Hydrothermal Liquefaction of Algae for Bio-oil Production

by

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Keywords: algae, hydrothermal liquefaction, bio-oil, catalyst, sodium carbonate, *Nannochloropsis, Isochrysis, Pavlova*

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Abstract

Increased demand of energy, depletion of fossil fuel resources, global warming and energy security issues have led to extensive research in renewable energy source. Among the different renewable energy sources, biomass is the only renewable energy source that can be converted to liquid fuels. This research is focused on the conversion of aquatic biomass (microalgae) to produce bio-oil through hydrothermal liquefaction pathways. Hydrothermal liquefaction (HTL) is a thermochemical process in which hot compressed water at a sub- or super-critical stage is used as a reaction medium to convert biomass to bio-oil and co-products (char, aqueous phase and gas). It is mostly suited for wet biomass such as algae.

In this study, effects of reaction temperatures and algal strains on HTL product yields were studied with and without using a catalyst (sodium carbonate). HTL was performed on three different algae strains viz. *Nannochloropsis, Pavlova a*nd *Isochrysis* at three reaction temperatures of 250, 300 and 350^{o}C, a holding time of 60 minutes and an algae loading of 14 solid wt.%. The selected algae strains were different from each other in terms of their biochemical composition. Although all the strains used had high protein content, *Nannochloropsis* had the highest protein content with a low carbohydrate and moderate lipid content, whereas *Pavlova* had high amount of carbohydrate in comparison to other algae. *Isochrysis* had a relatively equal distribution of biochemical composition.

The non-catalytic study showed that temperature influenced the liquefaction yields; an increase in temperature increased bio-oil and gas yield but reduced solid residue and water soluble product

However the variation of product yields depended on the algae strain. Maximum bio-oil yield (48.67 wt.%) was obtained for high protein containing algae strain (*Nannochloropsis*) at 350^{o}C. The bio-oil produced had high HHV (33-35MJ/kg), moderate pH (6-9), low density (900-950 kg/m^{3}), high TAN (30-65 mg KOH/g) and low moisture content (5-10 wt.% on wet basis). Apart from high nitrogen contents (4-6 wt.%), the elemental analysis resembled that of petroleum crude. A GC study showed that with the increased temperature organic acid content decreased while phenolic content increased. Nitrogen containing compounds like cyclic nitrogen and amides were also present in the bio-oil. A carbon and nitrogen balance showed that most of the carbon was distributed between the bio-oil (30-68 wt.%) and water soluble product (3-25 wt.%). The water soluble product also had a high initial nitrogen distribution, ranging from 3 to 37 wt.% with an increase of temperature.

Use of sodium carbonate had a significant role in decreasing the bio-oil yield of protein rich algae and increased the yield of carbohydrate rich algae. Maximum bio-oil yield (47.05 wt.%) was obtained for algae rich in carbohydrate (*Pavlova*) at 350^{o}C. Physical analysis of the catalytic run resembled that of non-catalytic runs. The chemical composition of bio-oil obtained from catalytic runs at lower reaction temperatures had lower organic acid content and higher hydrocarbons resulting in a lower TAN compared to bio-oil from non-catalytic runs. In addition more cyclic compounds were present in catalytic runs. Water soluble product from catalytic runs contained 5 to 25 wt.% of total initial nitrogen and 6 to 21 wt.% of total initial carbon. Overall, the use of sodium carbonate did not influence bio-oil characteristics, but it did have a significant role in increasing or decreasing bio-oil yield depending on the algae composition.

Keywords: Hydrothermal liquefaction, Nannochloropsis, Isochrysis, Pavlova, Algae, Bio-oil.

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CHAPTER ONE

1. INTRODUCTION

1.1 Background

Population explosion and its thirst for energy have led to an exponential increase in energy demand. The major share of the world's energy demand is being met by fossil fuels. According to the International Energy Agency [1], fossil fuels contributed 82% of the total primary energy consumed in the world, and about 62% of the world's oil is consumed by the transportation sector. Therefore, substituting liquid transportation fuels obtained from fossil fuels with renewable sources significantly reduces dependence on petroleum oils [2]. Biomass is the only renewable source of energy, which can be converted to liquid hydrocarbons and is abundant worldwide [3]. After coal, oil and natural gas, biomass is the fourth largest energy resource [4] and provides about 14% of the world's energy needs [5]. Biomass energy can be used for various purposes such as power and heat generation and also for production of valuable chemicals [6]. Terrestrial biomass such as woody biomass and energy crops are being used as a feedstock for producing liquid biofuels. However, due to the need for arable land, fresh water and fertilizer for its growth, there is a concern whether terrestrial biomass feedstocks are the appropriate choice for biofuels. In this context, the use of aquatic biomass offers an alternative option for converting biomass to biofuels [2].

Algal biomass is in forefront when it comes to aquatic biomass, as it does not compete with food for land, fresh water, and fertilizer, and can be grown in marine or waste water. In addition, it has higher production yields (40-60 dry ton/ha-yr) [7] than other energy crops and has a high carbon sequestration rate (1.8 kg of CO_2/kg of dry algae) [8]. Microalgae can be converted to bio-fuels by conventional methods like lipid extraction followed by transesterification or hydrodeoxygenation, but these technologies need a high lipid content algal strain. Likewise, algae can also be liquefied by thermochemical methods such as pyrolysis, but this process needs dry algae, which result in increase in overall operating cost and makes the process expensive. These problems can be addressed by hydrothermal liquefaction (HTL). HTL is mostly suited to wet biomass because it converts wet feedstock into liquid biofuels, making the overall process comparatively less expensive. Thus, the energy intensive process of drying wet biomass can be skipped in HTL. It occurs at sub- or super-critical temperatures and pressures. Bio-oil produced from HTL has a high heating value and less oxygen content compared to the bio-oil produced from the fast pyrolysis process. However, certain properties of bio-oil from HTL such as high nitrogen content (~5 wt. %), high viscosity and high total acid number (TAN) restrict its widespread applications and make it immiscible with conventional hydrocarbon fuels. High nitrogen content in bio-oil is not environmentally friendly as it produces NO_x gases on combustion. High total acid number of bio-oil determines the corrosiveness of the liquid fuels that can corrode internal wall of engine and degrade it. High viscosity of bio-oil impedes handling, processing and transporting of liquid fuels. Moreover, it affects the quality of fuel. Therefore, to reduce nitrogen content, TAN and viscosity of HTL bio-fuel, it should be further upgraded. Upgrading of bio-oil can be done using different techniques available such as catalytic cracking, catalytic hydrodeoxygenation, catalytic

denitrification and solvent addition. Upgrading of bio-oil can increase the quality of the bio-oil by making its properties similar to those of petroleum liquid fuels.

1.2 Research Objectives

Hydrothermal liquefaction of algae is still at its infancy and more studies are needed to be done to fill the knowledge gap in the literature. Different types of algae have different biochemical compositions, which affect HTL products yield and bio-oil properties. Therefore, here in this study, three types of algae strains with different biochemical compositions were selected for hydrothermal liquefaction. The overall objective of this study was to produce bio-oil from hydrothermal liquefaction of algae and characterize bio-oil properties. The specific objectives of the study were (i) to evaluate the effects of temperature and algal strains on bio-oil yield and its characteristics; and (ii) to determine the effects of an alkali catalyst on hydrothermal liquefaction.

1.2.1 Effects of temperature and algal strains on the product yields and its product characterizations.

In this objective, HTL of three algae strains, *Nannochloropsis, Pavlova* and *Isochrysis* was performed at three temperatures (250, 300 and 350^{o}C) and at a fixed residence time (1 h). The effects of temperature and algal strains on HTL products yields were analyzed. Different products obtained from HTL were also analyzed for their physical and chemical properties.

1.2.2 Effect of an alkaline catalyst on hydrothermal liquefaction of the algae strains.

In this objective, HTL of three algae strains, *Nannochloropsis, Pavlova* and *Isochrysis* was performed at three temperatures and a residence time of 1 h with the addition of an alkaline catalyst, Na_2CO_3. The effect of adding Na_2CO_3 on bio-oil yield was analyzed. Different products

obtained from HTL were analyzed for their physical and chemical properties, and the effect of the catalyst on these properties was studied and compared with that of without adding any catalyst.

To summarize, this thesis studied the effects of temperature, algae strains and alkaline catalyst (Na_2CO_3) on bio-oil yield and its properties. A review of the literature on algae hydrothermal liquefaction is presented in Chapter 2. Chapter 3 presents the effects of temperature and alkaline catalyst on HTL of three different types of algae strains and discusses its product characterizations. Chapter 4 summarizes the overall study with key findings and presents directions for future work.

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CHAPTER TWO

2. LITERATURE REVIEW

2.1 Energy Scenario

The rapid growth in world's population and its subsequent effect on industrial and transportation sector has led to a steep rise in the energy demands [1]. Most of the world's energy demands are met by fossil fuels. According to International Energy Agency [2], fossil fuel contributed 82% of the total primary energy consumed in the world, and about 62% of the world's oil consumption was by transportation sector in 2011. The exploitation of fossil fuels has caused major strain on the fossil fuel reserves of the world and has been the chief contributor to greenhouse gases emission, which is responsible for global warming [3]. Carbon dioxide is the main greenhouse gas emitted from burning of fossil fuels. According to International Energy Outlook 2013 [4], the world's energy-related carbon dioxide emissions will increase from 31.2 billion metric tons in 2010 to 36.4 billion metric tons in 2020.

Liquid fuel plays a vital role in the United States energy systems and economy. Figure 2.1 shows the U.S. primary energy consumption by fuel from the year 1980 to 2040 [5]. It illustrates that the total primary energy consumption grows by 12%, from 95 quadrillion Btu in 2012 to 106 quadrillion Btu in 2040. The share of fossil fuel falls from 82% in 2012 to 80% in 2040 due to decline in petroleum-based liquid fuels and increase in use of renewable sources of energy such as liquid biofuels. The projected growth rate for the U.S. energy –related CO_2 emission has declined in each year since 2005. In 2012, the U.S. greenhouse gas emissions was 6526 metric tons of CO_2

equivalent which was a decrease of 3.4 percent from 2011 [6]. This may be due to the increase in use of renewable fuels such as biofuels and increase in efficiency of the energy systems. This elucidates that biofuels can be alternatives to fossil fuels as they reduces the dependence on foreign imported fuels and provide energy security, reduce greenhouse gas emission and stabilize the cost of the fuel.

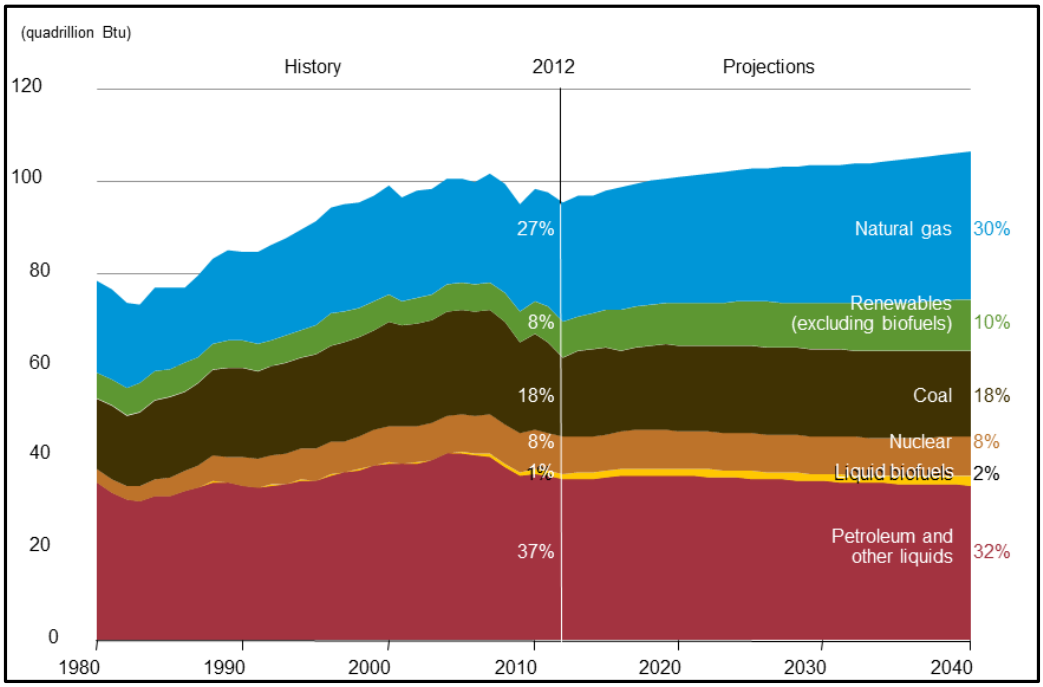

Figure 2.1. U.S. primary energy consumption by fuel, 1980-2040 [5]

According to the U.S. Energy Information Administration [5], biomass energy consumption grew more than 60% from 2002 to 2013. This growth was mostly due to the increase in the production of ethanol and bio-diesel. In 2013, biomass accounted for about 5% of the total U.S. energy consumption and about half of all renewable energy consumed.

2.2 Biomass and Biofuels

Biomass is an organic matter derived from biological species on a renewable basis [7]. Fuels derived from biomass sources like agricultural crops, herbaceous and woody energy crops, municipal organic wastes as well as manures are termed as biofuels. It is a promising alternative to fossil fuels as it is the only renewable source for the liquid fuels production and has a worldwide abundance [8]. After coal, oil and natural gas, biomass is the fourth largest energy resource [9] and provides about 14% of the world's energy needs [10]. Biomass energy can be used for various purposes such as power and heat generation and also for production of valuable chemicals [11]. The significance of biomass energy are: (1) it is CO_2 neutral as it does not increase CO_2 concentration in the atmosphere; (2) CO_2 released during combustion of biofuels is removed from the environment by photosynthesis during the production of biomass and (3) environmentally benign because it contains very low sulfur and nitrogen; hence the fuel obtained from biomass is clean with very minimal emission of NOx and SOx [12]. In addition, biomass are evenly distributed over the earth's surface so they increase scope for diversification and decentralization of energy supplies and hence achieve energy self-sufficiency at a local, regional and national levels [8].

Different types of biomass sources such as agricultural crops, herbaceous and woody energy crops, municipal organic wastes and manure are used for biofuel productions. Biofuels produced from food source or agricultural crops like corn, soybeans, canola oil etc. are termed as first generations biofuels [13]. Crops like sugarcane, corn etc. are used for producing bio-ethanol while soybean, rapeseed etc. are used for bio-diesel production. Today, almost 50 billion liters of the first generation biofuels are commercially produced annually [14]. But the main disadvantages of first generation biofuels are the use of food crops for energy production and their environmental

impacts and carbon balances [14]. To overcome the limitations of first generation biofuels, non-food crops such as lignocellulosic biomass, agricultural and municipal waste, and manure are used for biofuel production which are termed as second generation biofuels [13]. The examples of second generation biofuels are cellulosic ethanol and Fischer-Tropsch fuels [14]. At present, the production of second generation biofuels are not cost effective and are non-commercial due to number of technical barriers that need to overcome [14]. Third generation biofuels are fuels derived from algal biomass and to a certain extent to utilization of CO_2 as feedstock [15]. The main merit of algae as biomass is that it provides much higher yields of biomass and fuels than comparable energy crops and can be grown under conditions, which are unsuitable for conventional crop production. Thus it relieves food-versus-fuel pressure on agricultural land [16, 17]. However, the use of large volumes of water and presence of protein in the algae which imparts a significant amount of nitrogen content in the algal biofuel are the problems associated with its utilization. At present, the production of third generation biofuels are still at research level.

2.3 Algae as Biomass

Among many source of biofuels, algae has recently got into prominence because of its advantages over other biomass types. Algae provides much higher yields of biomass and fuels than comparable energy crops, and are capable of fixing CO_2 in the atmosphere thus resulting in the reduction of increasing atmospheric CO_2 levels [18].

Algae are autotroph having a large and diverse group of over 36000 species, ranging from unicellular microalgae to macroalgae such as seaweeds [19]. Based on their pigment, macroalgae are broadly divided into three groups; brown seaweed (*Phaeophyceae*), red seaweed (*Rhodophyceae*) and green seaweed (*Chlorophyceae*) [20]. In terms of abundance, microalgae are broadly divided into three groups; the diatoms (*Bacillariophyceae*), the green algae

(*Chlorophyceae*), and the golden algae (*Chrysophyceae*) [20]. Generally, microalgae have a high lipid concentration but a low carbohydrate concentration and vice versa for the macroalgae. As a result, microalgae are preferably used for biodiesel and macroalgae for ethanol synthesis.

Algaculture is a form of aquaculture which involves algae farming [21]. Microalgae can be grown both in fresh water and marine environment but macroalgae can grow only in marine environment. Algae can be cultivated in photobioreactor (as shown in Figure 2.2 b) which is a closed system or in an open pond (as shown in Figure 2.2 a) which is an open system. Open pond system is the simplest cultivation methods with the sun as source of light and energy, and the air as source of carbon dioxide. This is the natural method of growing algae which was used and favored by NREL's Aquatic Species Program (ASP) [19]. The benefit of the open pond system is that it is cheap to build but it has its own limitation too. This system has less control over the light, temperature, CO_2 concentration, nutrition and is prone to contamination by other extremely quick reproducing algal strain. Photobioreactor is a closed system cultivation of algae. Most of the deficiencies of the open system cultivation can be addressed by photobioreactor. Photobioreactor reduces contamination and gives better control over the guiding parameters of the system such as temperature, light, carbon dioxide intake etc. Photobioreactors can be tubular or flat on the basis of the shape.

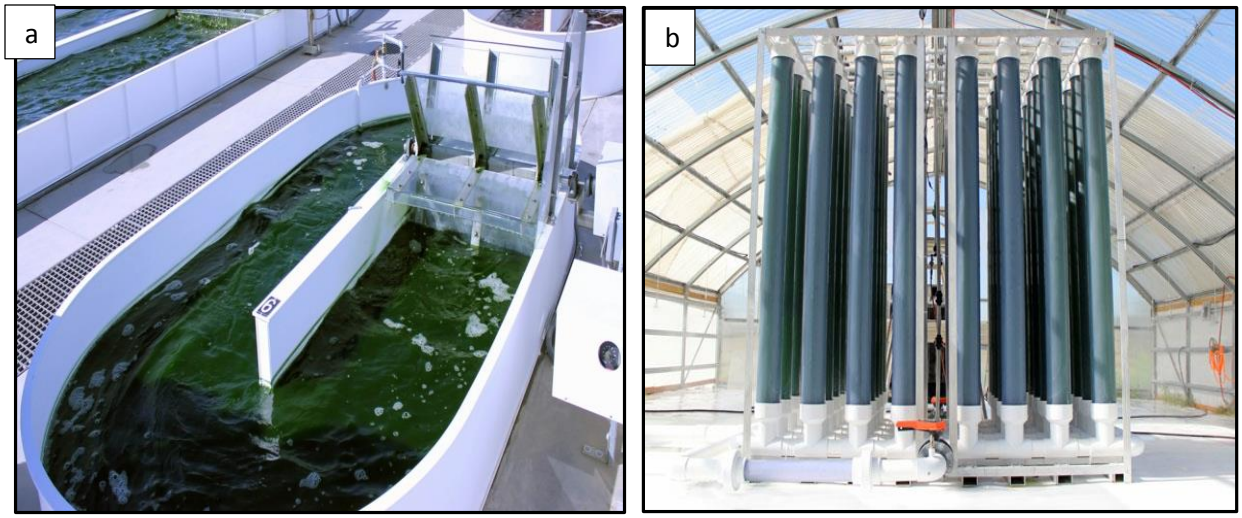

Figure 2.2. a) Open pond system [22]. b) Photobioreactor system [23].

Harvesting of algae is one of the most expensive, challenging and energy demanding processes in the production of algal biodiesel. Much of the microalgae are unicellular and motile so it becomes very tough to harvest. In comparison to other energy crops, the growth rate of algae and its subsequent harvesting rate is very high. Filtration, froth flotation, centrifugation, flocculation, sedimentation etc. are some of the algae harvesting methods. The harvesting method of algae depends on species, growth medium, algae production, end product and production cost benefit [24]. The main challenges in harvesting of algae are its size and motility, long harvesting period of high oil containing algae and high cost of flocculants [24].

The extraction process of algae is not well defined. Extraction of oil from algae is very difficult as it has very high moisture content and the cell membrane of algae does not rupture easily like that of seeds [25]. Pressing, chemical solvent, supercritical fluids and ultrasonic are some of the methods that are being used for algae oil extraction [25]. The choice of extraction methodology can affect the design of production process or vice versa.

The oil extracted from the algae can now be used as oil for the diesel engine but the efficiency is very low due to the high viscosity, lower volatility and reactivity of unsaturated hydrocarbon chain of the algae oil [1]. Triacylglycerides (TAGs) present in the algal oil can be converted into biodiesel through transesterification [26]. If the level of fatty acids in the oil is high, pretreatment of the oil should be done before transesterification and the process is termed as esterification [27]. In this process, the algal oil is reacted with alcohol in the presence of a strong acid catalyst (sulfuric acid), converting the free fatty acids into alkyl esters [27]. In transesterification, TAGs are converted into methyl esters of fatty acid (biodiesel) and glycerin by reacting it with alcohol (methyl alcohol) in the presence of strong base such as sodium hydroxide or potassium hydroxide [26, 27]. As the solubility of glycerol is low, it is easily separated by a settling tank or a centrifuge [27]. The excess methanol is extracted from the product by the addition of soft hot water as it will carry out all the methanol content from the product [27]. The biodiesel thus produced are used only after it has met ASTM D6751 standard [27].

Algae fuel can also be processed by thermochemical pathways. Hydrothermal processing is one of the innovative ways of thermochemical conversion of algae oil into biofuels. Other thermochemical processing such as combustion, pyrolysis and conventional gasification are also used but needs drying of algae before processing, which is a major drawback of these processes [28]. However, in hydrothermal processing wet algae can be used to produce oils which can be upgraded and used [29]. Moreover this process not only produces oil from lipids, as in case of conventional lipid extraction method, but also from carbohydrate and proteins, thereby increasing the total oil production with respect to initial lipid percentage [29, 30]. High throughput, high energy content, ability to use varied feedstocks like waste and lignocellulose and no need of maintaining specialized microbial cultures or enzymes are some of the added advantages of this

process [31]. Water plays an important role in hydrothermal processing, and its properties should be understood in order to understand hydrothermal processing.

2.4 Sub- and Super-critical Water Properties

Water is an ecologically safe substance that is spread throughout nature [32]. Water plays an essential role in hydrothermal liquefaction. It acts as a reaction medium, reactant and catalysts precursor [33]. The water used herein is heated and compressed simultaneously [12]. A phase diagram of pure water is displayed in Figure 2.3.

