Biofuel Production from Hydrothermal Liquefaction of Algae and Its Subsequent Upgrading

by

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Abstract

Algae are considered as a promising feedstock for biofuel production. The conversion of algae to biofuel was investigated in this study. The study was concentrated in two main areas: hydrothermal liquefaction of algae into bio-oil, and catalytic upgrading of the bio-oil produced from hydrothermal liquefaction of algae to improve the chemical properties of the bio-oil.

First, hydrothermal liquefaction (HTL) of nine different algae species was performed to understand the influence of their biochemical composition on product yields and properties at two reaction temperature (280 and 320^{o}C). The biochemical composition of the selected algae species showed a broad range of lipids (13 to 55 wt.%), carbohydrates (9 to 54 wt.%) and proteins (7 to 63 wt.%). The bio-oil yields obtained at 320^{o}C were higher than that obtained at 280^{o}C. The maximum bio-oil yield (66 wt.%) was obtained from the HTL of high lipid containing algae *Nannochloropsis* sp. at 320^{o}C. A predictive relationship between bio-oil yields and biochemical composition was developed and showed a broad agreement between predictive and experimental yields. The HTL bio-oils obtained from nine algae species were characterized for higher heating value (HHV), total acid number (TAN), ash content, moisture content, boiling point distribution, and elemental composition. The heating values of the bio-oils ranged from 31-36 MJ/kg. The maximum percentage of the bio-oils was in the vacuum gas oil range while high lipid containing algae *Nannochloropsis* sp. contained a significant portion (33-42%) in the diesel

range. The aqueous phase from HTL had a high amount of TOC (12-43 g/L) and COD (35-160 g/L) and showed the potential for carbon recovery. On the other hand, the high amount of ammonium (0.34-12 g/L), and phosphate (0.7-12 g/L) showed the possibility of nitrogen, phosphate and magnesium recovery via struvite production.

The bio-oil produced from HTL of algae cannot be used as "drop-in" fuel or blended with petroleum crude because it has high nitrogen content, high oxygen content and it is highly viscous. Thus, upgrading of the bio-oil is necessary to make it applicable as fuel. Second, upgrading of bio-oil produced from HTL of *Nannochloropsis* sp. was performed. Upgrading was performed with five different catalysts (Ni/C, ZSM-5, Ni/ZSM-5, Ru/C and Pt/C) at two reaction temperatures of 300 and 350^{o}C at a weight hourly space velocity (WHSV) of 0.51 g/g_{cat}.h. The upgraded bio-oil yields obtained were higher at 300^{o}C when compared to 350^{o}C. However, better quality fuel was obtained at 350^{o}C. The maximum upgraded bio-oil yield (61.5 wt.%) at 350^{o}C was obtained using Ni/C, and the lowest yield (47.19 wt.%) was obtained using ZSM5. Among the different catalysts used, Ru/C and Pt/C gave a better-quality fuel. Around 35-40% of the upgraded bio-oils were in the diesel range, and no vacuum residue fraction was found. Hydrogen consumption was the highest for noble metal catalysts. Overall, the catalytic upgrading of algae bio-oil was effective in improving the quality of the bio-oil.

Comparing the upgraded bio-oil obtained at 350^{o}C (our study) with the other published literature, which used higher temperatures (>400^{o}C), the properties of the upgraded bio-oil were observed to be similar. However, the drawback of our study was the use of longer residence time (10 h). Residence time is one of the process parameters that is directly related to the energy input and affects the overall process economics. Thus, in our

third study, the effect of residence time (2, 4, 6 and 10 h) on the upgrading product yields and its properties were investigated. Upgrading was performed at 350^{o}C using 5% Ru/C catalyst at a catalyst loading of 16.67 wt.%. The maximum upgraded bio-oil yield (60.20 wt.%) was observed at 4 h residence time. The properties of the upgraded bio-oils improved with the increase in residence time. The maximum higher heating value (44.32 MJ/kg), the lowest TAN, viscosity, and nitrogen content was observed at 10 h. However, the maximum energy recovery (44.37%) was obtained at 4 h residence time. The maximum hydrogen consumption (39.87 mg/g of bio-oil) was observed at 10 h residence time.

Also, the effect of a binary mixture of CO_2 and H_2 cold pressure on the upgrading of the bio-oil was investigated. Upgrading was performed at 350^{o}C for 4 h residence time using 5% Ru/C as a catalyst at a catalyst loading of 16.67 wt.%. The cold pressures of the binary mixture were 100 psi CO_2 + 900 psi H_2, 200 psi CO_2 + 800 psi H_2, and 300 psi CO_2 + 700 psi H_2. The upgraded bio-oil yield and properties obtained at 300 psi of CO_2+700 psi of H_2 were similar to that obtained using 1000 psi of H_2 (without CO_2), except for the TAN and HHV. The higher heating value decreased with the introduction of CO_2 cold pressure and was lowest at 300 psi of CO_2+700 psi of H_2. Similarly, TAN increased with the introduction of CO_2. The concentration of H_2S in the gaseous fraction increased with the increase in CO_2 pressure. The use of CO_2 in the upgrading reaction did not significantly change the upgraded bio-oil quality, but its incorporation in the upgrading system added benefit in terms of process safety as it expands the non-explosive regime.

Keywords: *Algae, Hydrothermal liquefaction, Upgrading, Catalysis, Supercritical CO_2.*

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List of Abbreviations

HTL	Hydrothermal Liquefaction
HHV	Higher Heating Value
TAN	Total Acid Number
TOC	Total Organic Carbon
TN	Total Nitrogen
COD	Chemical Oxygen Demand
WSP	Water Soluble Product
WHSV	Weight Hourly Space Velocity
LHSV	Liquid Hourly Space Velocity
UBO	Upgraded Bio-oil
VGO	Vacuum Gas Oil
ANOVA	Analysis of Variance
HDS	Hydrodesulfurization
HDN	Hydrodenitrogenation
HDO	Hydrodeoxygenation
FTIR	Fourier-Transform Infrared
GCMS	Gas Chromatography Mass Spectrometry
BET	Brunauer-Emmett-Teller
SEM	Scanning Electron Microscopy
EDS	Energy Dispersive X-Ray Spectroscopy

Chapter 1

Introduction

1.1. Background

According to International Energy Outlook 2016, the world liquid fuel consumption increased from 90 million barrels per day in 2012 to 95 million barrels per day in 2015 and is estimated to increase to 121 million barrels per day in 2040 [1]. This increase in consumption of fossil fuel has a detrimental effect on the environment such as an increase in greenhouse gases (GHGs). In 2016, the carbon dioxide (CO_2) level in the atmosphere stood at 403 ppm, which was significantly higher than the pre-industrial level of 280 ppm [2]. This increase in CO_2 level in the atmosphere is mostly related to anthropogenic activities. Over the last decade, the amount of CO_2 in the atmosphere has increased at an unprecedented rate (2 ppm per annum) [2]. At this rate, it will hit 500 ppm within 50 years putting us on route to increase the global temperature more than 3 ^oC above pre-industrial levels, which is higher than that aimed (hopefully 1.5^oC) at the 2015 Paris Agreement [2–4]. The increase in the CO_2 level caused by anthropogenic activities can be addressed by substituting the use of fossil fuels with the renewable, sustainable and environment-friendly forms of energy. According to Annual Energy Outlook 2017, the U.S. renewable energy production increased from 8.80 quadrillion Btu in 2014 to 10.22 quadrillion Btu in 2016 [5] and is expected to increase as the U.S. attempts to meet emission reduction targets and transition toward a diverse energy portfolio.

Among the different forms of renewable energy, biofuel is one of the viable options for reducing GHG emissions. Also, biomass is the only source which can be converted to liquid hydrocarbons and is abundant worldwide. Biomass as a source of energy has gained significant attention in the last decade due to the desire to develop an alternative source of energy, which can provide environmental and national energy security benefits. According to Renewable Fuel Standard (RFS) mandate within Energy Independence and Security Act of 2007 (EISA), the U.S. must produce at least 36 billion gallons of renewable transportation fuels by 2022 of which 15 billion gallons can be produced by corn-based ethanol and the rest from advanced biofuels [6]. Advanced biofuels can be produced from different types of biomass sources such as agricultural residue, herbaceous and woody energy crops, municipal organic wastes and manure, and algae. In the last decade, the interest in sustainable use of aquatic biomass such as algae has gained significant attention for the production of renewable biofuels and valuable chemicals.

1.2.Algal Feedstock

Algae are diverse organisms ranging from unicellular microalgae to macroalgae such as seaweeds. The main merit of using algae as a biofuel feedstock is that it provides much higher yields of biomass (40-60 dry ton/ha-yr) [7] when compared to terrestrial feedstock, and has cultivation flexibility, i.e., can be grown on non-arable land and can use wastewater, thus relieving food-versus-fuel pressure. Also, algae are capable of fixing CO_2 in the atmosphere (1.8 kg of CO_2/kg of dry algae) [8] thus, resulting in the reduction of atmospheric CO_2 levels, and their biochemical compositions can be tuned according to the need. The primary biochemical composition of algae is lipids, carbohydrates, and proteins. The biochemical composition of algae varies not only according to the species but also

according to the different growth parameters [9]. The variation in the composition makes them suitable for multiple applications. For example, *Dunaliella tertiolecta* is produced commercially for β-carotene [10], *Haematococcus pluvialis* is used for astaxanthin production [11], *Botryococcus braunii* and *Nannochloropsi*s are rich in lipids and can be used as biomass for biodiesel production [7, 12]. In an effort to commercialize biofuel production, a variety of algae to biofuel conversion technologies have been explored. In the conventional method, algae are converted into biodiesel via lipid extraction followed by transesterification. This approach suffers from several disadvantages such as it requires high lipid accumulating strains to improve economic viability [13]; however, high-lipid algae have low productivity [8]; requires drying of the wet algae feedstock which is an energy intensive process and requires a solvent for extraction of triglycerides [14, 15]. Likewise, the remnants or lipid extracted part (such as proteins and carbohydrates) are not recovered as liquid fuels. The lipid extracted parts are converted into biogas via anaerobic digestion [13, 16], chemicals via fermentation [17] and animal feeding supplements [18]. Various thermochemical conversion technologies which utilize whole algae biomass such as pyrolysis and gasification have come to the fore, but a significant amount of energy associated with drying of the feedstock have negative effect on these processes. However, these wet feedstocks can be converted to biofuels using a process called hydrothermal liquefaction.

1.3. Hydrothermal Liquefaction

Hydrothermal liquefaction (HTL) is a promising route for producing renewable fuels and chemicals from wet biomass such as algae. It is a thermochemical process and utilizes water at sub- or super-critical temperatures and pressures as a reactant and reaction

medium, thus eliminating energy intensive drying process. In addition, HTL not only converts lipid portion but utilizes whole algae for bio-oil production. HTL of algae produces a black viscous liquid fraction also known as bio-oil or bio-crude, a solid residue fraction, a gaseous fraction and a water fraction containing some polar organic compounds also known as an aqueous phase.

To date, substantial research on HTL of algae has been conducted due to the synergistic relationship between algae and hydrothermal liquefaction process. Various process variables such as temperature [15, 19-21], residence time [19, 20, 22], solid loading [19, 22, 23] and reaction rate [24] have been studied using different types of algae strains as feedstock. The bio-oil obtained from algae is a complex mixture of compounds with diverse chemical and molecular structures. Thus, it not only serves as fuels but also serves as a precursor for other products such as polymers, lubricants, and asphalts. As a fuel, the bio-oil has positive attributes such as high heating value (32-36 MJ/kg) and comparatively lower oxygen content (7-10 wt.%) [21]. However, on the downside, it is highly viscous (40-68 cP at 60^{o}C) [23], acidic (29-118 mg of KOH/g) [21] and has high nitrogen (2-9 wt.%) [21, 23], all of which are undesirable as fuel. Although the quality of algae bio-oil is similar to that of petroleum crude, the presence of high nitrogen content makes it unsuitable for blending. Therefore, to make it more desirable as fuel, upgrading of the bio-oil is required.

1.4. Upgrading/Hydroprocessing

As discussed above, the bio-oil obtained from HTL of algae needs to be upgraded for it to be used as “drop-in” fuel or blended with petroleum crude. The upgrading process improves the physical and chemical properties of the bio-oil. The upgrading process

discussed here is catalytic hydroprocessing. In the catalytic hydroprocessing, hydrogen is added to the bio-oil in the presence of a catalyst at a high temperature (300-500 ^{o}C) [25] and a high pressure (up to 30 MPa) [26]. Catalytic hydroprocessing has been used in the petroleum refinery for many years, and the process is well established. During catalytic hydroprocessing, several reactions such as hydrodesulfurization (HDS), hydrodenitrogenation (HDN), hydrodeoxygenation (HDO), hydrocracking, and hydrogenation occur simultaneously [27]. The oxygen content in the bio-oil is removed in the form of CO_2, CO or water. Nitrogen and sulfur are removed as NH_3 and H_2S, respectively [28, 29]. The most common industrially used catalysts for the heteroatom removal are CoMo-based and NiMo-based catalysts [30].

Different types of catalysts such as Ru/C [31, 32], Pt/C [31–34], Pd/C [31, 32, 35], HZSM-5 [31], Ni/C [31], NiMo/Al_2O_3[32], CoMo/Al_2O_3 [36], Ru/Al_2O_3 [32] have been tested for upgrading of algae bio-oil. The upgrading process significantly improved the quality of the bio-oil. Apart from the upgraded bio-oil, this process also produces coke and gases. The formation of coke results in catalyst deactivation and poisoning leading to a short catalyst life, which is a major problem associated with upgrading. The other disadvantages of catalytic hydroprocessing are the need of a high-pressure reactor system and a large amount of hydrogen.

1.5. Problem Statement

As discussed in section 1.3, the HTL product yields and its properties depend on different variables such as reaction temperature, residence time, solid loading and heating rate. Apart from these process variables, the biochemical composition of algae also influences the product yields and properties. Algae constitute of different biochemical

compositions like lipids, proteins, and carbohydrates. During HTL, these chemical compositions undergo complex reaction to form bio-oil, char, water-soluble product, and gas. Therefore, understanding the behavior of the biochemical composition during HTL and its effect on product distribution is very important for optimization of the whole process. Despite extensive studies on HTL of algae, only a handful of studies [37–39] have discussed the effects of its biochemical compositions on the bio-oil yields and composition. Those studies have focused more on the bio-oil and have not thoroughly investigated its effect on other byproducts. Thus, a detailed study on the effect of these biochemical compositions on the overall yields and product compositions are necessary for commercializing algae based biorefinery.

In the past, many bio-oil upgrading studies have been performed to improve its properties and make it applicable as fuel. Most of the upgrading studies [31, 33–35] have been performed in a supercritical water environment. Although the use of water during upgrading gives higher carbon and hydrogen, the oxygen removal is less effective. Also, the use of water possesses process challenges as it may contain organics, and requires wastewater treatment before its disposal into waste streams. Only a handful of studies [26, 36, 40] on dry bio-oil upgrading have been performed. Those studies are performed at high temperatures $\geq 400^{o}C$ and have studied only a few catalyst types. Temperature and residence time are the operating parameters that are related to the energy input and affects the overall energy efficiency. Thus, a parameter optimization study for the upgrading of dry bio-oil and search for a suitable catalyst is necessary for overall process economics.

Apart from temperature, residence time and catalyst loading, the parameter that affects the overall economics of the upgrading is the amount of hydrogen input. During

upgrading, mass transfer limitation occurs due to the low solubility of hydrogen in bio-oil, which decreases the efficacy of the process. Thus, a high hydrogen pressure is used during upgrading to enhance the solubility of hydrogen in the bio-oil and catalysts. The use of high hydrogen pressure can be overcome with the use of supercritical fluids. Although the use of supercritical water in the upgrading of algae bio-oil has been studied, the use of supercritical water has its disadvantages as discussed above. The use of other supercritical fluids such as CO_2 and propane on the upgrading of algae bio-oil can be explored as they have not been employed in bio-oil upgrading studies yet.

1.6. Research Objectives

Biofuels from algae are the product of the series of processes involving both upstream processes such as strain selection, algae growth, harvesting, and downstream processes such as hydrothermal liquefaction, and upgrading of fuels. This research addresses some of the fundamental process engineering issues surrounding the downstream process of hydrothermal liquefaction of algae and upgrading of the algae bio-oil to produce biofuels. The overall goal of this study was to develop biofuels from algal biomass. The specific objectives of the study were as follows:

Objective 1: To examine the influence of biochemical composition during hydrothermal liquefaction of algae on product yields and fuel properties

In this study, HTL of nine different algae species having varied biochemical compositions were performed at two temperatures (280 and 320^{o}C) and reaction time of 30 min to compare the effect of their biomass composition on product yields and properties. Details of this study are given in Chapter 2.

Objective 2: To evaluate the influence of different heterogeneous catalysts on the upgrading of algal bio-oil

In this study, the influence of different heterogeneous catalysts on the upgrading of algae bio-oil was studied. The bio-oil obtained from HTL of *Nannochloropsis* sp. was upgraded by catalytic hydrotreatment. Upgrading was performed with five different catalysts (ZSM-5, Ni/ZSM-5, Ni/C, Ru/C and Pt/C) in the presence of hydrogen at 300^{o}C and 350^{o}C, and all the experiments were conducted at a weight hourly space velocity (WHSV) of 0.51 g/g_{cat}.h. Chapter 3 summarizes the experiments, results, and discussion of this study.

Objective 3: To investigate the effect of residence time on the upgrading product yields and its properties

In this study, upgrading of the bio-oil obtained from hydrothermal liquefaction of *Scenedesmus* sp. was performed at different residence time of 2, 4, 6 and 10 hours using 5% Ru/C catalyst at a catalyst loading of 15 wt.% and at a reaction temperature of 350^{o}C to investigate the effect of residence time. The details of this study are summarized in Chapter 4.

Objective 4: To analyze the influence of binary mixture of CO_2 and H_2 on catalytic upgrading of bio-oil produced from HTL of algae

In this study, the influence of the binary mixture of CO_2 and hydrogen on the catalytic upgrading of algae bio-oil was investigated. Upgrading was performed at 350^{o}C using 5% Ru/C catalysts, at a residence time of 4 h and the cold pressures of the binary mixtures were 100 psi CO_2 + 900 psi H_2, 200 psi CO_2 + 800 psi H_2, and 300 psi CO_2 + 700 psi H_2. The details of this study are summarized in Chapter 5.

The overall conclusions of the study and recommendation for future work are summarized in Chapter 6.

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Chapter 2

Influence of Biochemical Composition during Hydrothermal Liquefaction of Algae on Product Yields and Fuel Properties*

Abstract

Hydrothermal liquefaction (HTL) of nine algae species were performed at two reaction temperatures (280 and 320^{o}C) to compare the effect of their biomass composition on product yields and properties. Results obtained after HTL indicate large variations in terms of bio-oil yields and its properties. The maximum bio-oil yield (66 wt.%) was obtained at 320^{o}C with a high lipid containing algae *Nannochloropsis*. The higher heating value of bio-oils ranged from 31 to 36 MJ/kg and around 50 % of the bio-oils was in the vacuum gas oil range while high lipid containing algae *Nannochloropsis* contained a significant portion (33-42%) in the diesel range. A predictive relationship between bio-oil yields and biochemical compositions was developed and showed a broad agreement between predictive and experimental yields. The aqueous phases obtained had high amount of TOC (12-43 g/L), COD (35-160 g/L), TN (1-18 g/L), ammonium (0.34-12 g/L) and phosphate (0.7-12 g/L).

Keywords: *Algae; Hydrothermal liquefaction; Biochemical composition; Bio-oil; Aqueous phase.*

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2.1. Introduction

Biomass is considered to be the only renewable source of energy that can be converted into liquid hydrocarbons and is abundant worldwide [1]. Among many sources of biomass, algae is a promising biomass feedstock because of its higher yield and faster growth rate [2, 3]. Algae can be converted into biofuels by various processes such as pyrolysis [4, 5], gasification [6, 7] and conventional extraction process [3]. However, the major complication of those processes is that they require dried biomass. Algae, a wet feedstock, needs a huge amount of energy for drying, which makes the process uneconomical. An alternative process that is best suited for producing biofuels from wet biomass or algae is hydrothermal liquefaction (HTL). HTL uses water at a sub- and super-critical temperature (250-380^{o}C) and pressure (7-30 MPa) as reactant and reaction medium. This process not only eliminates the energy-intensive drying process but also allows the utilization of whole algae for bio-oil production [8].

To date, numerous studies on HTL of algae have been conducted due to the synergistic relationship between algae and liquefaction process. Extensive studies were conducted to investigate the effect of different process variables such as temperature [9–14], residence time [9, 10, 13] and heating rate [15] on the bio-oil yield and its quality. Apart from these process variables, the biochemical composition of algae (which constitutes lipids, proteins, and carbohydrates) is an important factor found to influence the product yield and quality. Despite extensive studies on HTL of algae, there has been only a handful of studies [15–17] on the effects of biochemical compositions of algae on the overall yields and composition. Biller and Ross [18] studied the effect of the biochemical composition in HTL using different model chemical compounds and microalgae species

and reported that the conversion of lipids and proteins to bio-oil was more efficient compared to the conversion of carbohydrates. This finding was further confirmed by the work of Teri et al. [17], who studied HTL of model chemical compounds of protein, carbohydrate, and lipid alone and in mixture. The authors reported that the conversion of model lipid showed the highest yield (>90 wt.%) followed by protein and carbohydrate. They also performed binary and ternary mixtures of model compounds, and found that the bio-oil produced from the mixtures of polysaccharides and proteins exceeded the averaged bio-oil yield with an individual component. Barreiro et al. [16] performed HTL of different microalgae species to examine the influence of strain-specific parameters like cell structure, biochemical composition and growth environment on product yields and properties. Less variation in the bio-crude yields (45.6–58.1 wt.%) within species was obtained at a higher temperature of 375^{o}C, but significant differences in yields were observed at a lower temperature of 250^{o}C (17.6–44.8 wt.%). The difference in yields was due to the cell wall structure of algae and severity of the reaction conditions. In an alternative approach, Sheehan and Savage [19] incorporated algae HTL studies in their kinetics-based reaction model to study the effect of biochemical composition on product yields and found the activation energy required for biocrude formation was the lowest for proteins. To the best of our knowledge, only a limited number of studies were performed to evaluate the effect of algal biochemical compositions on bio-oil yields and its characteristics during HTL process. However, those studies were either focused on model compounds or a limited number of algae species with narrow variation of biochemical composition. Hence, additional data on HTL using different type of algal species with varying biochemical composition will provide how the biochemical composition will affect

HTL products yield and properties, which is very important in commericializing the algae based biorefinery.

Hence, the main objective of this study was to perform HTL of nine types of algae species to investigate the influence of biochemical compositions on bio-oil yields and fuel characteristics. In addition, models for predicting the bio-oil yields from HTL of algae at two temperatures of 280 and 320^{o}C were also developed Last, a comprehensive investigation of the aqueous phase obtained from HTL of different algae species was conducted for further aqueous phase utilization studies.

2.2. Materials and Methodology

2.2.1. Hydrothermal Liquefaction of algae

Nine algae species were used for HTL experiments. Algae species were obtained from Arizona Center for Algae Technology and Innovation (AzCATI), Mesa, Arizona and Reed Mariculture, Inc. (California). HTL of algae species were performed in a high-pressure experimental unit consisting of a batch reactor having 1 in. internal diameter and 100 ml internal volume, and equipped with electrical heating unit as shown in Appendix Fig. A.7 [12]. The reactor was loaded with approximately 10 g of algae (dry weight basis) at a solid loading of 15 wt.%, and purged with helium gas (> 99% purity, Airgas Inc., Charlotte, NC) to remove residual air and create an inert headspace. After purging, the reactor was pressurized at 100 psig with helium gas at room temperature. The reactor was then heated to a desired temperature (280^{o}C and 320^{o}C) at heating rate of 30^{o}C min^{-1}. After holding the reactor at the reaction temperatures for 30 minutes, it was cooled down to room temperature using cold water. Then the residual pressure created by gas formation was vented off and was not analyzed in this study. Product separation procedure and yield

equations were adopted from the previous paper [12]. In this study, bio-oil, solid residue and gaseous yields were gravimetrically measured using Eq. (1), and the remaining balance was considered as WSP (water soluble phase).

$$\text{Yields}_{\text{ oil/solid residue}} = \frac{Weight\ of\ the\ product\ (dry\ basis)}{Weight\ of\ the\ biomass\ (dry\ basis)} \times 100 \quad [1]$$

2.2.2. Biomass and Product analysis

Algae biomass were analyzed for their moisture content, ash and elemental composition using the methods described in the previous paper [12] whereas biochemical composition was provided by the suppliers. Fatty Acid Methyl Esters [FAME] analysis was performed according to the National Renewable Energy Laboratory's laboratory analytical procedure [20].

Bio-oils were characaterized for water content, ash content, higher heating value (HHV), total acid number (TAN), elemental analysis and chemical composition according to the method described by Shakya et al. (2015). Fourier Transform Infrared (FTIR) spectroscopic analysis of bio-oils was performed by using Thermo Nicolet iS10 (Thermo Scientific, Waltham, MA). The samples were analyzed for 34 scans over a range of 400-4000 cm^{-1} wavenumbers. Simulated distillation was performed as according to the method described by [21]. Energy recovery for the bio-oil obtained from different algae species was calculated using Eq. (2).

$$\boldsymbol{Energy\ recovery} = \frac{\boldsymbol{HHV_{BIO-OIL} \times Mass_{BIO-OIL}}}{\boldsymbol{[HHV_{ALGAE\ BIOMASS} \times Mass_{ALGAE\ BIOMASS}]}} \times \mathbf{100}\% \quad [2]$$

The aqueous phase produced from HTL of different algae species was analyzed for its pH, total organic carbon (TOC), chemical oxygen demand (COD), total nitrogen (TN), phosphate (PO_4^{3-}), ammonium (NH_4^+), chemical composition and metal content. TOC and TN were determined using a TOC-L analyzer attached with TNM-L unit (Shimadzu Corp.,

Japan). For the measurement, aqueous phase samples were filtered using 0.2 µm filter paper to remove any suspended particles. The filtrate samples of 50 dilution factor were prepared using ultra high purity water and kept in auto-sampler for measurement. The COD of the aqueous phase was measured according to the standard method [22]. Ammonium, phosphate, and magnesium were determined using YSI reagent kits according to the supplier's protocol. The aqueous phase was also characterized for its chemical composition using GC-MS. 10 ml of dichloromethane was mixed with 5 ml of the aqueous phase and the dichloromethane extracted/soluble part was used for GC-MS analysis using the same DB 17901 capillary column and heating program as described by Shakya et al. [12]. The trace metal concentration in the aqueous phase was measured using an Optima 5300 DV inductively coupled plasma spectrometer (ICP) with optical emission spectrometry (Perkin Elmer, Cambridge, UK). Approximately 0.05 g of the aqueous phase was digested by 25 ml of nitric acid overnight and then adjusted to 100 ml with ultrahigh purity (Type 1) water (Synergy Ultrapure Water Systems, EMD Millipore) and then filtered using 0.45 µm filter before the analysis.

