Effect of Temperature and Na₂CO₃ Catalyst on Hydrothermal Liquefaction of Algae

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Abstract

Hydrothermal liquefaction (HTL) has been identified as an innovative technique to convert aquatic or wet biomass such as algae into biofuels. In this study, HTL was performed on three algae strains viz. *Nannochloropsis*, *Pavlova* and *Isochrysis* at three temperatures of 250, 300 and 350 °C, with and without using Na₂CO₃ as catalyst and a holding time of 60 minutes. The effect of temperature on the HTL product yields and their properties were studied for both catalytic and non-catalytic HTL. Maximum bio-oil yield for non-catalytic (48.67 wt.%) and catalytic (47.05 wt.%) HTL was obtained at 350 °C from *Nannochloropsis* and *Pavlova*, respectively. Compared to non-catalytic HTL, Na₂CO₃ increased the bio-oil yield for high carbohydrate containing algae (*Pavlova* and *Isochrysis*) at higher temperatures (300 and 350°C) whereas for high protein containing algae (*Nannochloropsis*) the yield was higher only at lower temperature (250 °C). Total acid number, pH, density, higher heating value (HHV), ash, moisture and elemental composition were measured for bio-oils produced. The bio-oil obtained had the HHV in the range of 32 to 37 MJ/kg, which was comparable to heavy crude oil. Proximate and ultimate analyses were performed to characterize solid residue, and aqueous fraction was analyzed for acidity, total organic carbon and total nitrogen.

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1. Introduction

Hydrothermal liquefaction (HTL) is a promising route for producing renewable fuels and chemicals from wet biomass [1]. It uses water at sub- or super-critical temperatures and pressures as a reactant and reaction medium [2]. It is mostly suited to wet biomass such as algae because it obviates the need to dry biomass as required in other processes such as gasification, pyrolysis, transesterification, *etc.* and reduces the energy requirements while increasing the overall energy efficiency of the system [3]. The advantages of algae over terrestrial biomass feedstock are high biomass productivity (40-60 dry ton/ha-yr) [4], high carbon sequestration rate (1.8 kg of CO₂/kg of dry algae) [5], and they can be grown under conditions which are unsuitable for conventional crop production, thus relieving the food-versus-fuel pressure on agricultural land [6]. HTL of algae produces a black viscous liquid fraction also known as bio-oil or bio-crude, a solid residue fraction, a gaseous fraction and a water fraction containing some polar organic compounds also known as aqueous phase. In the case of HTL, the total oil yield is higher than the original lipid content in the algal biomass, which suggests that not only lipids but also whole algae (protein, lipids, and carbohydrates) gets converted to bio-oil [7, 8].

Over the last decade, extensive research on algae HTL has been conducted in subcritical water conditions. Various process variables such as temperature [9-13], residence time [9, 11-13] and heating rate [14] have been studied, using different types of algae strains as feedstock for HTL process. The primary focus of these studies has largely been on improving the bio-oil yield. Till date, the highest bio-oil yield reported is 79 wt.% [14]. Most of the HTL studies are performed at temperature range of 250 °C to 350 °C with 5 to 60 min residence time and majority in a batch reactor. In addition, most of the studies [10, 12, 13] have reported increase in bio-oil yield with

increase in temperature till 350 °C and subsequently decrease in the yield thereafter. However, some studies have reported maximum yield at 320 °C [9] and 375 °C [11]. These variations in the reaction temperature observed for maximum bio-oil yield is mainly due to the different types of biochemical composition (proteins, lipids and carbohydrates) of the algae strains used. In addition, very few studies [15] have studied the effect of different bio-chemical composition on bio-oil yield. Moreover, there is a lack of understanding how the temperature would impact bio-oil yield of algae that have different biochemical compositions and many studies have not reported the effect of temperatures on the properties (e.g., acidity, density) of the bio-oil obtained.

Apart from non-catalytic HTL, introduction of different types of catalysts in the HTL process have also been reported [16-26]. Although the bio-oil obtained from non-catalytic HTL has high heating value (32-39 MJ/kg), it has several negative attributes such as high oxygen (typically 10-20%) and nitrogen (4-6%), and high viscosity which makes it undesirable for use as a fuel. Therefore, together with maximizing bio-oil yield, improvement in the quality of bio-oil has been the interest of most of the catalytic work performed. A number of homogenous [12, 15, 19, 20, 24-26] and heterogeneous catalysts [22, 23] have been used in HTL to improve the bio-oil yield and its properties. Most of the studies have used Na₂CO₃ as a homogenous catalyst due to its previous usage in the HTL of lignocellulosic biomass for increasing bio-oil yield. However, some studies [18, 21] found the bio-oil yield to decrease while others [17, 19, 25] observed its yield to increase with the addition of Na₂CO₃ in different algal strains [27]. This conflict of results could be due to the varied liquefaction behavior of lipids, proteins and carbohydrates in the presence of Na₂CO₃. The HTL study of different model compounds and microalgae was performed by Biller *et al.* [15]. From the study performed with model compounds, it was

reported that the non-catalytic conversion of lipids and proteins to bio-oil was more efficient when compared to the lower yield with carbohydrates. In comparison, the use of Na_2CO_3 improved the conversion of carbohydrates and reduced the conversion of lipids and proteins. However, this study was limited to 350 °C. In addition, there is a lack of understanding of Na_2CO_3 and temperature effect on the bio-oil yield and its quality obtained from algae strains with different bio-chemical composition.

The objective of this study was to understand how algae strains that have different biochemical composition perform, in terms of products yield, at different HTL reaction temperatures and how the bio-oil quality changes when it is produced at different temperatures. In addition, the study examined the role of alkaline catalyst (Na_2CO_3) at different temperatures on the products yield and its effect on their properties for algae strains that are rich in carbohydrates and protein. The study hopes to fill the knowledge gap on how protein or carbohydrates rich samples perform with or without the use of Na_2CO_3 catalyst during HTL process.

2. Materials and Methodology

2.1. Materials

Algae samples of *Nannochloropsis*, *Pavlova* and *Isochrysis* were obtained in the form of slurry from Reed Mariculture Inc. (Campbell, CA.) and stored in a freezer until they were used. **Table 1** provides biochemical composition of the algae species which was provided by the supplier. Sodium carbonate (Na_2CO_3) was purchased from a chemical supplier (VWR, Atlanta, GA) and was used as received. Moisture content was determined by calculating the weight loss of a sample by heating it in an oven at 105 °C for 24 h. Ash content was determined according to ASTM E 1755 standard. Volatile matter was measured by using ASTM E 872. Higher heating value (HHV) was measured using an oxygen bomb calorimeter (IKA, model C2000). Elemental

composition of the algae samples was determined using an elemental analyzer (Perkin-Elmer, model CHNS/O 2400). Ultrapure (Type 1) water (Synergy Ultrapure Water Systems, EMD Millipore) was used for HTL experiments. High-purity (>99.99%) helium was purchased from Airgas Inc. (Charlotte, NC).