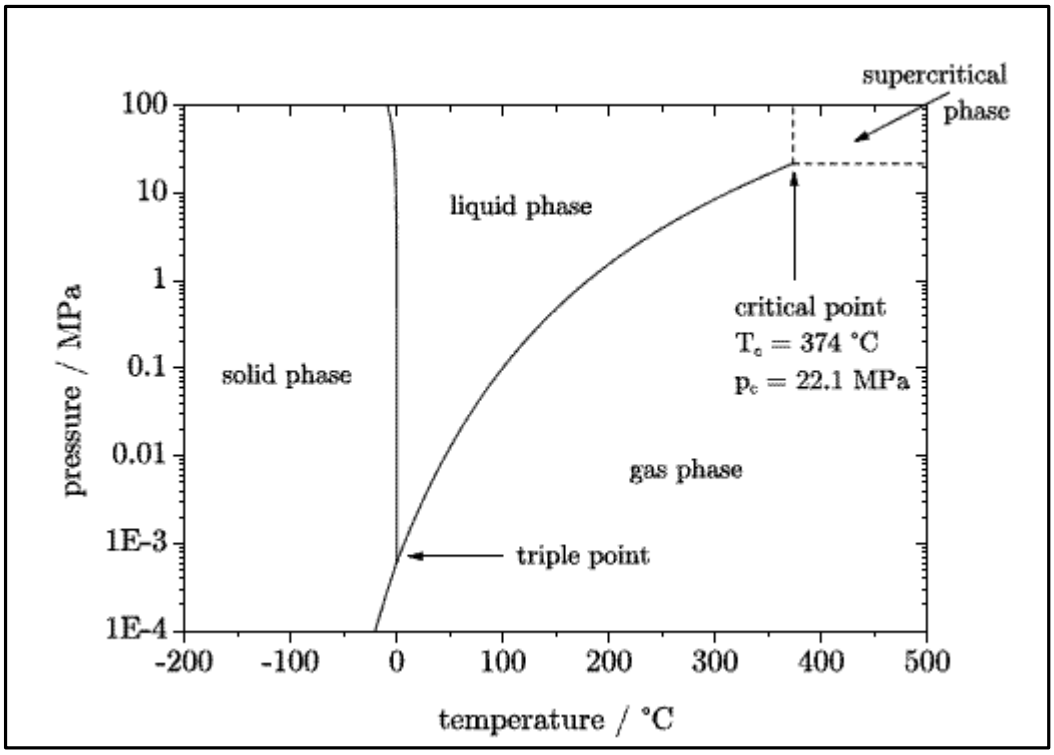

Figure 2.3. Pressure vs. temperature phase diagram of pure water. Adopted from [34].

Below the critical point, the vapor pressure curve separates the liquid from the gaseous phase but as the critical point approaches, the properties of both phases become increasingly similar and finally identical at the critical point [32]. Liquid water below the critical point is referred as subcritical water whereas water above the critical point is called supercritical water. Significant

attention has been drawn towards subcritical and supercritical water to perform chemical reactions due to its tunable properties and environmentally benign nature of the medium [12]. Water from ambient to supercritical conditions changes its characteristics from a solvent for ionic species to a solvent for non-ionic species due to the alteration in its properties like dielectric constant, ionic product, solubility etc.[12]. The different physical and chemical properties of water are discussed below.

2.4.1 Dielectric Constant

The dielectric constant (relative permittivity) of water can play a key role in influencing biomass reactions [12]. The dielectric constant decreases with increasing temperature and drops around critical point due to weaker hydrogen bonds as shown in the Figure 2.4 [35]. When thermal energy increases, the electronegativity of the oxygen molecule is reduced because the shared electron between oxygen and hydrogen atoms tends to circulate more evenly as a result water becomes less polar [36].

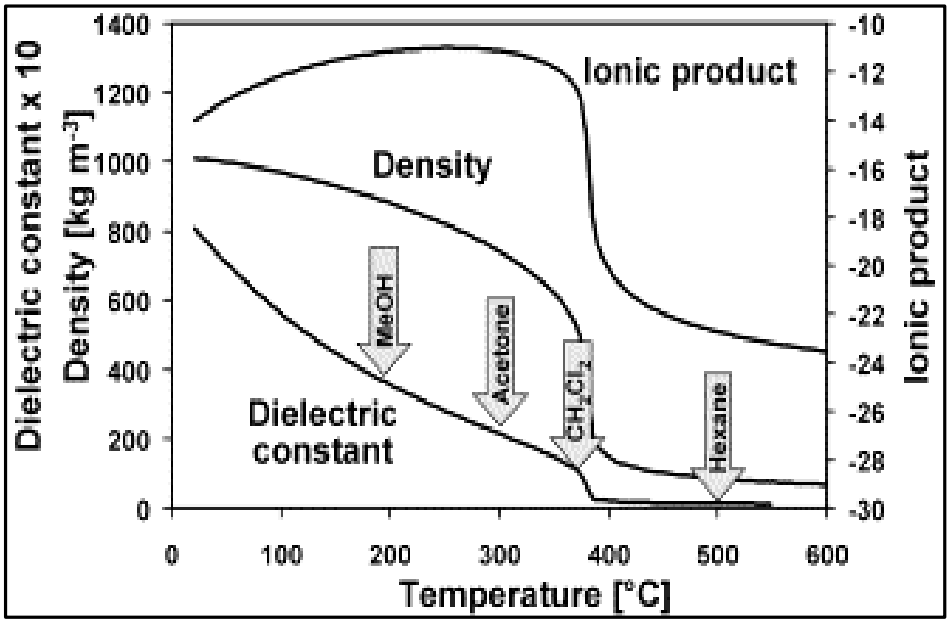

Figure 2.4. Physical properties of water with temperature, at 24 MPa [37].

Uematsu et al. [38] proposed the following empirical equation to correlate the experimentally measured dielectric constant for water with temperature and density:

$$\varepsilon_r = 1 + \left(\frac{A_1}{T^*}\right)\rho^* + \left(\frac{A_2}{T^*} + A_3 + A_4 T^*\right)\rho^{*2} + \left(\frac{A_5}{T^*} + A_6 T^* + A_7 T^{*2}\right)\rho\,{*}^3 + \left(\frac{A_8}{T^{*2}} + \frac{A_9}{T^*} + A_{10}\right)\rho\,{*}^4 \quad \textbf{(2.1)}$$

where,

ε_r is dielectric constant,

$T^* = \frac{T}{T_o}$, where T is temperature in K and T_o is 298.15K.

$\rho^* = \frac{\rho}{\rho_o}$, where ρ is density in kg/m^3 and ρ_o is 1000 kg/m^3.

A_i are the fitting parameters given in [38]. This equation is valid for temperature range of 273.15 K to 823.15 K and from density of 0 to 1150 kg/m^3. For example, at 403.15 K (density 900 kg/m^3), the dielectric constant is around 50, which is near that of formic acid and at 473.15 K (density 800 kg/m^3), it is 25, which is similar to that of ethanol. This polarity change makes water more affinitive to the organic hydrocarbons, most of which are nonpolar molecules [36].

2.4.2 Ionic Product

Ionic product is another important property of water that varies considerably with changes in temperature and density [33]. *Marshall and Franck* [39] proposed the following empirical equation to correlate the experimentally measured ion product of water (Kw in mol/kg^2) with temperature and density:

$$logK_W = A + \frac{B}{T} + \frac{C}{T^2} + \frac{D}{T^3} + \left(E + \frac{F}{T} + \frac{G}{T^2}\right)\log\rho \quad \textbf{(2.2)}$$

where T is temperature in kelvin, ρ is a density in g/cm^3 and A-G are fitting parameters [39].

The ionic product increases up to around 10^{-11} (by 1,000 folds) when liquid water is heated to near critical (200-300^{o}C) conditions [32]. Hence, near critical point, water provides an excellent acid/base environment because of high concentration of H^+ and OH^- ions, which can catalyze number of biomass-related reactions, without the need for adding external reagents [12]. The reason behind increment of ionic product at high temperature is that the self-dissociation of water is endothermic [32]. Above critical temperature, the ionic product decreases significantly with temperature but increases with pressure [32]. For example ionic product is about 9 orders of magnitude lower at 600^{o}C (and 25 MPa) than at ambient condition [12]. Accordingly free radical reactions dominates at these high temperature and low density conditions [33]. Antal *et al*. [40] proposed that ionic mechanisms are favored when $Kw > 10^{-14}$ and free –radical mechanisms are favored when $Kw << 10^{-14}$ [33]. Acid- or base-catalyzed reactions in hydrothermal medium shows a characteristic non-Arrhenius kinetic behavior near the critical point of water. The reaction rates increases with temperature below the critical temperature but at the critical point, reaction rates may increase or decrease drastically depending on the chemistry and properties [41]. Both the relative dielectric constant and the ionic product increases with pressure but the detailed mechanism of reactions are unknown [32].

2.1.3 Solubility

At standard conditions (T=25^{o}C, P=1 atm) water is poorly soluble with hydrocarbons and gases but acts as a good solvent for salts because of its high relative dielectric constant of 78.5 at a high density of 997 kg/m^3 [42]. However, at critical temperature and pressure, the relative dielectric constant is in the range of 10 which resembles to the dielectric constant of organic solvents and further decreases with the increase of temperature [42]. Therefore, supercritical water at low

densities is a poor solvent for ionic species like inorganic salts, whereas it is completely miscible with many organic compounds and gases [43]. At 300-400^{o}C solubility of salts reaches maximum and drops very rapidly after that with the increase of temperature [12, 44]. For example, solubility of NaCl is 38.99 wt.% at 100^{o}C and reaches 40 wt.% at 300^{o}C and decreases to 100 ppm at 450^{o}C, 25.3MPa [44]. At near critical point, water often does not exhibit complete miscibility, but an increased solubility of organic compounds with decrease solubility of inorganics salts. This can be correlated with the change of the relative dielectric constant [45].

2.1.4 Transport Properties

Under hydrothermal medium, diffusion rates are high and viscosity is low which give rise to efficient heat transfer and mass transfer for solid/liquid biomass liquefaction reactions [12]. At 15 MPa, viscosity of water at 25^{o}C is 0.89 cP, at 200^{o}C is 0.14 and at 300^{o}C is 0.09 cP [12]. The increase in the self-diffusivity of water with the increase of temperature and decreasing density is

Table 2.1. Comparison of ambient and supercritical water [46]

Properties	Ambient water	Supercritical water
Dielectric constant	78	<5
Solubility of organic compounds	Very low	Fully miscible
Diffusivity ($cm^2\ s^{-1}$)	10-5	10-3
Viscosity ($g\ cm^{-1}\ s^{-1}$)	10-2	10-4
Density ($g\ cm^{-3}$)	1	0.2 – 0.9

due to the breakage of hydrogen-bond network which reduces the barrier for translational and rotational motions [33]. Diffusivity increases by roughly an order of magnitude when the density changes from 1 to 0.1 g/cm^3 [33]. At supercritical stage, the diffusivity behavior is qualitatively

consistent with the kinetic theory of gases [33]. These transport properties can be used to reduce heating and mixing needs in the hydrothermal reactors [12].

2.2 Hydrothermal Processing

Hydrothermal processing (HTP) is a thermo-chemical reaction in which water at high temperature, higher than its boiling temperature, and high pressure, acts as a solvent involving one or different precursors in a closed system [47]. Figure 2.5 shows temperature and pressure range of water phase diagram at which different products from HTP of biomass are obtained. HTP utilizes different properties of hot and compressed water (as discussed in section 2.1) to convert the reactant to product. HTP is mostly suited for wet biomass (like algae) as it obviates the need to dry biomass

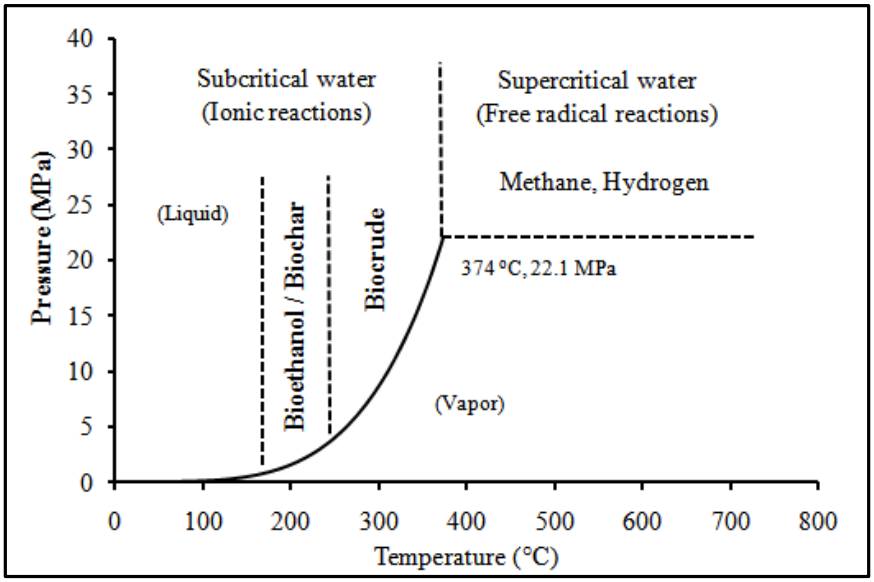

Figure 2.5. Hydrothermal treatment application referenced to pressure-temperature phase diagram of water [46]

and reduces the energy requirements while increasing the overall efficiency of the system [12, 36, 46, 48]. High throughput, high energy content, versatility of chemistry, ability to use varied feedstocks like waste and lignocellulose and no need of maintaining specialized microbial cultures or enzymes are some of the added advantages of this process [31]. Depending on the processing

temperature, pressure and other parameters, it is possible to tune the process in the direction of solid carbon (carbonization), liquid fuel (liquefaction) and synthesis gas (gasification) [48].

2.2.1 Hydrothermal Carbonization

Hydrothermal Carbonization (HTC) is a type of HTP in which biomass is heated in water at mild temperature of below 225^{o}C and a mild pressure of below 2 MPa to create a char product or biochar [49]. The main goal of this process is to increase the carbon-to-oxygen ratio in the product [49]. The product thus formed has potential to be used for synthesis gas conversion as a carbon neutral supplement to natural coal and also can be converted to industrial chemicals and soil nutrient amendments [49, 50]. Heilmann *et al.[49]* compared the properties of char produced from HTC of microalgae (*C. reinhardtii and D. Salina*) with natural coal and found it to be very similar. The carbon and hydrogen content were higher in algal chars produced from HTC, and are favorable for fuel synthesis [49]. The nitrogen content were also higher in the algal chars. The char produced from HTC of microalgae which are very rich in lipids can be reacted with alcohol to produce biodiesel [51]. Apart from the solid residue, the aqueous byproduct form HTC can also be converted to nutrients for algae growth as it contains considerable quantities of nitrogen-containing solutes [49].

2.2.2 Hydrothermal Liquefaction

Hydrothermal (direct) liquefaction (HTL) is a type of HTP in which biomass is converted into bio-crude/bio-oil using water at sub- or super-critical temperatures and pressures. The temperature and pressure range for a typical HTL are 280-380^{o}C and 7 – 30 MPa, respectively and sufficient to keep water in a liquid state [31]. HTL mimics the natural geological process thought to be involved in the production of fossil fuels [36]. The primary product of HTL is a black organic liquid called bio-crude or bio-oil and the main by-products are solid residue, aqueous products or water soluble

product and gases. The solid residue are also known as biochar. Bio-oils produced by this process has high oxygen content and higher nitrogen content (in case of algae as feedstock) although this makes the bio-oils generally unprocessable with petroleum feedstocks but it can serve as a starting material for valuable petroleum based fuels and products such as polymers, aromatics, lubricants etc. [31, 52]. Water soluble product or the aqueous phase of HTL is the solution of dissolved organics in the water.

HTL of biomass has been studied as a process for fuel production for last forty-five years. Oil production from biomass in hot water using alkali as catalyst were envisaged by Berl as early as 1920's [53]. The modern development of the process occurred following the Arab oil embargo of 1974 [31]. The concept of biomass (wood powder) to oil using high-pressure was developed by Appell and coworkers at the Pittsburgh Energy Research Center (PERC) and demonstrated at the Albany Biomass Liquefaction Experimental Facility at Albany, Oregon [31, 54]. But in this process, the reaction took place in an oil-rich phase instead of water-rich phase that is being used in today's researchers' process [31, 54]. Later researchers at Lawrence Berkeley Laboratory suggested the use of water-rich phase but employed subsequent alkaline and acid treatments [55]. Shell, the oil company, also developed the liquefaction process known as "Hydrothermal Upgrading", or the HTU process in the 1980s but abandoned the process in 1988 [31]. In the early 1980s, as the price of petroleum began to drop, HTL research was halted by the U.S. Department of Energy [31].

HTL of different lignocellulosic biomass such as wood, straws, stalks, shells and husks have been successfully performed and wealth of information about these can be found in literature [37, 56-59]. Recently, studies on hydrothermal liquefaction of aquatic plants such as algae in particular has been increasing and gaining interest as the process is mostly suited for conversion of wet

feedstock. In addition, the conventional approach (transesterification) for making biofuel from algae requires dewatering and drying of wet algae and also uses organic solvent for the extraction of lipids which makes it expensive [60]. Furthermore, in hydrothermal liquefaction of algae, not only the lipids but the whole algae is also used for producing bio-crude and the total yield is higher than the original lipids in the algal biomass, this suggests that oils are produced from non-lipid components of the algal biomass as well [31, 61].

Dote *et al.* (1994) were the pioneer in HTL of microalgae. Since then various researchers have worked on hydrothermal liquefaction of algae [30, 36, 62-69]. *Botryococcus braunni*, *Chlorella vulgaris, Dunaliella tertiolecta, Nannochloropsis sp., Microcystis viridis, Spirulina* etc. are some of the algae that has been studied for hydrothermal liquefaction. Large variances of bio-oil yield and quality indicate that either algal type or operating conditions, or both, significantly affect algal HTL. Table 2.2 lists the previous work performed on HTL of algae. There are studies performed on the effects of operating conditions on HTL of algae. Jena *et al.* [64] studied the effect of operating conditions on the HTL of *Spirulina platensis* and found out that the maximum yield was obtained at 350^{o}C for 60 min holding time and at 20% solids concentrations. This maximum yield at 350^{o}C was also observed by Anastasakis *et al.* [62] and Brown *et al*. [60]. Anastasakis *et al.* [62] studied the effect of operating parameters for *Laminaria saccharina* and observed the maximum yield at 350^{o}C for the residence time of 15 min and at 1:10 biomass: water ratio. Brown *et al*. [60] performed extensive study on the effect of temperature on *Nannochloropsis sp*. bio-oil yield and the study was carried out at temperature range of 200-500^{o}C for holding time of 60 min and observed that maximum bio-oil yield (43 wt %) was obtained at 350^{o}C. However, in contrast, Valdez *et al.* [68] observed the maximum yield of bio-oil at 300^{o}C at 20 min holding time for *Nannochloropsis sp.*. Alba *et al*. [30] also investigated the effect of temperatures on HTL of

Desmodesmus sp. at two different residence time of 5 min and 60 min and observed that the maximum yield (49 wt.%) was obtained at 375^{o}C at 5 min residence time. These variations in the optimum operating parameters could be due to the different types of biomass composition of different algae species used.

Catalytic study of HTL of algae have also been reported in several [29, 62, 63, 65-67, 69-74]. Table 2.2 also shows the catalysts used in HTL of algae. Both homogeneous and heterogenous catalysts have been used to study the HTL of algae. The most studied homogeneous catalysts for microalgae HTL is Na_2CO_3 [75]. Dote *et al.* [70], Inoue *et al.* [71], Minowa *et al*. [66], Shupping *et al*. [74] reported the increase of bio-oil yield with the increase of liquefaction temperature from 300^{o}C to 340^{o}C. But, in contrast, Yang *et al*. [73] and Ross *et al*. [67] observed the decrease in bio-oil yield with the introduction of Na_2CO_3. Ross *et al.* [67] studied the effect of alkaline and organic acids as catalysts in HTL of *Chlorella vulgaris* and found out that the bio-oil yield was maximum for organic acids. The bio-oil yields followed the trend of CH_3COOH > HCOOH > KOH > Na_2CO_3 for the use of catalysts [67]. Heterogeneous catalysts study on HTL have also been performed [63, 65]. Duan *and* Savage [63] studied the HTL of *Nannochloropsis sp*. using variety of common catalysts Pd/C, Pt/C, Ru/C, Ni/SiO_2-Al_2O_3, CoMo/γ-Al_2O_3 under hydrogen and helium atmosphere at 350^{o}C. In absence of added H_2, all the catalysts tested produced higher bio-oil yields of bio-oil in comparison to the bio-oil yields without catalysts and maximum bio-oil yield of 57% was obtained when Pd/C catalysts was used. The study also observed that the use of catalysts was largely insensitive to the presence or absence of hydrogen [63].

Although many studies have been done on HTL using homogenous catalysts but not much studies are done in case of heterogeneous catalysts and more work is needed to identify better heterogeneous catalyst for the process.

Table 2.2. Previous work done on hydrothermal liquefaction of algae

Year	Species	Catalysts	Observations	Reference
1994	*Botryococcus braunni*	Na_2CO_3	More oil was obtained than the amount of lipids contained in the algal cells. The experiments were conducted at the temperature range of 200-300^oC for 60 mins. The maximum oil yield was obtained at 300^oC was found to be 64 wt. %.	Dote *et al.* [70]
1994	*Botryococcus braunni*	Na_2CO_3	The properties of oil obtained by HTL are clarified. The oil was fractionated into three fractions of 5% of lower molecular weight hydrocarbons (MW, 197-281), 27.2% of botryococcenes (MW, 438-572) and 22.2% of polar substances (MW, 867-2209). At 200^oC, the maximum recovery of 78% was converted.	Inoue *et al.* [71]
1995	*Dunaliella tertiolecta*	Na_2CO_3	The experiments were conducted at the temperature range of 250-340^oC for time of 5 & 60 mins. The oil yield was about 37 wt.% on organic basis and had a calorific value of 36 MJ/kg. Viscosity of 150-330 mPa.s was found for the oil obtained from 340^oC and 60 min residence time. Na_2CO_3 had no catalytic effect on either the oil yield or its properties. The gas product was mostly CO_2.	Minowa *et al.* [66]

Table 2.2 continued.

Year	Species	Catalysts	Observations	Reference
1997	*Spirulina*	$Fe(CO)_5S$	This paper compared the oil yield of (*Spirulina*) species in different solvent medium with addition of $Fe(CO)_5$-S as catalyst and temperature range of 300-425^oC. Liquefaction in water as solvent gave more oil yield (78.3 wt.%) than organic solvent (52-68.9 wt.%) at 350^oC but the oxygen content in water as solvent was higher than in the organic solvent as a result the calorific value obtained were 26 MJ/kg and 32-36 MJ/kg respectively.	Matsui *et al.* [65]
1999	*Botryococcus braunni & D. tertiolecta*	Na_2CO_3	Both the species were compared for their oil yield and other properties. At 300^oC, the oil yield from *Botryococcus braunni* was found to be higher (64%) than *D. tertiolecta* (42 wt.%). The lower heating value was also found to be higher for *Botryococcus braunni* (45.9 MJ/kg) than *D. tertiolecta* (34.9 MJ/kg).	Sawayama *et al.* [72]

Table 2.2 continued.