The char from the HTL of algae was measured for its HHV, ash content and elemental composition by using the methods as described by Shakya et al. (2015). The char was also measured for BET (Brunauer-Emmett-Teller) surface area using a Quantachrome Autosorb- iQ with nitrogen absorption. Before measurement, all the samples were outgassed to 10^{-3} Torr at 300^{o}C for 10 h.

2.2.3. Predictive Modeling

Multiple linear regression of biomass composition parameters (lipids, carbohydrates, and proteins) against the corresponding bio-oil was performed using SAS to obtain a linear model for predicting bio-oil yield from HTL of algae. The confidence level of the regression was 95%, and the intercept was set at zero. Model validation was also performed comparing prediction with experimental results reported in other microalgae HTL literature.

2.3. Results and Discussion

2.3.1. Algal Characterization

Algae selected for this study had diverse biochemical composition; lipids ranging from 13–55 wt.%, carbohydrates ranging from 9-54 wt.% and proteins ranging from 7-63 wt.% as shown in **Table 2.1.** In the case of lipids, FAMEs analysis of the algae showed fatty acids (FAs) to range from C6 to C20. Based on the area percentage, palmitic acid (C16:0), palmitoleic acid (C16:1), linoleic acid (C18:2) and linolenic acid (C18:3) were the predominant FAs in all algae strains as shown in the appendix (Table A.8).

Table 2.1. Biochemical composition of different algae strains

Sample[¥] (Nomenclature)	Biochemical composition (wt.%)[a]		
	Lipids	Carbohydrates	Proteins
Chlorella (C-1)	15.78	16.10	46.80
Chlorella (C-2)	30.28	49.70	14.63
Nannochloropsis (N-1)	55.36	12.39	12.92
Nannochloropsis (N-2)	49.26	15.94	18.15
Nannochloropsis (N-3)	20.09	9.21	46.62
Nannochloropsis (N-4)	18.12	8.92	62.78
Pavlova (P-1)	13.88	28.00	46.94
Scenedesmus (S-1)	35.66	50.40	7.15
Scenedesmus (S-2)	17.83	54.17	30.06

Notes: [¥]All the algae, except for N-4 and P-1 were provided by AZCATI whereas; N-4 and P-1 were purchased from Reed Mariculture Inc. [a] values reported by AzCATI and Reed Mariculture are on a dry basis. AzCATI used ATP3 protocol for determination of the biochemical composition, and experiments were perfomed in duplicates (n=2) whereas Reed Mariculture obtained their values from Medallion laboratory (MN, USA).

Table 2.2. Proximate and ultimate analyses of algae strains. [¥]

Sample (Nomenclature)	Water Content (wt.%)	HHV (MJ/kg)[†]	Ash content (wt.%)	Elemental Analysis (wt.% on dry basis)[γ]			
				C	H	N	O*
Chlorella (C-1)	78.97	22.18	7.42	51.41	7.59	9.10	24.47
Chlorella (C-2)	72.36	23.82	2.99	54.34	8.04	2.50	32.12
Nannochloropsis (N-1)	64.05	29.44	6.65	62.51	9.24	1.76	19.85
Nannochloropsis (N-2)	76.50	28.92	7.37	62.12	9.36	2.64	18.52
Nannochloropsis (N-3)	74.14	22.72	8.28	50.76	7.35	7.37	26.24
Nannochloropsis (N-4)	68.88	24.02	3.42	56.83	9.32	10.13	20.30
Pavlova (P-1)	75.8	22.69	3.47	54.34	8.69	8.67	24.83
Scenedesmus (S-1)	75.11	25.45	2.02	56.31	8.32	2.01	31.35
Scenedesmus (S-2)	70.30	22.34	2.10	50.82	7.46	6.87	32.74

Notes: [¥]The reported values for water content, ash and HHV are average of two (n=2). [†] as received. [γ] The reported values are single data point (n=1). * by balance.

Table 2.2 illustrates proximate and elemental analyses of different algae strains. Ultimate analysis showed that the algae having higher lipids (N-1 and N-2) had higher C and H% resulting in higher heating values. Algae having higher protein (N-4) had the highest N% as expected. The sulfur content was not measured in this study since previous studies [12, 14] have reported their values to be less than 1%. Relatively low oxygen contents were observed with *Nannochloropsis* species (except N-3) compared to others.

2.3.2. Liquefaction Yields

The product yields (on dry weight basis) from HTL of nine algae strains at two reaction temperatures (280^{o}C and 320^{o}C) are presented in **Figure 2.1**. Duplicate experiments were conducted with selected species (S-1, C-1, N-3, P-1, and N-4) to demonstrate reproducibility of data. The variation in bio-oil yields was 1-5 wt.%, which was consistent with our previous work [12].

During HTL, different concentrations of lipids, proteins, and carbohydrates in the biomass undergo complex reactions processes of hydrolysis, depolymerization, condensation, re-polymerization and thermal cracking, etc. to form bio-oil, char, water

soluble product (WSP) and gaseous product. In this study, a higher bio-oil and gas yields and lower WSP yield were observed at 320^{o}C compared to that obtained at 280^{o}C. Relatively, less difference in char yields were observed at two temperatures when compared. An increase in bio-oil yields at 320^{o}C can be due to the attainment of activation energy required for bond cessation, thus resulting in extensive biomass decomposition and depolymerization to bio-oil [14]. *Nannochloropsis* species (N-1 and N-2) having high lipid contents yielded relatively large amount of bio-oil at 320^{o}C (56.47% for N-1 and 65.96% for N-2), which supports the hypothesis that more than 90% of lipids in the biomass is converted into products during HTL process [23]. In the biological system, triacylglycerides (TAGs) are the most common form of lipids [8], which can be hydrolyzed to fatty acids and glycerols in hydrothermal media. Generally, fatty acids contribute to bio-oil formation whereas water soluble glycerols contribute to the aqueous phase. According to [24], maximum glycerol concentration (4 – 6 wt.%) was observed in the aqueous phase which was produced at 260^{o}C HTL temperature. The amount of glycerol decreased with an increase in operating temperature which may support the reduced WSP of high lipid containing algae at a higher temperature. In the case of high protein containing algae (N-4), bio-oil yields increased from 32.92 wt.% at 280^{o}C to 46.41 wt.% at 320^{o}C. Proteins in hydrothermal media undergo hydrolysis and form amino acids and peptides [25]. An increase in WSP and a decrease in bio-oil at a lower temperature (280^{o}C) may be due to the presence of maximum amounts of hydrolyzed proteins and amino acids in the aqueous phase [8]. However, as the temperature and residence time increases, proteins in aqueous phase decomposes into bio-oil phase which results in an increase in the bio-oil and a decrease in WSP at higher temperatures [26]. Carbohydrates also get hydrolyzed during

HTL to water soluble products. The maximum glucose in the aqueous phase was obtained at 260^{o}C and reduced drastically over 300^{o}C [27]. Thus, this explains the high amount of WSP at a lower temperature for high carbohydrate containing algae. The glucose in the aqueous phase can convert into bio-oil under the harsher environment, and it can go further through secondary decomposition to form char and gaseous product, resulting in an increase in their yields.

Algae strains investigated in this study constitute all the biochemical components (lipid, protein, and carbohydrate) in varying proportions. Therefore, the overall bio-oil yields observed may not be only due to the sole effect of individual biochemical components, but also due to interaction effect between these components [17]. This cross-interaction between biochemical compounds may be the reason for high amounts of bio-oil yields obtained for algae S-1, which had both high carbohydrates and ample amounts of lipids. Although a detailed study on the interaction effects were not performed in this study, the formation of melanoidin compounds (formed by the interactions between proteins and carbohydrates) [28], and fatty acid amides (formed by the interaction between lipids and proteins) [29], in the bio-oil indicates the presence of interactions between different biochemical components.

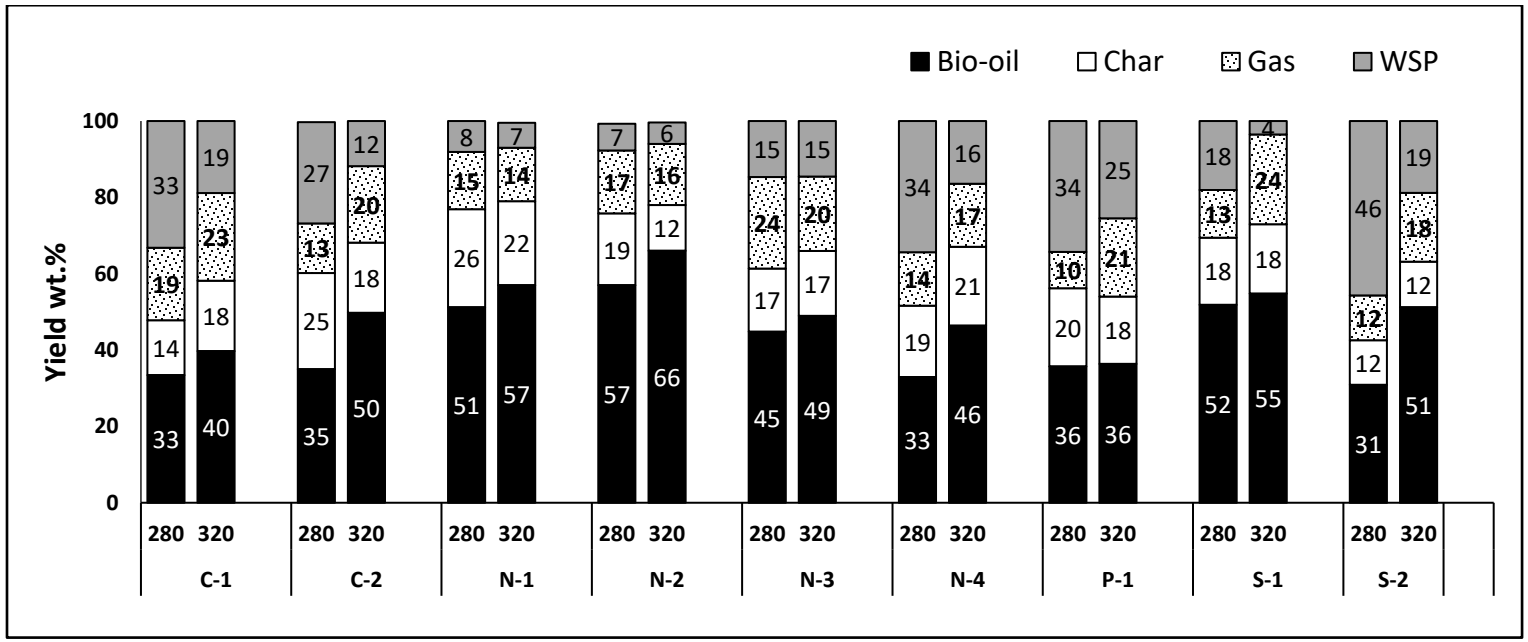

Figure 2.1. Product distribution from hydrothermal liquefaction of nine algae strains.
Note: HTL of all the algae species were performed in duplicates (n=2), except for C-2, S-2, N-1 and N-2.

2.3.3. Prediction of Bio-oil Yield

Equations (3) and (4) show the predictive models of the bio-oil yields obtained from the linear regression of the data obtained from fourteen HTL experiments performed at each temperature of 280 and 320°C, respectively.

$$Bio-oil\ Yield\ (wt.\%)_{280} = 0.90 \times L + 0.22 \times C + 0.32 \times P \quad (3)$$

$$Bio-oil\ Yield\ (wt.\%)_{320} = 0.96 \times L + 0.30 \times C + 0.43 \times P \quad (4)$$

where L, C and P are fractions of lipids, carbohydrates and proteins in the algae biomass. The predicted models explained about 97- 98% variation in the observed data as suggested by the adjusted R-square values. The coefficient of the respective component showed that the yield followed the trend of lipids > proteins > carbohydrates. The overall coefficients of lipids and proteins in this study were similar to other studies obtained by using model compounds [17, 18]. In contrast, the coefficient of carbohydrates were 5-7 times higher than the value reported. Higher conversion of carbohydrates may be due to its cross-interactions with other biochemical compounds, such as proteins, in the algae [28] or due to the effect of inorganics present in the algae, which might have catalyzed the carbohydrates to give higher yields [18, 30]. Also, algae have cell walls that are made up of bio-polymers like algenans, which can be converted into bio-oil. Apart from the model chemical compound studies, the carbohydrate conversion in this study was 2-2.5 times greater than that obtained by [31], who performed HTL of 10 batches of *Nannochloropsis*, having different biochemical compositions. This increase in carbohydrate conversion may be due to the use of different algae species in this study as compared to *Nannochloropsis* only in the previous work [31]. Different algae species have different cell wall thickness and have different proportion of cell matrices which could have altered yields [16, 32].

Although cross-interactions among various biochemicals and ash plays a vital role in the bio-oil yield, its incorporation in the model showed it to be insignificant, and collinear with the individual biochemical components which raised the multicollinearity problems. Additional information about model design is given in the Appendix A. In addition, the predictive model considering cross-interaction effect in other literature [31] showed poorer accuracy compared to the model considering the effect of a single model compounds . Therefore, the predictive models of Eq. (3) and (4) better predict HTL bio-oil yields from algae as it considered diverse algae species and different operating temperatures. Model validation was performed for the model obtained at 320^{o}C and given in the appendix (Table A.5). As only a few studies have been conducted at the same reaction conditions used in this study, and other literature with varied process conditions were used for the validation. The model obtained at 320^{o}C showed the difference in the predicted and experimental data to be ± 8%.

2.3.4. Bio-oil properties

Table 2.3 illustrates the properties of bio-oils obtained from different algae strains. The bio-oils obtained were a thick, black viscous layer. Bio-oils from all the algae strains were hydrophobic in nature. The water content of bio-oils was in the range of 4 – 9 wt.%. TAN in bio-oil is directly influenced by the amount of carboxylic acid [21] and also by phenolic present in the samples. Therefore, bio-oils obtained from high lipid (>30 wt.%) containing algae were observed to have maximum TAN (101-118 mg KOH/g). Bio-oils from high protein (>45 wt.%) containing algae strains had the lowest TAN (25–48 mg KOH/g). The decrease in TAN was observed for all the bio-oils obtained at 320^{o}C due to further degradation or decomposition of fatty acids in the severe environment.

The carbon and hydrogen contents were in the range of 69-79 wt.% and 10-14 wt.%, respectively which were higher than that of the original algae. An increase in carbon and hydrogen concentration in the bio-oil was observed with higher HTL temperature. The H/C ratio of the bio-oils was in the range of 1.8-2.2, which compared favorably with that of petroleum crude. Higher carbon and hydrogen content and lower oxygen content resulted in an increase in higher heating value (HHV) of the bio-oils. The HHV of bio-oils were in the range of 32 to 37 MJ/kg, which showed no trend with respect to biochemical compositions. As expected, the nitrogen content of the bio-oils from HTL of high protein containing algae was comparatively higher than others. The presence of relatively high amounts of nitrogen and oxygen makes the bio-oil unfavorable for blending with petroleum crude. Therefore, further upgrading of the bio-oil is required. Energy recovery in the bio-oil from algae samples were evaluated and higher energy (56-82%) was recovered at higher temperature compared to that (47-72%) at lower temperature. A maximum recovery of 83% was observed for a high lipid containing algae (N-2).

Table 2.3. Properties of bio-oil obtained from HTL of different algae strains. [¥]

Sample	Temp. (°C)	Water Content (wt.%)	TAN (mg KOH/g)[†]	Ash Content (wt.%)	HHV (MJ/kg)[†]	Elemental analysis (wt.% in daf)				Atomic ratio (mole/mole)		Energy Recovery (%)
						C	H	N	Balance*	H/C	N/C	
C-1	280	8.55	45.38	0.89	31.10	72.89	12.09	5.74	9.27	1.99	0.07	46.89
	320	9.88	27.44	0.25	33.11	77.67	12.72	7.34	2.27	1.97	0.08	59.31
C-2	280	9.10	101.61	0.22	32.92	77.80	13.64	2.50	6.05	2.10	0.03	48.79
	320	5.98	97.22	0.19	34.23	79.53	13.07	4.76	2.64	1.97	0.05	71.50
N-1	280	5.07	114.21	0.93	35.14	76.75	13.23	2.48	7.55	2.07	0.03	61.12
	320	6.32	96.60	0.30	35.83	79.11	12.90	5.37	2.63	1.96	0.06	68.72
N-2	280	6.63	118.71	0.67	36.44	77.34	14.19	3.74	4.74	2.20	0.04	72.25
	320	5.25	94.48	0.31	36.30	78.72	14.44	4.64	2.19	2.20	0.05	82.80
N-3	280	6.28	37.39	0.65	34.63	76.36	12.15	4.43	7.06	1.91	0.05	68.25
	320	7.41	30.11	0.22	35.46	78.53	12.98	6.66	1.84	1.98	0.07	76.37
N-4	280	7.75	28.91	0.69	34.82	76.24	10.01	5.24	7.83	1.57	0.06	47.72
	320	5.74	25.51	0.18	36.97	79.56	10.85	5.37	4.04	1.64	0.06	71.42
P-1	280	4.87	36.15	0.33	33.42	69.32	10.46	4.07	15.83	1.81	0.05	52.58
	320	4.22	30.42	0.19	35.29	76.81	10.50	3.81	8.68	1.64	0.04	56.54
S-1	280	5.96	118.29	0.59	34.34	77.95	12.28	2.61	7.17	1.89	0.03	69.97
	320	5.39	107.06	0.31	36.28	78.58	13.76	3.91	3.76	2.10	0.04	78.11
S-2	280	5.92	47.61	0.62	33.93	72.73	11.80	4.99	10.48	1.95	0.06	46.87
	320	9.96	29.70	0.29	34.37	76.49	12.53	5.76	5.21	1.97	0.06	78.79

[¥]The reported values for ash content, TAN, HHV, water content and elemental analysis are from average of two (n=2). daf: dry and ash free basis.
*Balance is the sum of % O and % S. [†] as received.

The distribution of major compounds in bio-oil produced from different algae strains are shown in **Figure 2.2**. Organic acids in the bio-oils obtained at both 280 and 320^{o}C were not only high for higher lipid containing algae (N-1 and N-2) but were also high for algae having relatively large amount of both lipids and carbohydrates (C-2 and S-1). This suggests that not only lipids but carbohydrates also contribute to the formation of organic acids. The bio-oils produced from all the algae species, except C-2 and S-1, had higher organic acids at 280^{o}C than at 320^{o}C. The high amount of organic acids at 280^{o}C may be due to the higher conversion of triglycerides into fatty acids at a lower temperature, whereas its reduction at 320^{o}C may be due to the decarboxylation of fatty acids to form hydrocarbons at a higher temperature [17]. In the case of S-1 and C-2, a slight increase in organic acid was observed at 320^{o}C, which may be due to the degradation and decomposition of carbohydrate in the algae to form formic acid, lactic acid and acetic acid [33]. These changes in organic acid are correlated with the TAN of the bio-oil. Hydrocarbons in the bio-oils were mostly aliphatic hydrocarbons formed due to decarboxylation of organic acids and decarbonylation of aldehydes and ketones. No specific trend was found with an increase in temperature for hydrocarbons. In the case of nitrogenated compounds, both amides and nitrogen heterocycles were observed in the bio-oils. The amino acids present in proteins undergoes decarboxylation and deamination reaction in hydrothermal media and produce amines, ammonia, carbonic acids and other organics [34]. As expected, algae having high initial protein content (C-1, N-3, P-1 and N-4) had a significantly high amount (42-48% of total peak area) of nitrogenated compounds present in bio-oil. Oxygenates and phenols were also present in the bio-oils, but no trend was observed with an increase in temperature. Oxygenates and phenols were formed

mainly due to decomposition of carbohydrates. Overall, the production of each chemical composition in bio-oil did not follow a single reaction pathway but multiple reaction pathways in this complex mixture.

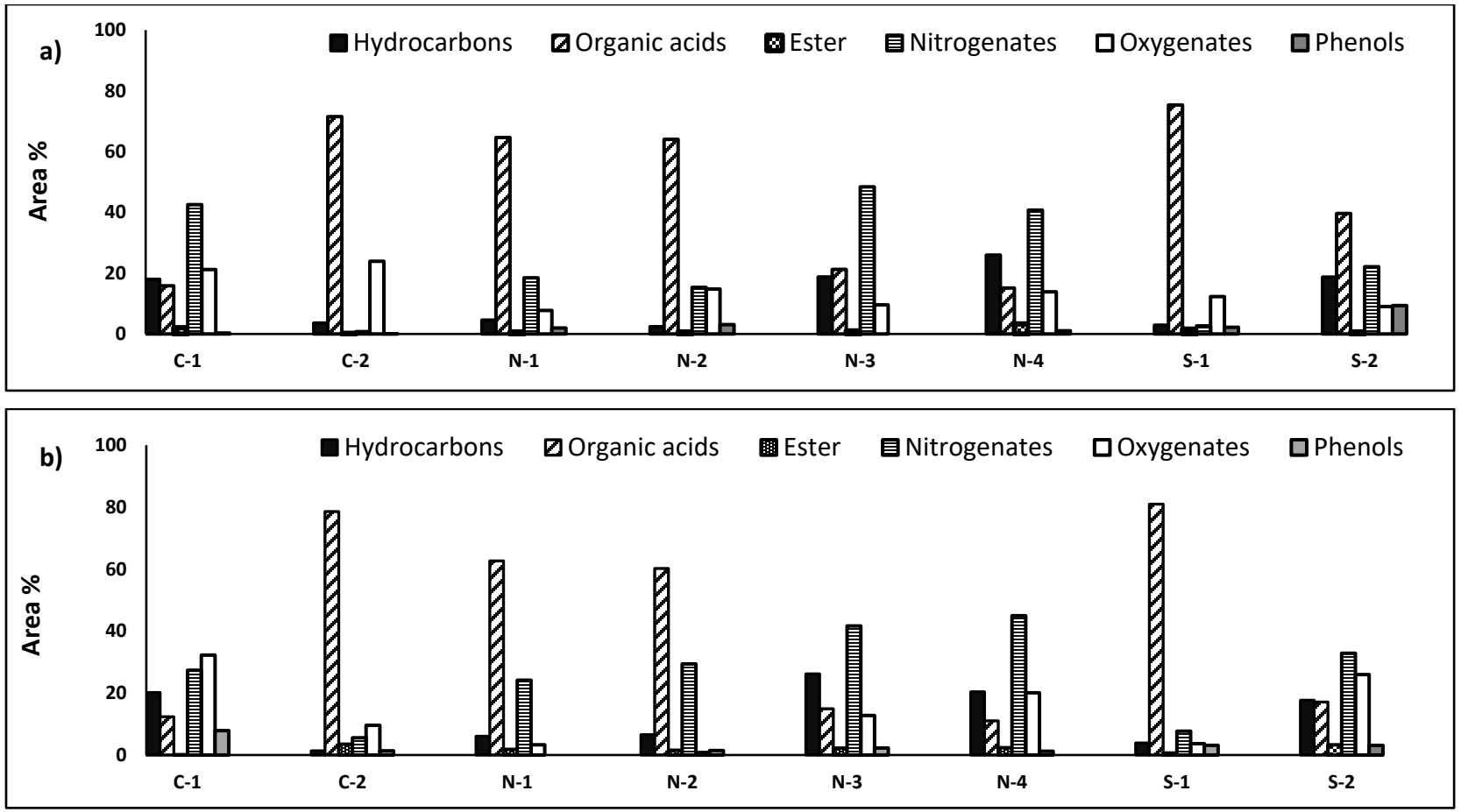

Figure 2.2. Distribution of chemical compounds in bio-oils obtained from HTL of different algae strains at a) 280°C and b) 320°C.

Figure 2.3 illustrates the bio-oil fraction from all the algae strains in different boiling point ranges. Boiling points were grouped [35]. The boiling points of bio-oils obtained from all the algae strains were predominantly (40-68%) in vacuum gas oil (VGO) range, except for N-1 and N-2, which had a maximum portion in diesel range (33-42%). An increase in diesel range for N-1 and N-2 may be due to the large amount of organic acids, mostly myristic acid and palmitic acid (55-75% of total lipids) present in them. The bio-oils from S-1 and C-2 also had high organic acids whereas they contained mostly stearic and oleic acid (50–75% of total lipids), which have higher boiling points than myristic and palmitic acids. All the bio-oils had a minimal amount of compounds in gasoline (0.5–5%) and kerosene (1-13%) ranges. About 9–30% of the bio-oils had a boiling point higher than 538^{o}C and N-4 (high protein containing algae) showed the maximum of 30%. An increase in the reaction temperature at 320^{o}C slightly increased the fraction of bio-oil in lower boiling points ranges and consequently decreased the fraction of bio-oil in vacuum residue range for all the bio-oils. However, the simulated distillation showed that the bio-oil fraction in the lower boiling point range was still very low, which requires a further upgrading of the bio-oil to be used as a transportation fuel substitutes or to blend it with conventional fuels.