Table 1: Biochemical composition analyses of algae species*

Strains	Biochemical Composition (wt. %)		
	Carbohydrate	Protein	Lipids
<i>Nannochloropsis</i>	8.92	62.79	18.12
<i>Pavlova</i>	28.00	46.94	13.88
<i>Isochrysis</i>	25.46	44.36	18.98

*Values reported by Reed Mariculture Inc. on dry basis

2.2. Experimental Setup and Procedure

HTL experiments were performed in a high pressure experimental unit as shown in **Figure 1**. The experimental unit consisted of a batch reactor of 1 in. internal diameter (i.d.) and 100 mL internal volume (High Pressure Equipment Company, Erie, PA) and equipped with an electrical heating unit. The temperature inside the reactor was continuously monitored using a 1/16th in. K-type thermocouple (Omega, Stamford, CT) attached to one end of the reactor. An in-line filter (pore size of 0.5 µm) was placed at the outlet of the reactor to prevent the entrainment of solids in the existing downstream system. The pressure in the reactor was measured using a pressure gauge, and the gas outlet line was connected to the moisture absorber cylinder installed prior to the gas collection chamber.

The reactor was loaded with approximately 10 g of algae (on a dry weight basis) at an algae: water ratio of 1:6 (14 wt.% solids) for all three algal feedstocks. With regards to catalytic HTL process, the reactor was additionally loaded with a Na₂CO₃ catalyst loading of 5 wt.% of the dry

solid for all samples. From here on, HTL process without using Na_2CO_3 is termed as non-catalytic HTL and processes using Na_2CO_3 is termed as catalytic HTL. In-line filter and valve assembly were connected to the reactor and securely tightened to seal the reactor. After the reactor was sealed, the headspace in the reactor was purged with helium gas by opening valve 3 to remove residual air and to create an inert environment for the reaction to occur. After purging with helium, valve 2 was closed, and the reactor was pressurized to an initial pressure of 35 psig after which valve 3 was closed.

Figure 1: Schematic representation of a high pressure experimental unit for hydrothermal liquefaction

The reactor was then heated to a desired temperature at the heating rate of $30\text{ }^{\circ}\text{C min}^{-1}$, and Figure 2 shows a typical temperature and pressure profiles during an HTL experiment. After holding the reactor at a desired temperature for an hour, the reactor was cooled down to room temperature using cold water and the residual pressure created by gas formation during the reaction was then released to a gas chamber by opening valve 2. Both the catalytic and non-catalytic experiments were performed in triplicates and statistical analysis of the data (ANOVA, Tukey's HSD) was performed to understand whether or not the obtained results were statistically different. All the statistical analyses were performed at 95% confidence interval.

Figure 2: Graphical representation of pressure and temperature variation in a typical HTL run at 250°C.

2.3. Product Separation

After the gas fraction was released to the gas chamber, the reactor was opened by removing the valve assembly to recover the liquid and solid fractions. The composition of the gas fraction was not analyzed in this study. The content in the reactor was poured in to a flask (F1) and in most of the cases, the products separated naturally in the flask into a bio-oil phase and an aqueous phase. The bio-oil phase appeared to float in the aqueous phase. The solid phase remained mixed with both the bio-oil phase and aqueous phase. The aqueous phase along with some solid fractions was then decanted to another flask (F2) but not all the aqueous phase decanted as some remained in the flask, F1, along with bio-oil and some solids. The procedure to separate different product fractions after the reaction is illustrated in **Figure 3**.

Figure 3: Process flow diagram of product separation after HTL

Equal amount of ultrapure water and acetone were used to rinse the reactor. Other solvents could be used to extract organic portion of the bio-oil such as dichloromethane (DCM). First, acetone was used to rinse, followed by the water; the rinsed acetone with bio-oil was collected in flask F1 while the rinsed water was collected in flask F2 along with decanted aqueous phase. The content in both of the flasks (F1 & F2) were vacuum filtered. Vacuum filtration was carried out using Whatman No. 5 filter paper (particle retention size of 2.5 μm) to recover the solid product. Content of flask (F2) was vacuum filtered to obtain solid residue and aqueous phase filtrate. The aqueous phase obtained was collected in a vial. Similarly, the content of the other flask (F1) was also vacuum filtered to recover solid products as residue and organic phase/acetone soluble phase as filtrate. The organic phase consisted of the bio-oil phase and acetone. The solid residues from both filtrations were mixed and weighed. The recovered solid residue product is termed as

char. The acetone from bio-oil in organic phase was separated using an IKA rotary evaporator that was operated at 40 °C and 556 mbar pressure to obtain acetone extracted bio-oil which is termed as bio-oil from here on.

A mass balance for each experiment was also performed to determine the relative content of different products in each phase. The mass of gas was calculated from the difference of total weight of the reactor before and after venting the gas. The product yields for non-catalytic experiments were calculated using Equation 1.

$$Yields_{\text{biooil/WSP/Solid residue}} (\text{wt. \%}) = \frac{\text{wt.of the products (dry basis)}}{\text{wt.of biomass (dry basis)}} \times 100 \quad (1)$$

For catalytic runs, the bio-oil and solid residue yields were calculated using Eqn. 1; whereas the water soluble product yield was calculated using Equation 2.

$$Yields_{\text{WSP}} (\text{wt. \%}) = \frac{\text{wt.of the products (dry basis)}}{\text{wt.of biomass (dry basis) + wt.of catalyst}} \times 100 \quad (2)$$

Na₂CO₃ is highly soluble in water (solubility=21.5 g/100 mL of water at 20 °C)[28]. In addition, very small amount of catalyst was used. So, in this study we assumed the catalyst to be in aqueous phase.

2.4. Product Analysis

Bio-oils produced from all the experiments were analyzed. The pH measurements were performed with a digital pH meter (Oakton, Model PC 510). Density was measured using a 2 mL calibrated density bottle (Cole-Parmer Model EW-34580-40). Water content of bio-oil samples was determined by Karl-Fischer (KF) analysis using a V20 Volumetric KF Titrator (Mettler Toledo, Columbus, OH). Ash content was measured according to ASTM E1755. Total acid number (TAN) was measured by ASTM D664 using T50 Titrator (Mettler Toledo, Columbus,

OH) in which 0.25 grams of the bio-oil sample was mixed with the titration solvent and titrated with potassium hydroxide. Elemental analysis of the bio-oil samples was performed using an elemental analyzer (Perkin-Elmer, model CHNS/O 2400).

Bio-oil chemical composition was analyzed with an Agilent 7890 GC/5975MS using a DB-1701 column. A bio-oil sample of approx. 100 mg was weighed and diluted to 10 mL with dichloromethane. The mixture was further diluted in ratio of 1:9 by dichloromethane. This diluted sample was injected into the GC in duplicates. The initial temperature of the column, 50°C was maintained for 2 min, and then increased to 250°C at 5°C/min, and held for 10 min. Helium (99.999%) was used as a carrier gas with flow rate set at 1.76 ml/min. Compounds were identified using NIST (National Institute of Standards and Technology) mass spectral library.

The aqueous phase produced from HTL of different algae strains at different temperatures was analyzed for its pH, total organic carbon (TOC) and total nitrogen (TN) content. TOC and TN measurements were conducted using a TOC-L analyzer attached with TNM-L unit (Shimadzu corp., Japan). To measure TOC and TN, aqueous phase samples were filtered using 0.2 µm filter to remove any suspended particles. The filtrate samples of 100 dilution factor were prepared and kept in an auto-sampler for measurement. Solid residue produced from HTL was also analyzed for its proximate and ultimate analyses by using the methods as described above.