Year	Species	Catalysts	Observations	Reference
2004	*Microcystis viridis*	Na_2CO_3	This study found out that oil yield depends upon the reaction temperature, holding time and catalyst dosage. Increase in the reaction temperature (300°C–340°C) and use of catalyst (5 wt.%) increased the oil yield and decreased the oil yield as the holding time increased from 30 min to 60 min. Aqueous phase, gas phase, solid residue phase were also studied. Carbon dioxide and methane constituted most of the gas phase. Nitrogen (998-1157 mg/l) and phosphate (2.45-5.38 mg/l) contents were present in the aqueous phase. Solid residue decreased as catalyst was introduced.	Yang *et al*. [73]
2010	*Dunaliella tertiolecta*	Na_2CO_3	This study was conducted at temperature range of 280-380°C at various residence time of 10-90 min with catalysts dosages of 2.5-10 wt%. Maximum oil yield 25.8 wt.% was obtained at 360°C and a residence time of 50 min using 5% catalyst. The heating value was found to be 30.74 MJ/kg.	Shupping *et al.* [74]

Table 2.2 continued.

Year	Species	Catalysts	Observations	Reference
2010	*Chlorella vulgaris, Spirullina*	Alkaline (Na_2CO_3 & KOH) Organic acids (CH_3COOH & HCOOH)	The study compares the outcome of HTL using organic acids (formic acid and acetic acid) and alkali (sodium carbonate and potassium hydroxide) as catalysts at temperatures of 300^oC & 350^oC for two low lipid containing species *Chlorella vulgaris* and *Spirulina*. The oil yield was high for organic catalysts and higher for *Chlorella* than *Spirulina* with increase in temperature but the oxygen and nitrogen content in biocrude for organic catalyst was higher than alkali catalysts. Heating value was highest for chlorella at 350^oC when using KOH as catalysts.	Ross *et al.* [67]
2010	*Enteromorpha prolifera*	Na_2CO_3	The marine macroalgae was liquefied in a batch reactor at temperatures of 220 - 320^oC with 5 wt.% Na_2CO_3 and reported that the bio-oil yield (23 wt.%) and HHV (30 MJ/kg) of bio-oil was highest at 300^oC with the reaction time of 30 min but decreased at 320^oC. The study also showed that there was little effect of using sodium carbonate on bio-oil yield. Acetic acid and glycerol along with various nitrogen-containing compounds were found in water soluble organics.	Zhou *et al.* [69]

Table 2.2 continued.

Year	Species	Catalysts	Observations	Reference
2010	*Nannochloropsis sp.*	None	This study was carried out at temperature range of 200-500^{o}C for holding time of 60 min. Bio-oil yield was highest (43 wt.%) at 350^{o}C and heating value was found to be 39 MJ/kg. Fatty acids, alkanes, alkenes, sterol-related compounds and heterocyclic N-containing compounds were produced at lower temperatures and the higher temperatures produced mostly PAHs. The oil yield decreased after 350^{o}C due to the initiation of gasification process and there was decrease in the value of H/C and O/C ratios decreased from 1.73 and 0.12 respectively at 200^{o}C to 1.04 and 0.05 respectively.	Brown *et al.* [60]
2011	*Laminaria saccharina*	KOH	This study investigated the influence of reactor loading, residence time, temperature and catalyst loading on HTL products. A maximum bio-oil yield of 19.3 wt% was obtained at 350^{o}C at a residence time of 15 min and at 1:10 biomass: water ratio without the presence of catalysts. HHV of 36.5 MJ/kg was obtained.	Anastasakis *et al.* [62]

Table 2.2 continued.

Year	Species	Catalysts	Observations	Reference
2011	*Desmodesmus sp.*	None	The study investigated the effect of temperatures (175-450^{o}C) and reaction times of up to 60 minutes. The oil yield (49 wt.%) was found to be maximum at 375^{o}C at 5 minutes reaction time, recovering 75% of the calorific value and with cell analysis they found out that the major cell wall rupture took place when going from temperature range of 225^{o}C to 250^{o}C. The oxygen yield decreased as the temperature increases but the nitrogen content gradually increased with the increase of temperature which was due to more protein conversion to liquid fuel.	Alba *et al.* [30]
2011	*Spirulina platensis*	None	This study investigated the influence of operating conditions for producing biocrude. The operating conditions include temperatures (200-380^{o}C), holding times (0-120 min) and solid concentrations (10-50%). The highest bio-oil yield (39.9 wt.%), representing 98.3% carbon conversion efficiency was produced at 350^{o}C, 60 min holding time and at 20% solids concentration.	Jena *et al.* [64]

Table 2.2 continued.

Year	Species	Catalysts	Observations	Reference
2011	*Nannochloropsis sp.*	Pd/C, Pt/C, Ru/C, Ni/SiO_2, CoMo, Zeolite	Studied the effect of six different heterogeneous catalysts on the production of bio-oils from microalga (*Nannochloropsis sp*.) under inert (helium) and high pressure reducing (hydrogen) conditions at 350^{o}C. In absence of added H_2, all the catalysts tested produced higher yields of bio-oil (max. being 57 %) and gas from the liquefaction but the elemental compositions and HHV (38 MJ/kg) of the oil were largely insensitive to the catalysts used. Presence of Ni catalyst reduces sulfur content beyond the detection limits but there was no significant decrease in nitrogen content in the bio-oil with all the catalysts.	Duan *et. al.* [63]
2011	*Spirulina* *Swine manure* *Digested anerobic sludge*	None	This paper compared the HTL product of different biomass and its properties at 300^{o}C, 10-12 MPa and 30 min reaction time. Spirulina gave the highest oil yield of 32.6% although similar higher heating value (32-34.7 MJ/kg) were estimated. Bio-oil molecular wt. was in the order of Spirulina< swine manure < digested sludge.	Vardon *et al.* [76]

Table 2.2 continued.

Year	Species	Catalysts	Observations	Reference
2011	*Chlorella vulgaris*, *Nannochloropsis occulata*, *Porphyridium cruentum*, *Spirulina*	Na_2CO_3 & HCOOH	This study compares bio-oil properties obtained from HTL of the different algae species with other biochemical compounds like soya protein, albumin, starch, glucose and sunflower under hydrothermal conditions at 350^{o}C. Depending upon the biochemical composition, the yields of bio-crude are 5-25 wt % higher than the lipid content of the algae. Bio-oil formation was most efficient for lipids and protein than carbohydrates and followed the trend lipid> protein> carbohydrates. Lipid and proteins converted most efficiently without Na_2CO_3 but improved the conversion of carbohydrates with it.	Biller *et al.* [29]
2013	*Spirulina platensis*	None	This study compared the bio-oil produced from HTL and pyrolysis (reaction condition: 350^{o}C and reaction time of 60 min). HTL resulted in higher bio-oil yields (41 wt.%), lower char yields (6.3 wt.%) and lower energy consumption ratio compared to pyrolysis. Bio-oil from HTL had superior fuel properties like thermal, storage stabilities and heating value compared to pyrolysis bio-oil.	Jena *et al.* [77]

Table 2.2 continued.

Year	Species	Catalysts	Observations	Reference
2012	*Nannochloropsis sp.*	None	This paper also studied the effect of process variables on HTL of *Nannochloropsis sp*. The variables include temperatures (250-400^{o}C), times (10-90 min), water densities (0.3-0.5 g/ml) and biomass loadings (5-35 wt.%). Liquefaction yielded a light and heavy fraction of bio-crude and on increasing the biomass loading increased the overall biocrude from 36-46 wt.%. However, water densities did not affect the yield and properties. Detailed analyses of the by-products were also performed.	Valdez *et al.* [68]

Bio-oils are also obtained from pyrolysis. HTL and pyrolysis are the two main types of thermochemical processes to obtain liquid fuels. Although both processes' end product is liquid fuel, the physicochemical properties of the oil obtained from these two processes varies for same biomass too. Fast pyrolysis, a type of pyrolysis, is performed at high temperature under atmospheric pressure for very short residence time of < 2 sec [31]. Bio-oil from fast pyrolysis typically have much higher oxygen content and moisture content, as compared to oil from HTL, and also contain a large proportion of polar organic compounds [31]. In addition, oil content from HTL has more desirable qualities than fast pyrolysis oils; however, fast pyrolysis oils have the advantage of short residence times and lower capital costs. Table 2.3 shows the comparison between HTL oils and fast pyrolysis oil.

Table 2.3. Comparison of physicochemical properties of fossil oil and bio-oils from HTL and from fast-pyrolysis of algae

Properties	HTL bio-oil*	Fast Pyrolysis [78]	Fossil Oil [78]
C (wt.%)	70-75	61	83-87
H (wt.%)	8-10	8.50	10-14
N (wt.%)	4-6.3	9.79	0.01-0.7
S (wt.%)	0-1	n.d.	0.05-1
O (wt.%)	8-19	20.19	0.05-5
Density (kg/m^3)	900-980	1160	750-1000
Heating Value (MJ/kg)	35-39	29	42

*Data were compiled from the above given literature in Table 2.2. n.d.: not determined.

2.2.3 Hydrothermal Gasification

In hydrothermal media, fast hydrolysis of biomass leads to rapid degradation of polymeric structure of biomass and the subsequent reactions are also fast which results in gas formation at lower temperatures compared to dry processes [79]. The main products of gasification are methane, hydrogen, carbon dioxide etc. Hydrothermal gasification can be performed at near or above critical temperature and pressure, with or without the use of catalysts, which is much lower than conventional atmospheric gasification [31]. It can be divided into catalytic hydrothermal gasification and higher temperature hydrothermal gasification or supercritical water gasification (SCWG). Catalytic hydrothermal gasification occurs at near-critical temperatures up to about 500^{o}C in the presence of catalyst and SCWG occurs at temperatures above 500^{o}C with or without catalyst [31]. Several works (Brown *et al.* [60], Schumacher *et al.* [80], Onwudili *et al*. [81] etc.) have been performed on hydrothermal gasification of algae species. Brown *et al*. [60] investigated the characteristics of the product from both liquefaction and gasification of *Nannochloropsis sp*. and found out that the gas composition changed with the temperature, the gas yield (32.6%) of hydrocarbons (CH_4) increased with the increase of temperature. Schumacher *et al*. [80] studied the amount of gas yield, composition of gas and amount of water soluble compounds produced by hydrothermal gasification of different seaweeds (*Fucus serratus, Laminaria digitata, Alaria esculenta and Bifurcaria bifurcate*) at 500^{o}C at the reaction time of one hour. Hydrogen, methane and carbon dioxide were the major gaseous product. The amount of gases produced was high, which may be attributed to the catalytic reaction of inorganic salt contained in the seaweed. The hydrogen yield was between 12-16 g/kg seaweed, which was higher than the lignocellulosic biomass [82], and methane yield between 39-104 g/kg seaweed. Onwudili *et al*. [81] investigated the gaseous composition and yield of three different algae species, two being a microalgae

Chlorella vulgaris and *Spirullina platensis* and other being a macroalgae *Saccharina latissima* at 500^{o}C, 36 MPa and batch reaction time of 30 minutes in the presence/absence of NaOH and Ni-Al_2O_3. They reported that NaOH catalyst increased hydrogen gas yield by double and decreased tar yields by up to 71 wt.%. The highest hydrogen gas yield was given by macroalgae, as the carbohydrate content of macroalgae was higher than the microalgae. It has been shown that the biomass with more carbohydrate content were more suitable for hydrogen production under hydrothermal conditions than the biomass with higher proteins and lipids contents [62, 81, 83, 84]. In addition, the presence of protein could inhibit gas-forming reactions favorable for hydrogen production [81, 83].

2.3 Effects of Operating Parameters on HTL

Bio-oil and other products from HTL of biomass depends upon various operating parameters. The operating parameters include reaction temperature, residence time, biomass loading, pressure, catalyst and solvent used. The various effects of these operating parameters on different product of HTL are discussed below.

2.3.1 Effect of Temperature on HTL

Temperature is a critical parameter for the conversion of reactant to product in HTL. Many studies [30, 60, 62, 64-66, 68] have been performed to study the effect of temperature on HTL of algae biomass. Typical operating temperatures reported in the literature are in the range of 250-375^{o}C, in which bio-oil yield increases with increasing temperature, and then decreases as the temperature is further increased above 350^{o}C [60, 62, 64, 68]. As the temperature increases, the ionic product of water increases drastically near the critical point, and water in this condition has ability to hydrolyze complex compounds catalyzed by the H^+ and OH^- ions [12, 32, 74], as a result these complex proteins, carbohydrates and lipid macromolecules undergo isomerization,

defragmentation/depolymerization and condensation reactions to form bio-oil [77]. But whenever the temperature goes above critical temperature, ionic product decreases and free radical reactions dominates [12]. Recombination of these free radical reactions leads to char formation due to their high concentrations [85]. In addition, at high temperature secondary decompositions (cracking) and boudouard gas reactions also becomes active which leads to the formation of gases [86]. Thus, at temperature higher than critical temperature there is significant decrease in bio-oil yield. The change in temperature not only changes the bio-oil yield but also affects its properties; increase in the temperature also leads to a decrease in oxygen content and consequently higher HHV [60, 62, 64, 66].

Different studies have reported results for bio-oil yield as a function of temperature [30, 60, 62, 64-66, 68]. Anastasakis *et al*. [62] found that bio-oil yield obtained from liquefaction of *L. saccharina* was highest at 350^{o}C (19.3 wt.%) and decreased as the temperature increased further (17-18 wt.% at 374^{o}C). The gas yield increased and the char formation decreased as the temperature increased for *L. saccharina*. Increase in temperature from 250^{o}C to 350^{o}C resulted in increase in carbon content in bio-oil (from 76.6 wt.% to 82 wt.%) and decrease in Oxygen (10.3 wt.% to 5.4 wt.%) content in the bio-oil, resulting in higher HHV (34.5 to 36.5 MJ/kg at 350^{o}C). Nitrogen content in the oil increased first and then decreased as the temperature increased further. Alba *et al.* [30] also reported that the bio-oil yield from *Desmodesmus sp.* depended on the reaction temperature between the range of 175-450^{o}C, with the increase of temperature, the yield increased at first and decreased on further increase in temperature. The maximum oil yield of 49 wt.% was obtained at 375^{o}C at 5 min reaction time and decreased thereafter.

2.3.2 Effect of Residence Time on HTL

The time period during which the assigned temperature is maintained for the reaction is called residence time. Many studies [30, 62, 64, 68, 74] have been performed to study the effect of residence time on HTL of algae. Residence time may define the composition of products and overall conversion of biomass. The hydrolysis and decomposition rate is relatively fast in supercritical process [87]. So, normally high temperature reaction require low holding time and vice versa to achieve higher conversion [88]. In hydrothermal media, the heavier intermediates can convert into liquids, gases or residues as a result of secondary and tertiary reactions [85] which may occur at higher residence time. Boocock *et al.*[89] reported that longer residence times suppressed the bio-oil yield except for very high biomass to water ratios. Alba *et al*. [30] observed with the use of sodium carbonate catalyst, there was negligible increase in bio-oil yield for the residence time range of 5-60 min at 300^{o}C but there was significant increase in bio-oil yield at 200^{o}C. Anastasakis *et al.* [62] reported increasing the residence time resulted in a decrease of the bio-oil yield, indicating re-polymerization or re-condensation of the newly formed compounds, the maximum bio-oil yield (19.3 wt.%) was obtained at 15 min residence time. Jena *et al*. [77] observed the bio-oil yield to increase until 60 min and thereafter decreased with further increase in reaction time. This may be attributed to the conversion of the lighter hydrocarbon compounds in the bio-oil into gaseous product. In addition, Karagoz *et al*.[90] observed the decomposition products were not similar for longer and shorter residences times both for 180^{o}C and 250^{o}C.

2.3.3 Effect of Biomass Loading

Biomass loading expressed in percentage is defined as the mass ratio of dry algae to feedstock. Many researchers [62, 64, 68] have investigated the potential effect of biomass loading on hydrothermal liquefaction. Water acts as both a hydrogen donor and as a solvent for hydrolyzing

complex compounds present in the biomass [62]. In general, for the production of liquid and gas, high amount of water is suitable due to enhanced extraction by denser solvent medium [91] and due to the availability of optimum quantities of H^+ and OH^- ions to catalyze the organic components [64]. High biomass to water ratios can suppress dissolution of biomass components and reaction rate due to the relative interactions among molecules of biomass and that of water becoming less influential [85]. According to Jena *et al.* [64], bio-oil yield from *Spirullina* increased from 32.5 wt.% to 39.9 wt.% when the solids concentrations increased from 10 wt.% to 20 wt.% and then remained more or less constant with further increase to 50 wt.%. Water soluble yield i.e., the dissolved organics in water, dropped as the solid concentration increased from 10-50 wt.% but gas and char yields remain moreover constant [64]. Valdez *et al.* [68] observed increase of bio-oil yield from 36 to 46 wt% as the *Nannochloropsis* concentration in the slurry increases from 5 to 35 wt.% which contradict the conclusion reported by Jena et *al.*[64]. However, both conclusion may be valid as different species of algae might have behaved differently during hydrothermal liquefaction. Anastasakis *et al.* [62] observed that the bio-oil yield increased as the biomass to water ratio increased from 2/30 to 3/30 and had no influence when the ratio was further increased. Gas and residue formation was favored at lower biomass loading [62]. For the HTL process to be energy and economically efficient (for pumping biomass slurry), the target biomass loading should be 15-20 wt.% [31].

The effects of operating parameters on hydrothermal liquefactions are summarized above. From the above studies we can explain that temperature affects bio-oil yield and its characteristics and many studies have been performed to study the effects of temperatures on HTL of algae [30, 60, 62, 64-66, 68]. But, the optimum temperature for bio-oil production from algae HTL varies case by case due to the different chemical composition and operating conditions. In case of the other

effects like biomass loading ratio and residence time for algae biomass, some studies have been performed but there are not much literature to fully understand the effects of those parameters and moreover it depends upon the type of algae strains. Therefore, these area needs to be studied more with different variety of algae strain, in order to fill the gap of knowledge in these area.

2.4 Effects of Biochemical Composition of Algae

Bio-oil yield and its properties depend not only on operating parameters but also on biomass composition. As algae is the biomass under study, here we are mainly focusing on the effect of biochemical composition of algae on the product yield and its characteristics. The main biochemical composition of algae are: carbohydrates, lipids and proteins. These biochemical components undergoes decomposition/ de-polymerization, degradation, re-polymerization etc. to form products. Here, we review the conversion pathways of various biochemical components of algae to understand the properties of algal bio-oil products.

2.4.1 Conversion of Carbohydrates

Polysaccharides cellulose, hemicellulose and starch are the most abundant carbohydrates in biomass [92]. Carbohydrates, under hydrothermal media, undergo rapid hydrolysis to form glucose and other saccharides, which subsequently further degrades [92]. Cellulose is a polysaccharide composed of straight chain of glucose, connected by β –(1→4)-glycosidic bonds which enables formation of strong intra-and inter-molecular hydrogen bonds and makes them crystalline, resistant to swelling in water and resistant to attack by enzymes [31]. However, water at high temperature and pressure can both rapidly break up the crystalline structure and hydrolyze the glycosidic bond to form glucose monomers [31]. But the competing reactions acts as a hindrance to high glucose yield, moreover glucose itself degrades to form other compounds [31]. Figure 2.6 illustrates the degradation and decomposition of glucose to form other compounds.

According to Sasaki *et al.* [93], below supercritical region glucose degradation rate was higher than cellulose hydrolysis rate but as the temperature approached and entered supercritical region cellulose hydrolysis rate proved to be higher than glucose degradation rate. Rogalinski *et al.* [94] observed that rapid heating is important to avoid some biopolymers to depolymerize and start degrade before the reaction temperature is reached. The cellulose hydrolysis rate increased ten fold between 240 and 310^{o}C in water at 25 MPa also at 280^{o}C within 2 minutes, 100% cellulose conversion was achieved [94]. Glucose and fructose in water dissolve and exists in three form: an open chain, a pyranose ring and a furanose ring [31]. For both glucose and fructose, the rate of inter-isomerization is slower than degradation rates [31]. The rate of glucose isomerization to fructose is much important than fructose to glucose because fructose reacts faster than glucose [95]. This may be due to the much lower abundance of acyclic form of glucose relative to the acyclic form of fructose in hydrothermal condition which may be driving the lower reactivity of glucose [95]. Different major products are formed from glucose and fructose. Glucose degrades mostly to glycolaldehyde, pyruvaldehyde, glyceraldehyde etc. while fructose degrades to form 5-hydroxymethylfurfural (5-HMF) [96]. Although aromatic compounds are often assumed to originate from lignin portion of lignocellulosic biomass, it can also be formed from the cellulosic sugars; fructose degraded to form 1,2,4-benzenetriol [97]. This was also justified by Nelson *et al*. [98] who reported the formation of aromatic compounds from hydrothermal reactions of pure cellulose at 250-400^{o}C. According to Srokol *et al*. [96], during dehydration, acidic conditions favored production of 5-hydroxymethylfurfural and basic conditions favored the formation of fragmented products, such as glycolaldehyde and glycerolaldehyde which on further decomposition and dehydrations leads to the formation of a variety of low molecular weight compounds such as formic acid, acetic acid, acrylic acid etc.

Hemicellulose is a heteropolymer composed of various monosaccharides, including xylose, mannose, glucose and galactose [31]. It is not crystalline and does not possess resistant structure due to the lack of repeating β-(1→4) glycosidic bonds and the random nature of the hemicellulose polymer [31]. Therefore, it is more susceptible to hydrolysis and are easily soluble at temperatures above 180^{o}C [99]. Mok and Antal [100] reported that at 230^{o}C, 2 min and 34.5 MPa, almost 100% of the hemicellulose of biomass was hydrolyzed. The open chain form of xylose produce glyceraldehyde, pyruvaldehyde, lactic acid, glyceraldehyde, formic acid and acetol which are fragmentation by-product in furfural production [40]. The dehydration product of hemicellulose also degrades in similar way as cellulose dehydration products [92].

Starch is a polysaccharide consisting of glucose monomers connected by α-(1→4) and α-(1→6) bonds [31]. Starches can be easily hydrolyzed in hydrothermal condition without adding acid or enzymes but the reported yield of glucose are relatively small than conventional enzymatic method, which may be due to further decomposition of glucose or degradation of starch into other oligomers [31]. Nagamori and Funazukuri [101] observed that starch completely solubilized already after 10 min in 180^{o}C but glucose yield were very negligible and found the highest yield of 63% both at 200^{o}C and reaction time of 30 min and at 220^{o}C and 10 min. The yield was low at more severe condition due to the degradation of glucose and the main degradation product was 5-HMF.

