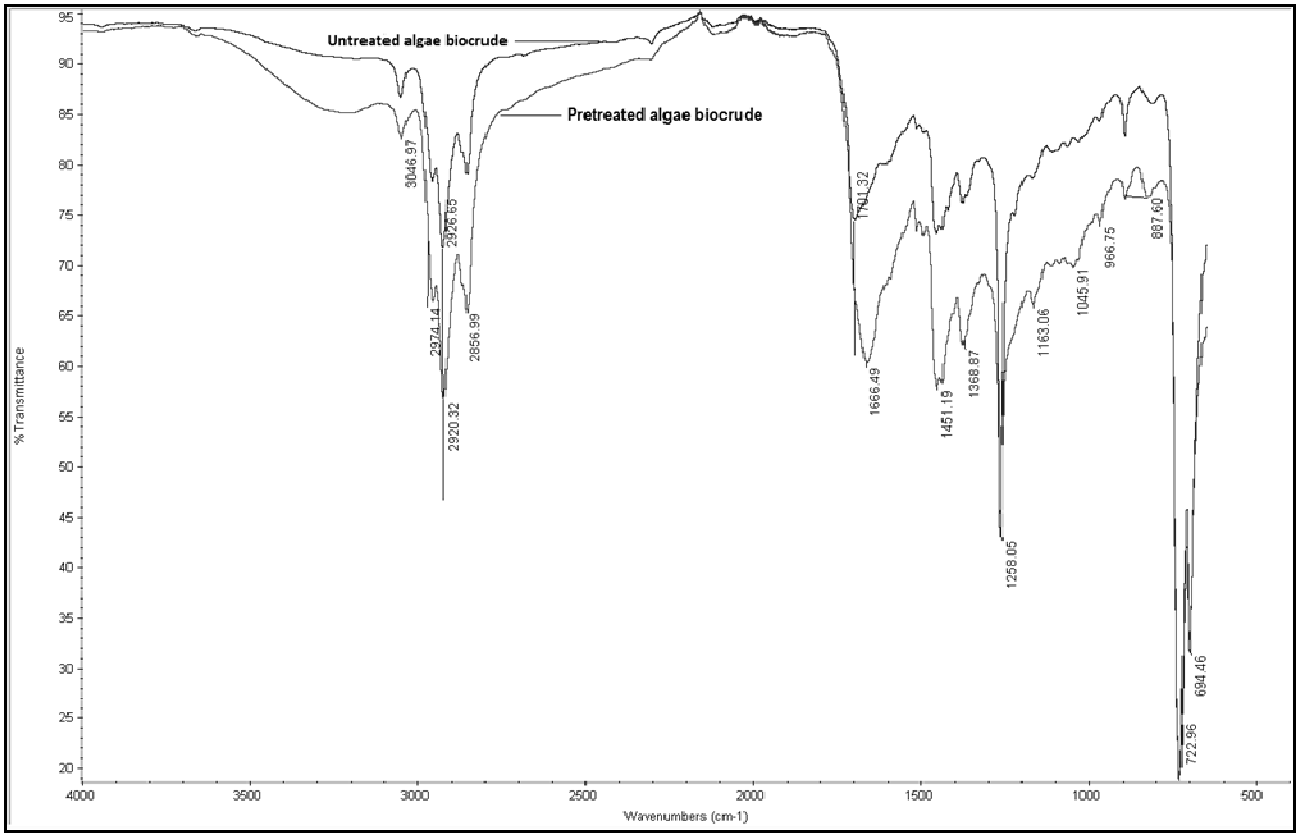

Figure7: FT-IR spectral of biocrudes produced from the hydrothermal liquefaction of untreated and pretreated algae.