3. Result and Discussion

3.1. Algae Characterization

Table 2 and **Table 3** illustrate proximate and ultimate analyses of algae, respectively. All three algae strains had relatively similar ash content, but, *Nannochloropsis* had the highest volatile

matter (22.51 wt.%) and HHV content (24.02 MJ/kg). Ultimate analysis was relatively similar for all algae strains.

Table 2: Proximate analysis and heating value of algae samples

Strains	Moisture (wt.%)	Ash (wt.%)	Volatile matter (wt.%)	HHV (MJ kg ⁻¹)
<i>Nannochloropsis</i>	68.88±1.24	3.42±0.38	22.51±1.28	24.02±0.07
<i>Pavlova</i>	75.80±0.42	3.47±0.33	17.74±0.77	22.69±0.07
<i>Isochrysis</i>	73.93±1.44	3.39±0.29	18.20±1.01	22.97±0.02

Table 3: Ultimate analysis of algae feedstocks

Strains	Ultimate Analysis (wt. % in dry & ash free basis)				
	C	H	N	S	O*
<i>Nannochloropsis</i>	56.83±0.33	9.32±0.06	10.13±0.06	0.37±0.19	19.93±0.26
<i>Pavlova</i>	54.34±1.36	8.69±0.41	8.67±0.21	0.82±0.09	24.01±2.07
<i>Isochrysis</i>	55.76±1.14	8.7±0.34	7.96±0.06	0.62±0.10	23.57±1.65

*By difference

3.2. Liquefaction Yields

3.2.1. Effect of temperature on liquefaction yields

Figure illustrates product yields from non-catalytic and catalytic HTL, and the yields were calculated on dry weight basis. Maximum bio-oil yield was obtained at 350 °C for all the tested algae strains. *Nannochloropsis* showed the highest bio-oil yield of 48.67 wt.%, which was higher than the value (43 wt.%) reported by Brown *et al.* [10] for the same reaction temperature and algal strain. However, this difference might be due to different operating conditions such as biomass loading (5 to 16 solid wt.%), initial pressure (69 kPa), and the biochemical composition

(carbohydrates-12 wt.%, proteins-52 wt.%, lipids-28 wt.%) of the species used in their study. Bio-oil and gas yields increased as the temperature increased from 250 °C to 350 °C; whereas, char and water soluble fractions decreased. Similar trends in product yields were noticed for other algae strains at different temperatures [10, 12, 13]. Based on the Tukey's HSD test, there was significant increase in bio-oil yield from 250 °C to 300 °C for *Nannochloropsis* (p-value = 0.0002) and from 300 °C to 350 °C for *Isochrysis* (p-value = 0.0117). This could be due to increased hydrolysis or decomposition/depolymerization reactions of the biomass into smaller compounds to form bio-oil. At higher temperatures, the activation energy required for bond cessation is easily overcome, resulting in extensive biomass depolymerization and higher bio-oil yields [10]. In the case of *Nannochloropsis*, bio-oil yield was observed to be significantly lower at 250 °C than at higher temperatures. This was probably due to the high protein content in this type of algal strain. At lower temperatures, the peptide bonds in proteins are more stable than glycosidic bonds in carbohydrates. Therefore, slow hydrolysis of proteins is achieved at low temperature [29, 30]. However, at higher temperatures (300 and 350 °C), proteins easily get hydrolyzed and results in the higher yield of bio-oil. Thus, *Nannochloropsis* had a higher yield at 350 °C, owing to the increased protein conversion. This also correlates with the GC-MS data in Figure 5 (a), which shows an increase in the relative abundance of nitrogenous compounds at higher temperatures.

Significant decrease in the solid residue was observed from 250 °C to 350 °C for *Nannochloropsis* (p-value = 0.0016) and *Pavlova* (p-value = 0.0188). However, no significant decrease was observed for *Isochrysis*. The decrease in solid residue likely occurred because of the severity of the reactions in which most of the solid fractions were converted to either bio-oil, water soluble or gaseous product when the temperature was increased. The water soluble

products (WSP) also decreased with increase in temperature. WSP decreased significantly with increase in temperatures for *Isochrysis* (p-value = 0.0005) and *Pavlova* (p-value = 0.0006) but for *Nannochloropsis*, it decreased significantly only from 250 °C to 300 °C. The decrease in WSP is probably due to more conversion of WSP to gaseous and bio-oil products with increase in temperature. Gas yield was observed to increase significantly with the increase of temperature from 250 to 350 °C. With the increase of temperature and residence time most of the products go through secondary decompositions (cracking) into lower molecular weight organic compounds [31, 32]. Complete closure of the mass balance was not possible due to experimental errors such as spilling of few drops of products or incomplete removal of bio-crude from the reactor during processing of algal HTL bio-oil.

Figure 4: Comparison between Non-catalytic and catalytic yields at different temperatures

3.2.2. Effect of alkaline catalyst (Na₂CO₃) on liquefaction yields

With the use of Na₂CO₃ as a catalyst, the bio-oil yield was in the order: *Pavlova* > *Isochrysis* > *Nannochloropsis* at higher reaction temperatures of 300 and 350 °C as shown in **Figure 4**. Maximum bio-oil yield of 47.07 wt.% was obtained for *Pavlova* at 350 °C. The bio-oil yield for *Pavlova* (p-value = 0.0026) and *Isochrysis* (p-value = 0.0444) increased with increase in the reaction temperature. For example, when compared to non-catalytic experiments at 300 °C, it increased by 21% and 11.7% for *Pavlova* and *Isochrysis*, respectively. This increase is probably due to the higher amount of carbohydrates present in these species, which gets more efficiently converted to bio-oil when using Na₂CO₃ as a catalyst [27]. In addition, Fang *et al.* [33] observed that the carbohydrate decomposition temperature shifted to a higher temperature due to the change in ion concentration (K_w) caused by Na₂CO₃, which correlates well with the results obtained in this study. However, a similar increase in the bio-oil yield was not observed for *Nannochloropsis* with increase in temperature. This could be directly related to the carbohydrate content in *Nannochloropsis*, which is significantly lower when compared to *Pavlova* and *Isochrysis*. On comparing with non-catalytic HTL, the bio-oil yield in catalytic experiment increased by 24.32% for *Nannochloropsis* at 250 °C. The use of Na₂CO₃ as a catalyst decreases the activation energy for protein hydrolysis, leading to proteins getting converted at a lower temperature to bio-oil compounds. This increase of protein hydrolysis is also supported by the GC-MS data of catalytic runs, which show higher amount of nitrogenous compounds in bio-oil produced at 250 °C when compared to non-catalytic HTL. However, due to the secondary decomposition of nitrogenous compounds to ammonia and water soluble compounds at higher temperatures [34] of 300 °C and 350 °C, the bio-oil yield from *Nannochloropsis* decreased by 20.6% and 23.8% when compared with non-catalytic HTL.