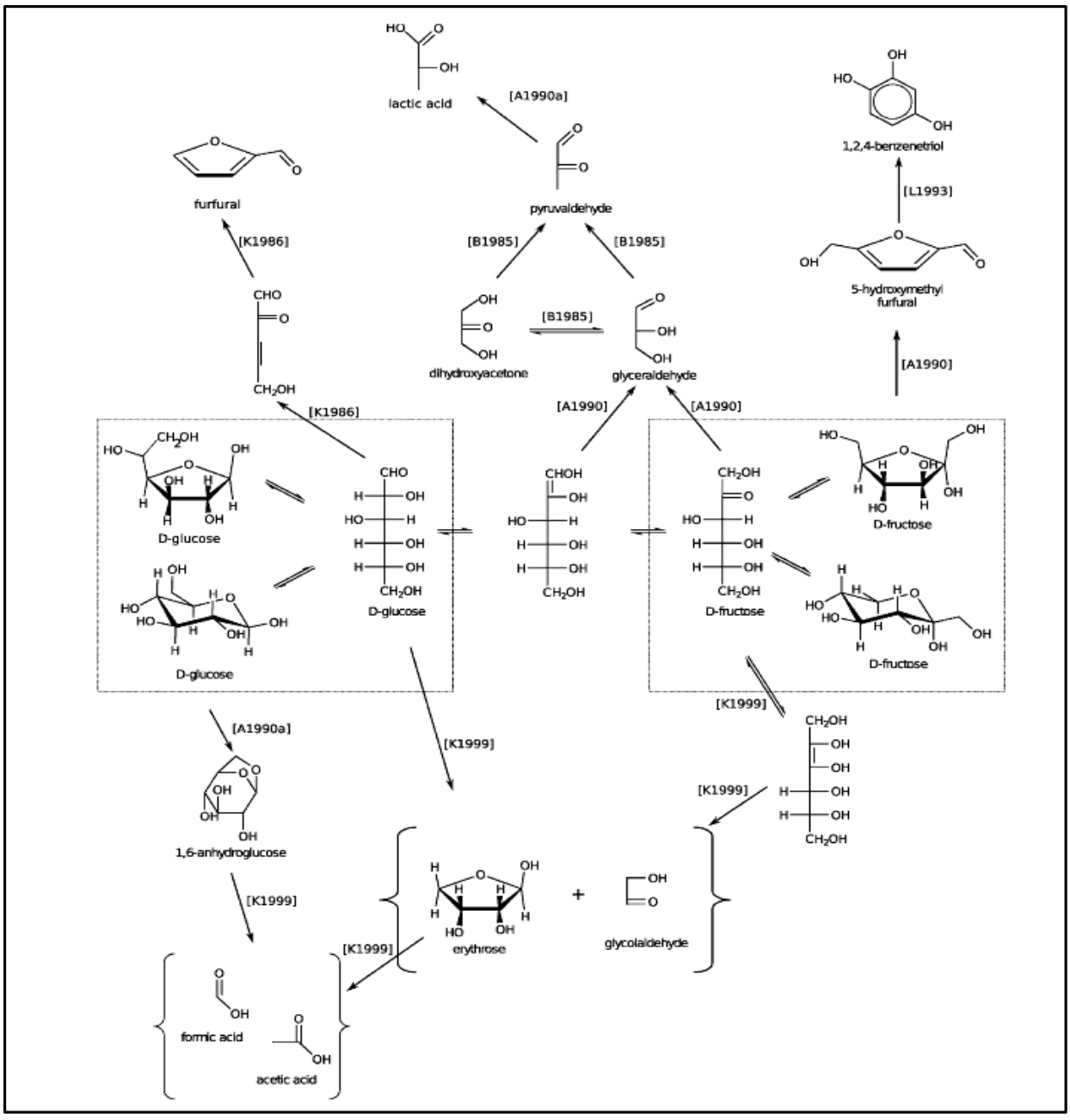

Figure 2.6. Pathways for the degradation of D-glucose and D-fructose. Adapted from Peterson et al.[31]

2.4.2 Conversion of Lipids

Lipids are naturally occurring fats and oils. They are non-polar compounds with mainly aliphatic character [92] which can undergo reactions that can convert them into ready substitutes for

conventional hydrocarbons [31]. Fats and oils are mainly in the form of triacylglycerides (TAGs), which consist of three fatty acids bound to a glycerol backbone [31]. They can be split into valuable intermediate such as fatty acids and glycerol by using hydrothermal media. Although being insoluble in water at ambient conditions, fats and oils are increasingly soluble at high temperature; the reasons being discussed in section 2.1.3. The reaction mainly happened in oil phase and the hydrolysis is dependent on the solubility of water in oil phase [102]. Khuwijitjaru *et al*. [103] studied the solubility of fatty acids in water from 60-230^{o}C at 50-150 bar and found exponential increase in solubility with temperature as shown in Figure 2.5. The Colgate-Emery process which use hydrothermal conditions of 250^{o}C and 5 MPa with TAG : water ratio of 2:1, are in use to hydrothermally split TAGs to free fatty acids and glycerol [31]. According to King *et al*. [104] rapid hydrolysis of fatty acids were achieved in liquid water at temperatures of 330-340^{o}C and water –to-oil ratios of 2.5 to 5 : 1 for 10-15 minutes, giving 90-100% yields of free fatty acids.

Fatty acids have high thermal stability but it can be partly degraded at hydrothermal conditions to produce long chain hydrocarbons, which have excellent fuel properties [92]. Watanabe *et al.* [105] observed the decarboxylation of stearic acid ($C_{17}H_{35}COOH$) to $C_{17}H_{36}$ and $C_{16}H_{32}$ hydrocarbons at supercritical conditions (400^{o}C, 25 MPa and 30 min). The alkane yield was suppressed in hydrothermal conditions, but with the addition of alkaline catalysts like NaOH and KOH, the decomposition increased and alkane yield was dominant [31]. On comparing the reaction at hydrothermal conditions with anhydrous pyrolysis of water –free stearic acid, the hot compressed water promotes the stability of fatty acids and suppresses the degradation [92]. In case of glycerol, during hydrothermal liquefaction, it is not converted to an oily phase but to water soluble compounds [92]. Thus, for bio-oil production, glycerol alone cannot be taken as a reactant. Buhler *et al*. [106] studied the decomposition of glycerol in a hydrothermal condition (349-475^{o}C, 25-45

MPa and 32-165 s) using a plug flow reactor and found yield as high as 31%. The main products were methanol, ethanol, acetaldehyde, propionaldehyde, acrolein and formaldehyde.

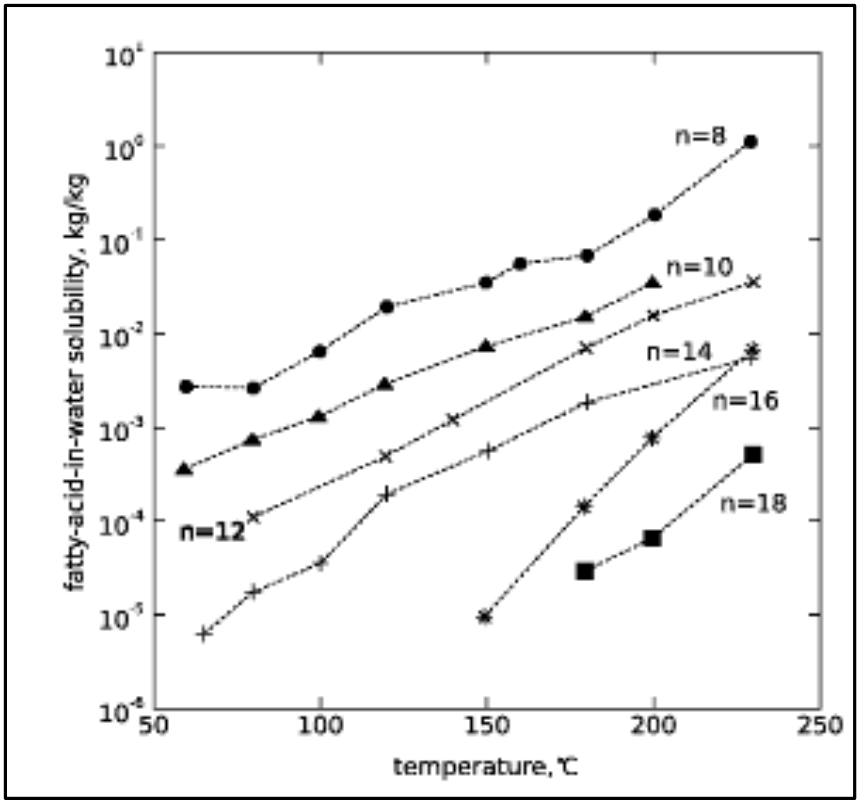

Figure 2.7. The solubility of saturated fatty acids in water at 15 MPa, where n is carbon number of each fatty acids, adapted from Khuwijitjaru et al. [103]

Normally, vegetables oil and marine species have unsaturated chains. Marine species mainly have a large number of polyunsaturated fatty acids (PUFA). Docosohexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are types of PUFAs. PUFA in algal oils could cause problems in the hydrothermal process due to its susceptibility of double bonds to attack in reactions and could lead to degradation of the oils to smaller compounds [107].

2.4.3 Conversion of Protein

Proteins are the major biomass components found in algae. Amino acids, the building block of protein are the chief source of nitrogen in the algae. Conversion of protein into bio-oil under HTL conditions leads to high nitrogen content in the bio-oil which are undesirable as it produce NOx gases.

Protein constitutes of polymers of amino acids structurally bonded by peptide bonds [31]. These peptide bonds regularly gets hydrolyzed to amino acids in hydrothermal conditions and a considerable fractions of nitrogen in protein gets into bio-oil, affecting smell, combustion and other properties of bio-oil [92]. Considering the stability, peptide bonds are more stable than glycosidic bonds and thus slow hydrolysis of protein is achieved at 250^{o}C [94, 108]. The amino acid yield is significantly lower than conventional low temperature acid hydrolysis because the decomposition rate of amino acid is relatively rapid compared to amino acid formation [31, 109].

According to Rogalinski *et al*. [110], the highest yield of amino acid from hydrolysis of bovine serum albumin (BSA) was obtained at 290^{o}C and 65 s but the total yields were low due to degradation of amino acids. There was complete decomposition of all amino acids at 330^{o}C and 200 s. Later Rogalinski *et al.* [94] observed that hydrothermal liquefaction of BSA at 250^{o}C, 25 MPa and 300 s in the presence of CO_2 increased the yield of amino acid form 3.7 to 15% and also reported that the catalytic influence of carbon dioxide decreases with increasing temperature. Amino acids undergo degradation through two main paths: a) decarboxylation reaction to produce carbonic acids and amines and b) deamination reaction to give ammonia and other organics [92]. Dote *et al*. [111] observed that two amino acids; asaparagine and glutamine were unstable at 300^{o}C and majority was decomposed to ammonia and converted to water soluble organic compounds but not to oil. Klinger *et al*. [112] reported hydrothermal decomposition of two of the simplest amino

acids; glycine and alanine. They observed that the primary mechanisms of decomposition of the amino acids were decarboxylation and deamination as shown in Figure 2.8. They found no effect of pressure (24-34 MPa, at 300-350^{o}C) on decomposition rate of the amino acids. The major decomposition products were acetaldehyde, diketopiperazine, ethylamine, methylamine, formaldehyde, lactic acid and propionic acid.

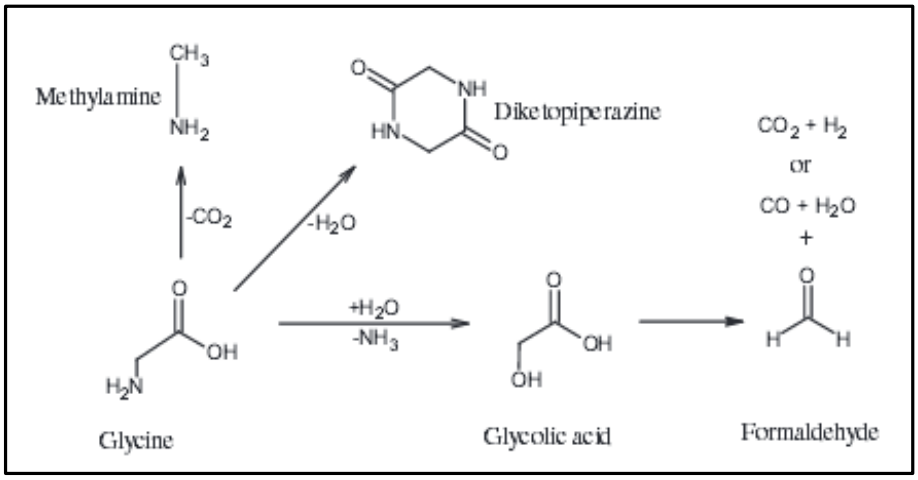

Figure 2.8. Reaction network of hydrothermal glycine decomposition, adapted from [112]

The biochemical composition of algae chiefly constitutes proteins. The presence of protein is undesirable in terms of bio-oil production as most of the protein also gets converted to nitrogen containing compounds of bio-oil which emits NOx gases when used. During hydrothermal liquefaction, the hydrolysis of biomass composition occurs together; amino acids and sugars are simultaneously formed and these can polymerize through Maillard reaction [113]. This leads to the formation of nitrogen containing cyclic organic compounds like pyridines and pyrroles which inhibits free radical chain reactions that are highly relevant for gas formation [113]. Biller and Ross [29] observed that sodium carbonate catalyst does not favor the efficient conversion of protein into bio-oil.

The effects of individual biochemical composition of algae in hydrothermal conditions were summarized above. The above studies were performed for particular processing parameters and the detailed behavior of biochemical components are yet to be studied at different processing parameters. Moreover, there is very little information on interaction between the different biochemical compositions on the bio-oil yield and its characteristics.

2.5 References

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CHAPTER THREE

3. EFFECTS OF TEMPERATURE AND ALKALINE CATALYST (Na_2CO_3) ON HYDROTHERMAL LIQUEFACTION OF THREE TYPES OF ALGAE AND ITS PRODUCT CHARACTERIZATION

Abstract

Hydrothermal liquefaction (HTL) is known as one of the most innovative ways to convert aquatic or wet biomass into bio-fuels. In this study, HTL was performed on three different algae strains viz. *Nannochloropsis sp.*, *Pavlova sp. a*nd *Isochrysis sp*. at three temperatures of 250, 300 and 350^oC, a holding time of 60 minutes, and algae loading of 14 solid wt.%. The HTL reactions were performed with and without using Na_2CO_3 as catalyst. The effect of temperature on the HTL product yields of the three algae strains were studied for both catalytic and non-catalytic HTL. Bio-oil and gaseous product were observed to increase with increased temperature and solid residue and water soluble product were observed to decrease. Maximum bio-oil yield for non-catalytic (48.67 wt.%) and catalytic HTL (47.05 wt.%) were both at 350^oC for *Nannochloropsis* and *Pavlova,* respectively. Na_2CO_3 as catalyst increased the bio-oil yield for high carbohydrate containing algae. Physical and chemical properties such as pH, density, higher heating value (HHV), ash content, moisture content, viscosity of bio-oils and char were analyzed for both non-catalytic and catalytic HTL. The bio-oil obtained had the HHV in the range of 32 to 37 MJ/kg, which was similar in nature to a heavy crude oil. The chemical compounds in bio-oil were determined using a gas chromatography mass spectroscopy. Result showed that an increase in reaction temperature decreased the organic acid in the bio-oil resulting in increased pH and

decreased in TAN. This was true for both catalytic and non-catalytic HTL. Co-product studies were also performed and found that solid residues contained high amounts of unconverted carbon and WSP (water soluble product) had higher amounts of nitrogen in them.

Keywords: Hydrothermal liquefaction, Nannochloropsis, Isochrysis, Pavlova, Algae.

3.1. INTRODUCTION

Hydrothermal liquefaction (HTL) refers to the process of converting biomass to liquid fuel (a.k.a. bio-oil or bio-crude) by using water at sub- or super-critical temperatures and pressures [1-6]. The density and dielectric constant of the water medium play a major role in solubilizing biomass at sub- and super-critical water conditions [7]. Hydrothermal liquefaction is mostly suited to wet biomass such as algae, as 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 [8]. The advantages of algae over terrestrial biomass feedstocks are: (i) algae have higher biomass productivity (40-60 dry ton/ha-yr) [9] than other energy crops; (ii) they have a high carbon sequestration rate (1.8 kg of CO_2/kg of dry algae) [10]; and (iii) they can be grown under conditions which are unsuitable for conventional crop production, thus relieving the food-versus-fuel pressure on agricultural land [11, 12]. An HTL product consists of 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 liquefaction, the total yield is higher than the original lipid in the algal biomass, which suggests that oils are produced from non-lipid components of the algal biomass as well [13, 14]. In other words, not only lipid but also whole algae (protein, lipids, and carbohydrates) get converted to bio-oil.

Many HTL studies have been performed using different types of microalgae strains such as *Botryococcus braunni* [3, 15, 16], *Nannochloropsis* sp. [1, 4, 17, 18], *Dunaliella tertiolecta* [16, 19, 20], *Chlorella vulgaris* [6], *Spirulina platensis* [5, 21], *Microcystis viridis* [22], and macroalgae such as *Laminaria saccharina* [2], *Enteromorpha prolifera* [23] with or without the use of catalysts. Large variances of bio-oil yield and quality indicate that either algal type, operating conditions, or both, significantly affect algal HTL. Many of the HTL studies [1, 3, 6, 19, 20, 22, 23] are typically performed at temperature range of 200^{o}C to 350^{o}C with 5 to 60 min residence time. Studies have shown that bio-oil yield increases with increasing temperature, however yield decreases as the temperature is increased beyond 350^{o}C [1, 2, 5, 18]. Brown *et al.* [1] investigated liquefaction and gasification yield of *Nannochloropsis sp.* at reaction temperatures ranging from 200^{o}C to 500^{o}C and reported the maximum bio-oil yield of 43 wt.% at 350^{o}C. However, Alba *et al.* [24] found the oil yield to be maximum (49 wt.%) at 375^{o}C and decreased thereafter for *Desmodesmus sp.*.

Published literature has shown the effects of the use of both homogenous [2, 3, 6, 15, 16, 19, 20, 22] and heterogeneous catalysts [17, 25] in HTL of algae. Most of the studies have used Na_2CO_3 as a homogenous catalyst due to its previous introduction in the HTL of lignocellulosic biomass was found to increase the bio-oil yield. Dote *et al.* [3] studied the HTL of *Botryococcus braunni* using Na_2CO_3 and reported maximum oil yield of 64 wt.% at 300^{o}C and 60 min residence time. Minowa *et al.* [19] also studied the effect of Na_2CO_3 (5 wt%) in HTL of *Dunaliella tertiolecta* and obtained a maximum yield of 37 wt.% on organic basis. In both cases, the use of Na_2CO_3 decreased the bio-oil yield with an increase of liquefaction temperature from 300 to 340^{o}C. Yang *et al.* [22] studied the effect of reaction temperature, holding time, and Na_2CO_3 dosage on the bio-oil yield of *Microcystis viridis*. The study reported that the increase in the reaction temperature (300^{o}C–

340^{o}C) and the use of catalyst (5 wt.%) increased the oil yield at lower holding time. However, this decreased the oil yield as the holding time increased from 30 min to 60 min. Shupping *et al.* [20] also investigated the effect of temperature, holding time and Na_2CO_3 dosage on the bio-oil yield from *Dunaliella tertiolecta* cake and reported that the maximum oil yield was obtained at 360^{o}C and a residence time of 50 min using 5 wt.% catalyst. The study also found that the increase in catalyst dosage until 5 wt.% increased the bio-oil yield and decreased beyond 5 wt.%. Ross *et al.* [6] studied the effect of alkali catalysts (KOH and Na_2CO_3) and organic acids (CH_3COOH and HCOOH) on the HTL of *Chlorella vulgaris* and *Spirullina* at 300^{o}C and 350^{o}C for 60 min. The study reported that the maximum bio-oil yield obtained followed the trend CH_3COOH > HCOOH > KOH > Na_2CO_3. Biller *et al.* [26] also investigated the effect of Na_2CO_3 and HCOOH catalyst on different types of model compounds and algae strains. The study reported that the bio-oil formation was more efficient for lipids and protein than carbohydrates when HTL was performed without using any catalyst; however, the use of Na_2CO_3 improved the conversion of carbohydrates but reduced the efficient conversion of lipids and proteins to bio-oil.

The overall goal of this study was to understand the influence of temperature and alkaline catalyst (Na_2CO_3) on production yields and their properties during the HTL process. The specific objectives of this study were to: (1) investigate the influence of temperature on the HTL of three algae strains (i.e., *Nannochloropsis sp*., *Pavlova sp.* and *Isochrysis sp*.) and characterize the products obtained from HTL; (2) to understand the effect of Na_2CO_3 catalysts on bio-oil yield of three algae strains at three temperatures and (3) to compare non-catalytic and catalytic reactions and products obtained.

3.2 MATERIALS AND METHODOLOGY

3.2.1. Materials

Algae samples of *Nannochloropsis sp.*, *Pavlova sp.* and *Isochrysis sp.* were obtained in the form of a slurry from Reed Mariculture Inc. (Campbell, CA.) and stored in a freezer until they were used. Table 3.1 provides biochemical compositions of the algae species which was provided by the supplier. Sodium carbonate (Na_2CO_3) was supplied by VWR (Atlanta, GA) and was used as received. Moisture content of the algae samples was determined by calculating the weight loss of a sample by heating it in an oven at 105^oC for 24 h. Ash content in the algae samples 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 was used for the experiments and obtained from Synergy Ultrapure Water Systems (EMD Millipore). High-purity helium was purchased from Airgas Inc. (Charlotte, NC), and all chemicals were purchased from VWR (Atlanta, GA) and were used as received.

Table 3.1 Biochemical composition analyses of algae feedstocks

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

*Values reported by Reed Mariculture Inc. on dry basis

3.2.2. Experimental Setup and Procedure

HTL experiments were performed in a high pressure experimental unit as shown in Figure 3.1. The experimental unit consisted of a batch reactor tube 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 $1/16^{th}$ in. (Omega, Stamford, CT) K-type thermocouple attached to one end of the reactor. An in-line filter (pore size of 0.5 micron) 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 1:6 algae: water ratio (14.29 wt.% solids) for all three samples. With regards to catalytic HTL process, the reactor was additionally loaded with a Na_2CO_3 catalyst dosage of 5 wt.% of the dry solid for all samples. From here on throughout the literature, 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 body 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 the helium, valve 2 was closed and the reactor was pressurized to an initial pressure of 35 psig after which valve 3 was closed.