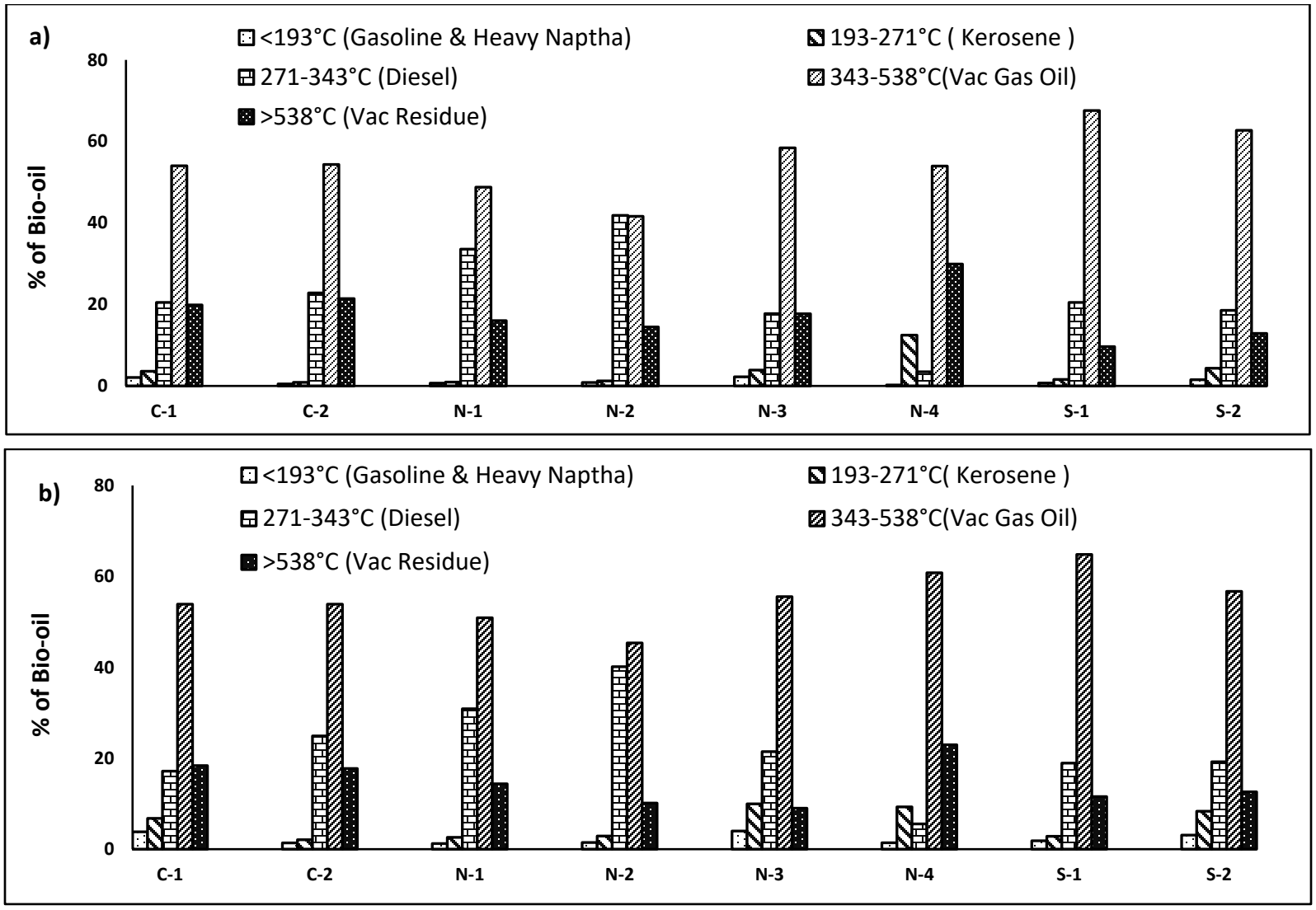

Figure 2.3. Boiling point distribution of bio-oil derived from HTL of different algae strains at a) 280°C and b) 320°C.

FTIR analysis of all the bio-oils was performed to study their functional group characteristics. The spectral band assignments and interpretation were based on the literature [36–38]. FTIR spectra of the bio-oils (S-2, N-1, N-4 and C-1) at 320^{o}C were selectively chosen for comparison as shown in Figure 2.4. The selected bio-oils of S-2, N-1 and N-4 species were for the highest amount of carbohydrate, lipids, proteins respectively, in their biomass. No significant difference was observed between the spectral band observed at 280^{o}C and 320^{o}C. All the bio-oil samples showed a broad-band in 3100-3650 cm^{-1}, which is possibly from O-H and N-H stretching vibrations indicating the presence of alcohols and carboxylic acids for O-H stretching and the presence of amine and amides (N-H stretching). This is supported by the presence of prominent N-H (amines and amides) bending peaks at 1600–1680 cm^{-1} and 1525-1575 cm^{-1}. Bio-oils obtained from high protein containing algae (N-4) had more absorption in the 3100-3500 cm^{-1} spectral region. The prominent C-H stretching (2800-3000 cm^{-1}) and –CH_3 bending (1360-1380 cm^{-1}) were observed in all the bio-oils. Bio-oils obtained from algae (N-1) having higher lipid content had prominent stretching in these regions, which may be due to the C-H stretching of fatty acids present in the bio-oils. This was supported by the prominent stretching observed for the bio-oils in the C=O stretching (1709 cm^{-1}) region. Apart from carboxylic acids, the presence of other compounds having C=O bonding such as ketones and aldehydes in the bio-oil also causes the stretching in 1709 cm^{-1} region. Some adsorption peaks were also observed at 500-900 cm^{-1}, which may be attributed to the C-H bending from aromatics such as naphthalene and phenols.

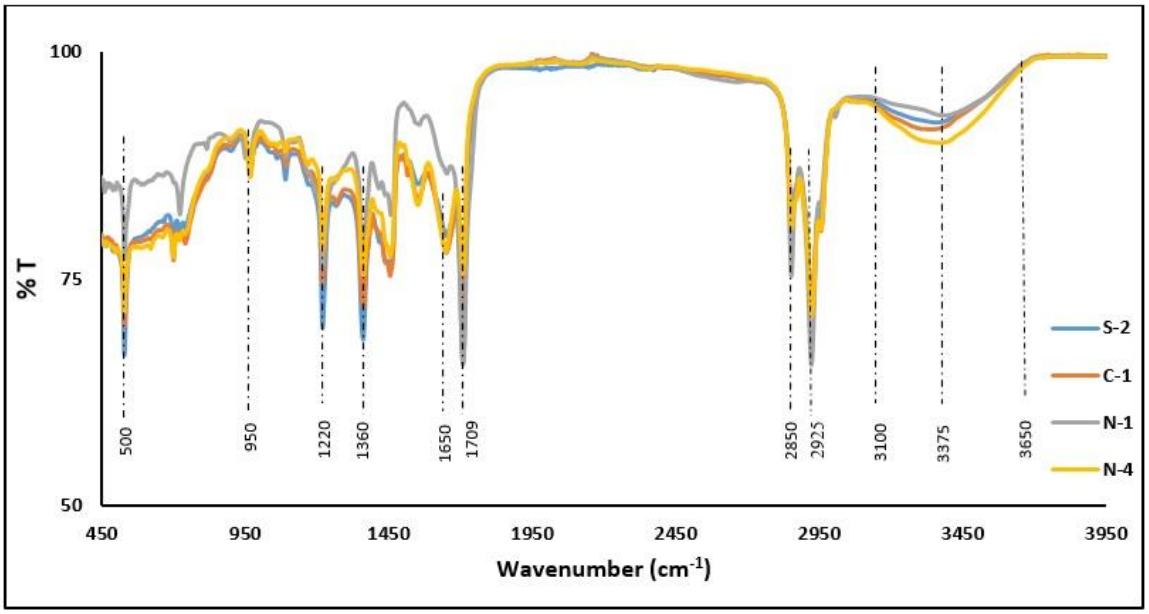

Figure 2. 4. FTIR spectra of the bio-oils obtained from selected algae samples at 320°C.

2.3.5. Solid residue analysis

Proximate and ultimate analyses of the solid residue obtained from HTL of different algae strains are summarized in Table 2.4. Ash content was observed to be in the range of 8-40 wt.% and followed the trend of original algae ash content. The HHV of solid residue were in the range of 15-32 MJ/kg which were in agreement with the previous studies [10, 12]. Ultimate analysis of the solid residue showed that it still had a significant portion of unconverted C, H and N. No significant trend in elemental values were observed at 280°C and 320°C. However, high C and H values of solid residue related to a higher HHV. The surface area of the solid residue obtained from HTL at 280°C for S-1 and C-2 were found to be 15.27 and 25.22 m^2/g, respectively whereas that obtained at 320°C were observed to be 13.33 and 13.69 m^2/g, respectively.

Table 2. 4. Proximate and ultimate analyses of solid residue of seven different algae.[¥]

Sample	Temp. (°C)	Ash Content (wt.%)	HHV (MJ/kg)[†]	Ultimate analysis (wt.% on daf basis)			
				C	H	N	Balance*
C-2	280	12.32	25.97	61.59	5.97	3.92	28.52
	320	13.48	27.53	66.32	4.85	4.60	24.23
N-1	280	19.05	29.86	57.97	8.96	0.43	32.64
	320	21.51	31.87	64.32	10.02	0.03	25.63
N-2	280	40.27	21.89	39.95	5.58	1.24	53.23
	320	40.54	20.19	39.76	6.15	0.52	53.57
N-3	280	33.66	20.72	36.43	4.90	2.25	56.42
	320	36.15	22.24	31.54	4.57	1.51	62.38
N-4	280	15.79	20.15	27.67	4.53	5.34	62.46
	320	21.71	22.87	35.79	5.27	2.10	56.84
P-1	280	24.88	18.61	40.05	4.77	4.29	50.89
	320	30.26	19.25	37.62	4.88	3.01	54.49
S-1	280	10.26	28.77	67.09	5.84	4.32	22.75
	320	8.89	31.49	70.72	6.32	4.17	18.79

Note: data for algae S-2 and C-1 were not shown due to less amount of char. [¥] The reported values for ash content, HHV, and ultimate analysis are single point data (n=1). *Balance is the sum of % O and % S. [†] as received. daf: dry and ash free basis.

2.3.6. Aqueous phase analysis

The aqueous phase is the major byproduct from HTL of algae, which is rich in nutrients and chemicals. Understanding the composition of the aqueous phase is critical to the overall economics of the HTL process. **Table 2.5** illustrates the total organic carbon, total nitrogen, phosphate, ammonium, magnesium and chemical oxygen demand of the aqueous product. The amount of total organic carbon (TOC) and chemical oxygen demand (COD) ranged between 12 to 43 g/L and 35 to 160 g/L, respectively, indicating a significant proportion of organic carbon distributed in the aqueous phase as dissolved organics. A slight decrease in organic carbon was observed with the increase in reaction temperature from 280°C to 320°C, which further supports the theory that at higher temperature the organics in the aqueous phase is converted to bio-oil or gaseous products. Shakya et al. [12] observed a similar trend in TOC for the aqueous phase from hydrothermal liquefaction of *Nannochloropsis*. On comparing with other algal feedstock, the HTL of high proteinaceous algae resulted in a high TOC (N-4: 43 g/L). As the COD values of the

aqueous phase are higher than the permissible wastewater discharge parameter (120 mg/L) set by the EPA [39], further recovery of the chemicals from the aqueous phase is required before dumping it into the wastewater streams. Due to the high amount of organic carbon (20-35 wt.%) in the aqueous phase, there is the potential of converting it into high-value chemicals and fuels. Elliot et al. [40] and Shanmugam et al. [41] demonstrated the production of methane from aqueous phase via catalytic gasification and anaerobic digestion, respectively.

Table 2.5. TOC, TN, COD, phosphate, ammonium and magnesium measurement of the aqueous phase at both temperature. ¥

Samples	Temperature (°C)	TOC (g/L)	TN (g/L)	COD (g/L)	PO_4^{-3} (g/L)	NH_4^+ (g/L)	Mg (g/L)
C-1	280	29.75±6.90	10.36±1.38	93.50±12.02	9.65±1.77	8.95±0.35	2.00±0.71
	320	23.62±1.57	9.77±2.37	70.50±47.38	5.95±1.12	9.30±0.71	0.90±0.07
C-2	280	12.62±0.82	1.49±0.01	52.00±24.04	9.04±1.46	0.13±0.01	1.45±0.07
	320	13.34±0.43	2.11±0.03	61.50±13.44	1.96±0.19	0.50±0.28	0.88±0.04
N-1	280	19.78±1.24	3.13±0.02	52.00±00	1.33±0.07	0.65±0.07	2.68±0.18
	320	12.71±0.85	3.07±0.07	52.00±00	0.94±0.21	0.90±0.42	1.85±0.14
N-2	280	16.75±0.32	2.51±1.98	35.50±23.33	2.06±0.16	0.65±0.07	1.48±0.46
	320	11.60±0.15	2.48±1.77	61.00±33.94	1.15±0.57	1.70±0.57	0.73±0.11
N-3	280	30.42±2.03	11.12±2.79	102.00±00	11.95±0.64	7.55±0.92	1.45±0.07
	320	24.14±1.44	10.48±3.17	71.00±00	8.87±1.51	9.75±0.35	1.22±0.11
N-4	280	43.10±4.15	15.01±0.18	160.50±12.02	11.70±0.57	9.00±1.41	1.25±0.21
	320	29.26±0.20	18.52±1.67	87.50±23.33	8.12±1.86	12.05±0.49	1.55±0.07
P-1	280	28.88±1.52	9.48±0.91	35.00±00	2.12±0.50	8.70±0.28	0.78±0.11
	320	26.01±2.79	9.50±2.03	54.00±24.04	6.26±0.62	9.20±0.14	1.02±0.04
S-1	280	14.62±0.94	0.98±0.90	35.00±00	0.72±0.04	0.08±0.03	1.98±0.39
	320	12.89±0.01	1.26±1.06	35.00±00	0.84±0.05	0.34±0.06	1.28±0.04
S-2	280	26.05±0.86	8.54±0.29	69.00±00	4.12±0.04	7.40±0.85	0.93±0.11
	320	20.64±0.01	8.48±0.07	37.00±00	5.02±1.32	4.00±0.14	0.32±0.11

¥ The reported values are the mean of duplicates (n=2).

Total nitrogen (TN) content in the aqueous phase ranged from 1 to 15 g/L and was similar to that obtained by Gai et al. [42]. The variation in TN in the aqueous byproduct is mostly due to the type of algal strains. Algae containing the highest proteins (N-4) gave the maximum TN (18 g/L) whereas that with the lowest protein content (S-1) resulted in the minimum TN (0.98 g/L). It is also known from the literature [13, 42] that around half to two-thirds of nitrogen in the algae is

retained in the aqueous phase. No significant trend was observed in total nitrogen content with the increase in temperature in this study. Ross et al. [25] reported a decrease in nitrogen concentration with an increase in temperature for *Spirulina* but reported no change for *Chlorella* which shows that change in nitrogen concentration with temperature depends on the algae strains. The ammonium in the aqueous phase ranged between 0.34 to 12.05 g/L, which were observed to be higher than that observed by Ross et al. [25]; this might be due to the use of catalysts in their work or the variation in the reaction temperatures (300 & 350^{o}C). From the observation of TN and NH_4^+ content, this study shows that most of the nitrogen in the aqueous phase are in NH_4^+ form rather than in NO_3^-. A higher level of ammonium in the aqueous phase demonstrates its potential for nitrogen recovery by algal growth as ammonium can be directly used to synthesize cellular N-compounds than the nitrate form in the algae [32]. The aqueous phase obtained were mainly alkaline (pH of 7.0-8.5) in nature due to the presence of a high amount of ammonium and other forms of basic nitrogenous compounds. However, the aqueous phase obtained from the algae having lower protein content (S-1 and C-2) were acidic (pH of 3.5 -5) in nature; in agreement with the work of Maddi et al. [43], who observed low pH in the aqueous phase obtained from high lipids and low nitrogen containing algae.

The chemical composition of the aqueous phase from HTL contained diverse chemical compounds like cyclopentenones, phenolics, carboxylic acids and nitrogen heterocycles as shown in the appendix (Table A.9). The chemical compounds present in the aqueous phase depended on the biochemical composition of the algae. The aqueous phase obtained from high protein containing algae had the maximum amount of nitrogenous compounds whereas maximum oxygenates were obtained from high carbohydrates containing algae. The high amount of

nitrogenous and phenolics compounds present have a wide variety of applications. High-value chemicals and drugs can be produced from pyridine, pyrazine, and their alkyl derivatives [43].

Phosphorus is one of the primary nutrients needed for algal growth [32, 44]. During HTL, the phosphorus in the algae also gets partitioned into different products. Phosphorus in the aqueous phase is mostly found in the form of phosphate [10, 13]. In this study, phosphate in the aqueous phase ranged from 0.7 to 11.95 g/L, which were in agreement with the values obtained by Maddi et al. [43]. The phosphate concentration slightly decreased with the increase in temperature for all the algae species except for P-1, S-1, and S-2, which increased slightly. Magnesium content in the aqueous phase was in the range between 0.7 to 2.6 g/L. Phosphate, ammonium, and magnesium in the aqueous phase can be recovered by precipitating it in the form of struvite (Magnesium ammonium phosphate), a slow releasing fertilizer, as shown by Shanmugam et al. [44]. According to Shanmugam et al. [44], around 0.10-0.12 g of struvite can be produced from 10 mL of aqueous phase, which had phosphate concentration of 3.9 g/L. The study also showed that more than 99% of phosphate and around 44-100% ammonia were recovered as struvite.

Apart from phosphorus and magnesium, the range of the other major trace elements in the aqueous phase was 24-758, 807-4151 and 209-1841 mg/L for Ca, K and Na, respectively as shown in the appendix (Table A.10). These data were consistent with the previous studies [45]. These variations in the metal elements, mainly attributed to their uptake by biomass during its growth. Other trace elements present in the aqueous phase were copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), etc. The presence of high amount of nutrients in the aqueous phase can not only be recovered as fuels or chemicals but can also be used for algal growth [45].

2.4. Conclusions

The effect of biochemical composition of nine algae species on the HTL bio-oil yields and properties at 280 and 320^{o}C were investigated. The bio-oil yields were higher at the higher temperature of 320^{o}C. The predictive yield model for bio-oil yield followed the trend lipid > protein > carbohydrate. A large concentration (40-68 %) of algae bio-oils were in VGO range. The aqueous phase had high amount of organic chemicals which could be recovered for fuels and chemicals. The high amount of phosphate, ammonia, and magnesium in the aqueous phase showed a potential of struvite production.

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Chapter 3

Catalytic Upgrading of Bio-oil Produced from Hydrothermal Liquefaction of *Nannochloropsis* sp.

Abstract

Hydrothermal liquefaction (HTL) is an attractive process to convert algae into bio-oils. However, the bio-oils obtained from HTL of algae cannot be directly used as fuel or blended with petroleum crude because of its high viscosity, high oxygen content and high nitrogen content, which are undesirable properties for transportation fuel. Therefore, upgrading of algae bio-oils is needed for using it as a fuel. In this study, the bio-oil obtained from HTL of *Nannochloropsis* sp. was upgraded by catalytic hydrotreatment. Upgrading was performed with five different catalysts (Ni/C, ZSM-5, Ni/ZSM-5, Ru/C and Pt/C) in the presence of hydrogen at 300^{o}C and 350^{o}C, and all the experiments were conducted at a weight hourly space velocity (WHSV) of 0.51 g/g_{cat}.h. The upgraded bio-oils yield was higher at 300^{o}C; however, higher quality upgraded bio-oils were obtained at 350^{o}C. Among the five catalysts tested, Ni/C gave the maximum oil yield (61 wt.%) at 350^{o}C. However, noble metal catalysts (Ru/C and Pt/C) gave the better upgraded bio-oils in terms of acidity, heating values, and nitrogen values. The higher heating value of the upgraded bio-oils ranged from 40 to 44 MJ/kg, and the nitrogen content decreased from 5.37 to 1.29 wt.%. Simulated distillation of the upgraded oils showed that about 35-40 wt.% of the upgraded oil were in the diesel range. Overall, the catalytic upgrading of algae bio-oil was effective in improving the

quality of the bio-oil. The main components present in the gaseous product were CH_4, CO, CO_2 and lower alkanes. The surface area of spent catalysts was reduced significantly due to coking.

Keywords: *Algae; Hydrothermal liquefaction; Bio-oil; Catalytic upgrading; Hydrodenitrogenation.*

3.1. Introduction

Production of renewable fuels and valuable chemicals from aquatic biomass such as algae have recently gained significant attention. The main advantage of using algae for biofuel is their high photosynthetic efficiency, faster growth rate and higher yield relative to terrestrial biomass [1, 2]. In the conventional approach, lipids from algae are extracted using solvent and transesterified to produce bio-diesel. However, the major problem with this approach is the need of dry algae biomass for lipid extraction. As a result, a huge amount of energy is wasted drying the biomass which makes this process uneconomical. In addition, the solvent extraction process utilizes only lipid fraction of the algae and rest of the biomass are wasted, making the process unsustainable. There are other pathways of converting algae into renewable fuels, for example, pyrolysis [3, 4] and gasification [5, 6]. However, these thermochemical processes typically require dry feedstocks for the production of renewable fuels. Among the different pathways, hydrothermal liquefaction (HTL) is the best-suited process for producing renewable fuels from algae or wet feedstocks due to their acceptance of water as a reactant and reaction medium, which ultimately leads to the elimination of energy-intensive drying process [7]. Also, HTL not only converts the lipid portion of algae into bio-oil but also utilizes the whole algae.

Extensive research on conversion of algae into bio-oil through HTL route has been conducted over the last decade. The bio-oil produced from HTL of algae had an advantage over

other terrestrial biomass such as switchgrass, pine etc. [8, 9] because of its high heating value (31-37 MJ/kg) and lower water content (5-10 wt.%) [10–13]. However, on the downside, the bio-oil obtained from HTL of algae is acidic in nature (TAN: 25-118 mg of KOH/g), had a high viscosity (40-67 cP) and high amount of oxygen and nitrogen (2-9 wt.%) which are undesirable in fuel [11, 14, 15]. To overcome these drawbacks and improve the quality of fuel, upgrading of the bio-oil is necessary. To date, most of the studies on the upgrading of HTL bio-oil from algae are focused in a supercritical water environment. Duan and Savage performed several studies [16, 17] on the upgrading of the algae bio-oil in a supercritical water environment. In their optimization study [16], the authors studied four different factors; catalyst type (Mo_2C, Pt/C, and HZSM-5), temperature (430-530^oC), residence time (2-6 h) and catalyst loading (5-20 wt.%). The study showed that the temperature was the most influential factor affecting the upgraded bio-oil properties, whereas catalyst type was the most influential factor affecting the fraction of N, and O-containing compounds. In another study [17], the authors investigated the influence of Pt/C catalyst on the properties of bio-oil obtained from the upgrading of algae bio-oil in a supercritical water environment. The oxygen and nitrogen content of the bio-oil decreased from 6.5 to 4.3 wt.%, and from 4.9 to 2.2 wt.%, respectively, and the sulfur values were below detection. Bai et al. [18] performed an extensive catalytic screening study in a supercritical water environment and reported that Ru/C exhibited the best performance for deoxygenation, whereas Raney Ni and HZSM-5 showed the best performance for denitrogenation. Apart from supercritical water environment, some studies have tested the use of solvents in upgrading of algae bio-oil. Wang et al. [19] used four metal catalysts (Pt/C, Ru/C, Ni/C and Co/C) at 350^oC in the presence of hydrogen to upgrade algae bio-oil mixed with isoparaffin instead of water. They observed a significant heteroatoms reduction in the upgraded bio-oil, and the color of the upgraded bio-oil differed according to the

catalysts used. Apart from these studies, there are only a handful of studies on upgrading without using a solvent. Barreiro et al. performed a comparative study [20] on upgrading of the bio-oil obtained from two different algae sp. (*S. almeriensis* and *N. gaditana*) at 400^{o}C and 4 h residence time. In addition, the authors also investigated the effect of two different heterogeneous catalysts (Pt/Al_2O_3 and HZSM-5) and the influence of water addition to the reaction medium. The study found that starting materials (bio-oils obtained from different algae species) used for the upgrading process strongly affected upgraded bio-oil yields (50.4 wt.% and 61.7 wt.% for *S. almeriensis* and *N. gaditana,* respectively, with the use of HZSM-5 catalysts). In the case of catalytic activities, the study reported that the overall process was thermally controlled as no marked differences in properties were found between catalyzed and uncatalyzed reactions. Elliot et al. were the pioneer in continuous upgrading of algae bio-crude into biofuel either using 1-step or 2-step process [21]. In two step operation, the first step reaction was performed at 125-175^{o}C at LHSV of 0.66 h^{-1} and second step reaction was performed at 405^{o}C at LHSV of 0.18 h^{-1}, whereas in one step operation, the reaction was performed at 405^{o}C at LHSV of 0.2 h^{-1}. The study reported higher upgraded bio-oil yield (79-85 wt.%), higher H/C ratio (1.85-1.98) and significant denitrogenation when compared to other batch upgrading studies. Li et al. performed an optimization study using only HZSM-5 catalysts in the presence of hydrogen but without water[22]. The authors reported a high yield of aliphatic hydrocarbons at 400^{o}C while a high yield of aromatic hydrocarbons produced at 500^{o}C.

When comparing upgrading studies in a supercritical water environment to a water-less environment, the former gave a high amount of carbon and hydrogen, but the oxygen removal was less effective than the water-less environment [20]. Also, the use of water may possess processing challenges after upgrading, as the aqueous phase may have some water-soluble compounds that

are not safe for disposal and require wastewater treatment. However, upgrading studies of bio-oil from HTL of algae without adding any solvent/water is very limited. Thus, in this study, the bio-oil obtained from HTL of algae (*Nannochloropsis*) were upgraded in a water-less environment to produce biofuels. Five catalysts (ZSM-5, Ni/ZSM-5, Ni/C, Ru/C and Pt/C) were selected to study the hydrodeoxygenation, hydrodenitrogenation and hydrodesulfurization capability of the catalysts. In addition, two temperatures of 300 and 350^{o}C were used for upgrading to investigate the effect of low-temperature on the upgraded bio-oil properties.

3.2. Material and Methodology

3.2.1. Hydrothermal liquefaction of Algae

Hydrothermal liquefaction (HTL) of *Nannochloropsis* (purchased from Reed Mariculture Inc., CA) was performed to obtain bio-oil for upgrading studies. HTL experiments were performed using a batch reactor of 1000 ml internal volume and equipped with an electrical heating unit. The temperature and pressure inside the reactor were continuously monitored using Delta-V software. HTL was performed at 320^{o}C for a residence time of 30 minutes with a solid loading of 15 wt.%. The reactor was loaded with approximately 120 g of algae (on dry basis). Product separation procedure was followed as described in the published document [23]. Bio-oil obtained from HTL of *Nannochloropsis* had a fairly high water content (13 wt.%) and was further mixed with dichloromethane to remove water. The dichloromethane was then separated by vacuum-evaporation, and the bio-oil with low water content (1.22 wt.%) was obtained and was used for upgrading studies.

3.2.2. Catalyst preparation

Five heterogeneous catalysts (ZSM-5, Ni/ZSM-5, Ni/C, Ru/C and Pt/C) were tested in this study. The commercial Ru/C and Pt/C both having 5 wt.% of active metal were obtained from

Sigma-Aldrich. ZSM-5 (SiO_2/ Al_2O_3 mole ratio: 50:1, CBV5524G) was obtained from Zeolyst International and was calcined before use. Calcination was performed at 550^oC for 2 h. 10 wt.% Ni/C and Ni/ZSM-5 were prepared by impregnating nickel nitrate hexahydrate (Alfa Aesar) on activated carbon powder (Supelco, product No. 31616) and ZSM-5, respectively. The amounts of nickel nitrate solutions were adjusted to 10 wt.% of activated carbon and ZSM-5 on metal basis. The slurry was shaken on a shake-bed overnight (60^oC, 180 RPM) and kept in an oven for drying at 105^oC for 24 h. The dried catalysts were stored in a desiccator until they were further used.