The solid residue decreased with the increase in temperature from 250 °C to 300 °C but no significant change was observed thereafter. Comparing with non-catalytic HTL, the solid residue of *Pavlova* and *Isochrysis* decreased. This decrease is probably due to the catalytic effect of Na₂CO₃, which converted more carbohydrates to bio-oil and inhibited the formation of char. Decrease in WSP was also observed with increase in temperature for catalytic HTL. For *Isochrysis*, significant decrease in WSP was observed with increase in temperature. However for *Nannochloropsis* and *Pavlova*, significant decrease was observed only when temperature was increased from 250 °C to 300 °C. When compared with non-catalytic HTL, WSP was found to increase at all the reaction temperatures. In the case of gas yield, significant increase was observed from 250 °C to 300 °C for *Nannochloropsis*, whereas for *Pavlova* and *Isochrysis*, no change was observed.

3.3. Bio-oil Characterization

Properties of the bio-oil produced from non-catalytic and catalytic HTL of algae strains are given in **Table 4**. It is noteworthy mentioning that the properties of the bio-oil will be different if a different solvent is used. For example, supplementary information (Table S1) shows that under the same experimental conditions, the moisture content of the bio-oil could be different when DCM is used instead of acetone. In all cases, the bio-oils obtained were black, viscous and possessed a foul or smoky odor. The density of algal bio-oils (805-970 kg/m³) was similar to that of fossil crude (750-1000 kg/m³) [35], but lower than that of water and thus a clear phase separation between aqueous phase and bio-oil phase occurred. Temperature had no significant effect on the density of non-catalytic and catalytic algal bio-oils except for catalytic HTL of *Nannochloropsis*, where density decreased with increase in temperature. This decrease is

probably due to a larger distribution of lower molecular wt. materials at higher temperatures and also a lower moisture content of the bio-oil. Temperature had a significant effect on the pH of both non-catalytic and catalytic bio-oil. The pH value (6-9) increased significantly from 250 °C to 350 °C for the bio-oils from all the strains. This increase in pH is mostly due to the reduction of organic acids like acetic acid, formic acid, *etc.* at higher temperatures. Compared to the non-catalytic bio-oils, the use of Na₂CO₃ had no significant effect in pH of the bio-oils. The pH of the algal bio-oils observed in this study was similar to the previously reported values [35]. Total acid number (TAN) of bio-oils from non-catalytic HTL was observed to decrease as the HTL temperature increased as shown in **Table 4**. Higher TAN number in bio-oil leads to operational problems and may cause corrosion during storage and transportation, thus a low TAN is desired [36]. The observed TAN values for the bio-oils from *Nannochloropsis* (32-46) were lower than previously reported values of 256.5 and 59-74 obtained by Duan and Savage using DCM as extraction solvent [36] and Elliot *et al.* (direct oil recovery) [37], respectively. However, they were higher than the values reported by Yuan et al. [38] and higher than the maximum TAN value (0.5) of biodiesel as specified in ASTM D 6751-07a [36, 39]. TAN of the bio-oils produced from catalytic HTL of all the strains were observed to decrease significantly with the increase of temperature from 250 °C to 300 °C. Decrease in TAN was observed when compared to the non-catalytic bio-oil. Variation in TAN is mainly related to the increase and decrease of organic acids in the bio-oil.

The elemental composition of the bio-oils from both the non-catalytic and catalytic HTL were independent of change in temperatures as shown in **Table 4**. In addition, the use of Na₂CO₃ did not have significant effect on the elemental composition of the bio-oils as shown in Table 4. Carbon and hydrogen content were in the range of 72-81 wt.% and 11-13 wt.% respectively, and

325 similar with the values reported in the previous literature for *Nannochloropsis* [9, 10]. Oxygen
326 content in the bio-oils were in the range of 0.5-7.50 wt.% for both the catalytic and non-catalytic
327 bio-oils. Higher carbon and hydrogen content, and lower oxygen content resulted in higher
328 heating value of the bio-oils and were in the range of 32-37 MJ/kg. Sulfur content was observed
329 to be below 1 wt.% for all the algal biomass and bio-oils, whereas it varies between 0 and 3 wt.%
330 in the case of petroleum crude oil [43]. Nitrogen content in algal bio-oil is likely due to the
331 presence of pyrroles and indoles, which are difficult to decompose at these conditions [14, 42].
332 Nitrogen content in both the bio-oils was observed to be higher than that of petroleum crude (0-
333 0.8 wt.%) [40, 41]. Ross *et al.* [20] observed that Na_2CO_3 increased nitrogen in the bio-oil
334 whereas Jena *et al.* [19] reported the decrease in nitrogen content in bio-oil with the use of
335 Na_2CO_3 . It is worth mentioning that nitrogen is undesirable in the bio-oil because it produces
336 NO_x gases when it is combusted, and also results in catalyst deactivation while using
337 heterogeneous catalyst, as it blocks a number of active sites through coke formation, related to
338 the adsorption of the nitrogen containing hydrocarbon molecules [44]

339 While GC-MS detected more than 100 compounds, the bio-oil likely contained additional
340 compounds which were lost during solvent evaporation or some higher molecular weight
341 compounds were not evaporated at all. The compounds detected were arranged in decreasing
342 order of area percentage and compounds with a higher area percentage (the total of which
343 represented more than 60% of the total peak area) were chosen for analysis. The chemical
344 compositions of bio-oil were grouped into different categories such as hydrocarbons (HC),
345 phenolics (Ph), nitrogenated compounds (NC), oxygenates (OC) and organic acids (OA). The
346 compounds identified under organic acids were hexadecanoic acid (palmitic acid), tetradecanoic
347 acid (myristic acid), octanoic acid (caprylic acid), hexadecanoic acid methyl ester *etc.* The use of

Na₂CO₃ resulted in bio-oil with similar organic acids in addition to few cyclic organic acids like carbamic acid, methyl-3-methylphenylester-, 1H- Indole-3-propanoic acid etc. that were not present in non-catalytic bio-oils. The fatty acid and fatty acid esters are presumably obtained from hydrolysis of triacylglycerides present in the lipid portion of the algae strains [10] in the bio-oil. However, there might be other pathways to form fatty acids during HTL, as bio-oil from sawdust (which contains no triglycerides) was reported to contain palmitic acid [45]. Srokol *et al.* [46] reported the formation of formic acid, lactic acid, acetic acid, acrylic acid etc. which are a result of the breakdown of glucose under hydrothermal condition. Organic acids in the bio-oils of three algae strains were observed to decrease with the increasing temperature as shown in **5** (a) and (b). This correlated with the decrease in pH and TAN of bio-oil as the temperature was increased. At 250 °C organic acids were in the range of 20 to 45% but decreased to 0 to 5% at 350 °C. At higher temperatures, decarboxylation of fatty acids takes place and long chain hydrocarbons (such as pentadecane, heptadecane etc.) are formed [10]. This may be the cause of increase in the percentage of long chain hydrocarbons with the increase in temperature. However, the use of Na₂CO₃ lowered the organic acid yields at a lower temperature of 250 °C (12-15%) as well as at higher temperatures as shown in **Figure 5** (b). This reduction in organic acid is due to the catalytic effect of Na₂CO₃ which directly breaks lipids to alkanes [15]. The data from GC-MS (supplementary information Figure S1) showed that DCM extracted oil had comparatively lower carboxylic acid than acetone extracted oil but was higher in oxygenated compounds (aldehydes and ketones). Other than that, we observed relatively high amount of lower molecular weight compounds like pyrazine, styrene were mostly observed in DCM extracted oil.