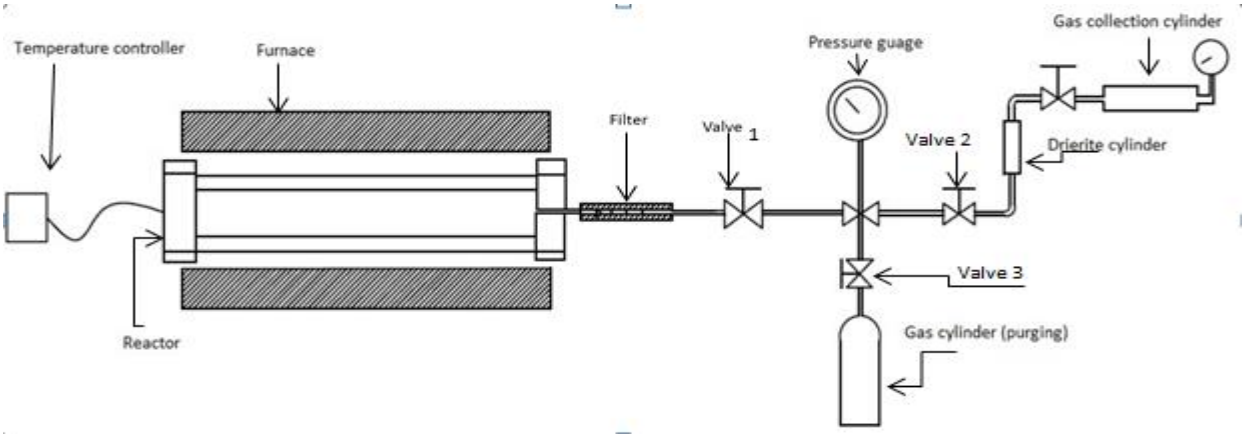


Figure 3.1 Schematic representation of a high pressure experimental unit for hydrothermal liquefaction

The reactor was then heated by keeping the furnace temperature higher than the reaction temperature to make up for thermal transfer inefficiencies and heating losses. HTL runs were performed at three different temperatures of 250ºC, 300ºC and 350ºC for 1 h residence times using three species. After completion of the reaction, the reactor was cooled down and the residual pressure created by gas formation during reaction was then released to a gas chamber by opening valve 2. For each sample at a particular temperature, triplicates of each run were performed and average values and standard deviations are reported.

3.2.3. 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 (Figure 3.2). The bio-oil phase appears to float in the aqueous phase. The solid phase remains mixed with both the bio-oil phase and aqueous phase. The aqueous phase along with some solid

fractions is then decanted to another flask (F2) but not all the aqueous phase gets decanted as some remains 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 3.3.

Equal amounts of ultrapure water and acetone were used to rinse the reactor. 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 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^{o}C and 556 mbar pressure.

Figure 3.4 illustrates the conversion of algae to different products from HTL. 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.

$$\text{Bio-oil yield (wt.\%)} = \frac{Mass\ of\ algal\ bio{-}oil\ in\ dry\ basis}{Mass\ of\ dry\ algae} \times 100 \quad (3.1)$$

$$\text{Solid residue (wt.\%)} = \frac{Mass\ of\ solid\ residue\ \ in\ dry\ basis}{Mass\ of\ dry\ algae} \times 100 \quad (3.2)$$

Water soluble product (WSP) (wt.%) = $\frac{Mass\ of\ dry\ water\ soluble\ organics}{Mass\ of\ dry\ algae} \times 100$ (3.3)

For HTL runs using Na_2CO_3, we assume that all the catalyst will be in the aqueous phase and thus the water soluble product is measured by following equation,

Water soluble product $(WSP)_{catalytic}$ (wt.%)= $\frac{Mass\ of\ dry\ water\ soluble\ organics}{Mass\ of\ (dry\ algae+catayst)} \times 100$ (3.4)

Figure 3.2 Separation of bio-oil and aqueous phase in a separatory funnel

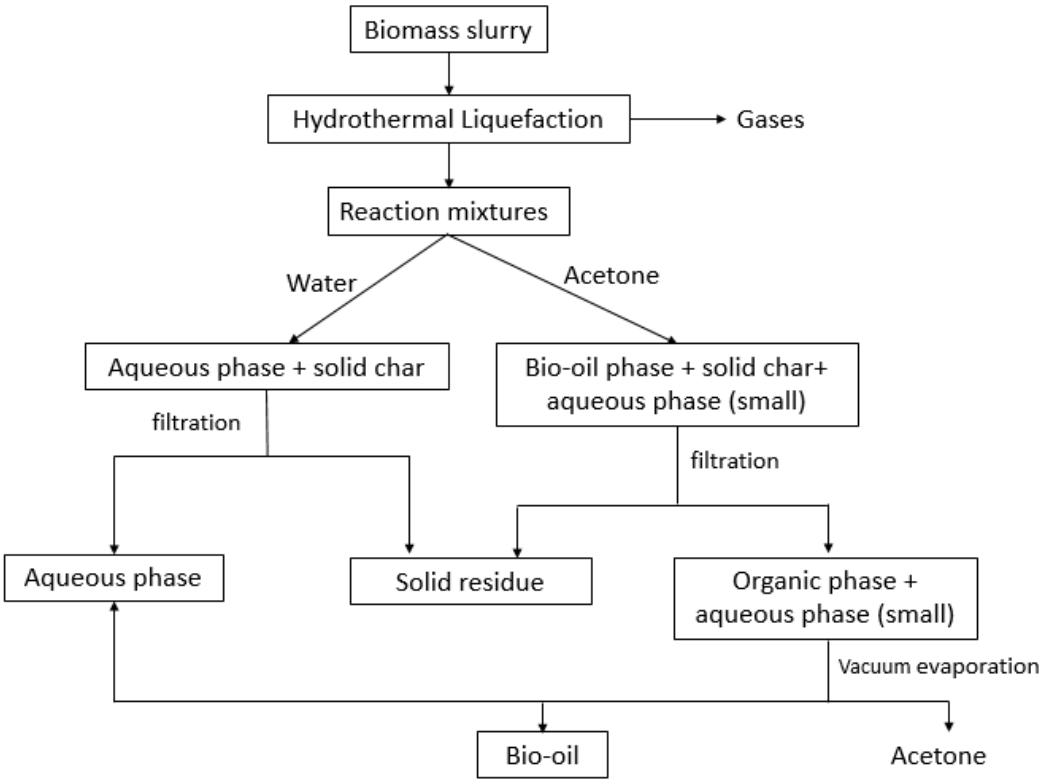


Figure 3.3 Process flow diagram of product separation after HTL

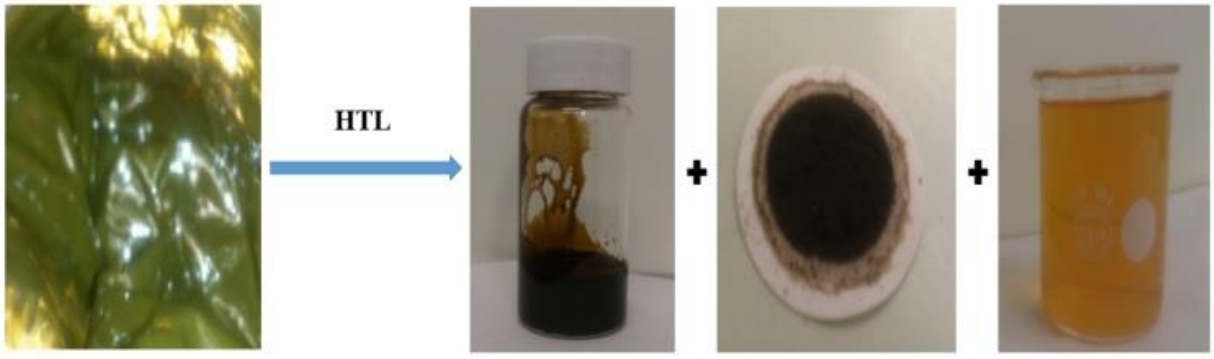


Figure 3.4 Products obtained from the HTL of algae

3.2.4. Product Analysis

Bio-oils produced from different algae strains at different temperatures using an HTL process were analyzed for their physical and chemical properties. Physical analysis of the bio-oil included pH, density, moisture, ash, volatile matter, total acid number (TAN), heating value, and viscosity determinations, while chemical composition was determined using gas chromatograph/mass spectrometer (GC/MS). 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 and volatile matter contents were measured according to ASTM E1755 and ASTM E872, respectively. Total acid number (TAN), which indicates the amount of acidic substance in the oil and is determined by the amount of potassium hydroxide in milligrams that is needed to neutralize the acids in one gram of crude oil, 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. The 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 100 mg was weighed and diluted to 10 ml with dichloromethane. The diluted sample was further diluted to 1:9 initial diluted sample to dichloromethane ratio. This diluted sample was injected into the column and each sample was injected twice. The initial temperature of the column, 50^{o}C was maintained for 2 min, and the temperature was heated to 250^{o}C at 5^{o}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 indicates the amount or the concentration of organic carbon content in a sample. TN indicates the amount or concentration of nitrogen content in a sample. TOC and TN measurements were conducted using a Shimadzu TOC-L analyzer attached with TNM-L unit. 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 was prepared and kept in an auto-sampler for measurement. Solid residue, or char, produced from HTL was also analyzed for its proximate and ultimate analyses by using the methods as described above.

C and N contained in the original biomass were distributed among the various product fractions. This elemental distribution of C and N was obtained by the following equation;

$$\text{Elemental Distribution (wt.\%)} = \frac{Mass\ of\ element\ in\ product\ fraction}{Mass\ of\ element\ in\ Biomass} \times 100 \qquad (3.5)$$

Full factorial experimental design was used to carry out these liquefaction experiments. A total of 27 runs were performed on the basis of two factors (i.e. algae strain and temperature) each having three levels and with three replications for each experiment. Statistical analysis was performed on the experimental data using JMP version 11. A one-way ANOVA analysis (95% confidence level) was performed to determine the p-value. The smaller p-value (<0.05) denotes the significant effect. For cases, when the p-values are less than 0.05, Tukey's HSD test was performed to analyze the effect of temperature at the three different levels.

3.3 RESULTS AND DISCUSSION

3.3.1. Algae Characterization

Tables 3.2 and 3.3 illustrate proximate and ultimate analyses of *Nannochloropsis*, *Pavlova* and *Isochrysis* algae strains, respectively. In terms of proximate analysis, ash content was relatively higher for *Pavlova* (3.4 wt.%). Higher ash content lowers the HHV for the biomass [26]. *Nannochloropsis* showed the highest HHV in comparison to the other two algae strains. Ultimate analysis (dry basis) was relatively similar for all algae strains. For example, carbon content varied from 54.34 wt.% to 56.83 wt.% with *Nannochloropsis* having the highest content. *Pavlova* has the highest sulfur content of 0.82 wt.%. *Nannochloropsis* (62.79 wt.%) had higher protein content than *Isochrysis* (44.36 wt.%) and *Pavlova* (46.94 wt.%) which correlated with high elemental nitrogen content in *Nannochloropsis*. Under HTL, considerable fractions of the nitrogen in protein gets converted into bio-oil resulting in smell, combustion and other properties of bio-oil [27]. Carbohydrate fractions were higher in *Pavlova* (28 wt.%) than in the other two species which correlates with the higher oxygen content.

Table 3.2 Proximate analysis of algae feedstocks

Strains	Proximate Analysis (wet basis)			
	Moisture (wt.%)	**Ash (wt.%)**	**Volatile matter (wt.%)**	**HHV (MJ kg^{-1})**
Nannochloropsis	68.88±1.24	3.42±0.38	22.51±1.28	24.02±0.07
Pavlova	75.80±0.42	3.47±0.33	17.74±0.77	22.69±0.07
Isochrysis	73.93±1.44	3.39±0.29	18.20±1.01	22.97±0.02

Table 3.3 Ultimate analysis of algae feedstocks

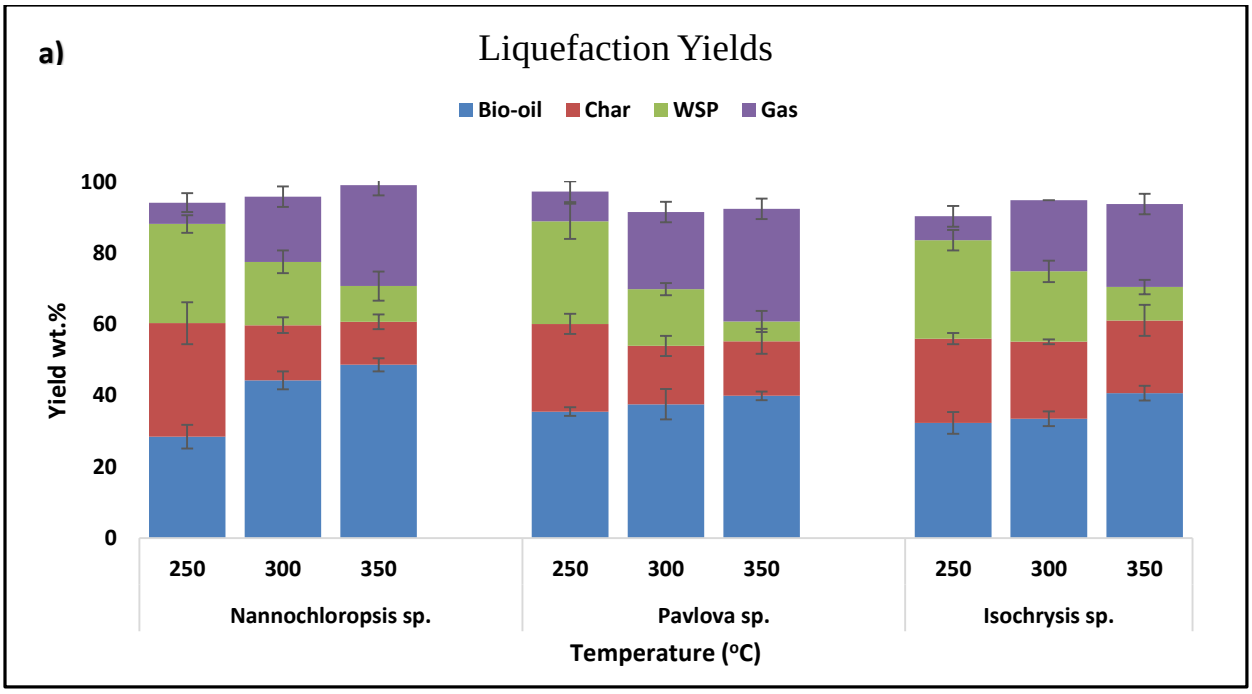
Strains	Ultimate Analysis (wt. % in dry basis)				
	C	H	N	S	O*
Nannochloropsis	56.83±0.33	9.32±0.06	10.13±0.06	0.37±0.19	23.35±0.26
Pavlova	54.34±1.36	8.69±0.41	8.67±0.21	0.82±0.09	27.48±2.07
Isochrysis	55.76±1.14	8.7±0.34	7.96±0.06	0.62±0.10	26.96±1.65

*By difference

3.3.2. Liquefaction Yields

Figure 3.5 (a) and (b) represents the product yields of non-catalytic and catalytic HTL of three algae species (i.e., *Nannochloropsis, Pavlova* and *Isochrysis)* carried out at temperatures of 250^{o}C, 300^{o}C and 350^{o}C for a residence time of one hour at 14 wt.% solid loading. Yields were calculated on dry basis using Equations (3.1- 3.4). For non-catalytic HTL, maximum bio-oil yield was found to be at 350^{o}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.* [1] for the same reaction temperature and algal strain. This difference may be due to different operating condition such as biomass loading (5 to 16 solid wt.%), initial pressure (69 kPa), and the differing biochemical composition (carbohydrates-12 wt.%, proteins-52 wt.%, lipids-28 wt.%) of the species used in their study. The bio-oil yield was found to be higher than the lipid content of algae biomass and this suggests that not only the lipids but also the carbohydrates and proteins were converted to bio-oil during HTL of algae. It was observed that gas and bio-oil yields increased as the temperature increased from 250^{o}C to 350^{o}C but char and water soluble fractions decreased. Similar trends in product yields were noticed for other algae strains at different temperatures [1, 2,

5]. Significant effects of temperature on bio-oil yield were observed for *Nannochloropsis* and *Isochrysis* as the p-values for the bio-oil data were <0.0002 and 0.0117, respectively. Although there was increasing trend in the bio-oil yield for *Pavlova,* the increase was not significant. Based on the Tukey HSD test, there was significant increase in bio-oil yield from 250^{o}C to 300^{o}C for *Nannochloropsis* and from 300^{o}C to 350^{o}C for *Isochrysis.* This increase in yields with increasing temperature is caused by the hydrolysis or decomposition/depolymerization reaction of the biomass into smaller compounds which form bio-oil. As the temperature increases, the energy to overcome activation energies for the bond cessation increases and extensive biomass depolymerization occurs resulting in higher bio-oil yields [1]. According to Biller et al. [26], oil formation follows the trend of lipids > proteins > carbohydrates. This suggests that not only temperature but also biomass composition affects bio-oil formation. In case of *Nannochloropsis,* bio-oil yield was observed to be significantly lower at 250^{o}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. So, slow hydrolysis of proteins is achieved at low temperature [29, 30]. However, at higher temperatures proteins easily gets hydrolyzed and more get converted to bio-oil. Thus, *Nannochloropsis* had a higher yield at a higher temperature. This correlates with the GC-MS data of nitrogenous compounds which are derived from protein, as shown in Figure 3.6 (a) in section 3.3.3. At lower temperature the nitrogenous compounds were very low in the bio-oil yield which increased as temperature increased.



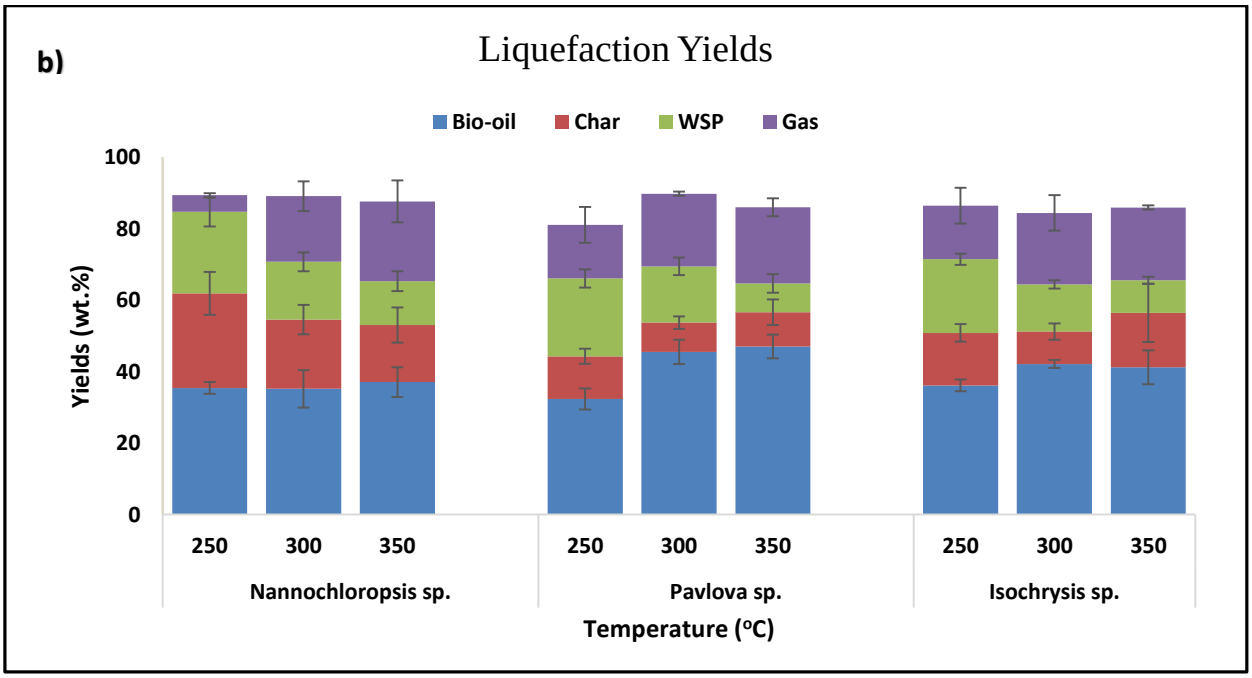


Figure 3.5 Liquefaction yields from three algae strains at different temperatures for (a) non-catalytic liquefaction and (b) catalytic liquefaction

With the use of Na_2CO_3 as catalyst, the bio-oil yield was in the order: *Pavlova* > *Isochrysis* > *Nannochloropsis* at higher reaction temperatures of 300 and 350^oC as shown in Figure 3.5 (b). At 250^oC, it was fairly constant for all the strains. Maximum bio-oil yield of 47.07 wt.% was obtained for *Pavlova* at 350^oC. The bio-oil yield result showed that with the use of Na_2CO_3 as catalyst, the highly proteinaceous algae (i.e., *Nannochloropsis*) gave a higher bio-oil yield (35.42 wt.%) at 250^oC compared to non-catalytic yield. The bio-oil yield did not increase significantly thereafter with the increase in temperature. The increase in bio-oil yield at lower temperature may be either due to the catalytic effect of Na_2CO_3, which causes the efficient conversion of carbohydrate to bio-oil as suggested by Biller *et al.* [26], or due to the catalytic effect of Na_2CO_3. Na_2CO_3 decreases the activation energy of protein hydrolysis, thus, more proteins hydrolyze to form bio-oil compounds resulting in a higher bio-oil yield. This increase of protein hydrolysis is supported by the GC-MS data of both non-catalytic and catalytic runs. The nitrogenous compounds found in bio-oil yield at lower temperatures is higher for catalytic bio-oils. However, at higher temperatures the nitrogenous compounds decreased. This decrease may be due to the secondary decomposition of nitrogenous compounds to ammonia which are easily dissolvable in aqueous phase.

The bio-oil yields were lower compared to non-catalytic HTL at 300^oC and 350^oC for *Nannochloropsis.* The decrease in bio-oil yield at higher temperature was probably due to inefficient conversion of protein into bio-oil or due to the secondary decomposition of nitrogenous compounds to ammonia, which are easily dissolvable in the aqueous phase, thus reducing bio-oil yield. According to Biller *et al.* [26], fewer proteins gets converted to bio-oil when using Na_2CO_3 as catalyst than without. Zhou *et al.* [23] reported that with Na_2CO_3 as catalyst, a slight increase in bio-oil yield with an increase in temperature was observed but, on comparing with the non-catalytic bio-oil yield at the particular temperature, the catalyst had little or no effect. In the case

of *Pavlova*, bio-oil yield significantly increased from 250°C (32.34 wt.%) to 300°C (45.49 wt.%) but no significant increase was seen at 350°C (47.05 wt.%). Comparing with the non-catalytic HTL, the bio-oil yields of this strain increased with the use of Na_2CO_3 at a higher reaction temperatures of 300 and 350°C. This increment of bio-oil yield is probably due to comparatively higher amount of carbohydrate present in this algal biomass. According to Biller *et al.* [26], carbohydrates are more efficiently converted to bio-oil when using Na_2CO_3 as a catalyst. For *Isochrysis*, temperature did not have significant effect on bio-oil yield during non-catalytic runs. Comparatively, bio-oil yield increased with temperature with the use of Na_2CO_3. This may also be due to the higher amount of carbohydrate in this algal biomass as well. A maximum bio-oil yield of 42.16 wt.% was obtained at 300°C for *Isochrysis*.