3.2.3. Upgrading of bio-oil

Bio-oil upgrading experiments were performed in a 450 ml Parr reactor equipped with a glass liner, controllable stirrer, cooling chamber and heating mantle. Ultrahigh purity grade (99.999%) hydrogen and nitrogen gases were purchased from Airgas Inc. (Charlotte, NC) and used in this study. In all the experiments, the weighted hourly space velocity (WHSV) of 0.51 g of bio-oil/g_{cat}.h was maintained as according to Eq (1). In a typical run, 8 g of catalyst was first loaded into the reactor, and repeated cycle of nitrogen purging was performed to create an inert headspace. After purging with nitrogen, hydrogen gas was purged to remove residual nitrogen, and the pressure was maintained at 1000 psig for catalyst reduction. Reduction of catalyst was carried out at 300^oC for a residence time of 1 h, and after reduction, the reactor was subsequently cooled down to room temperature, and again purged with nitrogen before opening. A known amount of bio-oil (approximately 40 g) was loaded in the reactor, and similar procedure of purging with nitrogen and hydrogen during reduction was followed. Hydrogen pressure was maintained at 1000 psig. This high pressure of hydrogen was maintained to ensure higher solubility of hydrogen in the oil resulting in an increase in reaction rate and further decreasing coke formation [24]. The reactor

was then heated to the selected temperature (either 300 or 350°C) while agitating bio-oil at 500 RPM for approximately 10 h residence time.

$$WHSV = \frac{Weight\ of\ biocrude\ (g)}{Weight\ of\ Catalyst\ (g) \times Reaction\ time\ (h)} \quad (1)$$

After the residence time, the reactor was cooled down. The residual pressure created by product gas formation during the reaction was then recorded, and the gas was collected in a gas chamber. Mass of the reactor, glass liner, catalyst and product fraction was recorded before and after each experiment. The reaction mixture obtained had two layers: light liquid and slurry mixed with catalysts. The light liquid fraction was collected first in a separate flask and vacuum filtered to recover the upgraded oil. The slurry portion of the reaction mixture was collected in another flask along with toluene, which was used to rinse the reactor. This mixture was vacuum filtered to recover catalyst as residue and organic phase/toluene soluble phase as filtrate. Toluene in the organic phase was separated using an IKA rotary evaporator that was operated at 60°C and 78 mbar pressure to obtain toluene extracted oil. A mass balance for each experiment was determined by the relative content of different products in each phase. The product yields for upgrading studies were calculated using Eq. (2). Hydrogen consumption during the catalytic upgrading was calculated according to Nam et al. [3]. All the experiments were performed in duplicates except for hydrogen alone.

$$\text{Yields}_{\text{ oil/solid residue(coke)}} = \frac{Weight\ of\ the\ product\ (dry\ basis)}{Weight\ of\ the\ algae\ bio-oil\ (dry\ basis)} \times 100 \quad (2)$$

3.2.4. Product analysis

3.2.4.1. Upgraded bio-oil characterization

The upgraded bio-oils produced from all the experiments were analyzed for water content, higher heating value (HHV), total acid number (TAN) and chemical composition according to the method described by Shakya et al. [23]. The viscosity of the upgraded bio-oils was measured

according to ASTM D445 using a calibrated Cannon-Fenske viscometer at 40ºC. Elemental analysis of upgraded oils was measured using EAI elemental analyzer. Fourier Transform Infrared (FTIR) spectroscopic analysis of bio-oils was performed by Thermo Nicolet iS10 (Thermo Scientific, Waltham, MA). The samples were analyzed for 34 scans over a range of 400-4000 cm^{-1} wavenumbers. Simulated distillation was performed as according to the methods previously described by Wang et al. [19]. Energy recovery for the upgraded bio-oils was calculated using Eq. (3).

$$Energy\ recovery = \frac{HHV_{UPGRADED\ BIO-OIL} \times Mass_{UPGRADED\ BIO-OIL}}{\left[HHV_{ALGAE\ BIOMASS} \times Mass_{ALGAE\ BIOMASS} + HHV_{H_2} \times Mass\ consumed_{H_2}\right]} \times 100\ \%$$

[3]

3.2.4.2. Gas analysis

The gas analysis was performed using a gas analyzer (Agilent 3000 Micro GC) equipped with four different modules (Mol Sieve 5A PLOT, 10 m × 0.32 mm × 12 µm; PLOTU 8 m × 0.32 mm × 30 µm; Alumina PLOT, 10 m × 0.32 m × 8 µm; OV1, 14 m × 0.15 mm × 2 µm). The gases were analyzed in duplicates to verify the reproducibility of the data.

3.2.4.3. Catalysts characterization

The catalysts both fresh and spent were characterized for BET (Brunauer-Emmett-Teller) surface area using a Quantachrome Autosorb- iQ with nitrogen absorption. Before measurement, all the samples were outgassed to 10^{-3} Torr at 300ºC. Both fresh and spent catalysts were also analyzed in a Zeiss DSM 940 scanning electron microscope with digital imaging (SEM-EDS).

3.2.4.4. Statistical Analysis

Each experiment at both temperatures was performed in duplicates to verify the reproducibility of data. Statistical analysis (ANOVA, Tukey's HSD) of all the data was performed

to understand if the obtained results were statistically different. All the statistical analyses were performed at 95% confidence interval.

3.3. Results and Discussion

3.3.1. Effects of catalyst on upgrading yields

Figure 3.1 shows the effect of catalysts on yields of various product fractions while upgrading at 300 and 350^{o}C. The bio-oil obtained from HTL of *Nannochloropsis* was converted to upgraded bio-oil, solid residue (coke) and gas. The total mass balance closure varied between 80 wt.% and 96.6 wt.%. Complete closure of mass balance was not possible due to experimental errors such as spilling of few drops of bio-oil while processing, loss of some oil compounds during solvent removal, during pressure relief procedure or due to the incomplete removal of oil slurry from the reactor walls.

Upgraded bio-oil obtained were reddish-brown, and no significant change in color was observed with the use of different catalysts. This was in contrast to a previous finding by Wang et al. [19], who observed variation in the upgraded bio-oil color with the use of various catalysts. The variation observed may be due to the effect of solvent (isoparaffin) used in their study. Figure 3.1 illustrates that the upgrading temperatures affected the product distributions. For all the runs, the upgraded bio-oil yields at 300^{o}C were significantly higher (p-value < 0.05) when compared to the yields at 350^{o}C. This decrease in bio-oil yield may be due to thermal degradation and decomposition of algae bio-oil or due to the catalytic activity of the catalyst which produces gaseous product via cracking, decarboxylation or decarbonylation reactions and also due to char formation from polymerization reaction at a higher temperature. Comparing the catalytic runs at 300^{o}C, the upgraded bio-oil yield was significantly higher (p-value=0.005) with the use of Ni/C and ZSM5 (75.25 wt.% and 74.69 wt.%, respectively) catalysts as compared to Ru/C and Ni/ZSM5

(60.47 wt.% and 61.31 wt.%, respectively) catalysts. At 350°C, the maximum upgraded bio-oil yield at 350°C was observed with Ni/C (61.50 wt.%), which was significantly higher (p-value=0.002) than the upgraded bio-oil yields from other catalysts. Compared to the catalytic runs, hydrogen only (non-catalytic) run gave a higher yield (64.15 wt.%) at 350°C. However, at 300°C, the upgraded bio-oil yield (72.69 wt.%) for hydrogen only run was similar to that obtained using Ni/C, ZSM5, and Pt/C.

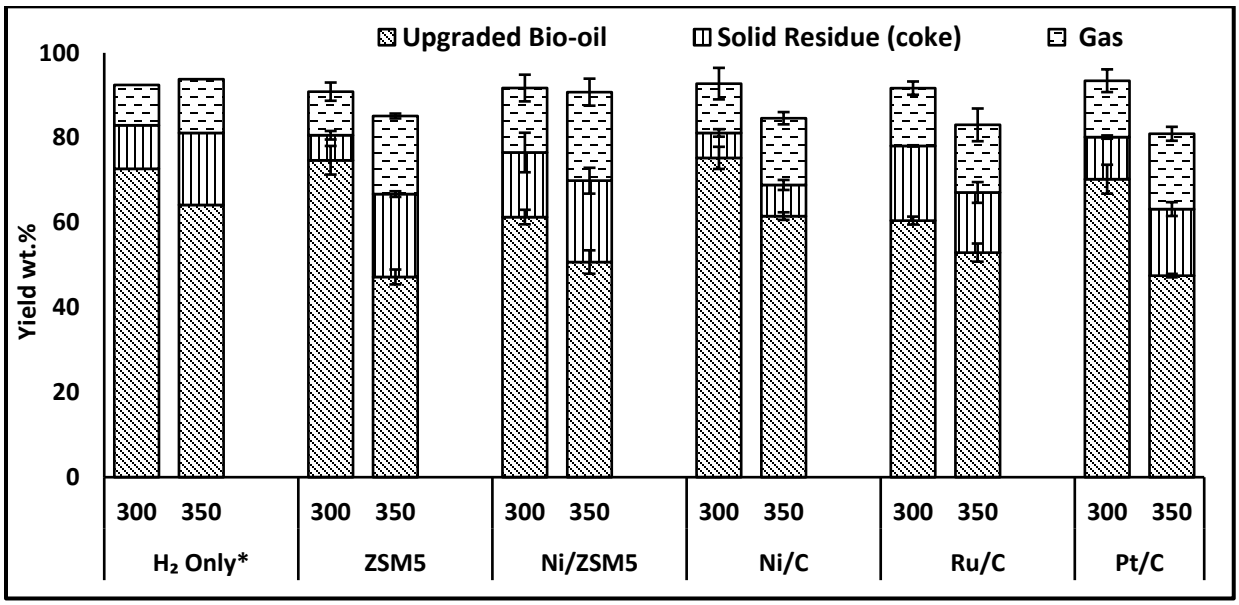

Figure 3. 1. Product fractions obtained from upgrading of algae bio-oil at 300 and 350oC (*denotes single run).

Solid residue or coke formation was observed at both 300 and 350°C, but it was more pronounced at 350°C. Solid residue or coke formation occurs due to increase in the rearrangement reaction and condensation reaction when organic compounds undergoes transformation over solid catalysts [25]. Solid residues obtained from ZSM5 (19.53 wt.%) and Ni/ZSM5 (19.16 wt.%) runs at 350°C were higher than hydrogen only (16.98 wt.%) run, indicating the catalytic effect of these catalysts on coke formation. In the case of Ni/ZSM5 (19.16 wt.%) and Ni/C (7.33 wt.%), the

former catalyst promoted more solid residue formation than the latter one (p-value=0.0361). This showed that ZSM5 played an important role in coke formation. The coke formation in this study was observed to be higher than other studies; this may be attributable to low upgrading temperatures, longer residence time and oil only environment (without the use of supercritical water or solvent medium). The gas yields were observed to increase with the increase of upgrading temperature from 300 to 350^{o}C. Ni/ZSM5 (20.84 wt.%) gave the highest gas yield at 350^{o}C. Compared to the hydrogen only run, the catalytic runs gave a higher gas yield. This result was contrary to the study by Bai et al. [18], which suggested that the gas yields were almost entirely thermal process with the sole exception of Ru/C catalyst. This variation may be due to the use of water, different reaction temperature (400^{o}C) and short residence time of 4 h in their study.

3.3.2. Effect of catalysts on properties of upgraded oil

Table 3.1 and 3.2 shows the properties of upgraded bio-oils at the upgrading temperatures of 300 and 350^{o}C, respectively. The upgraded bio-oils had water content below 1 wt.%. It is noteworthy to mention that all the catalysts produced some visible water droplets in the reactor wall and stirrer which were removed while removing toluene during solvent removal (at 77 mbar and 60^{o}C) process. The TAN of the upgraded bio-oils was observed to be lower than that of original algae bio-oil, indicating the decrease in some acidic compounds like fatty acids and phenols present in the algae bio-oil. At 300^{o}C, a modest decrease in TAN was observed for all the upgraded bio-oils. A significant reduction (p-value < 0.05) in TAN was observed for all runs at 350^{o}C when compared to 300^{o}C. At 350^{o}C, catalytic runs gave significantly lower TAN compared to hydrogen only run. Also, TAN was observed to be lower for metal catalysts; this may be due to the promotion of decarboxylation and decarbonylation reactions, leading to the conversion of organic acids to form alkanes with the use of metal catalysts [26]. TAN of the upgraded bio-oils obtained at 350^{o}C

decreased 71 to 98% of the original algae bio-oil. Substantial reduction in viscosity was observed after upgrading. The viscosity of upgraded bio-oils significantly reduced with the increase in upgrading temperature. The decrease in viscosity may be due to the reduction in branched alkane, chain length, and cycloalkanes present in the upgraded bio-oils which results in fewer chain entanglements, and fewer intermolecular interactions (London-force interactions) [27]. The viscosity of the upgraded bio-oils obtained at 350^{o}C reduced 81 to 96% of the original algae bio-oil. The viscosity was observed to be lower with the use of metal catalysts (Pt, Ru, and Ni) when compared to hydrogen only and ZSM5 runs.

The elemental composition of upgraded bio-oils showed that the C and H percentage increased for both catalytic and hydrogen only runs at 350^{o}C. The increase in C and H compared to original bio-oil was reflected on their heating values as well. H/C ratio was in the range of 1.50 and 1.76, which were lower than obtained by Elliot et al. [21] (1.85-1.98) in their continuous upgrading study but were similar (1.49-1.62) to that obtained by Barreiro et al. [20] at reaction temperature of 400^{o}C and residence time of 4 h with the use of HZSM-5 catalysts The decrease in H/C ratio was observed for H_2 only and ZSM5 runs when compared to original algae bio-oil. The reduction in H/C ratio may be due to increase in aromatics, unsaturated hydrocarbons and migration of H_2 atoms in the gaseous phase [22]. Heating values of upgraded bio-oils obtained at 350^{o}C were significantly higher (p-value <0.05) compared to that obtained at 300^{o}C for all runs, except for hydrogen only and Ni/ZSM5 runs. Upgraded oils obtained from metal catalysts (Pt/C, Ni/ZSM5, and Ru/C) had the higher heating values (43.36 to 44.57 MJ/kg) which were similar to that of petroleum diesel fuel (44.8 MJ/kg). Hydrogen only run also had a significant improvement in the heating value when compared to original algae bio-oil. Overall, higher heating values increased 9-20% of the original algae oil. The nitrogen content in original algae bio-oil is

comparatively higher than the bio-oil obtained from other terrestrial biomass due to the high protein content in algae. The decrease in nitrogen content was observed in the upgraded bio-oils obtained at both 300 and 350^{o}C, resulting in a decrease in N/C ratio. Decrease in N/C ratio indicated the hydrodenitrogenation (HDN) capability of the catalyst in use. Although complete HDN was not observed, Pt/C and Ru/C showed the best performance for HDN of the algae bio-oil. Hydrogen only run also gave a lower N/C ratio suggesting that temperature was also a major factor in HDN. We suspect a low level of sulfur content as hydrodesulfurization (HDS) occurs simultaneously with hydrodeoxygenation (HDO). The balance of the elemental analysis was assumed to be only oxygen, and oxygen content in the upgraded oils was lower than the original algae oil. All the catalysts tested showed catalytic activity for HDO, as the upgraded oils from the catalyzed runs had lower oxygen content. Removal of heteroatoms (i.e., S, N, and O) are critical during upgrading. The accepted heteroatom removal efficiency during upgrading occurs in the following order HDS> HDO> HDN [28]. Energy recovery for the upgraded bio-oils ranged between 35 and 54% of the algae biomass. It was higher for the upgraded bio-oils obtained at 300^{o}C for all the catalysts. This can be attributed to the high yield of upgraded bio-oils at 300^{o}C as the HHV was lower than that observed for the upgraded bio-oil obtained at 350^{o}C. Hydrogen only run gave the highest energy recovery at 350^{o}C.

Table 3. 1. Proximate and ultimate analyses of the upgraded bio-oil obtained at 300ºC.

Samples	Water Content (wt.%)	TAN (mg KOH/g)¥	Viscosity (cSt) ¥	HHV (MJ/kg) ¥	Energy Recovery (%)	Elemental Analysis (wt.% on daf basis)*				Elemental Ratios	
						C	H	N	Balance†	H/C	N/C
Bio-oil	1.25±0.19	23.26±0.55	68.83±2.84	36.44±0.64	68.95±0.43	79.56	10.85	5.37	4.04	1.64	0.06
H_2 Only	0.59±0.12 ^{a,b}	19.40±0.92 ^{a}	46.17±1.40 ^{a}	41.09±0.19 ^{a}	53.25±0.68 ^{a}	83.45	10.40	3.94	2.20	1.50	0.04
ZSM5	0.12±0.03 ^{b}	18.19±1.33 ^{a}	17.67±0.05 ^{b}	40.08±0.55 ^{a}	54.21±0.82 ^{a}	82.09	10.06	3.40	4.44	1.47	0.04
Ni/ZSM5	0.58±0.13 ^{a,b}	8.45±0.65 ^{b}	40.48±0.07 ^{c}	42.41±0.34 ^{b}	45.52±0.87 ^{b}	84.87	11.90	1.81	1.42	1.68	0.02
Ni/C	1.02±0.16 ^{a}	18.22±1.55 ^{a}	33.77±0.05 ^{d}	40.59±0.08 ^{a}	52.19±1.70 ^{a}	81.54	11.20	3.83	3.43	1.65	0.04
Ru/C	0.26±0.09 ^{b}	15.99±2.09 ^{a}	23.71±0.02 ^{e}	43.25±0.24 ^{b}	45.92±1.43 ^{b}	83.05	12.06	2.10	2.79	1.74	0.02
Pt/C	0.57±0.10 ^{a,b}	16.17±1.88 ^{a}	15.59±0.17 ^{b}	42.39±0.03 ^{b}	51.84±0.29 ^{a}	83.09	12.19	2.23	2.48	1.76	0.02

Different alphabets in the superscripts of each group (properties/column) denote the values are statistically significant at different catalyst type (rows) (α=0.05). numbers after ± symbol denotes standard deviation. ¥ as received. * denotes one-point data. daf: dry and ash free basis. † Balance is the sum of % O and % S

Table 3. 2. Proximate and ultimate analyses of the upgraded bio-oil obtained at 350ºC.

Samples	Water Content (wt.%)	TAN (mg KOH/g) ¥	Viscosity (cSt) ¥	HHV (MJ/kg) ¥	Energy Recovery (%)	Elemental Analysis (wt.% on daf basis)*				Elemental Ratios	
						C	H	N	Balance†	H/C	N/C
Bio-oil	1.25±0.19	23.26±0.55	68.83±2.84	36.44±0.64	68.95±0.43	79.56	10.85	5.37	4.04	1.64	0.06
H_2 Only	0.28±0.11^{y,z}	6.58±0.39^{x}	12.88±1.37^{x}	42.21±1.05^{z}	48.72±1.21^{x}	85.44	11.48	2.67	0.41	1.61	0.03
ZSM5	0.14±0.02^{z}	1.05±0.31^{y}	6.14±0.04^{y}	42.51±0.06^{y,z}	35.56±0.08^{w}	86.14	11.03	1.84	0.99	1.54	0.02
Ni/ZSM5	0.32±0.11^{y,z}	0.92±0.05^{y,z}	4.46±0.04^{y,z}	43.36±0.22^{x,y,z}	38.91±1.30^{w,z}	86.46	11.55	1.73	0.27	1.60	0.02
Ni/C	0.84±0.06^{x}	0.72±0.21^{y,z}	3.77±0.02^{z}	42.04±0.09^{z}	43.32±0.72^{y}	86.43	11.47	1.78	0.32	1.59	0.02
Ru/C	0.13±0.04^{z}	0.41±0.26^{z}	2.56±0.02^{z}	44.57±0.15^{x}	39.68±1.18^{y,z}	85.39	12.27	1.37	0.98	1.72	0.01
Pt/C	0.51±0.08^{y}	0.64±0.17^{y,z}	3.58±0.02^{z}	44.04±0.15^{x,y}	36.21±0.83^{w,z}	86.66	11.46	1.29	0.59	1.59	0.01

Different alphabets in the superscripts of each group (properties/column) denote the values are statistically significant at different catalyst type (rows) (α=0.05). numbers after ± symbol denotes standard deviation. ¥ as received. * denotes one-point data. daf: dry and ash free basis. † Balance is the sum of % O and % S

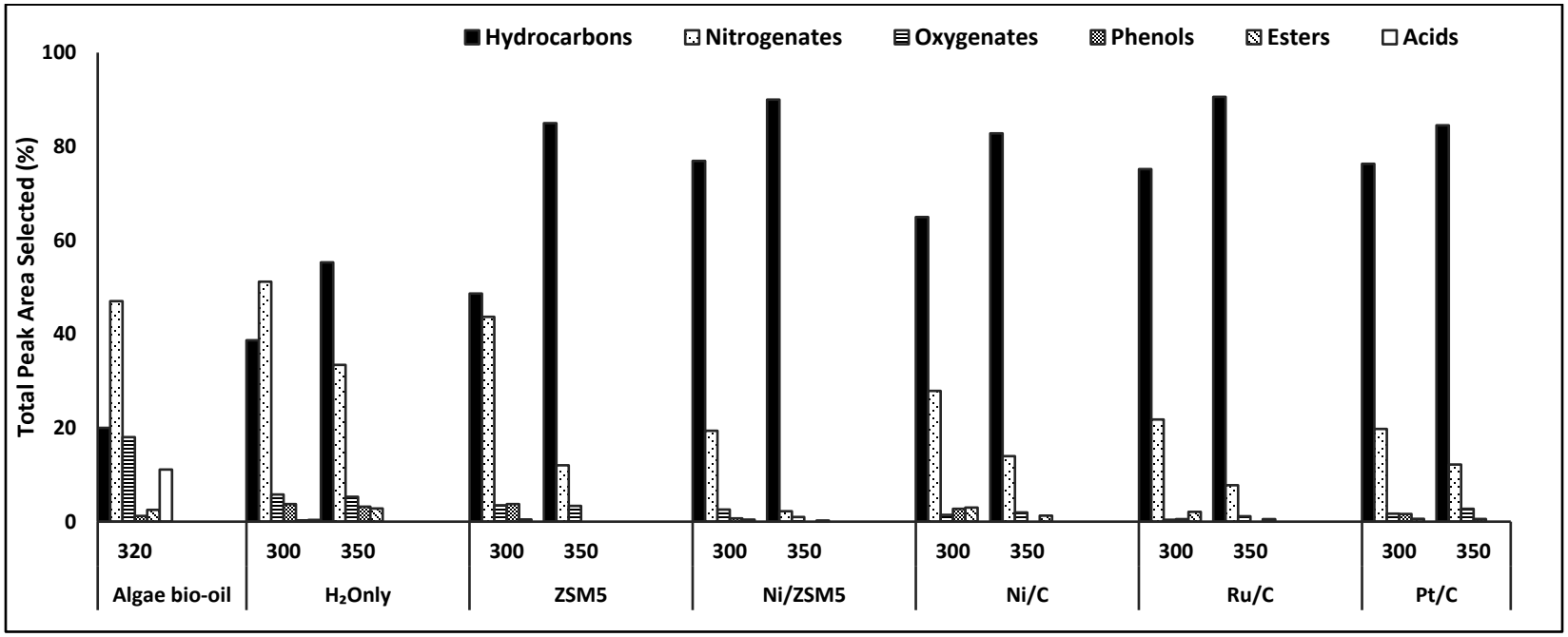

Figure 3. 2. Major chemical composition of algae bio-oil obtained at 320°C and upgraded bio-oils obtained at 300 and 350°C.

Figure 3.2 compares the peak area percentage of different chemical compound groups of the original algae bio-oil, hydrotreated oil (H_2 only) and catalytically upgraded bio-oils. The complete list of compounds under each group is listed in Appendix (Table B1). Due to the presence of high protein in *Nannochloropsis* biomass, the bio-oil produced had a very high nitrogenated compounds. The presence of nitrogenated compounds in the original bio-oil used for upgrading is not desirable because it not only promotes catalyst poisoning and deactivation, but also inhibits the HDS of sulfur-containing compounds through competitive adsorption [29]. The major nitrogenated chemical compounds present in the algae bio-oil were heterocyclic compounds such as indoles, pyridines, pyrrolidines, carbazoles, etc. and aliphatic amines, amides, and nitriles such as tetradecanamide, hexadecanamide, hexadecanitriles etc. According to [29], pyridine and piperidine were strong poisons for the hydrogenation pathways. Significant HDN was observed at both temperatures for the upgraded bio-oils when compared to original algae bio-oil. This can be observed with increase in hydrocarbons in the upgraded bio-oils. Some of the nitrogenated compounds undergo HDN to give alkanes and alkenes. For example, pyridine undergoes HDN to form intermediate piperidine followed by ring opening of piperidine to form pentylamine which subsequently forms pentane after nitrogen removal as ammonia [30]. However, complete HDN was not possible as some nitrogenated compounds like carbazoles which are least reactive N-compounds did not undergo HDN [29]. This may also be due to slow denitrogenation of nitrogen heterocyclic compounds when compared to aliphatic amine and nitriles, as the heterocyclic compounds require hydrogenation of ring containing nitrogen before hydrogenolysis of carbon-nitrogen bond [31]. Although, all the catalysts showed a reduction in nitrogenated compounds, the maximum reduction of nitrogenated compounds was observed for upgraded bio-oil using Ni/ZSM5 at 350^{o}C. Hydrogen only treated bio-oil at 350^{o}C also showed a significant decrease in

nitrogenated compounds in bio-oil. However, the decrease was not as substantial when compared to the catalytic runs.

Organic acids, phenols, and other oxygenated compounds are other compounds that are undesirable in biofuels due to the presence of oxygen atoms. Organic acids and phenols help to increase TAN, which promotes corrosion of engines. The original algae bio-oil contained organic acids. Organic acids undergo hydrodeoxygenation via decarboxylation reaction to give hydrocarbons. The major organic acids present in the original algae bio-oil were C14:0, C16:0, and C18:0. No traces of acids were observed in upgraded bio-oils, but a subsequent increase in hydrocarbons, mainly C15, C16, C17, and C18 were high as shown in Figure 3.3, which can be related to decarboxylation of fatty acids. On the other hand, organic acids might also have undergone reaction with amines to form amides [32, 33], which were present at both the reaction temperatures. Complete HDO of organic acids were observed in upgraded bio-oils from all the catalysts and in H_2 only treated oils. This is in agreement with the previous study [18] that decarboxylation of organic acids does not require catalysts and it is a thermal process. Although complete removal of organic acids was observed in the upgraded bio-oils, low TAN was still present. The presence of low amount of TAN was mainly due to the presence of phenols in the upgraded oil. At 300^{o}C, complete deoxygenation of phenols was not observed, instead they were concentrated in the upgraded bio-oil. This increase in phenols may be due to decomposition of ethers and alcohols. Formation of phenols is the first step (stabilizing step) during HDO of alcohols and ethers [28]. Similar behavior was observed at 350^{o}C for hydrogen only run. However, in the case of catalytic runs at 350^{o}C, complete hydrodeoxygenation of phenols was observed, resulting in an increase in aromatic hydrocarbons such as benzene and cyclohexane in the upgraded bio-oils. This complete hydrodeoxygenation of phenols might have been due to direct hydrogenolysis

of hydroxyl group or direct dehydration of saturated cyclic alcohol formed by hydrogen addition to the aromatic ring [34]. The varied proportion of oxygenated compounds were still present in the upgraded bio-oils; this may be due to residual oxygen product formed during HDO. Different compounds have different reactivity towards HDO, and according to Furimsky [28] reactivity of the various compounds followed the trend alcohols > ketones > alkyl esters > carboxylic acids > phenols.