Table 4: Properties of bio-oil with and without catalyst

Strains	Temperature (°C)	Ash (wt.%)		Moisture (wt.%)		HHV (MJ/kg)		pH		Density (g/ml)		TAN (mg KOH/g)	
		Non-cat.	Catalytic	Non-cat.	Catalytic	Non-cat.	Catalytic	Non-cat.	Catalytic	Non-cat.	Catalytic	Non-cat.	Catalytic
<i>Nannochloropsis</i>	250	0.18 ^A	0.69 ^A	7.63 ^A	9.43 ^A	33.40 ^A	33.43 ^A	6.61 ^A	6.88 ^A	0.93 ^A	0.97 ^A	45.88 ^A	37.66 ^A
	300	0.26 ^A	0.34 ^A	7.39 ^A	7.69 ^A	33.87 ^A	33.58 ^A	7.74 ^B	7.58 ^B	0.90 ^A	0.92 ^{A,B}	32.68 ^A	27.03 ^B
	350	0.26 ^A	0.39 ^A	7.00 ^A	7.07 ^A	34.82 ^A	33.71 ^A	7.70 ^B	7.62 ^B	0.90 ^A	0.90 ^B	32.45 ^A	32.09 ^{A,B}
<i>Pavlova</i>	250	0.29 ^A	0.47 ^A	10.14 ^A	8.15 ^A	33.90 ^A	33.86 ^A	6.22 ^A	6.55 ^A	0.93 ^A	0.94 ^A	64.27 ^A	49.27 ^A
	300	0.20 ^B	0.24 ^A	6.90 ^A	6.21 ^A	34.16 ^A	35.55 ^{A,B}	7.16 ^B	7.07 ^B	0.93 ^A	0.94 ^A	46.81 ^B	35.02 ^B
	350	0.44 ^C	0.19 ^A	5.59 ^A	6.55 ^A	32.20 ^A	36.93 ^B	7.10 ^B	7.39 ^A	0.91 ^A	0.94 ^A	41.11 ^B	37.40 ^{A,B}
<i>Isochrysis</i>	250	0.27 ^A	0.36 ^A	6.48 ^A	5.81 ^A	33.14 ^A	33.77 ^A	6.24 ^A	6.30 ^A	0.92 ^A	0.94 ^A	59.52 ^A	44.02 ^A
	300	0.27 ^A	0.19 ^A	10.28 ^A	4.64 ^A	34.66 ^A	35.61 ^A	6.91 ^B	7.36 ^B	0.94 ^A	0.85 ^A	30.81 ^A	30.80 ^B
	350	0.36 ^A	0.14 ^A	9.58 ^A	5.62 ^A	34.18 ^A	34.67 ^A	8.68 ^C	6.79 ^A	0.93 ^A	0.86 ^A	36.05 ^A	37.23 ^{A,B}
Strains	Temperature (°C)	Ultimate analysis (wt.% in dry basis)										Energy Recovery (ER)	
		C		H		N		S		O*		Non-cat.	Cat.
		Non-cat.	Cat.	Non-cat.	Cat.	Non-cat.	Cat.	Non-cat.	Cat.	Non-cat.	Cat.		
<i>Nannochloropsis</i>	250	72.55 ^A	75.20 ^A	14.15 ^A	12.27 ^A	5.13 ^A	5.25 ^A	0.49 ^A	0.33 ^A	7.50 ^A	6.24 ^A	39.62 ^A	49.30 ^A
	300	77.34 ^A	80.57 ^A	13.38 ^A	12.81 ^A	5.39 ^A	5.52 ^A	0.30 ^A	0.52 ^A	3.33 ^A	0.25 ^A	62.48 ^B	49.18 ^A
	350	79.33 ^A	76.34 ^A	11.96 ^A	12.68 ^A	5.26 ^A	5.02 ^A	0.17 ^A	0.58 ^A	3.01 ^A	4.97 ^A	70.56 ^C	52.03 ^B
<i>Pavlova</i>	250	79.75 ^A	80.17 ^A	12.42 ^A	12.40 ^A	4.01 ^A	4.54 ^A	n.d.	0.70 ^A	3.83 ^B	1.08 ^A	53.06 ^A	48.25 ^A
	300	80.02 ^A	75.43 ^A	13.21 ^A	11.59 ^A	5.02 ^A	4.64 ^A	0.46 ^A	0.56 ^A	1.09 ^C	7.54 ^A	56.60 ^B	71.27 ^B
	350	78.30 ^A	81.11 ^A	10.06 ^A	12.68 ^A	4.70 ^A	4.87 ^A	0.12 ^A	0.72 ^A	6.37 ^A	0.42 ^A	60.22 ^B	76.57 ^C
<i>Isochrysis</i>	250	75.23 ^A	79.11 ^A	12.34 ^A	12.32 ^A	3.92 ^A	4.09 ^A	0.47 ^A	0.57 ^A	7.77 ^A	3.54 ^A	47.17 ^A	53.14 ^A
	300	81.96 ^A	79.11 ^A	12.23 ^A	12.87 ^A	4.42 ^A	4.18 ^A	0.37 ^A	0.65 ^A	0.75 ^A	2.98 ^A	51.02 ^B	65.34 ^B
	350	77.36 ^A	79.68 ^A	11.24 ^A	13.23 ^A	4.39 ^A	4.37 ^A	0.25 ^A	0.96 ^A	6.39 ^A	1.63 ^A	61.24 ^C	62.18 ^B

*By difference. n.d., not detected. Different alphabets in the superscript of each group (or row of each species) denote the values are statistically different at different temperature level. Cat.- Catalytic

Nitrogenated compounds included cyclic nitrogen compounds like pyrazine, pyridine, indole, benzenamide *etc.* and branched amides like tetradecanamide, dimethyldecanamide *etc.* These compounds are formed by decarboxylation, deamination, dehydration, depolymerization and decomposition reactions of proteins [20, 47]. The amino acids undergo degradation through decarboxylation and deamination reactions to give amines, carbonic acids, ammonia and other organics [47]. Nitrogenated compounds in the bio-oils from three algae strains were observed to increase with an increase in temperature from 250 to 300 °C. For *Nannochloropsis*, the branched amides increased from 5 to 12 % from 250 °C to 300 °C and reduced to 7 % at 350 °C. Similar trend was observed for bio-oils from other strains. The increased percentage of branched amides may be due to the dehydration reactions of amines and carboxylic acids to form amides at higher temperatures. This can also be justified by the decrease in organic acids with the increase of temperature. Formation of nitrogen containing cyclic organic compounds like pyridines, indole, and pyrroles are due to the Maillard reaction as both sugars and amines formation takes place simultaneously. Formation of cyclic nitrogen compounds was high at higher temperatures. In the case of catalytic bio-oils, cyclic compounds like pyrazine, indole, pyridine *etc.* were higher in area percentage than amides (decanamides and acetamides). This shows that Na₂CO₃ produced more cyclic nitrogen compounds. In comparison to non-catalytic bio-oil, nitrogenous compounds decreased in the catalytic bio-oil.