For both the catalytic and non-catalytic HTL, solid residue decreased with the increase in temperature. This decrease in solid residue likely occurred because most of the solid fractions were converted to either bio-oil, aqueous or gaseous product when the temperature increased. In the case of non-catalytic HTL, the solid residue decreased significantly from 250°C to 300°C for *Nannochloropsis* and *Pavlova.* However, for *Isochrysis*, the decrease was not significant for solid residue. For catalytic HTL, solid residue of *Pavlova* and *Isochrysis* increased with temperature from 300°C to 350°C, although the increase was not significant. This increase with temperature is mainly due to the repolymerization through condensation or cyclization reactions of lower molecular wt. compounds to form char or solid residue [6]. Comparing non-catalytic HTL, the solid residue from catalytic HTL of *Pavlova* and *Isochrysis* decreased. This decrease in the solid residue is probably due to the catalytic effect of Na_2CO_3 which converted more carbohydrates to bio-oil. The water soluble product (WSP) also decreased with increasing temperature for both catalytic and non-catalytic HTL. For non-catalytic HTL, WSP decreased significantly with

increasing temperatures for *Isochrysis* and *Pavlova* but decreased significantly only from 250^{o}C to 300^{o}C for *Nannochloropsis*. For catalytic HTL, significant decrease in WSP was observed with increasing temperature for Isochrysis, however significant decrease was observed only from 250^{o}C to 300^{o}C for *Nannochloropsis* and *Pavlova*. The decrease in WSP is probably due to more conversion of WSP to gaseous product with an increase in temperature. With the increase of temperature most of the products go through secondary decompositions (cracking) into lower molecular weight organic compounds while the boudouard gas reaction also becomes active leading to more gas formation [31]. Thus, the increase in temperature reduces the solid residue and WSP yield while increasing the gas yield. For non-catalytic HTL, *Nannochloropsis* and *Pavlova* had significant increases in the gas yield at all temperatures whereas for *Isochrysis,* the gas yield was observed to increase when the temperature increased from 250^{o}C to 300^{o}C. Comparatively, in the case of catalytic HTL, significant increases in gas yield was observed from 250^{o}C to 300^{o}C for *Nannochloropsis*. In case of *Pavlova* and *Isochrysis,* increase in gas yield was not significant.

The mass balance closure for the product yields from non-catalytic HTL were in the range of 90 - 95 wt.% and that of catalytic HTL ranged from 85 -90 wt.%. The decrease in mass balance in catalytic HTL is mainly due to the use of a small amount of aqueous phase for TOC and TN determination. Complete closure of the mass balance was not possible due to the different types of errors such as spilling products or incomplete removal of bio-crude from the reactor during processing of algal HTL bio-oil.

3.3.3. Bio-oil Characterization

The physical and chemical properties of the bio-oil produced from non-catalytic and catalytic HTL of algae strains are given in Tables 3.4 and 3.5 respectively. The bio-oil obtained was black, viscous and possessed a foul or smoky odor. Bio-oils produced from algae strains had lower

density than water, and thus, a clear phase separation between aqueous phase and bio-oil phase occured. The density of algal bio-oil (805-970 kg/m^3) was similar to the density of fossil crude (750-1000 kg/m^3) [28]. Temperature had no significant effect on the density of non-catalytic algal bio-oil. In the case of catalytic HTL, there was no significant change in the density of bio-oil with change in temperature for *Isochrysis* and *Pavlova* except for *Nannochloropsis*, where density decreased with an 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^{o}C to 300^{o}C for the bio-oil 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-oil, the use of Na_2CO_3 had no significant effect in pH of the bio-oil. The pH of the algal bio-oils observed in this study were similar to the previously reported values [28]. Total acid number (TAN) of bio-oils from non-catalytic HTL were observed to decrease as the HTL temperature increased as shown in Table 3.3 (a). The highest TAN was observed for bio-oil from *Pavlova* (64.27) at 250^{o}C. For *Isochrysis*, the highest TAN (59.52) occurred at 250^{o}C while the lowest (30.81) was reported at 300^{o}C. For bio-oil from *Nannochloropsis*, TAN was observed to decrease significantly at 250^{o}C (45.88) to 350^{o}C (32.45). The observed TAN values for the bio-oil from *Nannochloropsis* were lower than the values 256.5 and 59-74 as reported by Duan and Savage [4] and Ellliot *et al.* [32], respectively but were higher than the maximum TAN value (0.5) of biodiesel as specified in ASTM D 6751-07a [4, 33]. With the use of Na_2CO_3, TAN of the bio-oils from all the strains were observed to decrease significantly from 250^{o}C to 300^{o}C. Minimum TAN was observed at 300^{o}C. There was a decrease in TAN when compared to the non-catalytic bio-oil. Increase and decrease of TAN is

mainly related to the increase and decrease of organic acids in the bio-oil. Higher TAN number in bio-oil leads to operational problems and may cause corrosion during storage and transportation, thus a low TAN is desired [4].

Temperature had no significant effect on the higher heating values of the bio-oils obtained from non-catalytic HTL. Observed higher heating values were in the range of 33-37 MJ/kg for all the three species. Higher heating value is due to the low oxygen present in the bio-oil. The use of Na_2CO_3 significantly increased the heating value of *Pavlova* bio-oil from 250^oC to 350^oC. However, it had no effect on the bio-oil from *Nannochloropsis* and *Isochrysis*. HHVs of all the three algal bio-oil were found to be lower than the petroleum crude (42.9 MJ/kg). HHVs for *Nannochloropsis* were observed to be less than that reported by Brown et al. [1] (37 – 39 MJ/kg) and Valdez et al. [18] (35 – 38 MJ/kg).

Table 3.3 shows the elemental analysis of the bio-oil from HTL for the three different algae strains. Nitrogen content in the bio-oil from the algal strains were in the order of *Nannochloropsis* > *Pavlova* > *Isochrysis* at all temperatures. This order is consistent with the biomass feedstocks. However, nitrogen content observed in the bio-oils from all strains were always less than that of the original algal biomass. Maximum nitrogen content was found to be maximum at 300^oC for all the algal bio-oils. Nitrogen content of *Nannochloropsis* was found to be higher than that reported by Brown et al [1], which was in the range of 3.9 and 4.1 wt.%. The algae feedstock that was used in their study also had lower nitrogen content (6.4 wt.%). The use of Na_2CO_3 did not have any significant effect on the nitrogen content of the bio-oil as shown in Table 3.4. Ross *et al.* [6] observed that Na_2CO_3 increased nitrogen in the bio-oil whereas Dote *et al.* [3] reported the decrease in nitrogen content of bio-oil produced from liquefying albumin using Na_2CO_3. It is worth mentioning that nitrogen is undesirable in the bio-oil because it produce NO_x gases when it is

combusted. In general, nitrogen content found in algal bio-oil is in range of 3 to 6 wt.% [1, 2, 6, 17, 19, 25, 26] and it is relatively high in comparison to petroleum crude (0-0.8 wt.%) [34, 35]. Nitrogen content in algal bio-oil is likely due to the presence of pyrroles and indoles, which are difficult to decompose at these conditions [26, 36]. Sulfur content was observed to be below 1 wt.% for all the algal biomass and bio-oils, whereas it varies between 0 and 3 wt.% in the case of petroleum crude oil [37]. Most of the sulfur in the algae bio-oil are likely due to the presence of dimethyl disulfide [36]. Carbon content of the algae bio-oil obtained without using Na_2CO_3 were in the range of 72-81 wt.% and were found to be independent of temperature. Carbon content of *Nannochloropsis* (72.55-79.33 wt.%) was the highest at 350^{o}C, but for *Isochrysis* (78.3-80 wt.%) and *Pavlova* (75.23-81.96 wt.%), it was observed to be highest at 300^{o}C. Use of Na_2CO_3 had no significant change on the carbon content of the bio-oils and which were found to be in the range of 75-81 wt.%. For both with and without Na_2CO_3 HTL runs, temperature did not have significant effect on the hydrogen content of bio-oils and were in the range of 11-13 wt.%. Hydrogen and carbon content of bio-oil from *Nannochloropsis* were found to be similar with the values reported by previous literatures [1, 18]. Elemental analysis of the algae bio-oil resembled that of petroleum crude oil, apart from its nitrogen and oxygen contents.

Table 3.4 Physical and ultimate analyses of bio-oil without catalyst

Strains	Temperature (ºC)	Physical analysis: Ash (wt.%)	Moisture (wt.%)	HHV (MJ kg^{-1})	pH	Density (g/ml)	TAN (mg KOH/g)
Nannochloropsis	250	0.18±0.02^{A}	7.63±4.42	33.40 ± 0.79^{A}	6.61 ± 0.01^{A}	0.93 ± 0.01^{A}	45.88 ± 2.81^{A}
	300	0.26±0.04^{A}	7.39±2.46	33.87 ± 0.34^{A}	7.74 ± 0.20^{B}	0.90 ± 0.01^{A}	32.68 ± 10.72^{A}
	350	0.26±0.00^{A}	7.00±3.19	34.82 ± 0.40^{A}	7.70 ± 0.10^{B}	0.90± 0.02^{A}	32.45 ± 5.71^{A}
Pavlova	250	0.29±0.02^{A}	10.14±6.16	33.90 ± 0.32^{A}	6.22 ± 0.06^{A}	0.93 ± 0.00^{A}	64.27 ± 3.25^{A}
	300	0.20±0.00^{B}	6.90±3.42	34.16 ± 1.18^{A}	7.16 ± 0.05^{B}	0.93 ± 0.02^{A}	46.81^{+B}
	350	0.44±0.00^{C}	5.59±1.17	32.20 ± 1.49^{A}	7.10 ± 0.01^{B}	0.91 ± 0.01^{A}	41.11 ± 1.23^{B}
Isochrysis	250	0.27±0.10^{A}	6.48±1.98	33.14 ± 1.00^{A}	6.24 ± 0.01^{A}	0.92 ± 0.00^{A}	59.52^{+A}
	300	0.27±0.06^{A}	10.28±2.09	34.66 ± 1.09^{A}	6.91 ± 0.04^{B}	0.94 ± 0.00^{A}	30.81 ± 4.1^{A}
	350	0.36±0.23^{A}	9.58±1.40	34.18 ± 1.49^{A}	8.68 ± 0.15^{C}	0.93 ± 0.00^{A}	36.05 ± 8.69^{A}

Strains	Temperature (ºC)	Ultimate analysis (wt.% in dry basis): C	H	N	S	O*
Nannochloropsis	250	72.55±8.06^{A}	14.15±0.95^{A}	5.13±0.49^{A}	0.49±0.09^{A}	7.68±6.71^{A}
	300	77.34±1.63^{A}	13.38±0.67^{A}	5.39±0.13^{A}	0.30±0.11^{A}	3.58±0.72^{A}
	350	79.33±1.28^{A}	11.96±0.63^{A}	5.26±0.15^{A}	0.17±0.20^{A}	3.28±0.61^{A}
Pavlova	250	79.75±2.02^{A}	12.42±0.70^{A}	4.01±0.04^{A}	n.d.	4.12±0.35^{B}
	300	80.02±0.42^{A}	13.21±0.15^{A}	5.02±0.66^{A}	0.46±0.08^{A}	1.29±0.32^{C}
	350	78.30±2.97^{A}	10.06±2.03^{A}	4.70±0.22^{A}	0.12±0.25^{A}	6.82±0.92^{A}
Isochrysis	250	75.23±1.60^{A}	12.34±0.27^{A}	3.92±0.10^{A}	0.47±0.02^{A}	8.04±1.98^{A}
	300	81.96±0.25^{A}	12.23±0.32^{A}	4.42±0.08^{A}	0.37±0.11^{A}	1.02±0.76^{A}
	350	77.36±2.69^{A}	11.24±1.43^{A}	4.39±0.37^{A}	0.25±0.10^{A}	6.76±4.58^{A}

*By difference. n.d.: not detected.+ single datum. Different alphabets in the superscript denotes the values are significant at α=0.05.

Table 3.5 Physical and ultimate analyses of bio-oil with catalyst

Species	Temperature (ºC)	Physical analysis (wet basis) Ash (wt.%)	Moisture (wt.%)	HHV (MJ kg^{-1})	pH	Density (g/ml)	TAN (mg of KOH/g)
	250	0.69±0.16^{A}	9.43±2.86^{A}	33.43±0.37^{A}	6.8±0.08^{A}	0.97±0.01^{A}	37.66±0.38^{A}
Nannochloropsis	300	0.34±0.21^{A}	7.69±4.42^{A}	33.58±0.48^{A}	7.58±0.13^{B}	0.92±0.01^{A,B}	27.03±0.21^{B}
	350	0.39±0.01^{A}	7.07±2.29^{A}	33.71±0.22^{A}	7.62±0.19^{B}	0.9±0.02^{B}	32.09±2.71^{A,B}
	250	0.47±0.13^{A}	8.15±1.56^{A}	33.86±0.32^{A}	6.55±0.27^{A}	0.94±0.00^{A}	49.271±3.93^{A}
Pavlova	300	0.24±0.08^{A}	6.21±2.34^{A}	35.55±0.31^{A,B}	7.07±0.03^{B}	0.94±0.01^{A}	35.02±0.21^{B}
	350	0.19±0.00^{A}	6.55±1.09^{A}	36.93±0.71^{B}	7.39±0.16^{A}	0.94±0.01^{A}	37.40±3.27^{A,B}
	250	0.36±0.02^{A}	5.81±1.12^{A}	33.77±0.7^{A}	6.30±0.18^{A}	0.94±0.02^{A}	44.02±1.6^{A}
Isochrysis	300	0.19±0.00^{A}	4.64±0.98^{A}	35.61±1.09^{A}	7.36±0.13^{B}	0.85±0.07^{A}	30.81±0.33^{B}
	350	0.14±0.06^{A}	5.62±1.11^{A}	34.67±0.8^{A}	6.7±0.09^{A}	0.86±0.01^{A}	37.23±2.82^{A,B}

Strains	Temperature (ºC)	Ultimate analysis (wt. % on dry basis) C	H	N	S	O*
	250	75.20±6.43^{A}	12.27±0.9^{A}	5.25±0.28^{A}	0.33±0.26^{A}	6.94±7.45^{A}
Nannochloropsis	300	80.57±3.58^{A}	12.81±0.24^{A}	5.52±0.47^{A}	0.52±0.06^{A}	0.58±4.22^{A}
	350	76.34±2.58^{A}	12.68±1.11^{A}	5.02±0.50^{A}	0.58±0.15^{A}	5.36±3.79^{A}
	250	80.1±2.77^{A}	12.40±0.16^{A}	4.54±0.2^{A}	0.7±0.04^{A}	2.27±3.10^{A}
Pavlova	300	75.43±6.37^{A}	11.59±0.99^{A}	4.64±0.39^{A}	0.56±0.06^{A}	7.78±7.78^{A}
	350	81.11±1.02^{A}	12.68±0.49^{A}	4.87±0.16^{A}	0.72±0.24^{A}	0.61±0.12^{A}
	250	79.11±0.19^{A}	12.32±0.08^{A}	4.1±0.32^{A}	0.57±0.03^{A}	3.90±0.18^{A}
Isochrysis	300	79.11±1.97^{A}	12.87±0.78^{A}	4.18±0.09^{A}	0.65±0.18^{A}	3.17±3.02^{A}
	350	79.68±2.53^{A}	13.23±0.42^{A}	4.37±0.23^{A}	0.96±0.1^{A}	1.77±3.29^{A}

*By difference. Different alphabets in the superscript denotes the values are significant at α=0.05.

While GC-MS detected more than 100 compounds, the bio-oil likely contained additional compounds which were lost during solvent evaporation. The compounds detected were arranged in decreasing order of area percentage and compounds with a higher area percentage representing more than 60% of the total peak area were chosen for analysis. The chemical composition of bio-oil was grouped into different categories such as hydrocarbons (HC), phenolics (Ph), nitrogenated compounds (NC), oxygenates (OC) and organic acids (OA) as shown in Table 3.6. Hydrocarbons represented straight chains, branched and cyclic (along with aromatic hydrocarbons). Phenolics included phenol and its derivatives (cresols and catechols). Nitrogenated compounds represented all the branched amides and compounds containing one or more nitrogen atoms. Oxygenates included compounds containing oxygen as a part of their chemical structure and organic acids constituted fatty acid, fatty acid esters and other organic acids.

The compounds identified under organic acids were hexadecanoic acid (palmitic acid), tetradecanoic acid (myristic acid), octanoic acid (caprylic acid), hexadecanoic acid methyl ester *etc*. The use of Na_2CO_3 resulted in bio-oil with similar organic acids but fewer cyclic organic acids like carbamic acid, methyl-3-methylphenylester-, 1H- Indole-3-propanoic acid etc. that were not found in non-catalytic bio-oil. The fatty acid and fatty acid esters are presumably obtained from hydrolysis of triacylglycerides present in the lipid portion of the algae strains [1] in the bio-oil. But there may be other pathways to form fatty acids during HTL, as bio-oil from sawdust (which contains no triglycerides) was reported to contain palmitic acid [38]. Srokol *et al.* [39] reported the formation of formic acid, lactic acid, acetic acid, acryclic acid etc. which are a result of the breakdown of glucose under hydrothermal condition. Hexadecanoic acid is one of the most frequent components found in bio-oil synthesized from algae [40]. Organic acids in the bio-oils of

Table 3.6 List of common compounds in bio-oil from the three algae strains identified from the MS library

Hydrocarbons	Phenolics	Organic acids and esters	Oxygenates	Nitrogeneous Compounds
Ethylbenzene	Phenols	Tetradecanoic acid	Tridecanedial	Pyridine
Napthalene, 2,3-dimethyl-	Phenol, 4-methyl-	n-Hexadecanoic acid	1-(1-Ethyl-2,3-dimethyl-cyclopent-2-enyl)-ethanone	Pyrazine, 2-ethyl-2,5-dimethyl-
Napthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	Phenol, 4-ethyl-	Hexadecanoic acid, methyl ester	Isophytol	Indole
2-Hexadecene, 3,7,11,15-tetramethyl-	Phenol, dimethyl-	Butanoic acid	1-Hexadecanol, 2-methyl-	1H-Indole, 3-methyl-
Heptadecane		Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester	Tropidine, 2-acetyl-8-demethyl-	1H-Indole, 2,3-dimethyl-
Styrene				Pyrrolidine, 1-(6-methyl-1-oxooctadecyl)-
Pentadecane				Quinoline, 4-methyl-
				Benzenamine, N-(1-methyl-2-propynyl)-
				Tetradecanamide
				N,N-Dimethyldecanamide
				N,N-Dimethyldodecanamide

three algae strains were observed to decrease with the increasing temperature as shown in Figure 3.6 (a) and (b). This correlates with the decrease in pH and TAN of bio-oil as the temperature is

increased. At 250^{o}C organic acids were in the range of 20 to 45% but decreased to 0 to 5% at 350^{o}C. At higher temperatures decarboxylation of fatty acids takes place and long chain hydrocarbons (such as pentadecane, heptadecane, tridecane etc.) are formed [1]. This may be the cause of increase in the percentage of long chain hydrocarbons with the increase in temperature. However, the use of Na_2CO_3 lowered the organic acid yields at a lower temperature of 250^{o}C (12-15%) as well as at a higher temperatures as shown in Figure 3.6 (b). This reduction in organic acid is due to the catalytic effect of Na_2CO_3 which directly breakdown lipids to alkanes [26].

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 [6, 27]. The amino acids undergo degradation through decarboxylation and deamination reactions to give amines, carbonic acids, ammonia and other organics [27]. Nitrogenated compounds in the bio-oil from three algae strains were observed to increase with an increase in temperature from 250 to 300^{o}C. The branched amides increased from 5 to 12 % from 250^{o}C to 300^{o}C and reduced to 7 % at 350^{o}C for *Nannochloropsis* and a similar trend was observed for bio-oil 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 are 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 Na_2CO_3 produced more

cyclic nitrogen. 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 while esters and organic acids were grouped separately. 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*. [23]. No trend of oxygenated compounds were observed with the increase in temperature. Catalytic bio-oils also had similar trends for oxygenated compounds as compared to non-catalytic bio-oils.

Phenolic compounds included phenol, phenol, 4-methyl-, phenol, 3-ethyl-5-methyl-. In the case of phenolic derivatives in bio-oil from three algae strains, phenols increased with the increase in temperature. This trend was also observed in catalytic runs. No significant changes in phenols were observed with the catalyst. A similar trend for phenolic derivatives was observed by Anastasakis *et al.* [2] in their bio-oil from *Laminaria Saccharina*. Phenolics increased from 0-3% at 250^{o}C to 10-15% at 350^{o}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 [1].