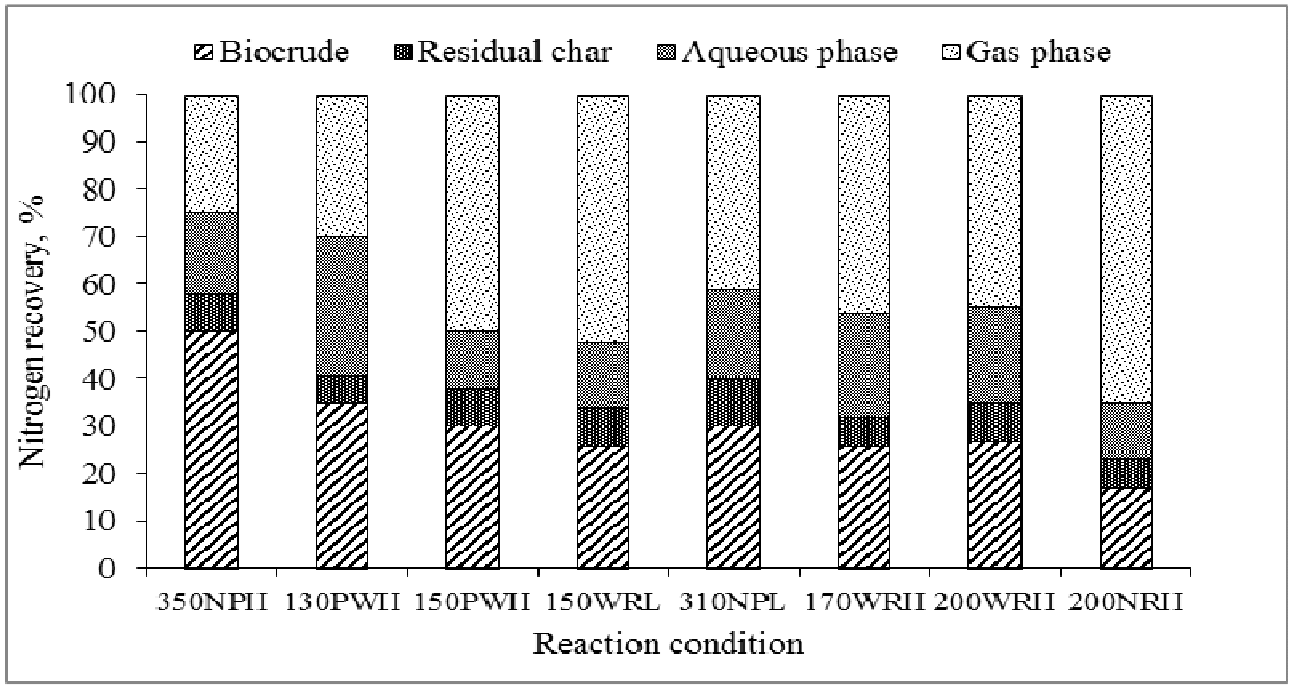

Figure 8: Nitrogen recovery in biocrude, residual char, aqueous and gas phases from liquefaction of untreated and pretreated algae

200PWH: Algae pretreated at 200°C, followed with HTL of pretreated algae with process water (PW) at high temperature (H) (350°C).

200DWH: Algae pretreated at 200°C, followed with HTL of pretreated algae with deionised water (DW) at high temperature (H) (350°C).

350NPH: HTL of untreated algae (no pretreatment) (NP)) at high temperature (H) (350°C)

130PWH: Algae pretreated at 130°C then HTL of pretreated algae at 130°C with process water (PW) at high temperature (H) (350°C)

150PWH: Algae pretreated at 150°C then HTL of pretreated algae with process water (PW) at high temperature (H) (350°C)

170PWH: Algae pretreated at 170°C then HTL of pretreated algae with process water (PW) at high temperature (H) (350°C)

150PWL: Algae pretreated at 150°C followed by HTL of pretreated algae with process water (PW) at low temperature (L) (310°C)

310NPL: HTL of untreated algae (no pretreatment) (NP)) at low temperature (L) (310°C)