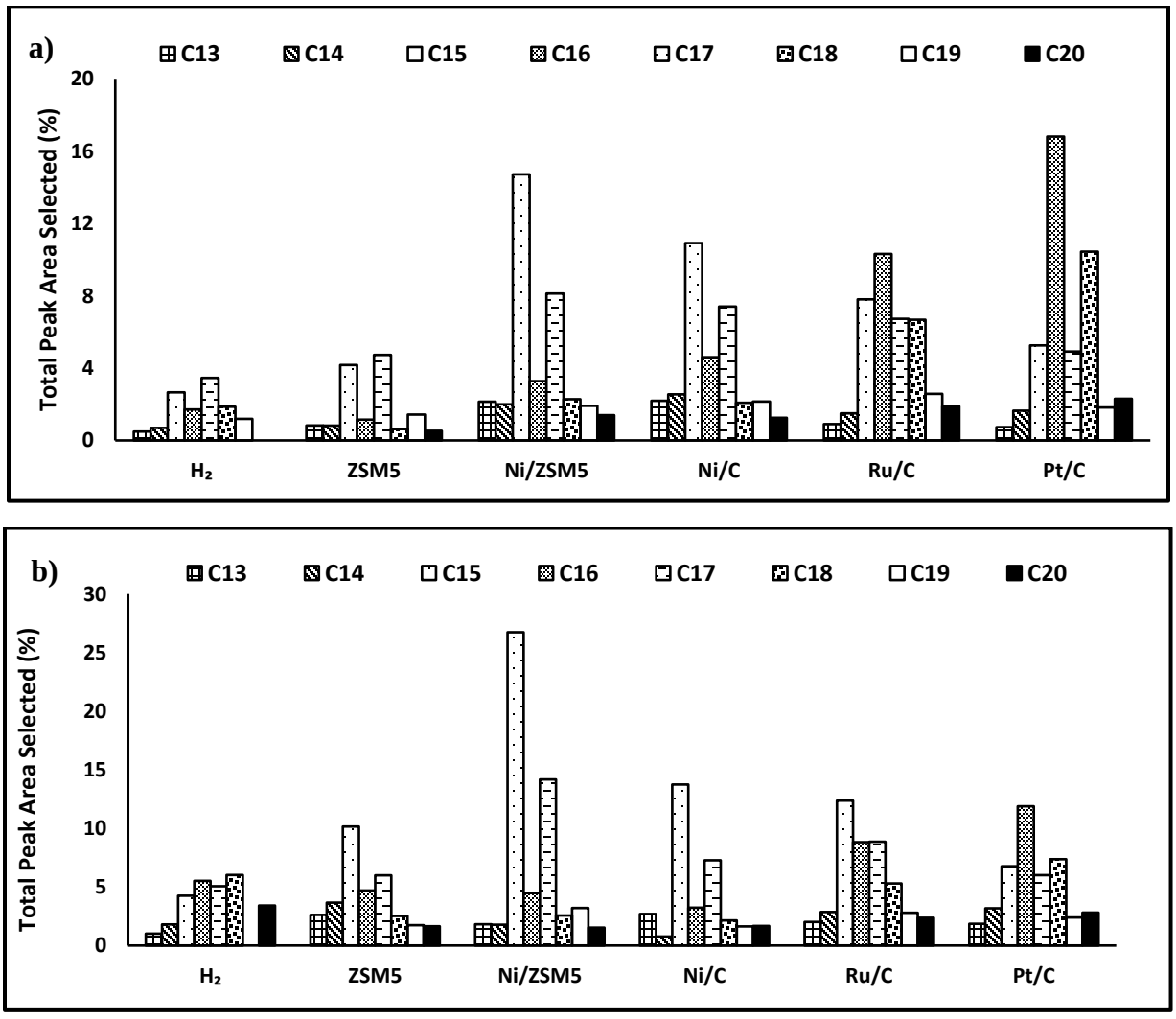

Figure 3. 3. Distribution of the major unbranched hydrocarbon in the upgraded bio-oils obtained using different catalysts at a) 300ºC and b) 350ºC.

A significant increase in hydrocarbons was observed in the upgraded bio-oils. HDO and HDN of oxygenated and nitrogenated compounds, respectively, increased hydrocarbon yields. Hydrocarbon increased in the range between 170-350% peak area when compared to the original bio-oil. All the catalytic runs gave a high amount of hydrocarbons when compared to hydrogen only run. This relates to the HDO ability of the catalysts in use. Large unbranched hydrocarbons from C10 to C29 were present in the upgraded bio-oils. The percentage of unbranched hydrocarbons in the upgraded bio-oils increased with the increase in upgrading temperature as shown in Figure 3.3. The upgraded bio-oils obtained from hydrogen only run and ZSM5 gave a lower percentage of unbranched hydrocarbons when compared to other catalysts in use. Comparison between aliphatic and aromatic hydrocarbon showed that the majority of hydrocarbons (about 58-80% peak area) were aliphatic hydrocarbons. The highest amount of aromatics (about 42% peak area) were obtained for the ZSM5 upgraded bio-oil.

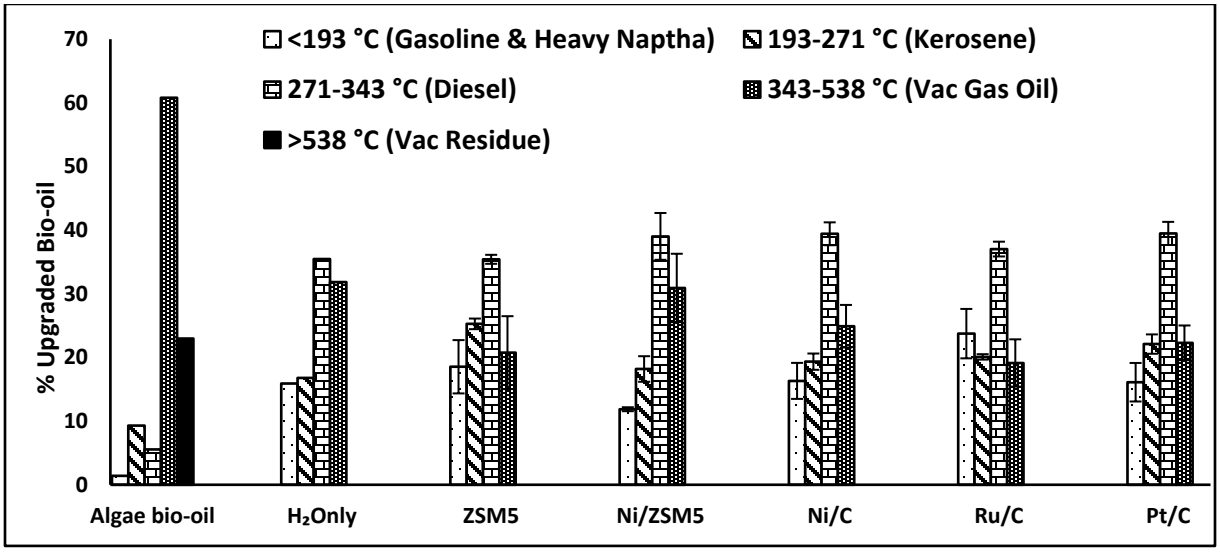

Figure 3. 4. Boiling point distribution of upgraded oils obtained at 350°C compared to the original algae bio-oil.

Figure 3.4 shows the simulated distillation of the original algae bio-oil and upgraded bio-oils at 350^{o}C. Boiling points were grouped as reported by Vardon et al. [35]. The boiling point distribution of the upgraded bio-oils showed the absence of bio-oil fraction in vacuum residue range, which was prominent (about 23%) in algae bio-oil. The algae bio-oil in vacuum residue range either may have been converted to lower boiling point fractions or may have contributed to coke formation. The original algae bio-oil had a maximum fraction in vacuum gas oil range (343-538^{o}C). However, upgraded bio-oils from all the experiments had a maximum fraction (35-40%) in diesel range (271-343^{o}C) which were similar to the result obtained by Elliot et al. [21] but where higher than that obtained by Duan et al. [36] in supercritical water environment. Among all the catalysts, Ru/C catalyst had a maximum fraction (24%) of upgraded bio-oil in gasoline and heavy naphtha range while the ZSM5 catalyst had a maximum fraction (25%) of upgraded bio-oil in kerosene range. Hydrogen only run had a maximum fraction of bio-oil in vacuum gas oil range. This shows that the catalyst helped in increasing the lower boiling point fractions in upgraded bio-oils. Overall, the simulated distillation revealed that the algae bio-oil which had very high fraction above 343^{o}C were upgraded to lower boiling point fraction bio-oil or it may have contributed to coking.

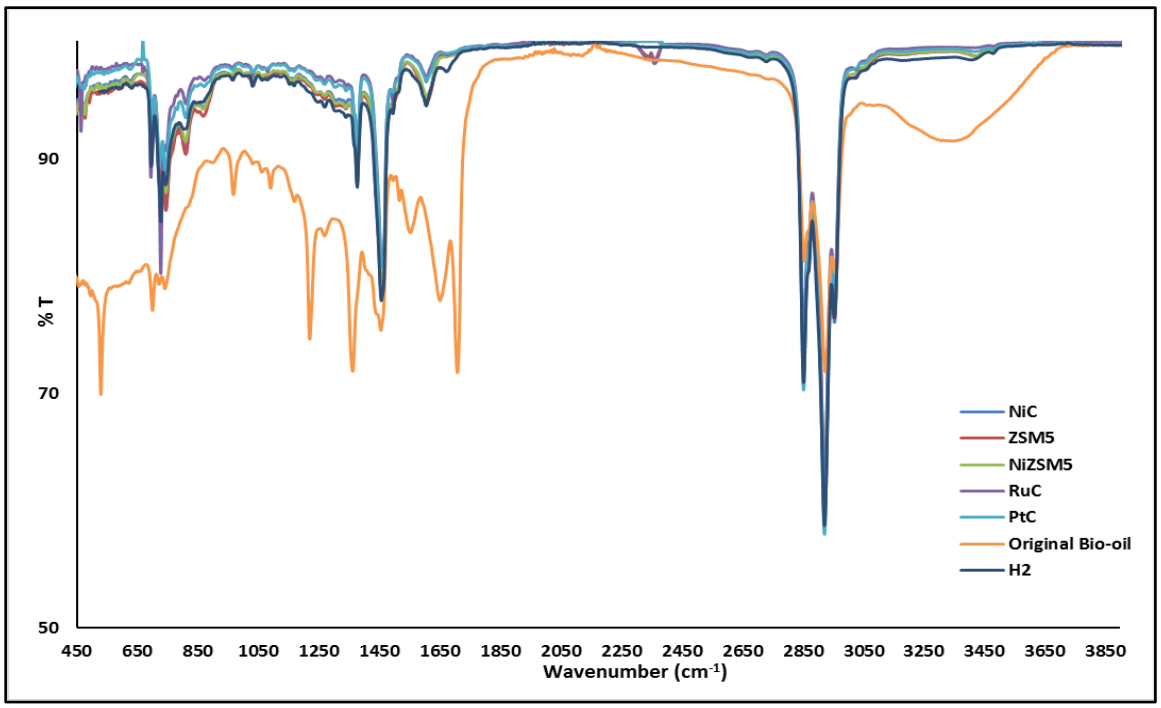

Figure 3. 5. FTIR spectra of algae bio-oil and upgraded bio-oils obtained at 350ºC.

FTIR analyses of the original algae bio-oil and upgraded bio-oils were performed to compare the changes in their functional groups before and after upgrading. The spectral band assignments were based on the literature [37]. FTIR spectra of upgraded bio-oils obtained at 350ºC are shown in Figure 3.5. Significant stretching observed for algae bio-oils at 3500-3100 cm^{-1} were absent or very insignificant in the case of upgraded bio-oils, indicating removal of O-H and N-H functional groups during the upgrading process. This correlates with GC/MS data of the upgraded bio-oil which show a significant decrease in phenols, alcohols, and nitrogenated compounds containing amines and amides. The decrease in amines and amides were also shown by the reduction in strength of bending peaks at 1680-1600 cm^{-1} and 1575-1525 cm^{-1} when compared to algae bio-oil peak. Hydrogen only run had the highest absorption at 1600-1680 cm^{-1} suggesting the presence of more amines and amides than the catalytic runs. The most prominent stretching was observed at 2800-3000 cm^{-1} which attributed to antisymmetric and symmetric stretching of

CH bonds in $–CH_3$, $-CH_2-$ of aliphatic compounds. The $-CH_2-$ scissor vibration and $–CH_3$ bending were also prominent at 1450-1475 cm^{-1} and 1370-1380 cm^{-1} regions, respectively. The other noticeable stretching observed for algae bio-oils at 1690-1730 cm^{-1} due to stretching of C=O bonds were completely absent in upgraded bio-oils. This may be due to decarboxylation of organic acids and decarbonylation of ketones and aldehydes during upgrading. Some absorption peaks observed at 600-900 cm^{-1} were attributed to the C-H bending from aromatics.

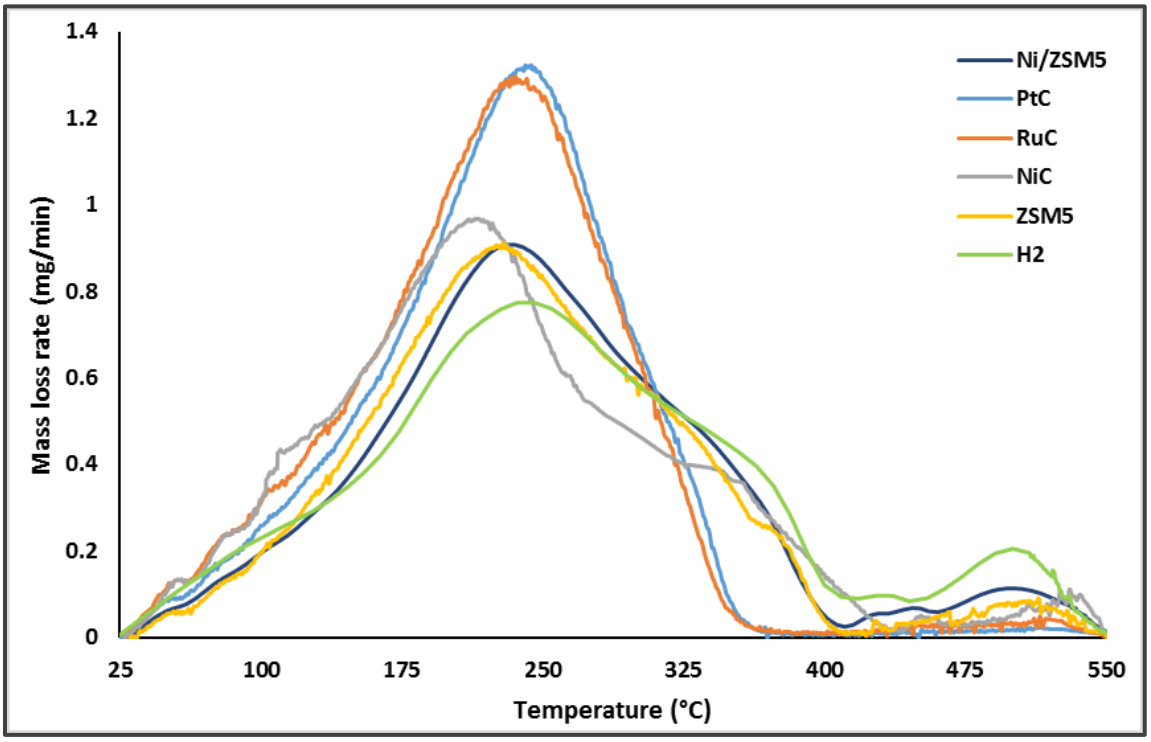

Figure 3. 6. DTG curves of upgraded bio-oils at 350°C.

Error! Reference source not found.6 shows the mass loss rate curves (derivative t hermograms-DTG curves) of upgraded bio-oils at 350°C. The mass loss rate of upgraded bio-oil differed with the use of catalysts. Pt/C and Ru/C gave the maximum mass loss rate in the temperature range between 25-350°C and were suppressed thereafter. This supports the findings from simulated distillation that the upgraded bio-oil obtained with the use of noble catalysts had

the maximum fractions below 350^{o}C. Shoulder peak (300-400^{o}C) on the right side of ZSM5, Ni/ZSM5, Ni/C and H_2 only (hydrotreated) upgraded oils were observed. This may represent the degradation of higher boiling point fractions found in the upgraded bio-oils. Hydrotreated oil had minimum mass loss rate below 350^{o}C but was maximum above 350^{o}C. This suggests that hydrotreated bio-oil had heavier compounds than catalytically hydrotreated bio-oil.

3.3.3. Gas composition analysis

Table 3.3 and 3.4 illustrates the hydrogen consumption, and gas composition at the upgrading temperatures of 300 and 350^{o}C. The amount of hydrogen consumed at 350^{o}C were significantly higher (p-value<0.05) than the amounts at 300^{o}C for all the catalyst used, except for Ni/ZSM5 whose values were not significantly different (p-value=0.118). Hydrogen consumption were higher with the use of noble metal catalysts compared to Ni or ZSM5. Apart from catalysts, hydrogen consumption also depends on the residence time as high residence time of 10 h was used in this study. Longer residence time leads to high hydrogen consumption [22]. For all catalysts, methane, carbon monoxide, carbon dioxide, and some higher alkanes (ethane and propane) were the major gases present in the gaseous product which were also observed by [20]. In addition, a considerable amount of nitrogen was also present in the gaseous product which may be produced from some nitrogenated compounds in the algae bio-oil. Both temperature and catalyst used had a significant impact on the gas composition. The gas became richer in hydrocarbons as temperature increased. The Pt/C gave the highest yields of methane and higher alkanes compared to other catalysts. Methane constituted the highest fraction of gaseous product. The higher amount of methane and a lower amount of CO and CO_2 might suggest that the methanation reaction (Eq. 4 & 5) was more prominent during the upgrading process. The presence of high amount of hydrogen during the reaction could also favor reverse water-gas shift reaction (Eq. 6) producing carbon

monoxide which subsequently undergoes methanation reaction. The presence of carbon monoxide and carbon dioxide are mainly due to decarbonylation and decarboxylation of bio-oil compounds during upgrading. Ru/C, Ni/C, and Ni/ZSM5 catalysts promoted the production of CO and CO_2 as compared to other catalysts. This was in agreement with the previous study [18] which suggested efficient removal of CO compounds with the use of Ru and Ni catalyst.

$$CO + 3H_2 \rightarrow CH_4 + H_2O \quad [4]$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad [5]$$

$$CO_2 + H_2 \leftrightarrow CO + H_2O \quad [6]$$

Table 3. 3. Hydrogen consumption and gas composition obtained at 300ºC.

Catalyst	Hydrogen consumed (mg/g feedstock)	Gas composition of the product (Mol% as received)						
		N_2	CH_4	CO	CO_2	C_2H_6	C_3H_8	C_4H_{10}
ZSM5	8.94±2.32[a]	1.6±0.51[a,b]	0.64±0.01[d]	0.13±0.01[d]	1.01±0.58[c]	0.46±0.01[a]	0.74±0.03[b]	0.16±0.01[c]
Ni/ZSM5	25.12±0.53[b]	0.71±0.01[a,b]	10.05±2.64[c]	4.57±0.51[a]	2.02±0.04[a]	3.70±1.80[a]	2.86±1.11[b]	0.65±0.31[c]
Ni/C	21.99±1.91[b]	0.16±0.10[b]	6.45±0.03[d]	3.09±0.01[b]	2.02±0.01[a]	2.22±0.01[a]	1.85±0.01[a,b]	0.69±0.00[ψ,b,c]
Ru/C	34.22±0.05[c]	1.33±0.69[a,b]	14.61±0.68[b]	1.07±0.07[c]	1.85±0.25[a]	3.45±0.01[a]	4.37±0.21[a]	1.01±0.03[a,b]
Pt/C	37.87±2.49[c]	1.01±0.49[a]	20.82±7.72[a]	0.95±0.28[c]	1.04±0.01[b]	6.52±4.40[b]	2.13±0.10[b]	1.23±0.62[a]

Different alphabets in the superscripts of each gas (column) denote the values are statistically significant at different catalyst type (rows) (α=0.05). numbers after ± symbol denotes standard deviation. nd: not detected; [ψ] standard deviation value is below one hundredth.

Table 3. 4. Hydrogen consumption and gas composition obtained at 350ºC.

Catalyst	Hydrogen consumed (mg/g feedstock)	Gas composition of the product (Mol% as received)						
		N_2	CH_4	CO	CO_2	C_2H_6	C_3H_8	C_4H_{10}
ZSM5	26.93±1.41[a]	1.09±0.16[a]	11.46±0.08[a]	2.88±1.06[a]	1.89±0.01[a,b]	5.36±0.02[a]	3.08±0.01[a]	1.05±0.01[a,b]
Ni/ZSM5	28.59±1.78[a]	1.13±0.62[a]	12.63±1.02[a]	4.99±0.07[b]	2.92±0.65[a,b]	5.33±0.48[a]	3.91±0.37[b]	0.96±0.14[b]
Ni/C	30.37±0.26[a]	0.34±0.02[a]	16.38±0.03[b]	3.36±0.01[a]	2.72±0.01[a]	6.75±0.02[b]	0.02±0[ψ,c]	nd
Ru/C	40.47±0.42[b]	1.26±0.26[a]	23.20±2.19[c]	1.24±0.10[c]	3.38±1.73[a]	7.50±0.49[b]	6.03±0.22[d]	1.14±0.09[a,c]
Pt/C	43.68±0.05[b]	0.58±0.05[a]	30.39±0.02[d]	1.51±0.01[c]	1.64±0.01[b]	9.71±0.01[c]	7.02±0.05[e]	1.24±0.01[c]

Different alphabets in the superscripts of each gas (column) denote the values are statistically significant at different catalyst type (rows) (α=0.05). numbers after ± symbol denotes standard deviation. nd: not detected; [ψ] standard deviation value is below one hundredth.

3.3.4. Catalyst characterization

BET surface area of both fresh and spent catalysts is given in Table 3.5. For all the catalysts, a significant decrease in surface area was observed. The decrease in surface area of catalyst was mainly due to the formation of non-desorbed heavy products called coke in the pores or on the outer surface of the catalyst. Comparatively, a higher decrease in surface area was obtained at 350^{o}C than at 300^{o}C. Except for Ru catalyst, all other catalysts observed decreased in surface area with an increase in temperature. The maximum decrease in surface area was observed for ZSM5 catalyst at 350^{o}C. This decrease in surface area for ZSM5 at 350^{o}C was due to coking of ZSM5 catalyst. The strong acid sites in ZSM5 play an important role in the formation of coke precursors, which subsequently undergo condensation reaction, rearrangement reaction, and various hydrogen transfer steps to produce large polynuclear aromatic molecules as coke [38]. Apart from the catalytic effects, thermal degradation of bio-oil compounds at high temperature and reaction time also might have caused coke formation. At high temperature, coke mainly constitutes of high polyaromatic compounds, however, at low temperature they are not polyaromatic [25]. The coke deposition in the catalyst was further supported by SEM-EDS study as shown in Appendix (Table B2). In addition to the deposition of coke, a considerable amount of other metals such as iron, chromium and nickel was also observed which could have been extracted from the algae bio-oil and the stainless-steel reactor parts. SEM pictures given in Appendix (Figure B3) showed some sinters of iron in the catalysts.

Table 3. 5. BET surface area of fresh and spent catalysts.

Catalyst	Surface Area (m^2/g)$^{\Psi}$		
	Original Catalyst	Spent Catalyst	
		300°C	350°C
10% Ni/C	588.57	270.30 (-54.0 %)*	89.66 (-84.7 %)
ZSM5	311.81	134.84 (-56.75 %)	21.82 (-93.00 %)
5% Ru/C	550.49	131.88 (-76.04 %)	182.03 (-66.93 %)
5% Pt/C	1162.92	473.43 (-59.29 %)	451.91 (-61.14 %)

* Values in parenthesis denotes % loss; $^{\Psi}$ denotes one-point data.

3.4. Conclusions

The effect of five heterogeneous catalysts (ZSM5, 10% Ni/ZSM5, 10% Ni/C, 5% Ru/C and 5% Pt/C) on the upgrading of algae bio-oil produced from hydrothermal liquefaction of *Nannochloropsis* sp. were studied at 300 and 350°C. The upgraded bio-oil yields were higher at 300°C, whereas a better-quality fuel was obtained at 350°C. Ni/C catalyst gave the maximum upgraded bio-oil yields (61 wt.%) at 350°C. Around 65 to 75% decrease in nitrogen content, 95 to 98% decrease in TAN was observed upon catalytic upgrading, whereas hydrogen only run gave 50% and 75% reduction of nitrogen content and TAN, respectively at 350°C.

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Chapter 4

Effect of Residence Time on Catalytic Upgrading of Bio-oil Produced from Hydrothermal Liquefaction of Algae

Abstract

Upgrading study of bio-oil produced from hydrothermal liquefaction of algae was performed to investigate the effect of residence time on the product yields and properties. The upgrading study was performed at a residence time of 2, 4, 6 and 10 h using 5% Ru/C at a catalyst loading of 16.67 wt.% and a reaction temperature of 350^{o}C. The upgraded bio-oil yields were observed to be maximum (60.2 wt.%) at 4 h and decreased as the residence time increased thereafter. Increase in the residence time improved the upgraded bio-oil properties. The maximum higher heating value (44.32 MJ/kg) and the lowest total acid number (0.95 mg of KOH/g), viscosity (2.65 cSt), nitrogen content (2.18 wt.%) and sulfur content (0.02 wt.%) were observed at 10 h. However, the maximum (44.37%) overall energy recovery was observed at 4 h. Simulated distillation of the upgraded bio-oils showed that the maximum fraction (46-54%) of the upgraded bio-oils was still in the vacuum gas oil range. Hydrogen consumption increased with the increase in the residence time.