In this study, ketones, ethers, and aldehydes were grouped under oxygenates. Examples of oxygenated compounds found in the bio-oil were tridecandial, ethanone, isophytol, 2-cyclopenten-1-one, 2,3-dimethyl-, 2-pentadecanone, 6,10,14-trimethyl-, *etc.* These oxygenates are mostly converted from polysaccharides and cellulose by hydrolysis, dehydration, cyclization *etc.* [16]. No trend of oxygenated compounds was observed with the increase in temperature for

both the catalytic and non-catalytic bio-oils. In the case of phenolic derivatives in bio-oil, phenols in both the catalytic and non-catalytic bio-oils increased with the increase in temperature. A similar trend for phenolic derivatives was observed by Anastasakis *et al.* [12] in their bio-oil from *Laminaria Saccharina*. Phenolics increased from 0-3% at 250 °C to 10-15% at 350 °C. Algal bio-oil do not possess lignin, therefore, the phenolics in algal bio-oil are likely produced from the carbohydrate portion of the algal biomass [10]. The carbohydrates hydrolyze to glucose, which degrades to form HMF, furfural and are ultimately condensed to phenols [48].

(a)

(b)

Figure 5: Distribution of major compounds in bio-oil produced from (a) non-catalytic and (b) catalytic HTL of different algae strains at different temperatures. HC = hydrocarbon, NC = nitrogenated compound, OC = oxygenated compound, OA = organic acids and esters, Ph = phenolics

Hydrocarbons are represented by straight chains like heptadecane, pentadecane, 1-tridecene, hexadecane, 2,6,10,14-tetramethyl- *etc.* and cyclic aromatics like ethylbenzene, benzene, 1-ethyl-2-methyl-, Napthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-, styrene *etc.* The increase in hydrocarbons in algal bio-oil with temperature is mainly due to decarboxylation of organic acids (myristic acid to tridecane) and decarbonylation of aldehydes to alkanes. An initial increase in hydrocarbons was observed with the increase in reaction temperature from 250 °C to 300 °C, however hydrocarbons decreased at a higher temperature (350 °C). This decrease might be due to further cracking of hydrocarbons and formation of gaseous compounds. Catalytic bio-oils yielded a higher percentage of hydrocarbons than non-catalytic bio-oils. In addition, the aromatic hydrocarbons had a fairly smaller area percentage with respect to aliphatic hydrocarbons in catalytic bio-oils. This is probably due to the catalytic effect of Na₂CO₃, which promotes direct breakdown of lipids to alkanes. Although most of the chemical compounds in all the three algae strains were similar, there were a few variations. For example, eicosene, the decomposition product of eicosapentaenoic acid was observed only in the bio-oil from *Pavlova*.

Energy recovery of bio-oil obtained from each algae strain at different temperatures is listed in Table 4 and was calculated using Eq. 3.

$$\text{Energy Recovery} = \frac{\text{HHV of bio-oil} \times \text{mass of bio-oil}}{\text{HHV of biomass} \times \text{mass of biomass}} \times 100\% \quad (3)$$

In the case of non-catalytic experiments, maximum energy recovery (70.56%) was achieved for the bio-oil from *Nannochloropsis* at reaction temperature of 350 °C. This can be attributed to the

higher yield obtained for *Nannochloropsis* at 350 °C as the HHV did not change significantly. Similarly, maximum recovery for *Pavlova* and *Isochrysis* was also obtained at 350 °C. In the case of catalytic experiments, *Pavlova* showed the highest energy recovery (76.57%) at 350 °C. This change was mainly due to catalytic effect of Na₂CO₃ which increased the yield of *Pavlova*.

3.4. Solid Residue Analysis

Proximate and ultimate analyses of solid residue obtained from both non-catalytic and catalytic HTL are summarized in **Table 5** and **Table 6**, respectively. In the case of non-catalytic HTL, ash content was found to increase significantly with temperature for *Nannochloropsis* (p-value=0.003) while no significant increase in the ash content was observed for other strains. However, in the catalytic HTL, ash content was observed to increase significantly for the solid residue of all the strains as the p-values was below than 0.05. The heating value of solid residue is higher than the algae or terrestrial biomass in many conditions.

Table 5: Proximate analysis and heating value of solid residue from non-catalytic and catalytic HTL of algae

Strains	Temperature (°C)	Proximate analysis			
		Ash (wt.%)		HHV (MJ/kg)	
		Non-catalytic	Catalytic	Non-Catalytic	Catalytic
<i>Nannochloropsis</i>	250	6.07 ^A	16.92 ^A	31.92 ^A	23.56 ^A
	300	20.35 ^B	41.76 ^B	25.89 ^A	17.66 ^B
	350	38.41 ^C	43.29 ^B	21.41 ^A	16.96 ^B
<i>Pavlova</i>	250	31.57 ^A	18.43 ^A	24.57 ^A	27.03 ^A
	300	40.15 ^A	28.12 ^B	21.75 ^A	26.49 ^A
	350	43.07 ^A	32.08 ^C	15.06 ^B	20.54 ^A
<i>Isochrysis</i>	250	15.06 ^A	14.89 ^A	31.68 ^A	15.82 ^A
	300	22.32 ^A	31.34 ^B	30.95 ^A	11.56 ^B
	350	24.04 ^A	39.16 ^C	21.77 ^A	6.68 ^B

Different alphabets in the superscript of each group (or row of each species) denote that the values are statistically different at different temperature levels.

Ultimate analysis of the solid residue from non-catalytic and catalytic HTL showed that it still had a significant portion of C, H and N. C and H were observed to be high in solid residue obtained at 250 °C for all the strains, which signifies that lower temperature conversion of the algae to bio-oil is not efficient as compared to higher temperature liquefaction. High C and H contents and heating values of solid residue make it an attractive feed for energy production. However, a decrease in C and H content was observed with increase in temperature, resulting in a lower higher heating value for the solid residue from non-catalytic and catalytic HTL. Apart from high C and H values, solid residues from both catalytic and non-catalytic HTL also had high N content, which makes it an attractive option for use as a bio-fertilizer.