a b

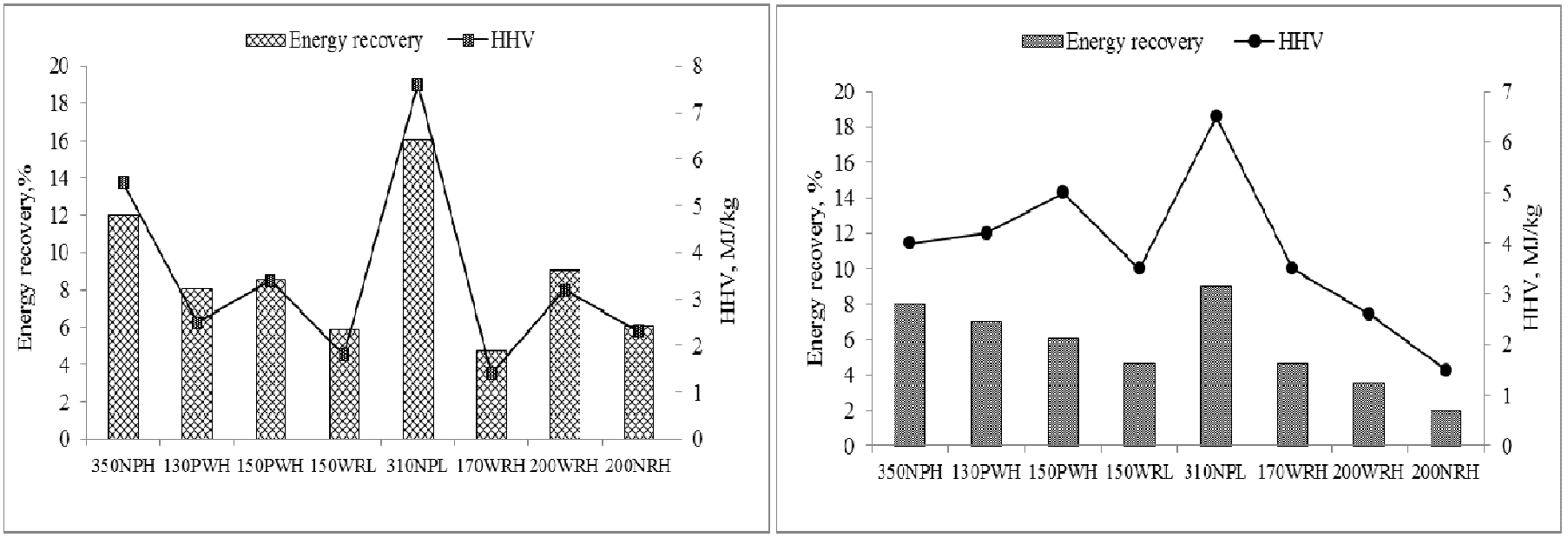

Figure 9: Energy recovery and HHV of (a) residual char and (b) aqueous phase obtained following HTL of pretreated and untreated algae at differenet reaction conditions

200PWH: Algae pretreated at 200°C, followed with HTL of pretreated algae with process water (PW) at high temperature (H) (350°C).
200DWH: Algae pretreated at 200°C, followed with HTL of pretreated algae with deionised water (DW) at high temperature (H) (350°C).
350NPH: HTL of untreated algae (no pretreatment (NP)) at high temperature (H) (350°C)
130PWH: Algae pretreated at 130°C then HTL of pretreated algae at 130°C with process water (PW) at high temperature (H)
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150PWL: Algae pretreated at 150°C followed by HTL of pretreated algae with process water (PW) at low temperature (L) (310°C)
310NPL: HTL of untreated algae (no pretreatment (NP)) at low temperature (L) (310°C)

Table 1: Elemental and biochemical composition of *Tetraselmis* sp.

	Tetraselmis sp. composition (wt%, afdw[a])		
Carbon (C)	42	Protein	58
Hydrogen (H)	6.8	Lipids	14
Nitrogen (N)	8.0	Carbohydrate	22
Sulfur (S)	3.0		
Oxygen[b] (O)	40.2		
H/C	1.94		
HHV[c] (MJ/kg)	19.2		

[a]:afdw: ash free dry weight; [b] determined by difference; [c] higher heating value.

Table 2: List of separate treatments with experimental variables.

Tag	HTL temperature (H/L)	Microalgae pretreatment (Y/N)	Pretreatment temp. (^{o}C)	Process water recycling (Y/N)
350NP*	H	N	N/A	N/A
310NP*	L	N	N/A	N/A
130PWH	H	Y	130	Y
150PWH	H	Y	150	Y
150PWL	L	Y	150	Y
170PWH	H	Y	170	Y
200PWH	H	Y	200	Y
200DWH	H	Y	200	N

*NP= No pretreatment, HTL= Hydrothermal liquefaction, H=High (350^{o}C), L= Low (310^{o}C), Y = Yes, N = No, N/A= Not applicable. e.g. 130PWH = microalgae pretreated at 130^{o}C followed with liquefaction of pretreated algae with recycled process water at high temperature (H) (350^{o}C).

350NPH: HTL of untreated algae (no pretreatment (NP)) at high temperature (H) (350^{o}C)

310NPL: HTL of untreated algae (no pretreatment (NP)) at low temperature (L) (310^{o}C)

130PWH: Algae pretreated at 130^{o}C then HTL of pretreated algae with process water (PW) at high temperature (H) (350^{o}C)

150PWH: Algae pretreated at 150^{o}C then HTL of pretreated algae with process water (PW) at high temperature (H) (350^{o}C)

150PWL: Algae pretreated at 150^{o}C followed by HTL of pretreated algae with process water (PW) at low temperature (H) (310^{o}C)

170PWH: Algae pretreated at 170^{o}C then HTL of pretreated algae with process water (PW) at high temperature (H) (350^{o}C)

200DWH: Algae pretreated at 200^{o}C, followed with HTL of pretreated algae with deionised water (DW) at high temperature (L) (350^{o}C).