Keywords: *algae, hydrothermal liquefaction, upgrading, residence time, Ru/C*.

4.1. Introduction

Conversion of biomass to renewable fuels and valuable chemicals have a significant potential in addressing environmental issues, national energy security, and employment opportunities. Among the different types of biomass, algae are considered as one of the promising feedstock for biofuel production because of its high productivity, flexibility in growing condition and its tunable biochemical properties [1]. Algae, being a wet feedstock can be converted to biofuels in a sustainable way through hydrothermal liquefaction process. Hydrothermal liquefaction (HTL) is a promising thermochemical process, which utilizes water at sub- and super-critical temperature (250-380^{o}C) and pressure (7-30 MPa) as reactant and reactions medium, thus eliminating energy-intensive drying process, to produce bio-oil. This process not only converts the lipid portion but utilizes the whole algae for bio-oil production [2].

The bio-oil produced from HTL of algae has positive attributes such as high heating value (32-36 MJ/kg) and comparatively lower oxygen content (7-10 wt.%) [3]. However, on the downside, the bio-oil is highly viscous (40-68 cP at 60^{o}C) [4], acidic (29-118 mg of KOH/g) [3] and has high nitrogen content (2-9 wt.%) [3, 5] which are undesirable as fuel. Thus, it can neither be blended with petroleum crude nor directly used as drop-in fuel. Therefore, catalytic upgrading is required to make it more applicable as fuel. In the last decade, several studies on the catalytic upgrading of algae bio-oils have been performed using different types of catalysts such as Pd/C, Pt/C, Ru/C, HZSM-5, Mo_2S, CoMo/γ-Al_2O_3, Ni/SiO_2-Al_2O_3, etc. [6–13]. Apart from the type of catalysts, the upgrading process depends on other operating parameters such as temperature, residence time and catalysts loading. Duan and Savage performed an optimization study in a supercritical water

environment using three catalysts (Mo_2C, Pt/C and HZSM-5), three reaction temperatures (430, 480 and 530^oC), three residence time (2, 4 and 6 h) and three catalyst loadings (5, 10 and 20 wt.%) [7]. The authors observed that the reaction temperature was always the most influential factor. They noted that the influence of the catalysts and reaction time to be the greatest on the upgraded oil properties and observed the lowest temperature of 430^oC to be the best for producing upgraded bio-oil with the highest hydrogen content and HHV. In another study using Pd/C as catalysts, Duan and Savage [6] investigated the effect of residence time (2, 4, 6 and 8 h) and catalyst loading (5 to 80 wt.%) in a supercritical water environment. They found that the longer residence time and a higher catalyst loading decreased the upgraded bio-oil yield [6]. The highest yield was observed at 4 h residence time and catalyst loading of 20 wt.%. Li et al. also performed optimization study (temperature: 400, 450 and 500^oC; residence time: 0.5, 1, 2 and 4 h; catalyst loading: 5, 10, 25 and 50 wt.%) in the presence of HZSM-5 and without adding water [13]. The authors reported the upgraded bio-oil yield to decrease from 75 to 44 wt.% with the increase in temperature and catalyst loading. However, the residence time had no significant effect on the upgraded bio-oil yields. At a lower temperature (400^oC), aliphatic hydrocarbons and some amines were prominent whereas at 500^oC aromatic hydrocarbons were found to be abundant and a complete removal of amines was observed.

The above-given studies were mostly conducted at a high-temperature (>400^oC). In chapter 3, we found that the upgraded bio-oil obtained at a lower temperature of 350^oC had similar values of H/C, O/C, N/C, and HHVs compared to that obtained at a high-temperature upgrading (>400^oC) [6, 13]. However, the drawback of the study was the residence time of 10 h employed, which was higher than that used in the other studies.

Apart from the temperature, residence time also affects the process economics as it contributes to the energy input. Hence, in this work, the influence of residence time during upgrading at 350^{o}C was investigated to find the optimum time for obtaining quality upgraded bio-oil.

4.2. Materials and Methodology

4.2.1. Materials

Algae (*Scenedesmus* sp.) biomass was provided by Arizona Center for Algal Technology and Innovation (Mesa, Arizona) in the form of a slurry (17 wt.% solids). The commercial 5% Ru/C catalyst was obtained from Sigma-Aldrich. Ultrahigh purity grade (99.999%) carbon dioxide, hydrogen, helium, and argon gases were purchased from Airgas Inc. (Charlotte, NC). HPLC grade acetone, dichloromethane, and toluene were purchased from a chemical supplier VWR (Atlanta, GA). De-ionized water was used for HTL experiments.

4.2.2. Hydrothermal liquefaction of algae

Hydrothermal liquefaction of *Scenedesmus* sp. was performed in a 1 L batch reactor system (Parr Instruments Co., Moline, PA) at 320^{o}C for a residence time of 30 minutes with a solid loading of 15 wt.%. Approximately, 100 g of algae (dry weight basis) was loaded into the reactor; the headspace was purged with helium to remove residual air and to create an inert environment. After purging with helium, the reactor was pressurized to an initial pressure of 100 psig and was heated to the desired reaction temperature. After the residence time of 30 minutes, the reactor was cooled down using cold water and the residual gas formed was vented off. The product separation procedure was followed as described in the previous paper [14]. The bio-oil obtained had a relatively high moisture content, and

dichloromethane was used to remove the aqueous phase. Finally, dichloromethane was separated by vacuum-evaporation, and bio-oil with low final moisture content (1.25 wt.%) was obtained which was used for upgrading studies.

4.2.3. Upgrading of bio-oil

Upgrading was performed in a 450 ml batch reactor (Parr Instruments Co., Moline, PA) at 350^{o}C and 5% Ru/C (catalyst loading of 16.67 wt.%). Four residence times of 2, 4, 6 and 10 h were selected to study the effect of residence time. In a typical experiment, 8 g of catalyst was first loaded into the reactor, and a repeated cycle of nitrogen purging was performed to create an inert headspace. After purging with nitrogen, hydrogen gas was purged to remove residual nitrogen, and the pressure was maintained at 1000 psi for catalyst reduction. Reduction of catalyst was carried out at 300^{o}C for a residence time of 1 h. After the reduction, the reactor was subsequently cooled down to room temperature and again purged with nitrogen and argon before opening. A known amount of bio-oil (approximately 40 g) was loaded into the reactor, and the similar procedure of purging with nitrogen during reduction was followed. After this, hydrogen was purged to remove residual nitrogen, and the hydrogen pressure was maintained at 1000 psig. This high pressure of hydrogen was maintained to ensure higher solubility of hydrogen in the oil resulting in an increase in the reaction rate and a further decrease in coke formation [15].

The reactor was then heated to the reaction temperature (350^{o}C) while agitating the mixture at 500 RPM for the given residence time. Product separation procedure was followed as described in section 3.2.3. The product yields for upgrading studies were calculated using Eq. (1). Hydrogen consumption and carbon dioxide consumption during

the catalytic upgrading was calculated according to Eq. (2). Energy recovery was calculated using Eq. (3).

$$\text{Yields}_{\text{oil/solid residue(coke)}} = \frac{Weight\ of\ the\ product\ (dry\ basis)}{Weight\ of\ the\ algae\ bio-oil\ (dry\ basis)} \times 100 \quad (1)$$

$$\text{Hydrogen/Carbon dioxide consumption} = \left[(P_i/T_i) - \left(X_{n,f} \times P_f/T_f\right)\right] \times V_{gas}/R \quad (2)$$

$P_{i\ or\ f}$ = initial/final reactor pressure

$T_{i\ or\ f}$ = initial/final reactor temperature at 298 K

$X_{n,f}$ = final mole fraction of hydrogen/carbon dioxide in a produced gas

V_{gas} = reactor volume occupied by gas

R= gas constant (8.314 J/K.mol)

$$\text{Energy recovery} = \frac{HHV_{BIO-OIL} \times Mass_{BIO-OIL}}{\left[HHV_{ALGAE} \times Mass_{ALGAE} + HHV_{H_2} \times Mass\ consumed_{H_2}\right]} \times 100 \quad (3)$$

4.2.4. Product analysis

All the upgrading products; upgraded bio-oils, gas and solid residue were analyzed as described in section 3.2.4.

4.2.5. Statistical Analysis

All experiments were performed in duplicates for each condition, except for hydrogen only experiment at 10 h residence time to verify the reproducibility of data. Statistical analysis (ANOVA, Tukey's HSD) of all the data was performed using statistical software (SAS) to evaluate if the obtained results were statistically different. All the statistical analyses were performed at 95% confidence interval.

4.3. Result and Discussion

4.3.1. Algae characterization and hydrothermal liquefaction

Algae (*Scenedesmus* sp.) used for HTL had protein, carbohydrate and lipid content of 30.06 wt.%, 54.17 wt.%, and 17.83 wt.%, respectively. The ultimate analysis of the biomass showed carbon, hydrogen, nitrogen, sulfur and oxygen content to be 51.51±0.61 wt.%, 7.26±0.07 wt.%, 10.97±0.56 wt.%, 0.77 wt.% and 30.26±0.78 wt.%, respectively. The bio-oil yield of 46.38±2.44 wt.% was obtained from the HTL of the algae biomass. The bio-oil obtained had a higher heating value of 34.51±0.18 MJ/kg which was comparatively greater than that obtained from HTL of lignocellulosic biomass [16]. However, the bio-oil still had a high viscosity (49.50±0.25 cSt), high TAN (18.53±0.27 mg of KOH/g) and high nitrogen content (5.74±0.56 wt.%) as shown in **Table 4.1** and **Table 4.2**.

4.3.2 Effect of residence time on upgrading product yields

The upgraded bio-oils (UBO) obtained were reddish-brown, and no significant change in color was observed with the increase in residence time. **Figure 4.1** shows the product distribution obtained from upgrading of the algae bio-oil at different residence time. The increase in residence time had a significant (p-value=0.001) effect on the upgraded bio-oil yield. The upgraded bio-oil yield decreased from 59.30 wt.% to 50.44 wt.% with the increase in residence time from 2 to 10 h, respectively. The upgraded bio-oil yield was maximum (60.2 wt.%) at 4 h residence time and decreased with the increase in residence time thereafter. The decrease in upgraded bio-oil yield may be due to the prominence of secondary reactions such as cracking to form a gaseous product at a longer residence time [17]. No significant change in the upgraded bio-oil yields was observed at

the lower residence time of 2 and 4 h, which was in agreement with the results obtained by Li et al. [13]. Duan and Savage also reported no change in the upgraded bio-oil yields at 2 and 4 h residence time in their upgrading optimization (in supercritical water environment) study using 5% Pd/C catalyst at 400^{o}C [6]. At 10 h residence time, the upgraded bio-oil yield was observed to be higher for a non-catalytic (hydrogen only) experiment (61.74 wt.%) when compared to the catalytic experiment. This decrease in the upgraded bio-oil yield with the use of catalyst further suggested that the catalytic cracking and bio-oil decomposition was prominent with the use of Ru/C catalyst at 350^{o}C.

A significant (p-value=0.034) decrease in solid residue or coking was observed with the increase in residence time from 2 to 10 h. The solid residue decreased from 16.6 wt.% to 12.98 wt.% with the increase in residence time from 2 to 10 h. Subsequently, a significant (p-value=0.042) increase in gaseous yield was observed with the increase in residence time from 2 to 10 h. The maximum (22.1 wt.%) gas yield was observed at a residence time of 10 h. This decrease in solid residue and increase in the gaseous yields at a longer residence time may be due to the increased catalytic cracking of the bio-oil. At 10 h residence time, the non-catalytic experiment gave a higher solid residue (19.45 wt.%) and a lower gaseous yield (14.46 wt.%) compared to the catalytic experiment. This decrease in solid residue and increase in gaseous yield suggested that the catalytic action of Ru/C prevented solid residue formation but intensified the formation of gaseous product at a longer residence time.

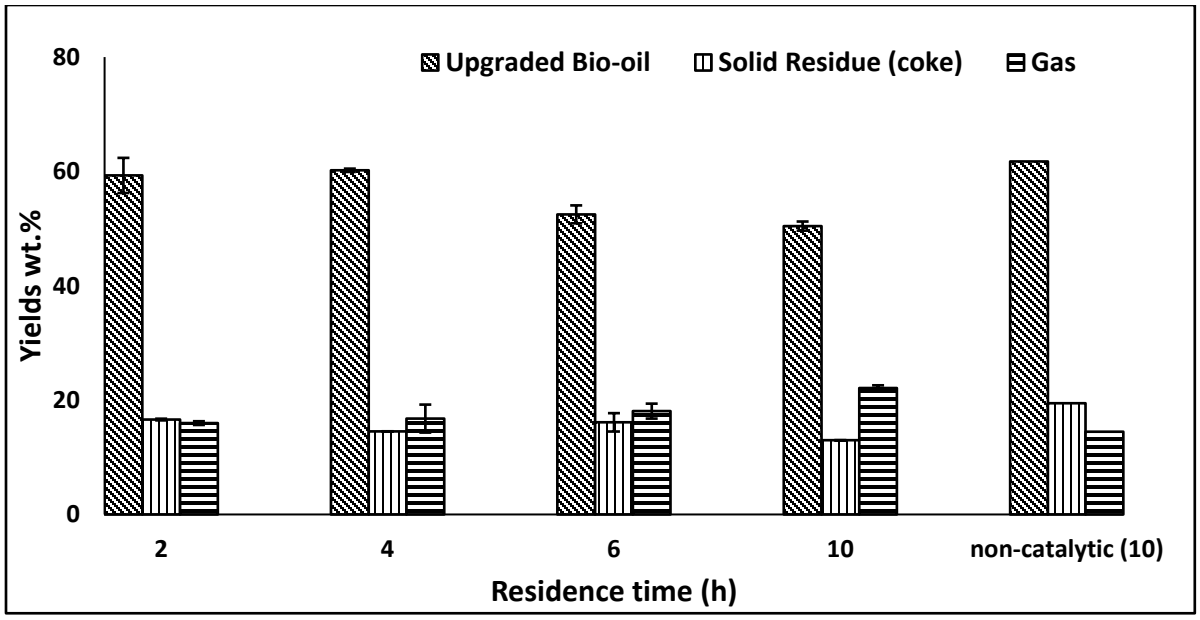

Figure 4.1. Product distribution from the upgrading of algae bio-oils at different residence time. (Note: All the experiments were performed in duplicates at 350°C. Only the non-catalytic experiment was a single point.)

4.3.3. Effect of residence time on Upgraded Bio-oil Properties

Table 4.1 shows the effect of residence time on the properties of upgraded bio-oil. The upgraded bio-oil had water content below 0.5 wt.%. A significant (p-value <0.05) increase in higher heating value was obtained for the upgraded bio-oils when compared to the original bio-oil. The heating values increased significantly (p-value=0.0001) with the increase in residence time from 6 h to 10 h. The heating value of the non-catalytic experiment was lower than that of the catalytic experiment. The total acid number (TAN) of the upgraded bio-oils were lower than that of the original bio-oil, indicating a decrease in acidic and phenolic compounds present in the original bio-oil. The TAN of the upgraded bio-oils decreased significantly (p-value=0.013) with the increase in residence time from 2 to 6 h. The TAN obtained in all the conditions were higher than the values (0.5 mg of KOH/g) for biodiesel as specified in ASTM D6751-07a [10]. The viscosity of the upgraded

bio-oils significantly (p-value<0.0001) decreased with the increase in residence time and ranged from 2.65 to 7.69 cSt for catalytic experiments. The catalytically upgraded bio-oils were more free-flowing than the original bio-oil and non-catalytic bio-oil obtained at 10 h residence time. Energy recovery was higher for non-catalytic runs. However, for catalytic runs, 2 and 4 h residence time gave the maximum energy recovery (43-44%).

Table 4.1. Properties of upgraded bio-oil obtained at different residence times.

Residence Time (hr)	Water Content (wt.%)	HHV (MJ/kg)†	TAN (mg of KOH/g)†	Viscosity (cSt) †	Energy Recovery (%)†
Biocrude	1.25±0.14	34.51±0.18	18.53±0.27	49.50±0.25	66.1±0.18
2	0.31±0.05^{a}	43.43±0.10^{a}	1.90±0.37^{a}	7.69±1.59^{a}	43.20±2.30^{a,b}
4	0.29±0.01^{a}	43.77±0.14^{a}	1.20±0.35^{a,b}	4.02±0.35^{a,b}	44.37±0.05^{a}
6	0.21±0.11^{a}	43.72±0.18^{a}	1.14±0.29^{b}	4.53±0.06^{b}	38.79±1.14^{b,c}
10	0.12±0.01^{a}	44.32±0.22^{b}	0.95±0.40^{b}	2.65±0.13^{c}	38.15±0.47^{c}
Non-catalytic (10 h)	0.12±0.10^{a}	42.82±0.17^{c}	1.74±0.05^{a}	12.40±0.0^{d}	44.76±0.17^{a}

Different alphabets in the superscripts of each group (properties/column) denote the values are statistically significant at different catalyst type (rows) (α=0.05). numbers after ± symbol denotes standard deviation. † as received.

The carbon and hydrogen content of the original bio-oil significantly increased upon upgrading as shown in **Table 4.2**. No significant change in carbon and hydrogen content was observed for the catalytically upgraded bio-oils obtained at different residence time, resulting in similar H/C ratio. However, the maximum carbon (85.43 wt.%) and hydrogen (11.94 wt.%) were observed at 10 h residence time which followed the trend of higher heating values. The carbon content of the upgraded bio-oils found in this study was greater than that observed by Duan and Savage in their optimization study [7] and was closer to the values for petroleum crude [18]. The nitrogen value of the original bio-oil drastically decreased upon upgrading and ranged between 2.18 to 3.02 wt.%. The decrease

in nitrogen and sulfur values were observed with the increase in residence time. This decrease in nitrogen and sulfur values indicated the increase in hydrodenitrogenation (HDN) and hydrodesulfurization (HDS) with the increase in residence time. Overall, improved quality of the upgraded bio-oil was observed at a longer residence time of 10 h.

Table 4.2. Elemental analysis of upgraded bio-oil obtained at different residence times.

Sample	Elemental Analysis (wt.% on dry basis)					Elemental Ratios		
	C	H	N	S¥	O*	O/C	N/C	H/C
Biocrude	73.46±0.26	9.48±0.08	5.74±0.56	0.81	11.77±0.38	0.12	0.07	1.55
2 h	84.60±0.0^{a}	11.75±0.07^{a}	3.02±0.24^{a}	0.09^{a}	0.99±0.22^{a}	0.01	0.03	1.67
4 h	84.86±0.10^{a}	11.91±0.02^{a}	2.64±0.09^{a,b}	0.05^{c}	0.88±0^{a}	0.01	0.03	1.68
6 h	84.29±2.27^{a}	11.81±0.36^{a}	2.42±0.03^{b}	0.04^{c}	1.74±2.43^{a}	0.02	0.02	1.68
10 h	85.43±0.63^{a}	11.94±0^{a}	2.18±0.10^{b}	0.02^{d}	0.50±0.43^{a}	0.00	0.02	1.68
Non-catalytic (10 h)	86.04±0.09^{a}	11.39±0.06^{a}	2.59±0.05^{a,b}	0.07^{b}	0.11±0.20^{a}	0.00	0.03	1.59

Different alphabets in the superscripts of each group (elemental group) denote the values are statistically significant at different catalyst type (rows) (α=0.05). numbers after ± symbol denotes standard deviation. * by balance, ¥ All the values had zero standard deviation.

Figure 4.2 compares the peak area percentage of different chemical groups present in the bio-oil, hydrotreated bio-oil (H_2 only) and catalytically upgraded bio-oils obtained at various residence time. The presence of high amount of protein in *Scenedesmus* biomass resulted in a high amount (60% peak area) of nitrogenated compounds in the HTL bio-oil. The major nitrogenated chemical compounds present in the *Scenedesmus* bio-oil were indoles, acridine, isoquinoline, pyrrolidines, piperazine, carbazoles, hexadecanamide, octadecanamide. The presence of nitrogenated compounds in the original bio-oil used for upgrading is not desirable because it gets adsorbed in the active sites of the catalyst and undergoes condensation and hydrogen transfer reaction to form coke [19–22]. The

formation of coke promotes catalyst poisoning, deactivation and also inhibits the hydrodesulfurization (HDS) of sulfur-containing compounds through competitive adsorption [19–22]. During upgrading process, most of the nitrogenated compounds underwent hydrodenitrogenation (HDN) resulting in a decrease of peak area % of nitrogenated compounds in the upgraded bio-oil. The nitrogenated compounds decreased from 22 % to 9 % with the increase in residence time from 2 to 10 h, respectively. No significant reduction in nitrogenated compounds was observed after 6 h, which suggested that the catalyst might have been poisoned/deactivated due to the presence nitrogenous compounds. No traces of acridine, isoquionolinone, or piperazine were found in the upgraded bio-oils as the compounds might have undergone HDN to produce hydrocarbons and ammonia. For examples, acridine undergoes aromatic ring hydrogenation to form intermediate 2-aminodicyclohexylamine followed by hydrogenolysis of C-N bond to give dicyclohexylmethane [23]. The major nitrogenated compounds observed at 2 h residence time were 2,7-dimethyl-indolizine, octadecanamide, hexadecanitrile, octadecanenitrile. A significant decrease in 2,7-dimethyl-indolizine, octadecanamide, hexadecanenitrile, and octadecanenitrile were observed with the increase in residence time as shown in **Figure 4.3a**. The appearance of new nitrogenated compounds was also observed in the form of 2,3,7- trimethylindole after 2 h residence time and it increased with the increase in residence time. However, other nitrogenous compounds like pentadecanenitrile, nonadecanenitrile were not altered. The aliphatic nitriles and amides may have under gone HDN to form hydrocarbons with the increase in residence time.

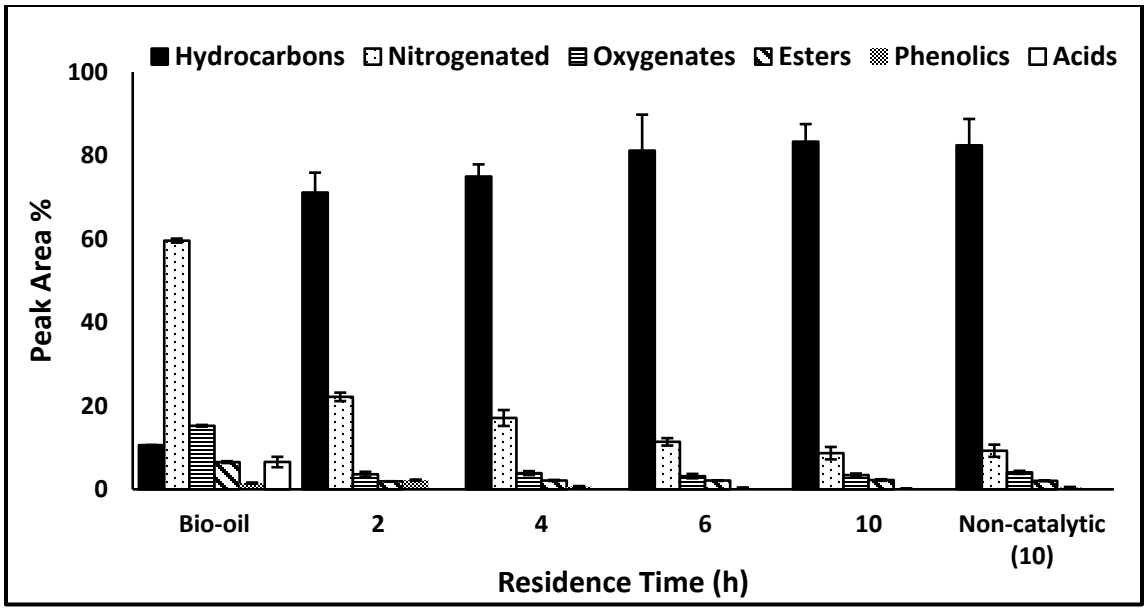

Figure 4.2. Chemical composition of the upgraded bio-oils obtained at different residence time.

Hydrocarbons in the upgraded bio-oils significantly increased when compared to the original bio-oil. The increase in the hydrocarbon is mainly due to the decrease in organic acids, oxygenates and nitrogenated compounds. Organic acids in the original bio-oil undergo decarboxylation and decarbonylation reaction to give long chain hydrocarbons [24]. For example, hexadecenoic acid in the bio-oil undergoes decarboxylation reaction to produce pentadecane. The other route of conversion of the organic acids to hydrocarbons is through hydrogenolysis of C-O bond to give aldehyde, followed by further hydrogenation to form alcohols and finally dehydration and hydrogenation to yield alkanes [24]. The upgraded bio-oils had higher aliphatic hydrocarbons than the aromatic hydrocarbons. **Figure 4.3c** shows the unbranched hydrocarbons observed in the upgraded bio-oils obtained at different residence time. Heptadecane was the most abundant unbranched alkane in the upgraded bio-oils, which may have formed due to HDN of octadecanamide or decarboxylation of oleic acid, as they were the primary compounds in

the original bio-oil. The other major unbranched hydrocarbons observed in the upgraded bio-oils were pentadecane, hexadecane, and octadecane. The increase in hexadecane and octadecane were observed with the increase in temperature. Overall, the unbranched hydrocarbons increased from 62 % to 73 % peak area with the increase in residence time from 2 to 10 h, respectively. In the case of aromatic hydrocarbons, no significant change in the peak area % was observed with the increase in residence time. However, decrease in ethyl benzene and 1,2,3,4-tetrahydro-1,5,8-trimethyl- naphthalene was observed at the longer residence as shown in **Figure 4.3 b**. Oxygenates in the upgraded bio-oils decreased significantly when compared to the original bio-oil. The longer residence time gave the minimum oxygenated compounds, indicating the activity of hydrogenation and hydrogenolysis reaction were prominent. The phenolics in the upgraded bio-oils slightly increased at 2 h residence time and decreased thereafter with the increase in residence time. This increase in phenolics at 2 h residence time shows that phenolics are the intermediate of different compounds and undergoes deformation with the increase in residence time. Formation of esters in the upgraded bio-oils is mainly due to the degradation of acids.