Table 6: Ultimate analysis of solid residue from non-catalytic and catalytic HTL of algae

Strains	Temp- erature (°C)	Ultimate analysis (wt. % dry & ash free basis)									
		C		H		N		S		O*	
		Non- cat.	Cat.	Non- cat.	Cat.	Non- cat.	Cat.	Non- cat.	Cat.	Non- cat.	Cat.
<i>Nannochloropsis</i>	250	66.68 ^A	51.84 ^A	9.23 ^A	7.20 ^A	4.59 ^A	4.80 ^A	0.24 ^A	0.10 ^A	13.16 ^A	19.13 ^A
	300	55.63 ^{A,B}	35.89 ^A	7.83 ^B	4.80 ^A	3.76 ^{A,B}	2.50 ^B	0.39 ^A	0.14 ^A	12.04 ^B	14.89 ^A
	350	50.24 ^B	35.26 ^A	6.96 ^B	4.72 ^A	2.22 ^B	1.68 ^B	0.28 ^A	0	1.88 ^B	15.04 ^A
<i>Pavlova</i>	250	54.94 ^A	60.61 ^A	6.04 ^A	10.58 ^A	4.83 ^A	3.06 ^A	0.20 ^A	0.36 ^A	2.41 ^A	6.95 ^A
	300	43.06 ^A	53.25 ^B	4.41 ^A	8.40 ^B	3.19 ^B	2.49 ^B	0.65 ^A	0.18 ^A	8.54 ^A	7.54 ^B
	350	37.35 ^A	52.78 ^B	3.58 ^A	10.03 ^A	2.58 ^B	2.30 ^B	0.67 ^A	0.26 ^A	12.75 ^A	2.54 ^B
<i>Isochrysis</i>	250	62.80 ^A	38.00 ^A	9.49 ^A	4.94 ^A	3.29 ^A	4.67 ^A	0.35 ^A	0.32 ^A	34.80 ^A	37.17 ^A
	300	58.71 ^{A,B}	25.99 ^B	8.49 ^{A,B}	2.69 ^B	2.74 ^B	2.43 ^B	0.29 ^A	0	7.44 ^{A,B}	37.55 ^B
	350	41.50 ^B	19.31 ^B	5.88 ^B	1.55 ^B	2.12 ^B	1.32 ^B	0.64 ^A	0	0.04 ^B	38.66 ^B

*By difference. Cat., catalytic runs. Different alphabets in the superscript of each group (or row of each species) denote that the values are statistically different at different temperature levels.

3.5. Aqueous Phase Analysis

Aqueous phase obtained had a very foul smell, especially due to the presence of ammonia and was observed to be semi-transparent. **Table 7** illustrates the total organic carbon, total nitrogen

and pH of the aqueous products obtained from non-catalytic HTL and catalytic HTL experiments. The aqueous phase was observed to be alkaline, which was probably due to the presence of ammonia (a weak base). The pH of the aqueous phase from catalytic runs (8-9.5) was found to be higher than that of the non-catalytic runs (7-8). This may be due to the presence of the alkali catalyst in the aqueous phase. This was in agreement with Ross *et al.* [20], who also found the same pattern when Na₂CO₃ was used as catalyst.

The amount of total organic carbon (TOC) in the aqueous phase indicates that during HTL, a significant proportion of the organic products are distributed in the aqueous phase as dissolved organics [12]. Zhou *et al.* [16] reported an abundance of acetic acid and glycerol in the aqueous phase from hydrothermal treatment of *Enteromorpha prolifera*. Presence of acetates and formates in the aqueous phase, which are likely from carbohydrate hydrolysis, was also reported by other studies [13, 20]. For non-catalytic HTL, the organic carbon in the aqueous phase obtained from *Nannochloropsis* and *Isochrysis* was observed to decrease with increase in temperature. This was expected because most of the carbon in aqueous phase gets converted to bio-oil and gases with increase in temperature. A similar trend was reported by Yang *et al.* [25] for the aqueous phase from hydrothermal liquefaction of *Mirocystis viridis*. On the other hand, the aqueous phase from HTL of *Isochrysis* did not show any trend with increase in temperature. In comparison to the non-catalytic aqueous phase, the catalytic aqueous phase was observed to have a higher TOC at higher temperatures (300 and 350 °C) for *Nannochloropsis* and *Pavlova*. This was due to the effect of Na₂CO₃, which shifted organic matter from the feedstock into the aqueous phase as non-volatile organic carbon [25] and reduced the formation of char. This was in agreement with Yang *et al.* [25], who reported increase in the organic matter in the aqueous phase with increase in temperature in presence of Na₂CO₃.

Table 7: Properties of aqueous phase from non-catalytic and catalytic HTL

Strains	Temperature (°C)	TOC (g/l)		TN (g/l)		pH	
		Non-cat.	Catalytic	Non-cat.	Catalytic	Non-cat.	Catalytic
<i>Nannochloropsis</i>	250	59.05 ^{*A}	36.56 ^A	14.90 ^{*A}	9.67 ^A	7.72 ^A	8.53 ^A
	300	33.79 ^B	66.16 ^A	6.69 ^B	8.16 ^A	8.28 ^B	8.54 ^A
	350	28.78 ^C	73.16 ^A	10.18 ^C	9.06 ^A	8.28 ^B	9.42 ^B
<i>Pavlova</i>	250	51.12 ^A	29.79 ^A	6.58 ^A	7.96 ^A	7.70 ^A	8.31 ^A
	300	37.15 ^A	31.72 ^A	8.48 ^A	8.31 ^A	8.16 ^B	7.90 ^B
	350	28.94 ^A	34.17 ^A	10.22 ^A	8.05 ^A	8.35 ^C	8.02 ^{A,B}
<i>Isochrysis</i>	250	15.80 ^A	31.41 ^A	4.76 ^A	8.30 ^A	8.07 ^A	8.06 ^A
	300	41.53 ^{*B}	37.51 ^A	6.85 ^{*B}	6.56 ^{A,B}	7.66 ^B	8.22 ^A
	350	17.24 ^A	24.12 ^A	8.08 ^B	7.56 ^B	8.12 ^A	9.08 ^B

* contains single datum. Different alphabets in the superscript of each group (or row of each species) denote that the values are statistically different at different temperature levels.

Total nitrogen (TN) content in the aqueous phase also varied according to algal strains. Nitrogen in algae is present in the form of proteins and most of it readily decomposes into water soluble amino acids and ammonia [9, 15]. In the case of non-catalytic HTL, an increase in the nitrogen content was observed with increase in temperature but it depended on type of algae strain. Increase in TN with temperature may be due to increase in the deamination reaction that converts amine and amide compounds in bio-oil into ammonia which gets dissolved in the aqueous phase. A similar trend was observed by Yang *et al.* [25]. For the aqueous phase from catalytic runs, TN did not show any significant trend with the increase in temperature. Ross *et al.* [20] reported a decrease in nitrogen concentration with an increase in temperature for *Spirulina*, but reported no change in nitrogen concentration for *Chlorella*. This shows that increase or decrease in nitrogen concentration with temperature depends on the type of algae strain. The use of Na₂CO₃ as catalyst increased the nitrogen concentration at lower temperature for *Isochrysis* and *Pavlova*, this may be due to the catalytic effect of Na₂CO₃ which increases hydrolysis of proteins which undergoes further decomposition to form water soluble compounds. However, at higher

temperature it did not have any influence on the concentration of nitrogen in the aqueous phase when compared to the aqueous phase from non-catalytic HTL as shown in **Table 7**.