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

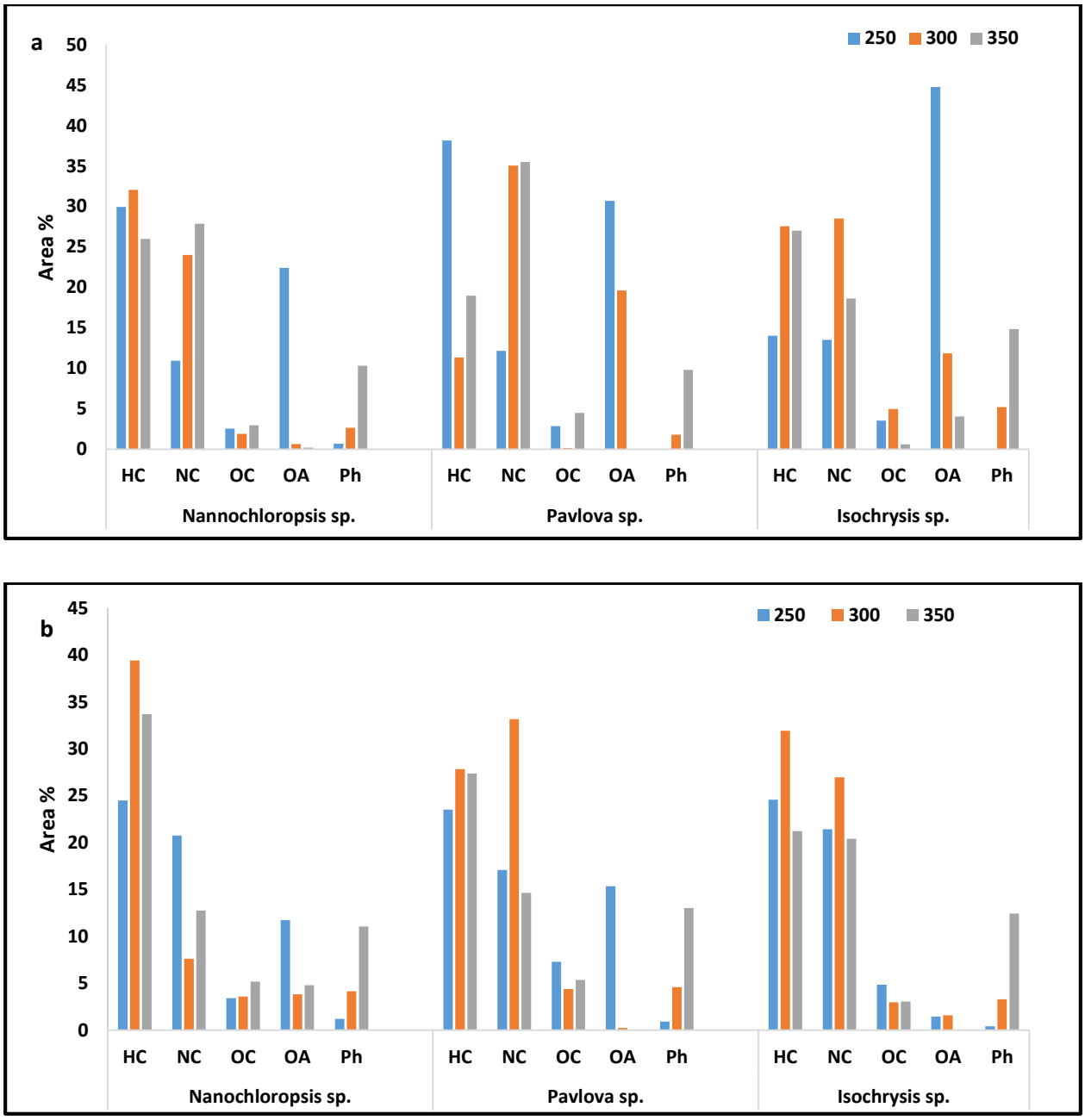


Figure 3.6 Distribution of major compounds in bio-oil produced from (a) non-catalytic and (b) catalytic HTL of different algae strains at different temperatures

(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^{o}C to 300^{o}C, however hydrocarbons decreased at higher temperature (350^{o}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 fairly smaller area percentage with respect to aliphatic hydrocarbons. This is probably due to the catalytic effect of Na_2CO_3 which breakdowns lipids to alkanes. Although most of the chemical compounds in all the three algae strains were similar but there were few variations. For example, eicosene, decomposed from eicosapentaenoic acid was observed only in bio-oil from *Pavlova.*

3.3.4. Solid Residue Analysis

Proximate and ultimate analyses of solid residue obtained from both non-catalytic and catalytic HTL were performed, and the values are reported in Tables 3.7 and 3.8 respectively. In the case of non-catalytic HTL, ash content was found to increase significantly with temperature for *Nannochloropsis* while no significant increase in the ash content was observed for other strains. However, in catalytic HTL, ash content was observed to increase significantly as the temperature increased from 250^{o}C to 350^{o}C for *Pavlova* and up to 300^{o}C for the solid residue of the other strains. The HHVs of char were observed to be higher for char obtained at lower reaction temperatures for both catalytic and non-catalytic runs and then for char obtained as the reaction temperature increased. For non-catalytic runs, the decrease was not significant for *Nannochloropsis* and *Isochrysis* but a significant decrease was obtained for *Pavlova* when the temperature increased from 300-350^{o}C. However, for catalytic runs, the HHVs of solid char were observed to significantly decrease from 250^{o}C to 300^{o}C for *Nannochloropsis* and *Isochrysis* as shown in Table 3.8. This decrease in heating value with increased temperature corresponds to a high amount of unconverted carbon and hydrogen content in the solid residue as shown by ultimate

analysis. The high energy partitioned in the solid residue fraction of the yield can be retrieved using the solid residue as feedstock for energy generation.

Table 3.7 Proximate and ultimate analyses of solid residue from non-catalytic HTL of algae

Strains	Temperature (°C)	Proximate analysis (wet basis) Ash (wt.%)	Moisture (wt.%)	HHV (MJ kg^{-1})
	250	6.07±0.73^{A}	8.09±9.27^{A}	31.59±0.0^{A}
Nannochloropsis	300	20.35^{B}	9.45±5.54^{A}	25.89±2.31^{A}
	350	38.41±1.62^{C}	16.67±2.92^{A}	21.41±4.91^{A}
	250	31.57±0.55^{A}	10.04±4.71^{A}	24.57±1.11^{A}
Pavlova	300	40.15±8.75^{A}	14.22±7.71^{A}	21.75±1.54^{A}
	350	43.07±9.80^{A}	12.59±3.10^{A}	15.06±0.35^{B}
	250	15.06±1.43^{A}	23.19±13.42^{A}	31.68±0.63^{A}
Isochrysis	300	22.32±7.86^{A}	16.51±1.56^{A}	30.95±3.99^{A}
	350	30.26±1.72^{A}	5.31±3.52^{A}	21.77±2.79^{A}

Strains	Temperature (°C)	Ultimate analysis (wt. % dry basis) C	H	N	S	O*
	250	70.34±5.17^{A}	9.44±0.29^{A}	4.91±0.45^{A}	0.12±0.18^{A}	15.2±5.73^{A}
Nannochloropsis	300	55.63±3.83^{A,B}	7.83±0.41^{B}	3.76±0.47^{A,B}	0.39±0.25^{A}	32.39±3.52^{B}
	350	50.24±1.82^{B}	6.96±0.28^{B}	2.22±0.13^{B}	0.28±0.01^{A}	40.3±2.23^{B}
	250	54.94±6.38^{A}	6.04±0.76^{A}	4.83±0.62^{A}	0.20±0.16^{A}	33.98±7.59^{A}
Pavlova	300	43.06±7.64^{A}	4.41±0.81^{A}	3.19±0.21^{B}	0.65±0.28^{A}	48.69±8.94^{A}
	350	37.35±3.44^{A}	3.58±0.73^{A}	2.58±0.14^{B}	0.67±0.13^{A}	55.82±4.15^{A}
	250	62.80±0.23^{A}	9.49±0.17^{A}	3.29±0.07^{A}	0.35±0.00^{A}	24.07±0.47^{A}
Isochrysis	300	58.71±2.16^{A,B}	8.49±0.47^{A,B}	2.74±0.05^{B}	0.29±0.07^{A}	29.76±2.66^{A,B}
	350	41.50±7.45^{B}	5.88±1.23^{B}	2.12±0.08^{B}	0.64±0.13^{A}	49.86±8.89^{B}

* By difference. Different alphabets in the superscript denotes the values are significant at α=0.05.

The ultimate analysis of the solid residue showed that solid residue still had a significant portion of C, H and N in it. 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. The low amount of C and H content at higher

temperatures suggests that most of the organic material has been converted from the solid algal biomass to other product fractions. A decrease in C and H content was observed with an increase

Table 3.8 Physical and ultimate analyses of solid residue from catalytic HTL of algae strains

Strains	Proximate analysis (wet basis)			
	Temperature (ºC)	Ash (wt.%)	Moisture (wt.%)	HHV (MJ kg^{-1})
Nannochloropsis	250	16.92 ± 2.34^{A}	2.9 ± 0.38^{A}	23.56 ± 0.7^{A}
	300	41.76 ± 2.2^{B}	7.51 ± 6.55^{A}	17.66 ± 1.03^{B}
	350	43.29 ± 9.48^{B}	13.07 ± 4.34^{A}	16.965 ± 1.37^{B}
Pavlova	250	28.58 ± 1.86^{A}	15.45 ± 2.81^{A}	27.03 ± 2.39^{A}
	300	42.71 ± 1.47^{B}	10.45 ± 4.09^{A}	26.49 ± 1.65^{A}
	350	53.04 ± 0.7^{C}	13.15 ± 5.19^{A}	20.54 ± 1.04^{A}
Isochrysis	250	14.89 ± 1.57^{A}	3.72 ± 0.96^{A}	15.82 ± 1.31^{A}
	300	31.34 ± 4.23^{B}	12.26 ± 1.94^{B}	11.56 ± 1.08^{B}
	350	14.91 ± 0.61^{A}	10.15 ± 1.91^{B}	6.68 ± 2.15^{B}

Strains	Temperature	Ultimate analysis (wt.% dry basis)				
	(ºC)	C	H	N	S	O*
Nannochloropsis	250	51.84 ± 0.23^{A}	7.20 ± 0.76^{A}	4.80 ± 0.69^{A}	0.10 ± 0.20^{A}	36.05 ± 1.89^{A}
	300	35.89 ± 7.66^{A}	4.80 ± 1.13^{A}	2.50 ± 0.30^{B}	0.14 ± 0.12^{A}	56.65 ± 9.21^{A}
	350	35.26 ± 7.60^{A}	4.72 ± 1.03^{A}	1.68 ± 0.13^{B}	0 ± 0.09^{A}	58.38 ± 8.66^{A}
Pavlova	250	60.61 ± 0.11^{A}	10.58 ± 0.25^{A}	3.06 ± 0.15^{A}	0.36 ± 0.05^{A}	25.38 ± 0.17^{A}
	300	53.25 ± 0.83^{B}	8.40 ± 0.09^{B}	2.49 ± 0.17^{B}	0.18 ± 0.03^{A}	35.67 ± 1.13^{B}
	350	52.78 ± 2.07^{B}	10.03 ± 0.51^{A}	2.30 ± 0.04^{B}	0.26 ± 0.11^{A}	34.62 ± 2.73^{B}
Isochrysis	250	38.00 ± 0.11^{A}	4.94 ± 0.12^{A}	4.67 ± 0.20^{A}	0.32 ± 0.05^{A}	52.06 ± 0.24^{A}
	300	25.99 ± 3.22^{B}	2.69 ± 0.68^{B}	2.43 ± 0.48^{B}	0 ± 0.11^{A}	68.91 ± 4.50^{B}
	350	19.31 ± 0.71^{B}	1.55 ± 0.07^{B}	1.32 ± 0.05^{B}	0 ± 0.23^{A}	77.91 ± 0.49^{B}

* By difference. Different alphabets in the superscript denotes the values are significant at $\alpha=0.05$.

in temperature. For solid residue of *Nannochloropsis*, C decreased from 70.34 wt.% at 250ºC to 50.24 wt.% at 350ºC and hydrogen decreased from 9.44 wt.% at 250ºC to 6.96 wt.% at 350ºC. Similarly for *Isochrysis* and *Pavlova*, there was decrease in the C and H values as the temperature increased as shown in Table 3.6. In comparison to non-catalytic HTL, the solid residue from catalytic HTL had lower C content which suggests that most of the organic material has been

converted to bio-oil with use of Na_2CO_3 as catalyst. The H content in the solid residue did not follow any trend but amounts were higher for *Pavlova* (8.4-10.58 wt.%) than the other two strains. Whereas for solid residue from non-catalytic HTL, an increase in H content was observed for *Pavlova sp.* but remained consistent for other algae strains residue. High C and H contents and heating values of solid residue make it an attractive feed for energy production. Apart from high C and H values, solid residues from both catalytic and non-catalytic HTL also have high N content for all strains. N content decreased with an increase in temperature for both types of runs as shown in Tables 3.6 and 3.7. High nitrogen content of solid residue also makes it an attractive option for use as a bio-fertilizer.

3.3.5. Aqueous Phase Analysis

The aqueous phase was measured for its total organic carbon (TOC), total nitrogen (TN) content and pH. For this analysis the aqueous phase was not dried. The aqueous phase obtained had very foul smell, especially due to the presence of ammonia and was semi-transparent. Table 3.9 and 3.10 illustrates the total amount of organic carbon, total amount of nitrogen and pH present in the aqueous product obtained from non-catalytic HTL and catalytic HTL respectively. The pH of the aqueous phase of HTL of algae was alkaline, which was probably due to the presence of ammonia (a weak base) in the aqueous phase. 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 was in agreement with Ross *et al.* [6], who also found the same pattern when Na_2CO_3 was used as catalyst. This may be due to the presence of an alkali catalyst in the aqueous phase.

Total organic carbon was obtained by deducting total inorganic carbon from total carbon of the aqueous phase. For non-catalytic HTL, the aqueous phase obtained from *Nannochloropsis* and *Pavlova* were observed to decrease with an increase in temperature. A similar trend with the

increase in temperature was reported by Yang et al.[22] for the aqueous phase from hydrothermal liquefaction of *Mirocystis. viridis*. This was obvious because most of the carbon was converted to bio-oil and gases with an increase in temperature. The TOC of *Isochrysis* on the other hand did not show any pattern with the increase in temperature. TOC of *Nannochloropsis*, *Isochrysis* and *Pavlova* ranged from 28.78 to 59.05 g/l, 28.94 to 51.12 g/l, and 15.80 to 41.53 g/l, respectively. In comparison to the non-catalytic aqueous phase, the catalytic aqueous phase was observed to have

Table 3.9 Properties of aqueous phase from non-catalytic HTL

Strains	Temperature (°C)	TOC (g/l)	TN (g/l)	pH
Nannochloropsis	250	59.05*^{A}	14.90*^{A}	7.72±0.01^{A}
	300	33.79±0.45^{B}	6.69±0.23^{B}	8.28±0.02^{B}
	350	28.78±0.06^{C}	10.18±0.25^{C}	8.28±0.1^{B}
Pavlova	250	51.12±14.61^{A}	6.58±2.25^{A}	7.70±0.00^{A}
	300	37.15±4.32^{A}	8.48±2.55^{A}	8.16±0.01^{B}
	350	28.94±0.38^{A}	10.22±0.06^{A}	8.35±0.01^{C}
Isochrysis	250	15.80±0.46^{A}	4.76±0.26^{A}	8.07±0.04^{A}
	300	41.53*^{B}	6.85*^{B}	7.66±0.02^{B}
	350	17.24±0.10^{A}	8.08±0.03^{B}	8.12±0.04^{A}

* contains single datum

a higher TOC at higher temperatures (300 and 350°C) for *Nannochloropsis* and *Pavlova*. This was in agreement with Yang *et al.* [22], who reported an increase in the organic matter in the aqueous phase with increased temperature and sodium carbonate addition. This was due to the effect of Na_2CO_3, which shifted organic matter from the feedstock into the aqueous phase as non-volatile organic carbon [22]. The amount of organic carbon in the aqueous phase indicates that, during HTL, a significant proportion of the organic products are distributed in the aqueous phase as dissolved organics [2]. Zhou et al. [23] reported an abundance of acetic acid and glycerol in the aqueous phase from hydrothermal treatment of *Enteromorpha prolifera* (macroalgae). Presence

of acetate and formates, which are likely from carbohydrate hydrolysis, were also reported in the aqueous phase by other studies [5, 6].

Table 3.10 Properties of aqueous phase from catalytic HTL

Strains	Temperature (ºC)	TOC (g/l)	TN (g/l)	pH
Nannochloropsis	250	36.56 ± 6.4^{A}	9.67 ± 1.65^{A}	8.53 ± 0.23^{A}
	300	66.16 ± 10.01^{A}	8.16 ± 0.41^{A}	8.54 ± 0.03^{A}
	350	73.16 ± 26.86^{A}	9.06 ± 0.8^{1A}	9.42 ± 0.01^{B}
Pavlova	250	29.79 ± 2.50^{A}	7.96 ± 0.43^{A}	8.31 ± 0.06^{A}
	300	31.72 ± 7.40^{A}	8.31 ± 0.06^{A}	7.9 ± 0.13^{B}
	350	34.17 ± 14.96^{A}	8.05 ± 0.88^{A}	$8.02\pm0.07^{A,B}$
Isochrysis	250	31.41 ± 3.46^{A}	8.30 ± 0.08^{A}	8.06 ± 0.28^{A}
	300	37.51 ± 10.05^{A}	$6.56\pm1.15^{A,B}$	8.22 ± 0.06^{A}
	350	24.12 ± 6.39^{A}	7.56 ± 0.09^{B}	9.08 ± 0.20^{B}

Different alphabets in the superscript denotes values are significant at α=5%

The concentration of total nitrogen (TN) content in the aqueous phase also varies according to algal strains. Nitrogen is present in the form of protein in the algal biomass so most of the protein readily decomposes into water soluble amino acids and ammonia [18, 26]. However, only a portion of N in the aqueous phase is present as ammonia [18] and other forms may be NO_3^- or other compounds [24]. In the case of non-catalytic HTL, the TN of *Nannochloropsis*, *Pavlova* and *Isochrysis* ranged from 6.69 to 14.90 g/l, 6.58 to 10.22 g/l and 4.76 to 8.08 g/l. Increase in nitrogen content was observed with an increase in temperature but it depended on type of algae strain. Increase in TN with temperature may be due to an increase in the deamination reaction which converts amine and amide compounds in bio-oil to ammonia gas that is then dissolved in the aqueous phase. A similar trend was observed by Yang *et al*. [22]. For the aqueous phase from catalytic runs, TN did not show any significant effect with the increase in temperature. TN of *Nannochloropsis*, *Pavlova* and *Isochrysis* ranged from 8.16 to 9.67 g/l, 7.96 to 8.31 g/l and 6.56

to 8.30 g/l respectively. Ross *et al.* [6] reported a decrease in nitrogen concentration with an increase in temperature for *Spirulina*, but reported no change in nitrogen concentration for *Chlorella*. This shows an increase or decrease in nitrogen concentration with temperature depends on the type of algae strain. The use of Na_2CO_3 as catalyst, however, 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 3.9.

3.3.6. Carbon and Nitrogen Balance

Carbon and nitrogen balance were performed to investigate their distribution in the product streams of HTL using Equation 3.4. 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. Figure 3.7 (a) and (b) illustrate the carbon distribution for both non-catalytic and catalytic runs respectively. For both catalytic and non-catalytic runs, the bio-oil fraction accounts for the maximum amount of carbon percentage. The carbon distribution increased in the bio-oil and gas and unaccounted portion but decreased in solid residue and water soluble products as the temperature increased. Other studies [2, 18, 22] have also reported similar trends with an increase in temperature. The carbon distribution in bio-oil was observed to increase significantly with the increase of temperature for *Nannochloropsis* and *Isochrysis* but did not increase significantly for *Pavlova*. This increase is mainly due to the increase in bio-oil yield and carbon conversion with an increased temperature. However for *Pavlova,* the bio-oil yield did not increase with temperature. With the use of Na_2CO_3, *Pavlova* and *Isochrysis* had a significant increase in the carbon distribution with temperature. This increase in carbon distribution for *Pavlova* and *Isochrysis* is mainly due to the catalytic effect of Na_2CO_3 which efficiently converts the carbohydrates in the algae to bio-oil. For *Nannochloropsis*,

the carbon distribution did not increase significantly with an increase in temperature but a higher carbon distribution at a lower temperature was observed when compared to the non-catalytic run. This increase in carbon distribution for *Nannochloropsis* at a lower temperature was mainly due to the catalytic effect of Na_2CO_3, which increased the bio-oil yield at lower temperature. In comparison to non-catalytic runs, carbon distribution in bio-oil for the catalytic run is higher for *Pavlova* and *Isochrysis* as the yield increases.

Much of the carbon in lower temperature HTL appears in the water soluble product and solid residue. The solid residue decreased with increased temperature for all algae strains. In the case of the non-catalytic run, solid residue from *Nannochloropsis* and *Pavlova* decreased significantly with an increase in temperature from 250 to 300^{o}C whereas that of *Isochrysis* decreased significantly from 300 to 350^{o}C as shown in Figure 3.7 (a). This decrease is due to the decrease in the yield and carbon conversion with increase in temperature. In the case of *Nannochloropsis*, it ranged from 10.69 to 39.44 wt.% of initial carbon, while for *Pavlova*, it ranged from 10.50 to 24.91 wt.% and for *Isochrysis*, it ranged from 15.21 to 26.63 wt.% of initial carbon. A comparatively lesser amount of carbon (1-10 wt.% of initial carbon) in solid residue was observed in other studies [2, 22, 26]. For the catalytic runs the solid residue decreased significantly with an increase in temperature from 250 to 300^{o}C. In comparison to solid residue from non-catalytic runs, catalytic runs had lower carbon distribution in solid residue at 250^{o}C. *Nannochloropsis, Pavlova* and *Isochrysis* had a solid residue of 39.44 wt.%, 24.91 wt.% and 26.63 wt.% respectively, at 250^{o}C without using Na_2CO_3 whereas it decreased to 24.14 wt.%, 13.34 wt.% and 9.98 wt.% respectively when using Na_2CO_3 as catalyst. This decrease is mainly due to the effective conversion of the carbohydrate portion of algae into bio-oil at a lower reaction temperature through the use of Na_2CO_3.

Carbon in the water soluble product (WSP) fraction also had a similar trend as that of solid residue with an increasing temperature. We observed less carbon distribution 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. The carbon distribution in WSP of *Nannochloropsis*, *Pavlova* and *Isochrysis* after non-catalytic reactions were found to be in the range of 5.62 to 24.17 wt.%, 2.96 to 25.45 wt.% and 5.73 to 18.61 wt.% of initial carbon respectively. The use of Na_2CO_3 had no significant change in WSP yield when compared with non-catalytic yield. Using Na_2CO_3 carbon distribution were found to be in the range of 9.25 to 20.93 wt.%, 6.33 to 19.09 wt.% and 6.57 to 16.44 wt.% of initial carbon for *Nannochloropsis*, *Pavlova* and *Isochrysis* respectively. High carbon distribution (40-45 wt.% of initial carbon) in the aqueous fraction was also observed by Valdez *et al*. [18] and Yang *et al*. [22] whereas a moderate carbon distribution (20-30 wt.% of initial carbon) was observed by Biller *et al.* [26] and Anastasakis *et al*.[2]. The higher amount of carbon in the aqueous fraction is probably due to the breakdown of a large amount of carbohydrates to polar water-soluble organics and not to non-polar hydrocarbon type structures [26]. The carbon in the gas and unaccounted fraction was determined by the difference. The amount of gaseous yield increased with the increase of temperature, which automatically increased the carbon distribution in gases. This can be suggested by the high amount of gas and unaccounted fraction obtained. The unaccounted fraction consists of losses during the processing (handling losses and low molecular wt. compounds loss during drying aqueous phase). Due to the high amounts of carbon distribution in the char and aqueous fractions, they can be used as feedstock for further energy production.