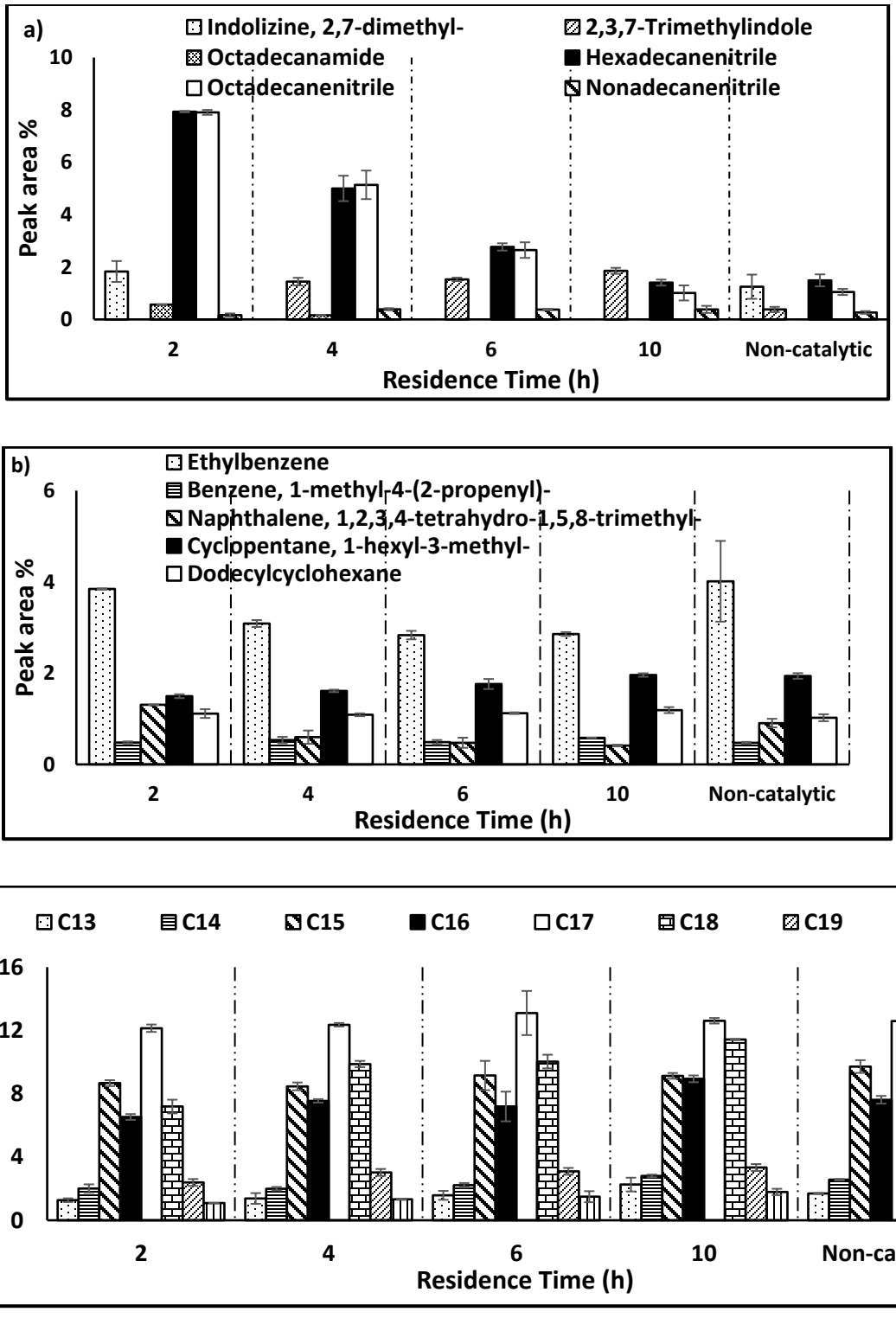

Figure 4.3. Distribution of a) nitrogenous compounds, b) aromatic hydrocarbons and c) unbranched hydrocarbons in the upgraded bio-oils obtained at different residence times.

Simulated distillation of the original bio-oil had a major fraction (70 %) in the vacuum gas oil range, and only a small fraction was in the diesel range which was in agreement with the results obtained by Shakya et al. [3] for *Scenedesmus* sp. No trace of vacuum residue (>538^{o}C) fraction was present in the upgraded bio-oils as shown in **Figure 4.4**. The absence of vacuum residue fraction in the upgraded bio-oil might be due to the hydrocracking of the higher boiling point compounds to lower boiling point fractions or it may have contributed to coke formation via rearrangement and condensation reaction. The major fraction of the upgraded bio-oils was in the vacuum gas oil range (47 – 54%) which was in contrast with the upgraded bio-oil obtained by Elliot et al. [12] and Patel et al. [25]. They observed the maximum fraction of the upgraded bio-oil to be in the diesel range. This difference in the result might be either due to the difference in various process parameters in which HTL of algae and upgrading of the bio-oil were performed or the difference in biochemical composition of the algae used. The vacuum gas oil (VGO) range decreased with the increase in residence time from 2 to 10 h. However, the lighter fractions such as gasoline, kerosene and diesel fractions increased with the increase in residence time. The increase in the diesel range fraction was in agreement with the major diesel range compounds present in the upgraded bio-oil; unbranched hydrocarbon such as C15-C20 and branched hydrocarbon such as 9-hexyl-heptadecane, 3-methyl-heptadecane etc., increased with the increase in residence time. The hydrogen only experiment (control experiment) also had the maximum fraction of upgraded bio-oil in vacuum gas oil range, but, except for the diesel range, all other lower fractions were similar to the original bio-oil. This shows that the Ru/C catalyst was helpful in increasing the lower boiling point fractions in the

upgraded bio-oils. However, the overall upgraded bio-oil still had a high fraction in vacuum gas oil range.

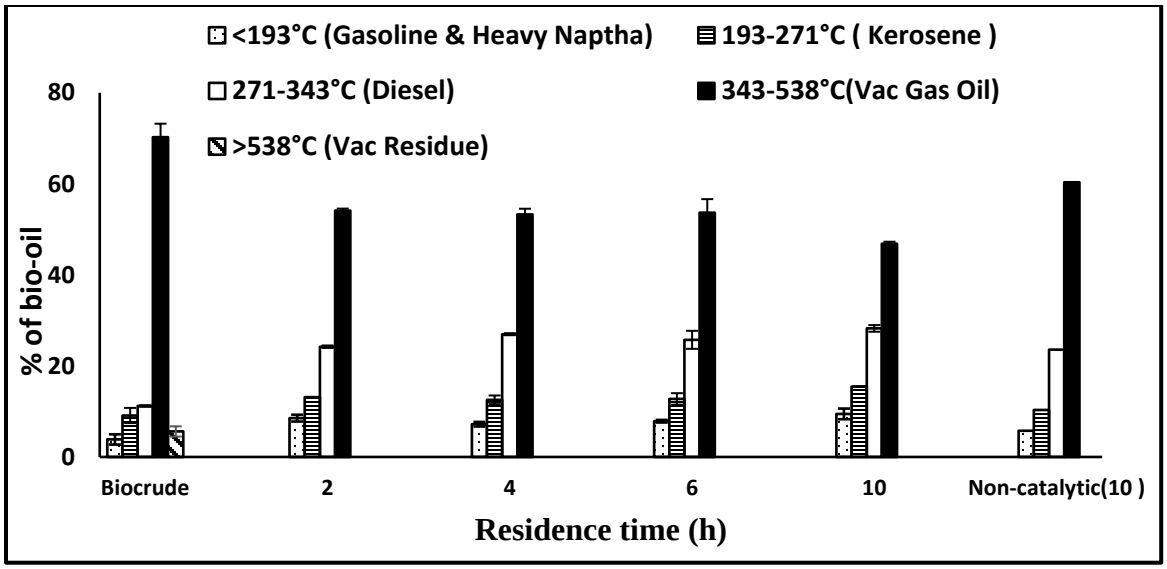

Figure 4.4. Boiling point distribution of the upgraded bio-oils produced at different residence time.

Figure 4.5 shows the FTIR analyses of the original bio-oil and upgraded bio-oils obtained at different residence time. FTIR analyses were performed to compare the changes in their functional groups. The spectral band assignments and interpretation were based on the literature [3, 26, 27]. A significant stretching in the region of 3100-3500 cm^{-1} which were observed for the original bio-oil was very small or insignificant in the case of upgraded bio-oils at all residence time. This indicates the removal of O-H and N-H functional groups during upgrading which can be correlated to the GCMS data, where a significant decrease in alcohols, carboxylic acids, amides, and amines were observed after upgrading. The reduction in strength of bending peaks at 1525-1575 cm^{-1} of the upgraded bio-oils when compared to original bio-oil also suggests decrease in amines and amides. A

significant decrease in stretching band in the region between 1600-1750 cm^{-1} was observed for the upgraded bio-oils, indicating a decrease in C=O bond containing compounds like carboxylic acids, ketones, and aldehydes. However, the upgraded bio-oil obtained at 2 h had a more prominent stretching in this region when compared to the upgraded bio-oils obtained at different residence time, indicating the presence of a lower amount of acids, ketones, aldehydes. The stretching observed at 2800-3000 cm^{-1} was comparatively more prominent for upgraded bio-oils which attribute to antisymmetric and symmetric stretching of CH bonds in $–CH_3$, $-CH_2-$ of aliphatic compounds. This suggested the formation of more aliphatic hydrocarbons after upgrading. This was supported by the $-CH_2-$ scissor vibration and $–CH_3$ bending, which was prominent at 1450-1475 cm^{-1} and 1370-1380 cm^{-1} regions, respectively. A significant decrease in bending was observed for the upgraded bio-oils at 730 cm^{-1} that correspond to C-H stretching for aromatics, indicating a decrease in aromatics during the upgrading process.

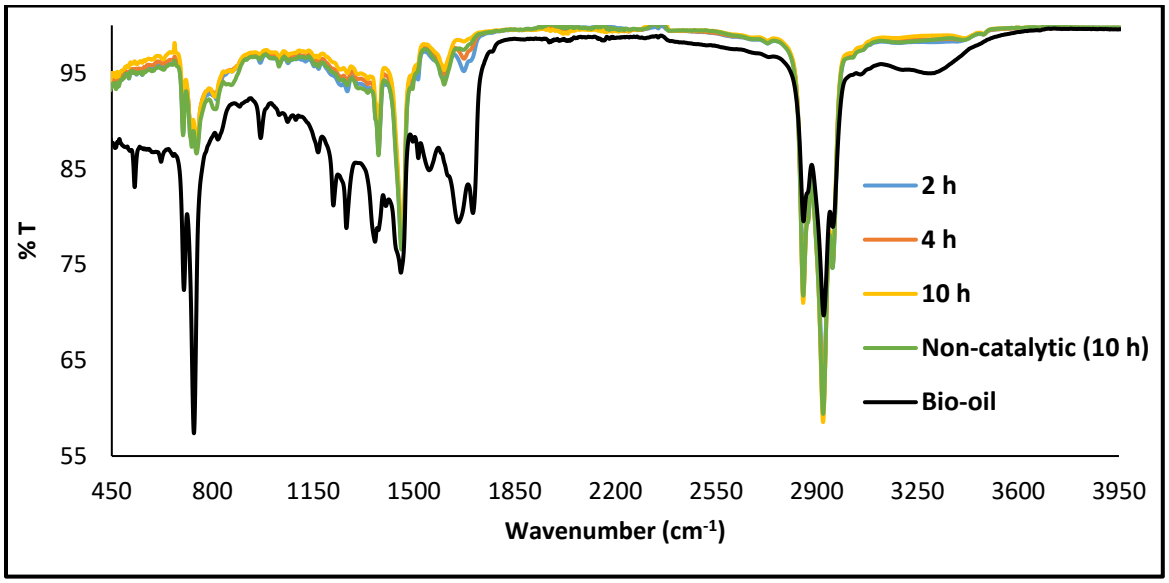

Figure 4.5.FTIR spectra of the original bio-oil and upgraded bio-oils obtained at different residence time.

4.3.4. Effect of residence time on gas composition

Table 4.3 illustrates the hydrogen consumption, product gas yields, and composition. Hydrogen consumption increased with the increase in residence time, which was in agreement with Li and Savage [13]. The maximum amount of hydrogen consumption (39.87 mg/g of bio-oil) was obtained at 10 h residence time. No significant change (p-value=0.092) in product gas yield was obtained with the increase in residence time. The major gases present in the gaseous product were methane, carbon monoxide and some higher alkanes (ethane and propane) which were similar to our previous findings in chapter 3. No traces of nitrogen were observed in the gaseous product of the catalytic experiments at all residence times, which was also in agreement with Duan and Savage [10] and suggested the formation of ammonia. A significant increase (p-value=0.0001) in hydrogen sulfide (H_2S) in the gaseous product was observed with the increase in residence time. It increased from 48.14±3.56 ppm to 90.18±0.80 ppm with the increase in residence time from 2 to 10 h. This shows that HDS was more prominent at a higher residence time. The amount of methane increased 73% with the increase in residence time from 2 to 10 h. This increase in methane and subsequent decrease in the carbon monoxide suggested that the methanation reaction is more prominent during the upgrading process. The presence of carbon monoxide and carbon dioxide are mainly due to the decarboxylation and decarbonylation of bio-oil compounds which gets promoted by Ru catalyst during upgrading [28, 29]. At all the residence time, carbon monoxide was observed to be higher than carbon dioxide; this suggested the reverse water gas shift reaction was favored during the upgrading process. However, as the residence time increased above 4 h, carbon monoxide decreased significantly which may be due to the methanation reaction of CO to

form methane. The presence of excess carbon monoxide may lead to its accumulation over the catalyst Ru surface resulting in low rates of reaction due to lower availability of active sites [30]. Apart from methane, other higher alkanes such as ethane, propane, and butane also increased with the increase in residence time. This increase in the higher alkanes might be due to increase in catalytic cracking and hydrogenolysis of bio-oil compounds at longer residence time.

Table 4.3. Hydrogen consumption, product gas formation, and gas composition during upgrading of algae bio-oils at different residence time.

Residence Time (h)	Hydrogen consumed (mg/g feedstock)	Product Gas (mmol/g feedstock)	Gas composition of the product (Mol %) (as received)						
			H_2	CH_4	CO	CO_2	C_2H_6	C_3H_8	C_4H_{10}
2	31.28±0.89^{a}	3.13±0.12^{a}	78.61±0.38^{a}	8.57±0.02^{a}	3.33±0.32^{a}	0.17±0 ^{a}	4.51±0.01^{a}	3.81±0.03^{a}	0.54±0.01^{a}
4	34.61±0.29^{b}	3.18±0.05^{a}	75.58±0.90^{b}	9.67±0.19^{a}	3.87±0.84^{a}	0.06±0.04^{c}	5.48±0.11^{b}	4.50±0.08^{b}	0.76±0.03^{b}
6	37.37±0.82^{c}	3.22±0.16^{a}	72.47±0.42^{c}	12.63±0.03^{b}	0.66±0.04^{b}	0.13±0.03^{a,b}	7.28±0.09^{c}	5.57±0.18^{c}	1.02±0.09^{c}
10	39.87±0.26^{c}	3.40±0.06^{a}	68.07±3.38^{d}	14.81±1.98^{c}	0.46±0.04^{b}	0.06±0.05^{b,c}	8.81±1.03^{d}	6.26±0.42^{a}	1.25±0.06^{d}

Different alphabets in the superscripts of each group (column) denote the values are statistically significant at different catalyst type (rows) (α=0.05). numbers after ± symbol denotes standard deviation.

4.4. Conclusions

The effect of residence time (2, 4, 6 and 10 h) on the upgrading of algae bio-oils was studied. The upgraded bio-oil yields were observed to be maximum at 4 h residence time and decreased as the residence time increased thereafter. The upgraded bio-oils at 10 h residence had the maximum HHV (44.32 MJ/kg), the lowest TAN, nitrogen content, and viscosity. However, the energy recovery was observed to be maximum at 4 h residence time for the catalytic upgrading experiments. Most of the upgraded bio-oils at all the residence time were in the vacuum gas oil range. Hydrogen consumption increased with the increase in residence time. Thus, comparing the properties of the upgraded bio-oils and its yields at different residence time, and considering a high energy input penalty associated with a longer residence time, we can conclude that 4 h residence time was optimum for the upgrading of the algae bio-oil.

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Chapter 5

Influence of Binary Mixture of CO_2 and H_2 on Catalytic Upgrading of Bio-oil Produced from Hydrothermal Liquefaction of Algae

Abstract

Upgrading of bio-oil produced from hydrothermal liquefaction of algae was performed to study the influence of the cold pressure of a binary mixture of CO_2 and H_2. Upgrading was performed at 350^{o}C using 5% Ru/C at a catalyst loading of 16.67 wt.% and a residence time of 4 h. The cold pressures of the binary mixture were 100 psi CO_2 + 900 psi H_2, 200 psi CO_2 + 800 psi H_2, and 300 psi CO_2 + 700 psi H_2. The upgraded bio-oil properties and the yields obtained at 300 psi of CO_2+700 psi of H_2 were comparable to that obtained using 1000 psi of H_2, except for the total acid number and higher heating value. The total acid number increased and the higher heating value decreased with the increase in CO_2 cold pressure. The nitrogen content was the lowest (2.40 wt.%) at the maximum CO_2 cold pressure. Simulated distillation of the upgraded bio-oils showed the maximum fraction (64-66 %) of the upgraded bio-oils was still in vacuum gas oil range. The use of CO_2 in the upgrading reaction did not significantly change the upgraded bio-oil quality, but its incorporation in the upgrading system added benefit in terms of process safety as it expands the non-explosive regime.

Keywords: *algae, hydrothermal liquefaction, upgrading, CO_2 expanded liquid, Ru/C, bio-oil*.

5.1. Introduction

The conversion of algae into bio-oil via hydrothermal liquefaction (HTL) is a promising biomass conversion process because of its ability to process feedstocks with high moisture content [1]. The bio-oil obtained from HTL of algae is a complex mixture of compounds with diverse chemical and molecular structure. Thus, it not only serves as fuels but also serves as a precursor for other products such as polymers and lubricants. The bio-oil has positive attributes of fuels such as high heating value (32-36 MJ/kg) and comparatively lower oxygen content (7-10 wt.%) [2]. However, it is highly viscous (40-68 cP at 60^{o}C) [3], acidic (29-118 mg of KOH/g) [2], has high nitrogen (2-9 wt.%) [2, 3] and is unstable. Thus, it can neither be used as a drop-in fuel nor it can be blended with petroleum crude. Therefore, to make it more desirable as fuel, upgrading of the bio-oil is necessary.

In conventional upgrading process, three phases; solid (catalyst), liquid (bio-oil) and gas (hydrogen), reaction occurs, and most of the reactions are mass transfer limited due to the low solubility of hydrogen in bio-oil, which decreases the efficacy of the process. To enhance the solubility of hydrogen in the bio-oil and catalysts, a high hydrogen pressure (30 MPa) is required, which may offset overall process economics [4, 5]. Thus, to overcome this problem, supercritical fluids such as water, carbon dioxide, and propene [6–9] have been used because of their higher miscibility with reactive gases such as hydrogen, lower viscosity, higher diffusion rates, and their pressure-tunable physical properties. Use

of water as a supercritical fluid has been extensively used for the upgrading of algae bio-oil [7, 8, 10], but the process challenges such as water removal after processing and its disposal have been an issue as the wastewater may contain organics, and this require treatment as wastewater before disposal into waste streams. Among several supercritical fluids, CO_2 is the most favorable supercritical fluid because it is highly miscible with hydrogen, non-flammable, non-toxic, inexpensive and has a mild critical temperature (31.1^oC) [11–13]. Supercritical CO_2 ($scCO_2$) dissolves into the organic liquid/solvent, causing it to expand/swell considerably in volume, thus resulting in a low viscosity of the liquid, increased solubility of hydrogen in the liquid phase, and a decreased mass transfer limitation because of increased hydrogenation efficacy [14]. As the $scCO_2$ dissolves into an organic solvent, the solvent becomes a CO_2-expanded liquid (CXL).

Several studies [6, 15–24] on model compounds hydrogenation have been performed using CXL as the reaction medium. Arunajatesan et al. investigated hydrogenation of cyclohexene to cyclohexane in a continuous fixed bed reactor using CO_2 and a solid catalyst Pd/C [19]. They observed a stable catalytic activity at near-critical temperature and pressure of 70^oC and 13.6 MPa, respectively, with cyclohexene conversion exceeding 80%. Devetta et al. compared the hydrogenation of unsaturated ketones using Pd/Al_2O_3 catalyst with and without $scCO_2$ as a solvent and observed an increase in the reaction rate with the use of $scCO_2$ [25]. Hydrogenation of nitrogen-containing compounds like benzonitrile to benzylamine in the $scCO_2$ environment was performed by Chatterjee et al., who reported a high conversion (90.2%) and selectivity (90.9%) using Pd/MCM-41 catalyst at 8-10 MPa [20]. They observed that the selectivity and conversion were a function of increased CO_2 pressure. Similarly, Zhao et al.

investigated the hydrogenation of nitrobenzene with different transition metal catalysts such as Ru, Rh, Pt, and Pd in scCO_2 medium and in ethanol medium. [21]. The authors observed 100% yields of aniline in scCO_2 medium (14 MPa) with 5% Pd/C and 5% Pt/C catalysts at 35^oC.

Apart from hydrogenation of these model compounds, several studies on biodiesel production using CO_2 as reaction medium also have been studied. Bertoldi et al. investigated the effect of CO_2 as a cosolvent on the production of fatty acid ethyl esters from soybean oil via transesterification in the continuous catalyst-free process [26]. The authors reported a decrease in the yield of ethyl esters with an increase of CO_2 to the systems, whereas considerable reaction yields were achieved at 350^oC, 10 MPa, oil to ethanol molar ratio of 1:40, and a CO_2 to substrate mass ratio of 0.05:1. Ma et al. also investigated transesterification of soybean oil using an acid catalyst (H_2SO_4 and $NaHSO_4$) in CO_2 expanded methanol liquids to form biodiesel [9]. The goal of adding CO_2 in the system was to increase the reaction rate, shorten the reaction time, reduce the methanol consumption in traditional acid catalysis method or decrease the high-temperature and pressure in the supercritical methanol method. They reported a complete oil conversion at 10 MPa, 70^oC with methanol to oil ratio of 12:1 and H_2SO_4 of 4%.

The above-discussed studies suggested CO_2 to be a suitable reaction medium for catalytic reactions, as it enhances reaction rate, decreases viscosity and reduces mass transfer limitation. Apart from acting as a reaction medium, CO_2 also acts as a reactant during the upgrading process. To the best of our knowledge, to date, none of the catalytic upgrading of bio-oil work have employed CO_2 as a reaction medium although some model compounds have been tested. Thus, this study proposes to explore the influence of CO_2 on

catalytic hydrotreatment/upgrading of bio-oils. We hypothesize that the use of CO_2 as reaction medium during the upgrading process will significantly improve hydrogenation of bio-oil compounds and improve the overall quality of the upgraded bio-oil. Hence, the objective of this study was to investigate the influence of a binary mixture of CO_2 and hydrogen on the catalytic upgrading of algae bio-oil.

5.2. Materials and Methodology

5.2.1. Materials

Algae (*Scenedesmus* sp.) biomass was provided by Arizona Center for Algal Technology and Innovation (Mesa, Arizona) in the form of a slurry (17 wt.% solids). The commercial 5% Ru/C catalyst was obtained from Sigma-Aldrich. Ultrahigh purity grade (99.999%) carbon dioxide, hydrogen, helium, and argon gases were purchased from Airgas Inc. (Charlotte, NC). HPLC grade acetone, dichloromethane, and toluene were purchased from a chemical supplier VWR (Atlanta, GA). De-ionized water was used for HTL experiments.

5.2.2. Hydrothermal liquefaction of algae

Hydrothermal liquefaction of *Scenedesmus* sp. was performed in a 1 L batch reactor system (Parr Instruments Co., Moline, PA) at 320^{o}C for a residence time of 30 minutes with a solid loading of 15 wt.%. Approximately 100 g of algae (dry weight basis) was loaded into the reactor; the headspace was purged with helium to remove residual air and to create an inert environment. After purging with helium, the reactor was pressurized to an initial pressure of 100 psi and was heated to the desired reaction temperature. After the residence time of 30 minutes, the reactor was cooled down using cold water and the residual gases formed were vented off. The product separation procedure was followed as described

in the previous paper [2]. The bio-oil obtained had a relatively high moisture content, and dichloromethane was used to remove the aqueous phase. Finally, dichloromethane was separated by vacuum-evaporation, and bio-oil with low final moisture content (1.25 wt.%) was obtained which was used for upgrading studies.

5.2.3. Upgrading of bio-oil

For a typical upgrading studies, 40 g of bio-oil was upgraded in a 450 ml batch reactor (Parr Instruments Co., Moline, PA) at 350^{o}C and using 5% Ru/C catalyst (catalyst loading of 16.67 wt.%) for a residence time of 4 h. The cold pressure of the binary mixture of CO_2 and H_2 was incorporated using the following conditions: 100 psi CO_2 + 900 psi H_2, 200 psi CO_2 + 800 psi H_2, and 300 psi CO_2 + 700 psi H_2. Also, a control run with 1000 psi cold pressure of H_2 was performed to compare the results.

In a typical experiment, 8 g of catalyst was first loaded into the reactor, and a repeated cycle of nitrogen purging was performed to create an inert headspace. After purging with nitrogen, hydrogen gas was purged to remove residual nitrogen, and the pressure was maintained at 1000 psi for catalyst reduction. Reduction of catalyst was carried out at 300^{o}C for a residence time of 1 h. After the reduction, the reactor was subsequently cooled down to room temperature and again purged with nitrogen and argon before opening. A known amount of bio-oil (approximately 40 g) was loaded into the reactor, and the similar procedure of purging with nitrogen during reduction was followed. After this, CO_2 was added into the reactor, and the pressure was maintained as according to the experimental condition for 15 min. After 15 min, H_2 was topped to make the initial cold pressure of 1000 psi. In a control run, only H_2 was added into the reactor and the cold pressure was maintained at 1000 psi. Then, the reactor was then heated to the reaction

temperature (350°C) while agitating the mixture at 500 RPM for the given residence time. Product separation procedure was followed as described in section 3.2.3. The product yields for upgrading studies were calculated using Eq. (1). Hydrogen consumption and carbon dioxide reacted or loss during the catalytic upgrading was calculated according to Eq. (2) as used by Nam et al. [28]. Energy recovery was calculated using Eq. (3).

$$\text{Yields}_{\text{oil/solid residue(coke)}} = \frac{Weight\ of\ the\ product\ (dry\ basis)}{Weight\ of\ the\ algae\ bio-oil\ (dry\ basis)} \times 100 \quad (1)$$

$$\text{Hydrogen/Carbon dioxide reacted or loss} = \left[(P_i/T_i) - \left(X_{n,f} \times P_f/T_f\right)\right] \times V_{gas}/R \quad (2)$$

$P_{i\ or\ f}$ = initial/final reactor pressure

$T_{i\ or\ f}$ = initial/final reactor temperature at 298 K

$X_{n,f}$ = final mole fraction of hydrogen/carbon dioxide in a produced gas

V_{gas} = reactor volume occupied by gas

R= gas constant (8.314 J/K.mol)

$$\text{Energy recovery} = \frac{HHV_{BIO-OIL} \times Mass_{BIO-OIL}}{\left[HHV_{ALGAE} \times Mass_{ALGAE} + HHV_{H_2} \times Mass\ consumed_{H_2}\right]} \times 100 \quad (3)$$

5.2.4. Product analysis

All the upgrading products; upgraded bio-oils, gas and solid residue were analyzed as described in section 3.2.4.