1 **Table 3: Elemental and trace metals in process water following pre-treatment.**

Elemental composition %w/w		Trace Elements	Concentration	
			mg/kg	mmol/kg
C	15.02	Na	9129.30	396.90
H	3.15	Mg	389.49	16.20
N	1.23	Al	13.30	0.50
S	0.70	K	569.90	14.60
HHV(MJ/kg)	9.00	Ca	150.30	3.50
		Mn	1.00	0.02
		Fe	14.40	0.30
		Ni	3.20	0.10
		Cu	16.80	0.30
		Zn	38.70	0.60

2

3

1 Table 4: Chemical composition of biocrude from untreated and pretreated algae following liquefaction at 310^{o}C

S/N	Compounds	Chemical formula	[a]Molecular weight, g/mol	Retention Time, min	Area, %	
					Untreated algae biocrude	Pretreated algae biocrude
1	Pyrrole	C_4H_5N	137	10.28	1.80	bdl
2	Heptadecene, 17 chloro	$C_{17}H_{33}Cl$	272	12.27	bdl	4.0
3	Egtazic acid	$C_{14}H_{24}N_2$	380	12.61	3.3	bdl
4	Indole	C_8H_7N	117	14.25	2.4	bdl
5	2-Heptacosanone	$C_{27}H_{54}O$	394	14.22	3.8	bdl
6	Tetradecanoic acid, 2- hydroxy-	$C_{14}H_{28}O_3$	244	15.1	1.5	bdl
7	Phenol, 2-cyclohexyl – 4, 6 dinitro	$C_{12}H_{14}O_5$	234	16.14	2.9	bdl
8	9-Hexadcenoic acid, methyl ester	$C_{17}H_{34}O_2$	270	16.25	15.6	bdl
9	8-Octadecenal	$C_{18}H_{34}O$	266	16.82	1.7	bdl
10	Hexadecane	$C_{16}H_{30}$	226	16.28	bdl	4.9
11	Pentadecanoic acid, 14-methyl	$C_{15}H_{30}O_2$	242	17.18	-	21.8
				17.27	13.6	-
12	Hexadecanedioc acid, 3-methyl-, dimethyl ester	$C_{17}H_{32}O_4$	361	18.41	4	bdl
13	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	18.92	38.5	-
14	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	18.97	-	65.5
15	Oleic acid	$C_{18}H_{32}O_3$	296	19.17	5.0	bdl
16	8-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	19.27	0.6	bdl
	Glycidol sterate	$C_{21}H_{40}O_3$	340	19.63	2	bdl
17	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	19.82	1.3	bdl
18	Oxiraneoctanoic acid	$C_{19}H_{36}O_3$	312	20.63	bdl	3.8
19	Eicosanoic acid	$C_{20}H_{40}O_2$	312	23.28	2.0	bdl

2 bdl: below detection level, [a]: calculated using atomic mass of respective elements (C: 12, H: 1, N: 14, Cl: 35, O: 16).

3

Table 5: Comparison of energy analyses for biocrude derived from untreated and pretreated algae at different reaction conditions.

Condition	Heat input (E_{in})					Heat output (E_{in})		H/C atomic ratio	Energy Recovery, %	ECR
	Cultivation (MJ/kg oil)[a]	Harvesting (MJ/kg oil)[b]	Pre-treatment (MJ/kg oil)	HTL (MJ/kg oil)	Total E_{in} MJ/kg	E_{out}=HHV oil MJ/kg	$\Delta E=E_{in}- E_{out}$ MJ/kg oil			
350NPH	9.78	6.15	NP	10.28	26.21	34	7.79	1.41	64.9	0.30
130WRH	"	''	3.33	14.1	33.36	32.1	-1.26	1.55	52.4	0.57
150WRH	''	''	3.7	16.36	35.99	31.8	-4.19	1.33	46.7	0.60
170WRH	''	''	3.5	15.48	34.91	32.3	-2.61	1.57	49.9	0.61
150WRL	''	''	2.95	10.86	29.74	30	0.26	1.3	54.9	0.49
310NPL	''	''	NP	13.92	29.85	32.1	2.25	1.03	37.3	0.43
200WRH	''	''	3.53	15.65	35.11	30.3	-4.81	1.42	46.6	0.66
200NRH	''	''	"	29.25	48.71	28	-20.71	1.32	23.8	1.20

HHV: higher heating value; HTL: hydrothermal liquefaction; NP: no pre-treatment; [a]: Sawayama *et al*.[9]; [b]: estimated in accordance to Lee *et al*.[57] and Shelef *et al*.[58].

Oil ≡ biocrude.

Total Heat input [E_{in} (MJ/kg)] = Energy for cultivation + harvesting (electroflocculation and centrifugation) + pre-treatment + liquefaction

Heat output [E_{out}=HHV oil MJ/kg] = Energy produced from biocrude estimated based on the CHNS data.

Net energy balance (ΔH) =E_{in}- E_{out} (MJ/kg oil)

ECR: energy consumption ratio