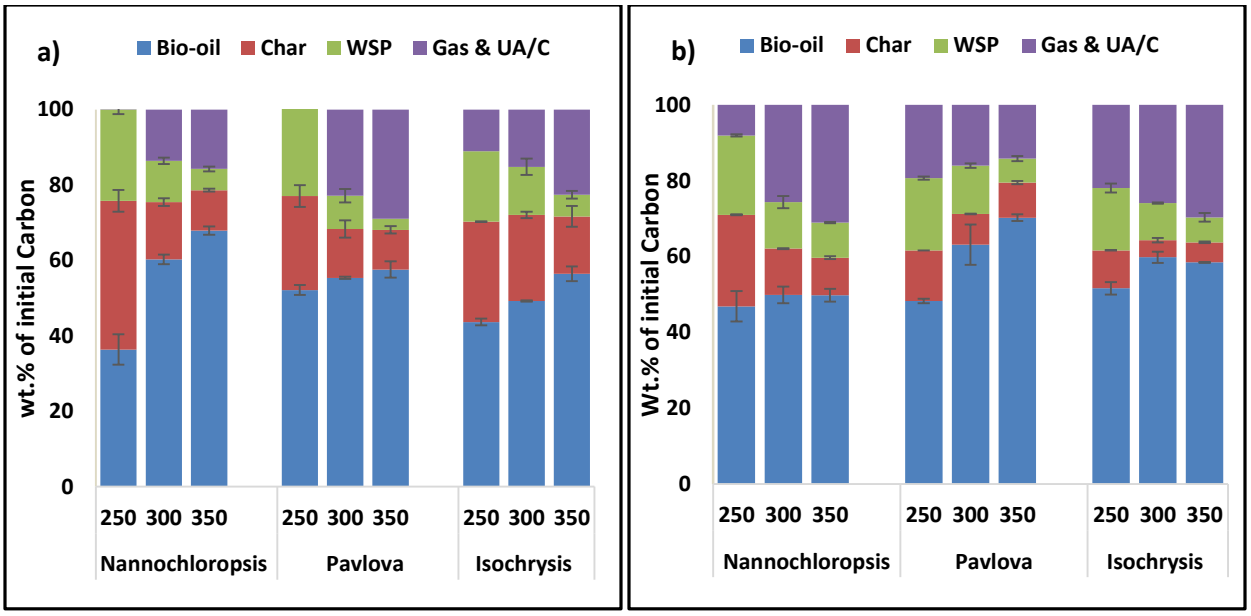


Figure 3.7 Carbon distribution in the HTL product streams (a) non-catalytic and (b) catalytic

Figures 3.8 (a) and (b) illustrate the nitrogen distribution for both non-catalytic and catalytic runs, respectively. For the non-catalytic runs, nitrogen fraction in the bio-oil for *Pavlova*, *Isochrysis* and *Nannochloropsis* were observed to be in the range of 16.42 to 21.77 wt.%, 15.94 to 22.45 wt.% and 14.43 to 25.26 wt.% of initial nitrogen, respectively. Significant increase in nitrogen distribution with the increase in temperature was observed for *Nannochloropsis* and *Isochrysis* but this was not true for *Pavlova*. Nitrogen in bio-oil increased with the increase in temperature because of decomposition of proteins into ammonia compounds, which further decomposes to form amides and nitrogen heterocyclic compounds. Increase in nitrogen distribution was also due to the increase in the total yield of bio-oil. Bio-oil without nitrogen is most desirable as it does not produce NO_X gases when combusted. The use of Na_2CO_3 increased the amount of total initial nitrogen percentage in bio-oil at 250^{o}C when compared to non-catalytic bio-oils. *Nannochloropsis*, *Pavlova* and *Isochrysis* were observed to have 18.35 wt.%, 18.17 wt.% and 19.84 wt.% of total initial nitrogen, respectively, when using Na_2CO_3. This increase is 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*. [2] reported the distribution of about 40 wt.% of initial nitrogen in bio-oil whereas Biller *et al.* [26] observed it to be only 10-25 wt.% of initial nitrogen. Nitrogen in bio-oil is mainly in the form of cyclic nitrogen like pyridine, pyrrole, indole etc. and amides like dimethyldcanamide, hexadecanamide etc.

Nitrogen fractions in solid residue and WSP were observed to decrease with increase in temperature. Without using Na_2CO_3, the nitrogen fraction in solid residue was observed to be in the range of 4.55-13.72 wt.%, 5.44- 9.77 wt.% and 2.66-15.44 wt.% of initial nitrogen for *Pavlova, Isochrysis* and *Nannochloropsis,* respectively. Nitrogen distribution decreased with increased temperature for all algae strains. This is probably due to the conversion of protein to bio-oil and aqueous phase with an increased temperature. Nitrogen found in solid residue is probably due to the unconverted proteins. With the use of Na_2CO_3, the nitrogen distribution was higher at low temperatures (<12 wt. %) and lower (<3 wt.%) at higher temperatures when compared with non-catalytic runs as shown in Figure 3.8 (a) and (b). Nitrogen distribution in the water soluble product also showed a similar trend with an increase in temperature. For non-catalytic runs, nitrogen fractions in the WSP were found to decrease with temperature and were in the range of 28.58 to 6.89 wt.%, 37.16 to 3.07 wt.% and 29.83 to 7.37 wt.% for *Nannochloropsis, Pavlova* and *Isochrysis*, respectively. A similar trend was observed with the use of Na_2CO_3 as catalyst and fractions were in the range of 25.34 to 8.49 wt.%, 22.25 to 5.66 wt.% and 20.53 to 7.73 wt.% of the initial nitrogen, for *Nannochloropsis*, *Pavlova* and *Isochrysis*, respectively. The opposite trend of nitrogen in aqueous phase with increased temperatures was observed by Valdez et al. [18]. This is because Valdez et al. have analyzed whole aqueous phase rather than WSP for nitrogen distribution. The decrease in N distribution in aqueous phase with increased temperature was also

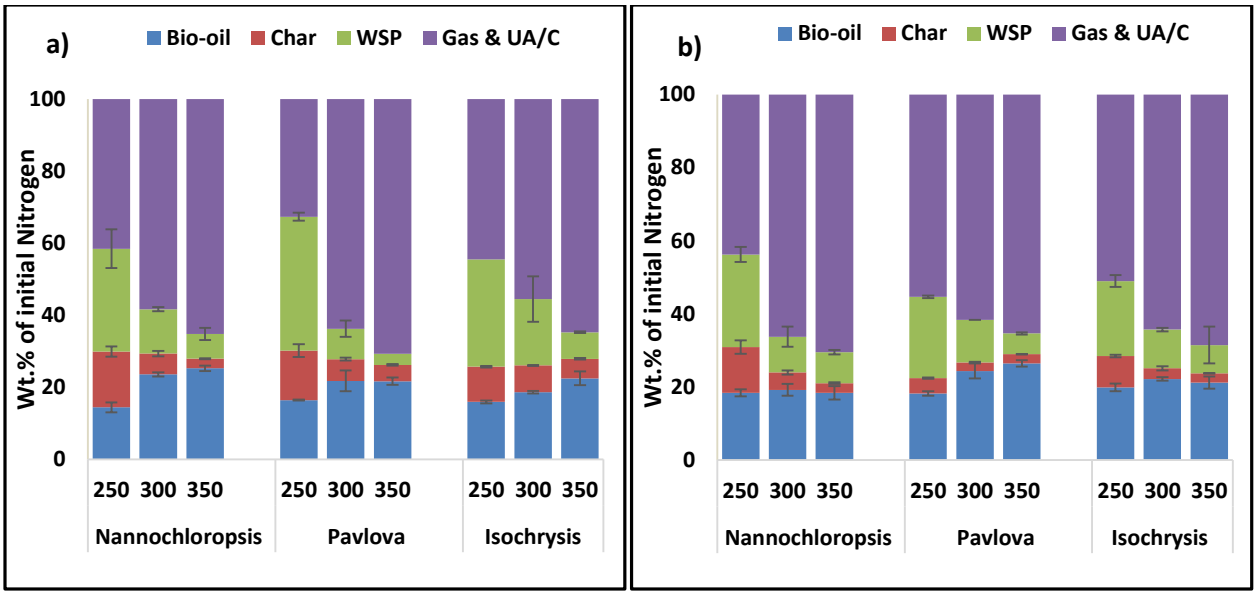


Figure 3.8 Nitrogen distribution in the HTL product streams (a) non-catalytic (b) catalytic

observed by Ross *et al.* [6] with the use of alkali catalysts (Na_2CO_3 & KOH). Ammonia is mostly present in the aqueous phase because at higher temperature reactions converting organic nitrogen to ammonia becomes more favorable [18]. Therefore, while evaporating the aqueous phase for WSP most of the ammonia might have evaporated, thus reducing the total nitrogen distribution in WSP. Due to the high amount of nitrogen distribution in the aqueous phase, it can be recovered and further used as nutrient for algal cultivation.

3.4 Conclusions

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 a 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 for non-catalytic HTL. Use of Na_2CO_3 as a catalyst also had similar effect with increased temperature but maximum bio-oil yield followed the order of *Pavlova* > *Isochrysis* > *Nannochloropsis.* At a lower temperature, Na_2CO_3 helped in selectively increasing the yield of

high protein containing algae and also favored conversion of carbohydrate to bio-oil. The bio-oil produced had a high heating value in the range of 33 to 35 MJ/kg, had a low density, and were alkaline in pH for all three strains. The bio-oil produced resembled that of petroleum crude except for its high nitrogen and oxygen content 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 physical and chemical 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 are highly distributed in these phases. The solid residue or char had high C content at lower temperatures and decreased as the temperature increased. The aqueous product had a high amount of organic carbon and nitrogen content. Presence of high carbon in the aqueous phase makes it a suitable biomass feedstock and can be further used for fuel generation purposes and high nitrogen in the aqueous phase can be recovered for use as a nutrient for algal growth.

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CHAPTER FOUR

4. SUMMARY AND FUTURE DIRECTIONS

4.1 Summary

Bio-oil production from algae biomass via hydrothermal liquefaction has received great attention. Hydrothermal liquefaction is a thermochemical process in which hot compressed water at sub- or super-critical stage is used as a reaction medium to convert biomass to bio-oil, gases, water-soluble products and solid residue often termed as char. The main objective of this study was to produce bio-oil from hydrothermal liquefaction of algae and to study the properties of the bio-oil along with its co-products. The main objective was further divided into two specific objectives: (1) to study the effect of temperatures on the product yields from hydrothermal liquefaction of algae and its properties; and (2) to understand the effect of an alkaline catalyst, Na_2CO_3, on hydrothermal liquefaction of algae.

Three kinds of algae (*Nannochloropsis sp.*, *Pavlova sp.*, and *Isochrysis sp.*) that have different biochemical compositions were selected for hydrothermal liquefaction (HTL). HTL was performed at three temperatures of 250^{o}C, 300^{o}C and 350^{o}C, a residence time of one hour with solid percentage of 14-15 wt.% in a batch reactor, with or without using Na_2CO_3 as catalyst at 5 wt.% of dry algae. Maximum bio-oil yield for non-catalytic (48.67 wt.%) and catalytic HTL (47.05 wt.%) were both obtained at 350^{o}C for *Nannochloropsis* and *Pavlova*, respectively. This may be because, at a high temperature, water's dielectric constant decreases and it easily solubilizes the organic compound of the biomass. In addition, the ionic product of water increases drastically at

high temperature and water has the ability to hydrolyze complex compounds catalyzed by H^+ and OH^- ions. As a result complex molecules breakdown to form bio-oil compounds. The study also showed that not only lipids but also the other fractions in algae such as proteins and carbohydrates got converted to bio-oil. Although the bio-oil yield of *Nannochloropsis* during non-catalytic reaction was the highest at 350^{o}C compared to all strains of algae used, its yield was the lowest at 250^{o}C. This may be because *Nannochloropsis* had high protein content, and at lower temperature the peptide bond of amino acids, which are the building blocks of protein, are more stable than the glycosidic bonds of glucose resulting in lower yield of bio-oil. In the case of catalytic HTL reactions, at higher temperatures, increased carbohydrate content (*Pavlova* and *Isochrysis*) resulted in a higher bio-oil conversion than *Nannochloropsis.* Bio-oils obtained from HTL were analyzed for their ash, water content, density, HHV, TAN, pH and also for their elemental composition. Na_2CO_3 had no significant catalytic effect on the HHVs, density, pH of the bio-oil produced from all the strains and were in the range of 33 to 37 MJ/kg, 805 to 970 kg/m^3, 6 to 9, respectively. But at lower temperatures TAN decreased for catalytic runs when compared to non-catalytic runs. The decrease in TAN may be due to the catalytic effect of Na_2CO_3, which increases the direct formation of alkanes from triglycerides which was reflected on GCMS study. Ultimate analysis showed that the bio-oil from both catalytic and non-catalytic runs had a high percentage of carbon (72-82 wt.%) and hydrogen (10-14 wt. %) content. The downside of the bio-oil was that, it had a high nitrogen content of between 4-5 wt.%. Solid residue decreased with increase in temperature.

Analysis of solid residue also showed that it had a high carbon distribution for both catalytic and non-catalytic runs and could be further used as energy feedstock. The water-soluble products (WSP) of aqueous phase decreased with the increase in temperature. The aqueous phase had a pungent smell, which was due to the ammonia present in it. Nitrogen distribution in the WSP was

high (40 wt.%). Because of this, there is a possibility of recovering nutrients from the aqueous phase and utilizing them for algae cultivation.

4.2 Future Directions

Hydrothermal liquefaction is still at an early stage of development. This research on hydrothermal liquefaction of algae has tried to fill the knowledge gap in the literature about how temperature can influence bio-oil properties from different strains of algae and the role alkaline catalysts play on bio-oil yields and its properties. With the knowledge gained from this research, the study on the intricacies of hydrothermal liquefaction can be expanded. The following topics are recommended for future studies:

- Co-products Study:

  Hydrothermal liquefaction gives three main co-products apart from the main product: solid residue, gas and water soluble product. Although limited characterization of the co-products was performed in this research, a more detailed study on the co-product analysis (such as metal analysis in both aqueous and solid char, analysis of nitrogen containing compounds in aqueous phase) should be done. The aqueous phase, which accounts for 25-30 wt.% of total liquefaction yield, consists of high carbon and nitrogen distribution. Therefore, the presence of different valuable carbon containing compounds and nitrogenous compounds in the aqueous phase should be identified. This will provide clear understanding on how the aqueous phase can be utilized. For example, several studies [1, 2] have voiced the use of aqueous phase in algae cultivation. Moreover, due to the amount of carbon content in aqueous phase, it has also been seen as an energy feedstock. Therefore, proper knowledge of the aqueous phase can help us decide the scope of its use. In the case of solid residue, a detailed study on the chemical constituent of the residue should be

performed to understand the effect of solid residue formation and its further use. This research did not include a study of the gas phase, and it should be performed to find out the gas composition of algae HTL.

- Overall Effect of Operating Parameters on HTL of Algae:

  This research was focused only on the effect of reaction temperature and algae strain on the liquefaction behavior. However, other parameters such as residence time, biomass loading, catalyst dosage, initial pressure etc. are believed to play a key role in determining the liquefaction yields. Few studies [3-5] have been performed to see the overall effect of some operating parameters but some of the findings contradict with one another. Therefore, for better understanding of the liquefaction behavior, the effect of these parameters should be further examined.

- Study of the Model Compounds:

  Algae differ from one strain to another in their biochemical composition. This difference makes it tough to predict the yields and properties of algae bio-oil. Therefore, the study of the model biochemical compounds allows us to better understand the reaction mechanism and perform kinetic studies. Although few studies [6-9] have been performed on model compounds, more study on this topic can help to reduce the knowledge gap in the existing literature.

- Effect of other Catalysts on HTL Yield and Upgrading of Algal Bio-oil:

  This research studied the effect of only one alkaline catalysts (Na_2CO_3) but other homogenous (KOH, CH_3COOH, HCOOH) and heterogeneous catalyst (Pd/C, Pt/C, Ru/C, Ni/SiO_2-Al2O_3, CoMo/γ-Al_2O_3 and Zeolite) can also be taken under consideration for the study. Few studies [10-13] have been performed on the effect of heterogeneous catalysts

but further study on different catalysts could help reduce the knowledge gap. Upgrading of the bio-oil from algae can also be performed. Therefore, further studies can be done to explore various facets of catalytic HTL of algae and upgrading of algal bio-oil.

- Study the Effect of other Solvents as Reaction Media:

  This research was performed using water as a reacting medium. Water at high temperature and pressure behaves as a non-polar solvent and it extracts the oil and other compounds from the algae. Although other solvents such as ethanol, acetone etc. can extract some oil at normal temperature and pressure without performing HTL, it would be interesting to see the effect of using these solvent as a reacting medium instead of water. Moreover, both water and these solvents can also be used at variable ratios and a study of this effect on the bio-oil yield and quality may be performed.

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APPENDIX A: Data for Graphs

Table A.1 Data for Figure 3.5 (a). HTL liquefaction yields from three algae strains at different temperatures.

	Nannochloropsis		
	250 ºC	**300 ºC**	**350 ºC**
Biooil	28.49±3.32	44.31±2.54	48.67±1.85
Char	31.86±5.93	15.48±2.2	12.1±2.05
WSP	27.88±2.48	17.79±3.18	10.01±4.07
Gas	6±2.64	18.33±2.88	28.33±2.88
	Pavlova		
	250 ºC	**300 ºC**	**350 ºC**
Biooil	35.52±1.2	37.6±4.29	39.96±1.22
Char	24.64±2.82	16.38±2.84	15.29±3.52
WSP	28.81±4.97	15.94±1.71	5.58±3
Gas	8.33±2.89	21.67±2.89	31.67±2.89
	Isochrysis		
	250 ºC	**300 ºC**	**350 ºC**
Biooil	32.37±3.07	33.50±2.07	40.69±2.06
Char	23.65±1.56	21.66±0.69	20.44±4.37
WSP	27.69±2.87	19.77±3.01	9.39±2.04
Gas	6.67±2.89	20±0	23.33±2.88

Table A.2 Data for Figure 3.5 (b) HTL yields from three algae strains at different temperature using Na_2CO_3 as catalyst

Nannochloropsis			
	250ºC	**300 ºC**	**350 ºC**
Biooil	35.42±1.65	35.18±5.24	37.07±4.18
Char	26.47±5.99	19.37±4.12	15.95±4.89
WSP	22.80±4.09	16.18±2.65	15.22±2.78
Gas	4.67±0.57	18.33±4.16	22.33±5.86
Pavlova			
	250 ºC	**300 ºC**	**350 ºC**
Biooil	32.34±2.98	45.49±3.42	47.05±3.3
Char	11.96±2.09	8.25±1.76	9.53±3.57
WSP	21.77±2.56	15.69±2.47	8.06±2.59
Gas	18.33±2.89	20.33±0.57	21.33±2.52
Isochrysis			
	250 ºC	**300 ºC**	**350 ºC**
Biooil	36.15±1.65	42.16±1.12	41.21±4.76
Char	14.66±2.46	9.07±2.29	15.23±8.11
WSP	20.63±1.60	13.19±1.14	9.12±1.02
Gas	15±5	20±5	20.33±0.58

Table A.3 Data for Figure A.1 Distribution of major compounds in bio-oil produced from (a) non-catalytic and (b) catalytic HTL of different algae strains at different temperatures

a. Strains	Compounds (Area %)	Temperatures		
		250	300	350
Nannochloropsis	HC	29.95	32.06	25.9959
	NC	10.93	24.01	27.86
	OC	2.56	1.89	2.95
	OA	22.42	0.65	0.21
	Ph	0.66	2.65	10.3
Pavlova	HC	38.14	11.34	18.97
	NC	12.15	35.04	35.5
	OC	2.85	0.13	4.5
	OA	30.7	19.6	0
	Ph	0	1.79	9.81
Isochrysis	HC	14.02	27.56	27.01
	NC	13.51	28.51	18.64
	OC	3.55	4.98	0.61
	OA	44.75	11.85	4.04
	Ph	0	5.19	14.86

b. Strains	Compounds (Area %)	Temperatures		
		250 °C	300 °C	350 °C
Nanochloropsis	HC	24.50	39.43	33.70
	NC	20.75	7.61	12.76
	OC	3.39	3.58	5.18
	OA	11.72	3.82	4.80
	Ph	1.19	4.15	11.04
Pavlova	HC	23.51	27.83	27.36
	NC	17.08	33.14	14.624
	OC	7.30	4.38	5.33
	OA	15.34	0.23	0
	Ph	0.92	4.56	13.02
Isochrysis	HC	24.56	31.93	21.21
	NC	21.44	26.95	20.40
	OC	4.85	2.97	3.045
	OA	1.44	1.58	0
	Ph	0.41	3.28	12.43

Table A.4 Data for Figure 3.7. Carbon distribution in the HTL product streams (a) non-catalytic and (b) catalytic

a. Carbon Balance (wt %):

	Temp. (ºC)	Bio-oil	Char	Aqueous	Gas & UA/C[1]
Nannochloropsis	250	36.37±4.04	30.85±2.90	24.17±1.18	0.02
	300	60.30±1.27	15.15±1.04	10.92±0.84	13.62
	350	67.94±1.10	10.69±0.39	5.62±0.61	15.74
Pavlova	250	52.13±1.32	24.91±2.89	25.45±2.12	-
	300	55.37±0.29	12.98±2.30	8.82±1.77	22.84
	350	57.58±2.19	10.51±0.97	2.96*	28.94
Isochrysis	250	43.67±0.93	26.63±0.09	18.61*	11.07
	300	49.24±0.15	22.80±0.84	12.78±2.16	15.16
	350	56.45±1.95	15.21±2.73	5.73±0.99	22.59

b. Carbon Balance (wt %):

	Temp. (ºC)	Bio-oil	Char	Aqueous	Gas & UA/C[1]
Nannochloropsis	250	46.86±4.01	24.14±0.11	20.94±0.28	8.06
	300	49.87±2.21	12.23±0.13	12.27±1.61	25.62
	350	49.81±1.68	9.90±0.36	9.26±0.18	31.04
Pavlova	250	48.27±0.60	13.34±0.02	19.09±0.42	19.30
	300	63.14±5.33	8.08±0.10	12.75±0.59	16.02
	350	70.24±0.88	9.26±0.44	6.34±0.66	14.17
Isochrysis	250	51.66±1.64	9.98±0.03	16.44±1.18	21.91
	300	59.80±1.49	4.50±0.56	9.79±0.19	25.90
	350	58.47±0.14	5.27±0.19	6.58±1.12	29.68

1: By difference; * one datum

Table A.5 Data for Figure A.2 Nitrogen distribution in the HTL product streams (a) non-catalytic (b) catalytic

Nitrogen Balance (wt %):					
	Temp. (°C)	**Bio-oil**	**Char**	**Aqueous**	**Gas & UA/C[1]**
Nannochloropsis	250	14.43±1.38	15.44±1.42	28.58±5.39	41.54
	300	23.59±0.57	5.74±0.71	12.34±0.60	58.32
	350	25.27±0.74	2.65±0.16	6.90±1.69	65.18
Pavlova	250	16.43±0.16	13.73±1.77	37.17±1.11	32.67
	300	21.77±2.86	6.03±0.40	8.44±2.27	63.75
	350	21.66±1.03	4.55±0.25	3.06*	70.70
Isochrysis	250	15.94±0.40	9.78±0.21	29.83*	44.46
	300	18.60±0.35	7.47±0.13	18.40±6.33	55.52
	350	22.46±1.90	5.44±0.22	7.38±0.22	64.72

Nitrogen Balance (wt %):					
		Bio-oil	**Char**	**Aqueous**	**Gas & UA/C[1]**
Nannochloropsis	250	18.36±0.97	12.54±1.81	25.34±2.06	43.76
	300	19.17±1.62	4.79±0.58	9.78±2.72	66.26
	350	18.39±1.85	2.64±0.20	8.49±0.62	70.47
Pavlova	250	18.17±0.60	4.23±0.21	22.25±0.34	55.35
	300	24.35±2.05	2.37±0.16	11.59±0.00	61.69
	350	26.44±0.88	2.52±0.05	5.67±0.30	65.36
Isochrysis	250	19.84±1.06	8.60±0.38	20.53±1.61	51.02
	300	22.16±0.47	2.95±0.58	10.57±0.52	64.32
	350	21.20±1.67	2.52±0.11	7.73±5.05	68.54

1: By difference; * one datum