3.6. Carbon and Nitrogen Balance

Carbon and nitrogen balance were performed to investigate their distribution in the product streams of HTL. The carbon and nitrogen distribution in the product phases were calculated using the elemental analysis values from bio-oil, solid residue and water soluble product and the difference was assumed to be the gas phase and unaccounted portion. The elemental composition of water soluble product is presented in supplementary information (Table S2). **Figure 66** (a) and (b) illustrate carbon distribution for both non-catalytic and catalytic runs respectively. For both non-catalytic and catalytic runs, the bio-oil fraction accounts for the maximum amount of carbon percentage. The carbon distribution in bio-oil was influenced by the increase in bio-oil yield with increase in temperature. The carbon distribution in the bio-oil obtained from *Nannochloropsis* and *Isochrysis* significantly increased from 36% to 68% and from 43% to 56% of initial carbon in feedstock, respectively with the increase of temperature from 250 to 350 °C. However, there was no significant increase observed for *Pavlova* (53-58% of initial carbon). With the addition of Na₂CO₃, the carbon distribution in the bio-oil from *Pavlova* and *Isochrysis* significantly increased from 48% to 70% and from 51% to 59% of initial carbon in feedstock, respectively with the increase from 250 to 350 °C. However, it did not increase significantly for *Nannochloropsis* and ranged from 46% to 50% of initial carbon in feedstock. This change in carbon distribution was mainly due to the catalytic effect of Na₂CO₃ on the bio-oil yields which were discussed in Section 3.3. In comparison to non-catalytic runs, carbon distribution in bio-oil for the catalytic run was higher for *Pavlova* and *Isochrysis* at higher temperatures (300 °C and 350 °C).

Much of the carbon in lower temperature HTL appears in the water soluble product and solid residue. The carbon distribution in solid residue depended upon the yield and carbon conversion. It was observed to decrease with the increase in temperature. In non-catalytic experiments, the carbon distribution in solid residue of *Nannochloropsis*, *Pavlova* and *Isochrysis* were found to be in the range of 10.69 to 39.44 wt.%, 10.50 to 24.91 wt.% and 15.21 to 26.63 wt.% of initial carbon respectively. However with the addition of Na_2CO_3 , the carbon distribution in solid residue were found to be in the range of 10 to 24 wt.%, 9 to 13 wt.% and 5 to 24 wt.% for *Nannochloropsis*, *Pavlova* and *Isochrysis*, respectively. Carbon in the WSP was observed to be less for all the algae species. This was due to the loss of low molecular weight compounds during the drying of aqueous phase to obtain WSP. When compared to non-catalytic WSP, no change in the carbon distribution was observed for all the strains. High carbon distribution (40-45 wt.% of initial carbon) in the aqueous fraction was also observed by Valdez *et al.* [9] and Yang *et al.* [25], whereas a moderate carbon distribution (20-30 wt.% of initial carbon) was observed by Biller *et al.* [15] and Anastasakis *et al.* [12]. The carbon in the gas and unaccounted fraction (called as Gas & UA/C) was determined by the difference.

Figure 6: Carbon distribution in the HTL product streams (a) non-catalytic and (b) catalytic

Figure 7 (a) and (b) illustrate nitrogen distribution for both non-catalytic and catalytic runs, respectively. For the non-catalytic runs, nitrogen fraction in the bio-oil from all the strains ranged from 14 to 25 wt.% of initial nitrogen in the feedstock. Increase in the nitrogen distribution in bio-oil was observed with temperature and this was due to the increase in the total yield of bio-oil and conversion of protein to bio-oil. With the use of Na_2CO_3 , the nitrogen fractions in the bio-oil from all the strains were observed to be in the range of 18 to 26 wt.% of initial nitrogen in the feedstock. The amount of total initial nitrogen percentage distributed in the bio-oils obtained at 250°C was higher than that of the non-catalytic bio-oils at the same temperature. This increase was mainly due to the catalytic effect of Na_2CO_3 which induces efficient conversion of protein to nitrogenous compounds at lower temperatures. Anastasakis *et al.* [12] reported the distribution of about 40 wt.% of initial nitrogen in bio-oil whereas Biller *et al.* [15] observed it to be only 10-25 wt.% of initial nitrogen.

(a)

(b)

Figure 7: Nitrogen distribution in the HTL product streams (a) non-catalytic (b) catalytic

Nitrogen distribution in the solid residue was observed to decrease with increase in temperature.

With the use of Na_2CO_3 , the nitrogen distribution in solid residue were observed to be in the

range of 2.64 to 12.54 wt.%, 2.53 to 4.23 wt.% and 2.52 to 8.6 wt.% of initial nitrogen in the

feedstock for *Nannochloropsis*, *Pavlova* and *Isochrysis*, respectively. It was observed that at low

temperature, lower distribution of nitrogen was obtained for catalytic solid residue. Nitrogen

distribution in the water soluble product also showed a similar trend with an increase in

temperature. For the non-catalytic runs, nitrogen fractions in the WSP were found to decrease

with temperature and were in the range of 6.89 to 28.58 wt.%, 3.07 to 37.16 wt.% and 7.37 to

29.83wt.% for *Nannochloropsis*, *Pavlova* and *Isochrysis*, respectively. A similar trend in the

nitrogen distribution was observed with the use of Na_2CO_3 as catalyst. The opposite trend of

nitrogen in aqueous phase with increased temperatures was observed by Valdez *et al.* [9]. This

could be because Valdez *et al.* have analyzed whole aqueous phase rather than WSP for nitrogen

distribution. Jazrawi *et al.* [49] reported that the nitrogen in aqueous phase increase at low

temperature and high retention time and decreased at high temperature and high retention time.

The decrease in N distribution in aqueous phase with increased temperature was also observed by Ross *et al.* [20] with the use of alkali catalysts (Na_2CO_3 & KOH). Due to the high amount of nitrogen distribution in the aqueous phase, it can be recovered and further used as nutrient for algal cultivation or can be recovered as fertilizer.

4. Conclusions and Remarks

HTL of three different algae strains with and without catalyst of Na_2CO_3 were performed at three different temperatures of 250°C, 300°C and 350°C. It can be concluded that with the increase in temperature the bio-oil yield increased, and for non-catalytic experiment, maximum yield of bio-oil (48.67 wt.%, 39.96 wt.% and 40.69 wt.%) was obtained at 350°C for *Nannochloropsis*, *Pavlova* and *Isochrysis*, respectively. Use of Na_2CO_3 as a catalyst also had a similar effect with increased temperature but maximum bio-oil yield followed the order of *Pavlova* > *Isochrysis* > *Nannochloropsis*. Na_2CO_3 helped in selectively increasing the yield of high protein containing algae at low temperatures as in the case of *Nannochloropsis* and shifted the conversion of carbohydrate to higher temperature. The bio-oil produced had a high heating value in the range of 32 to 37 MJ/kg, had a low density, and was alkaline in pH for all three strains. The bio-oil produced resembled that of petroleum crude except for its high nitrogen (4-5 wt.%) and oxygen content (1-8 wt.%) which made it unsuitable for blending with petroleum crude. Apart from the product yield, the use of Na_2CO_3 had no significant effect on the properties of the bio-oil when compared to non-catalytic bio-oil properties. Analysis of other by-products such as solid residue and aqueous phase showed that the carbon and nitrogen fractions of the original biomass were distributed in them and their distribution depended on the reaction temperature and catalyst used. The aqueous product had a high amount of organic carbon and nitrogen content. Higher carbon content in the aqueous phase makes it a suitable biomass feedstock and can be further used for

fuel generation purposes and nitrogen in the aqueous phase can be recovered for use as a nutrient for algal growth.

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