5.2.5. Statistical Analysis

All experiments were performed in duplicates for each condition to verify the reproducibility of data. Statistical analysis (ANOVA, Tukey's HSD) of all the data was performed using statistical software (SAS) to evaluate if the obtained results were statistically different. All the statistical analyses were performed at 95% confidence interval.

5.3. Result and Discussion

5.3.1. Algae characterization and hydrothermal liquefaction

Algae (*Scenedesmus* sp.) used for HTL had protein, carbohydrate and lipid content of 30.06 wt.%, 54.17 wt.% and 17.83 wt.%, respectively. The ultimate analysis of the biomass showed carbon, hydrogen, nitrogen, sulfur and oxygen content to be 51.51±0.61 wt.%, 7.26±0.07 wt.%, 10.97±0.56 wt.%, 0.77 wt.% and 30.26±0.78 wt.%, respectively. The bio-oil yield of 46.38±2.44 wt.% was obtained from the HTL of the algae biomass. The bio-oil obtained had a higher heating value of 34.51±0.18 MJ/kg which was comparatively greater than that obtained from HTL of lignocellulosic biomass [29]. However, the bio-oil still had a high viscosity (49.50±0.25 cSt) compared to No.2 diesel fuel (2.5-3.2 cSt) [30, 31] or even soybean biodiesel (4.2-4.6 cSt) [30, 32], high TAN (18.53±0.27 mg of KOH/g) compared to biodiesel from cooking oil (0.29 mg KOH/g) [33] or ASTM D6751 requirement (0.5 mg of KOH/g) [10] and high nitrogen content (5.74±0.56 wt.%). The carbon, hydrogen and oxygen content of the bio-oil were 73.46±0.26 wt.%, 9.48±0.08 wt.% and 11.77±0.38 wt.%, respectively.

5.3.2. Effect of CO_2 and H_2 cold pressure on upgrading product yields

Figure 5.1 shows the yields of various product fractions obtained after upgrading of the bio-oil in a binary mixture of CO_2 and H_2. The product fraction includes upgraded bio-oil, solid residue (coke) and a gaseous fraction. The total mass balance closure varied between 88 wt.% to 96 wt.% and this variability in mass balance was mainly due to difficulty in recovering the solid fractions from the reactor wall, and due to the loss of the upgraded bio-oil during solvent (toluene) extraction. The yield of the upgraded bio-oil obtained using the binary mixture ranged from 53 wt.% to 58 wt.%. No significant (p-

value=0.289) changes in the upgraded bio-oil yields were observed with the increase in CO_2 pressure. This shows that with lower hydrogen pressure and addition of CO_2 similar yields of upgraded bio-oil can be obtained. This similarity in the upgraded bio-oil yields at reduced hydrogen pressure may be due to the effect of CO_2, which might have reacted with hydrogen to form bio-oil compounds such as acetic acids and alcohols. For example, Kusama et al. [16] performed hydrogenation of CO_2 using promoted Rh/SiO_2 catalyst at 240^oC in a flow of premixed H_2/CO_2 ratio of 3 to produce ethanol. Yin et al. [35] performed hydrogenation of CO_2 using ruthenium complex to give formic acid. Although a clear indication as to why the upgraded bio-oil increased is still unknown, further study with the increase in CO_2 pressure is suggested to confirm the effect of CO_2.

A significant (p-value=0.001) increase in the solid residue (coke) was observed with the addition of CO_2 when compared to the control run. Increase in solid residue may be due to the decrease in initial hydrogen pressure [34]. Increase in condensation and coupling reaction during upgrading also leads to coke formation [36]. Increase in CO_2 pressure had no significant change (p-value=0.33) in the solid residue, and it ranged from 20 wt.% to 21.41 wt.%. In the case of gas yield, no significant change was observed. This shows that the increase in cold CO_2 pressure may have suppressed the gas formation.

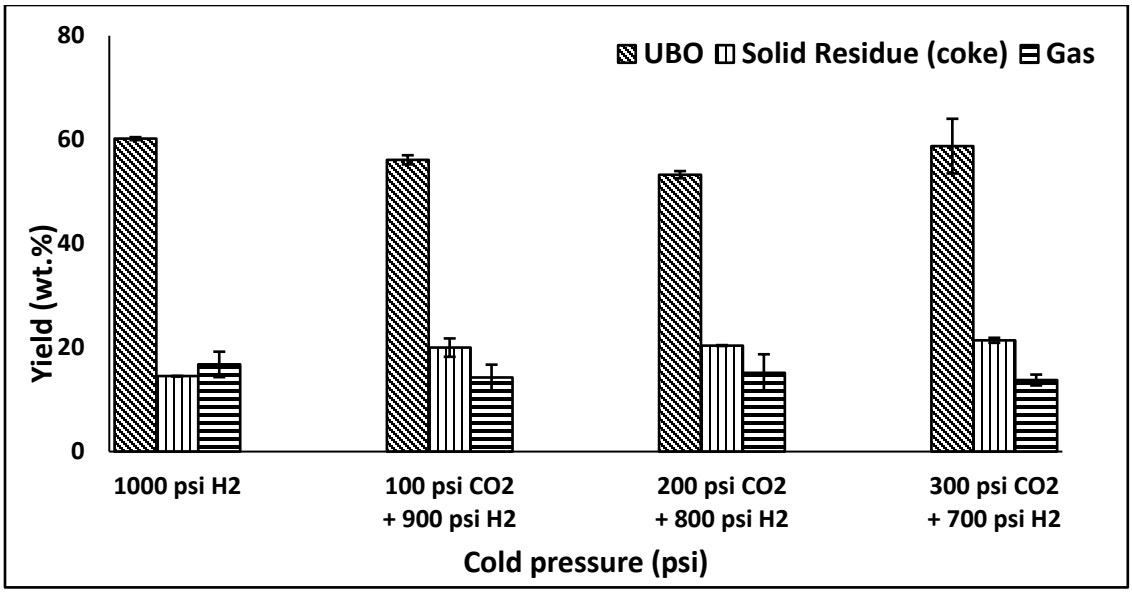

Figure 5.1. Product distribution obtained from the upgrading of bio-oils using a binary mixture of CO_2 and H_2 at different cold pressure levels.

5.3.3. Effect of CO_2 and H_2 cold pressure on upgraded bio-oil properties

Table 5.1 and **Table 5.2** shows the effect of CO_2 and H_2 pressure on the properties of the upgraded bio-oils. The water content of the upgraded bio-oils obtained with the addition of CO_2 ranged from 0.16 wt.% to 0.23 wt.% which was lower than the value observed for petroleum crude (<0.5%) [28]. A significant (p-value=0.0016) decrease in the viscosity of the upgraded bio-oils was observed with the increase in CO_2 pressure from 100 psi (5.00 cSt) to 200 psi (4.39 cSt). The viscosity of upgraded bio-oil produced from the control experiment was similar to that obtained at 200 and 300 psi of CO_2. The viscosity of the upgraded bio-oils was in between 4.16–5.00 cSt, which was within the range of 1.9-6 cSt required for the biodiesel standard of ASTM D445 [37]. The upgraded bio-oils obtained using binary mixture of CO_2 and H_2 had a higher TAN when compared to the control experiment. This increase in the TAN of the upgraded bio-oils might be due to the formation of organic acids and alcohols from the reaction of CO_2 with H_2 [35, 38].

However, no significant change in the TAN was observed with the increase in CO_2 cold pressure from 100 to 300 psi. Energy recovery was similar for the control experiments when compared to the upgraded bio-oils obtained using CO_2.

Table 5.1. Properties of the upgraded bio-oils obtained using the binary mixture of CO_2 and H_2.

Pressure	Water Content (wt.%)	HHV (MJ/kg) †	TAN (mg of KOH/g) †	Viscosity (cSt) †	Energy Recovery (%)
Biocrude	1.25±0.14	34.51±0.18	18.53±0.27	49.50±0.25	66.10±0.18
1000 psi H_2 (Control)	0.29±0.01[a]	43.77±0.14[a]	1.20±0.35[a]	4.02±0.35[a]	44.37±0.05[a]
100 psi CO_2 + 900 psi H_2	0.23±0.04[a]	43.42±0.05[b]	3.13±0.55[b]	5.00±0.27[b]	41.04±0.66[a]
200 psi CO_2 + 800 psi H_2	0.18±0.05[a]	43.24±0.02[b,c]	3.39±0.13[b]	4.39±0.33[a]	39.06±0.49[a]
300 psi CO_2 + 700 psi H_2	0.16±0.08[a]	43.19±0.12[c]	3.54±0.07[b]	4.16±0.02[a]	43.85±3.46[a]

Different alphabets in the superscripts of each group (properties/column) denote the values are statistically significant at different pressure levels (rows) (α=0.05). numbers after ± symbol denotes standard deviation. † as received.

The elemental composition of the upgraded bio-oils obtained using a binary mixture of CO_2 and H_2 showed no significant variation (p-value=0.594) in the C percentage when compared to the control experiment. No significant variation in the C values was observed with the increase of CO_2. However, a significant (p-value= 0.049) decrease in H content was observed with the increase of CO_2 pressure from 0 to 300 psi. This decrease in H values of the upgraded bio-oils at 300 psi of CO_2 was reflected in the higher heating values. The higher heating values ranged between 43.19 to 43.77 MJ/kg, and were similar to that of the petroleum diesel fuel (44.8 MJ/kg) [39]. H/C ratio was in the range of 1.57 to 1.63 which was less than No 1 diesel (1.84) and No 2 diesel (1.82) [30]. The O/C ratio (0.01) was constant for all the upgraded bio-oils obtained using a binary mixture of CO_2 and H_2.

No significant change in the nitrogen content was observed in the upgraded bio-oils for both control and binary systems. The nitrogen content ranged from 2.40 wt.% to 2.87 wt.% which was smaller or similar to that obtained by Duan and Savage [7]. Sulfur values in the upgraded bio-oils (both control and binary systems) were significantly lower than that of the original bio-oil. No change in the sulfur values of the upgraded bio-oils was observed with the increase of CO_2 cold pressure. The sulfur values ranged from 0.05 wt.% to 0.06 wt.% which was still higher than that of diesel fuel (0.034%) [30]. Overall, the inclusion of CO_2 did not improve the quality of the upgraded bio-oil significantly when compared to the control experiment, but it helped in process safety by expanding the non-explosive regimes in the gas phase.

Table 5.2. Ultimate analysis of the upgraded bio-oils obtained using the binary systems of CO_2 and H_2.

Pressure	Elemental Analysis (dry basis)					Elemental Ratios		
	C	H	N	S[¥]	O*	O/C	N/C	H/C
Biocrude	73.46±0.26	9.48±0.08	5.74±0.56	0.81	11.77±0.38	0.12	0.07	1.55
1000 psi H_2 (Control)	84.86±0.10[a]	11.91±0.02[a]	2.64±0.09[a]	0.05[a]	0.88±0[a]	0.01	0.03	1.68
100 psi CO_2 + 900 psi H_2	85.16±0.23[a]	11.55±0.08[a, b]	2.87±0.15[a]	0.06[a]	0.61±0.29[a]	0.01	0.03	1.63
200 psi CO_2 + 800 psi H_2	84.94±0.63[a]	11.41±0.25[a, b]	2.79±0.63[a]	0.06[a]	0.99±0.26[a]	0.01	0.03	1.61
300 psi CO_2 + 700 psi H_2	85.56±0.81[a]	11.21±0.19[b]	2.40±0.05[a]	0.06[a]	0.95±1.16[a]	0.01	0.02	1.57

Different alphabets in the superscripts of each group (elemental group) denote the values are statistically significant at different pressure levels (rows) (α=0.05). * by balance, [¥] All the values had zero standard deviation.

Figure 5.2 shows the peak area percentage of the chemical compounds present in the upgraded bio-oils obtained using the binary mixture of CO_2 and H_2. The peak area of nitrogenated compounds of the upgraded bio-oils obtained using the binary mixture of CO_2

and H_2 ranged from 17 to 19%. The overall nitrogenous compounds of the upgraded bio-oils obtained using the binary mixture was observed to be the lowest at 300 psi CO_2+700 psi H_2, and were similar to that of the control experiment. The primary nitrogenous compounds present in the upgraded bio-oils obtained using the binary mixture were hexadecanenitrile, octadecanenitrile, 2-myristynoyl pantetheine, pyrimidin-5-carboxamide etc. as shown in **Figure 5.3a**. No traces of 2,3,7 -trimethylindole, N-(4-Methoxyphenyl)-2-hydroxyimino-acetamide were observed in the upgraded bio-oil obtained using the binary mixture. Nitriles (hexadecanenitrile and octadecanenitrile) significantly increased with the introduction of CO_2 into the system. However, it decreased with the increase in CO_2 cold pressure. The appearance of amide (octadecanamide) was observed at 200 and 300 psi of CO_2 pressure. This appearance of amides and resulting reduction in the nitriles may be due to the hydrogenation of nitriles to amines [17] and its subsequent reaction with acids to form amides [17, 18, 40]. Moreover, Ru also helps in conversion of CO_2, H_2 and secondary amines to corresponding formamides via two steps- CO_2 hydrogenation to formate compounds followed by dehydration to formamide [15].

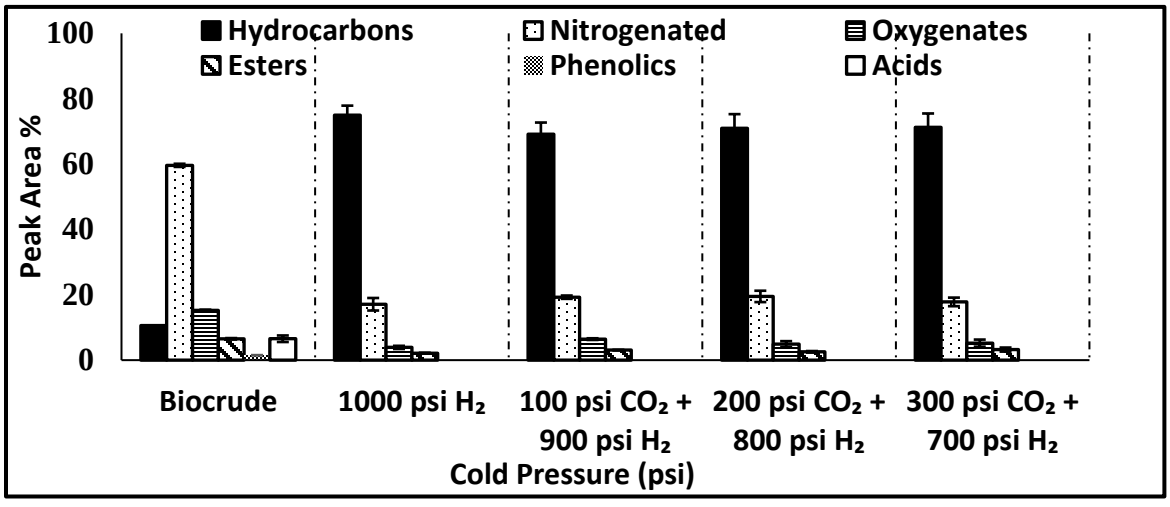

Figure 5.2. Chemical composition of the upgraded bio-oils obtained at different cold pressure levels of CO_2 and H_2.

Hydrocarbons present in the upgraded bio-oils decreased with the introduction of CO_2 as shown in **Figure 5.2**. However, no significant change in hydrocarbons was observed with the increase in CO_2 cold pressure from 100 to 300 psi. Aromatic hydrocarbons such as ethylbenzene, 1,2,3,4-tetrahydro-1,5,8-trimethyl-naphthalene decreased with the increase in CO_2 cold pressure, whereas 1-hexyl-3-methyl-cyclopentane increased as shown in **Figure 5.3b**. This increase in the saturated cyclic ring although at reduced hydrogen pressure may be due to the effect of CO_2, which expands the bio-oil resulting in an increased surface area for the hydrogenation of the unsaturated aromatic hydrocarbons. Apart from the hydrogenation of unsaturated aromatic hydrocarbons, other unsaturated aromatic oxygenates also might have converted to saturated aromatic hydrocarbons [24]. For example. Hitzler et al. [24] selectively hydrogenated acetophenone at 300^{o}C in the presence of 5% Pd AP Deloxan catalyst to ethylcyclohexane. The aliphatic hydrocarbons were the major hydrocarbons present in the upgraded bio-oils. The major aliphatic hydrocarbons present are shown in **Figure 5.3c**. No significant change in the lower aliphatic hydrocarbons (C_{11} to C_{15}) were observed with the increase of CO_2 cold pressure. The decrease in C_{16} and C_{18} were observed for the upgraded bio-oils obtained using the binary mixture. The addition of CO_2 increased the peak area % of oxygenates when compared to that of the control experiment. This increase in the oxygenates may be due to the formation of alcohols from alkenes by hydroformylation with CO_2 [41] or due to hydrogenation of CO_2 to form ethanol and other higher alcohols [16]. No phenolics and acids were observed in the upgraded bio-oils.

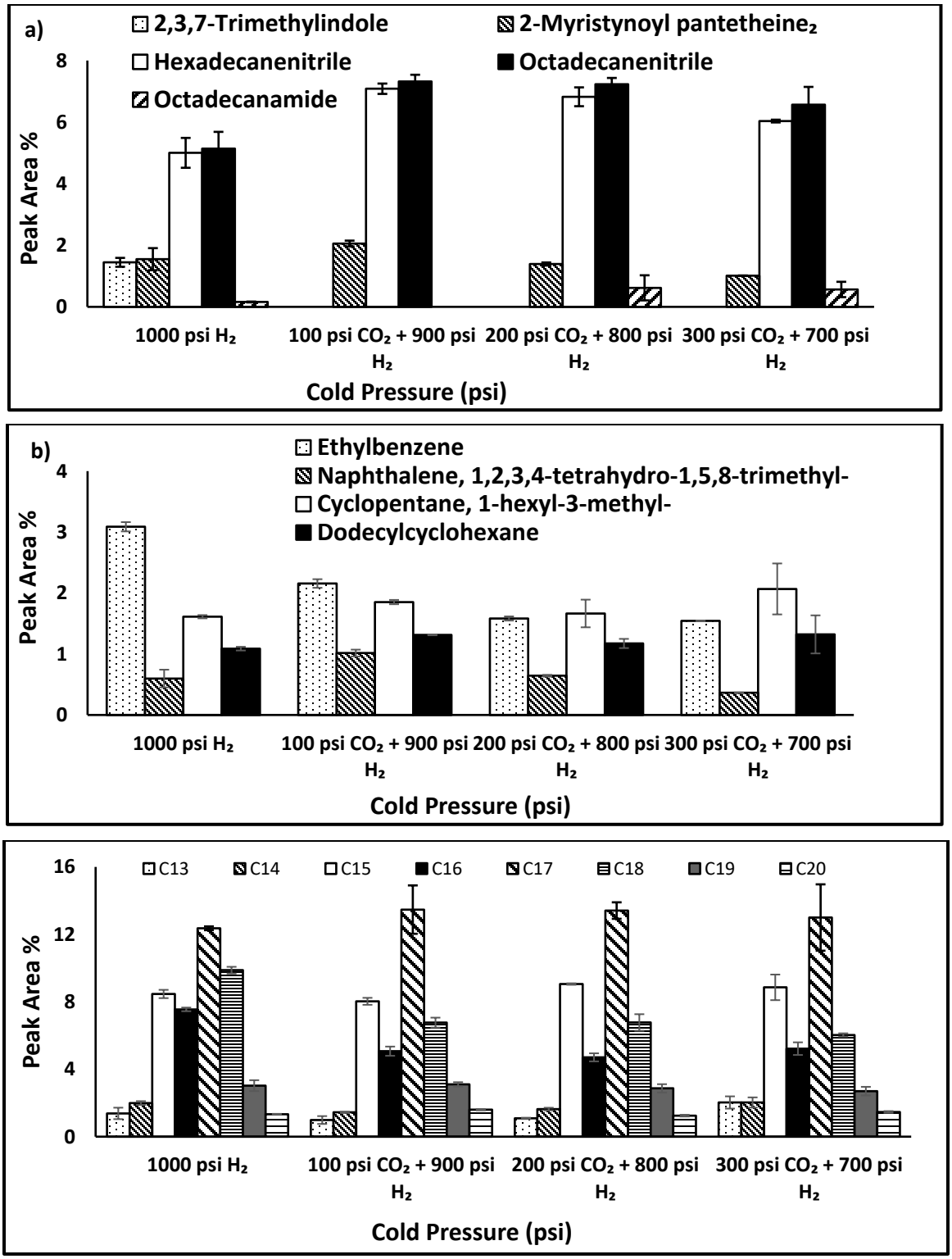

Figure 5.3. Distribution of a) nitrogenous compounds, b) aromatic hydrocarbons and c) unbranched hydrocarbons in the upgraded bio-oils obtained at different cold pressure levels of CO_2 and H_2.

Figure 5.4 shows the comparison of boiling point distribution of the upgraded bio-oils obtained using the binary mixture and the control experiment. The significant fraction (65-66.5%) of the upgraded bio-oils obtained using the binary mixture was in the vacuum gas oil range. Around 22-25% increase in the vacuum gas oil range was observed with the introduction of CO_2 upon upgrading when compared to the control experiment. This increase in the vacuum gas oil range may be due to the reduction of hydrogen available for hydrogenation of the compounds or it may also be due to the increase in coupling reaction [11] and Friedel-crafts alkylation reaction [6] which gets pronounced due to the solvent property of $scCO_2$. No significant change in the vacuum gas oil range was observed with the increase in CO_2 cold pressure. Diesel and kerosene fractions of the upgraded bio-oils decreased 18-19% and 29-30%, respectively, with the addition of CO_2 when compared to the control experiment. No significant change in the diesel and kerosene fractions were observed with the change in the CO_2 cold pressure. The maximum fraction (22%) of the diesel range compound was observed at 300 psi of CO_2. Drastic reduction in the lower boiling point range (gasoline and naphtha) was observed with the addition of CO_2. This reduction in lower boiling point range compounds may be due to the loss of high volatiles while depressurizing the reactor system or due to being soluble in CO_2.

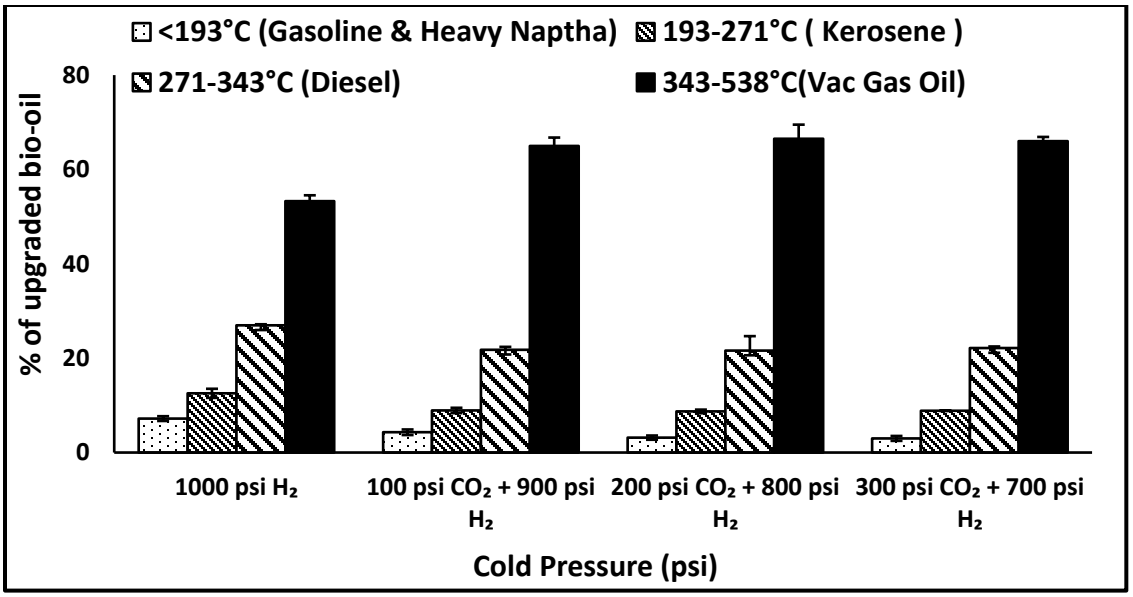

Figure 5.4. Boiling point distribution of the upgraded bio-oils obtained at different cold pressure levels of CO_2 and H_2.

FTIR analyses of the upgraded bio-oils obtained from the control experiment and that obtained using a binary mixture of CO_2 and H_2 were performed to compare the changes in the functional groups as shown in **Figure 5.5**. The spectral band assignments and interpretation were based on the literature [42–44]. A significant reduction in stretching around the region of 3100-3500 cm^{-1} was observed for the upgraded bio-oils when compared to the original bio-oil. This suggests the removal of O-H and N-H functional groups during upgrading. However, it is to be noted that with the introduction of CO_2, a slight increase in the stretching in the region is observed; this can be related to GCMS findings in which we observed a slight increase in nitrogenated compounds and alcohols. A prominent stretching was observed for both (control and binary mixture) the upgraded bio-oils in the region between 2800-3000 cm^{-1} which are attributed to antisymmetric and symmetric stretching of CH bonds in $–CH_3$, $-CH_2-$ of aliphatic compounds. This demonstrates the formation of more aliphatic hydrocarbons after upgrading. This was

supported by the -CH_2- scissor vibration and –CH_3 bending, which was prominent at 1450-1475 cm^{-1} and 1370-1380 cm^{-1} regions, respectively. However, the vibration and bending were more prominent for the upgraded bio-oils obtained for the binary mixture of 300 psi CO_2+700 psi H_2. A significant decrease in the region 1600-1750 cm^{-1} was observed for the upgraded bio-oils, indicating the decrease in C=O bond containing compounds like carboxylic acids, esters, ketones, and aldehydes. The upgraded bio-oils obtained using 300 psi CO_2+700 psi H_2 had a more prominent stretching in the region (1600-1750 cm^{-1}) when compared to other upgraded bio-oils. This increase in stretching may be due to the presence of esters in the upgraded bio-oils, which gets pronounced with the increase in CO_2 cold pressure as shown by GCMS observation. A significant decrease in bending was observed for the upgraded bio-oils at 730 cm^{-1} that correspond to C-H stretching for aromatics indicating a decrease in aromatics during the upgrading process. However, the bending increases for the upgraded bio-oils obtained with the increase in CO_2 cold pressure. This increase in bending may be due to the lack of hydrogen for hydrogenation of aromatic rings.

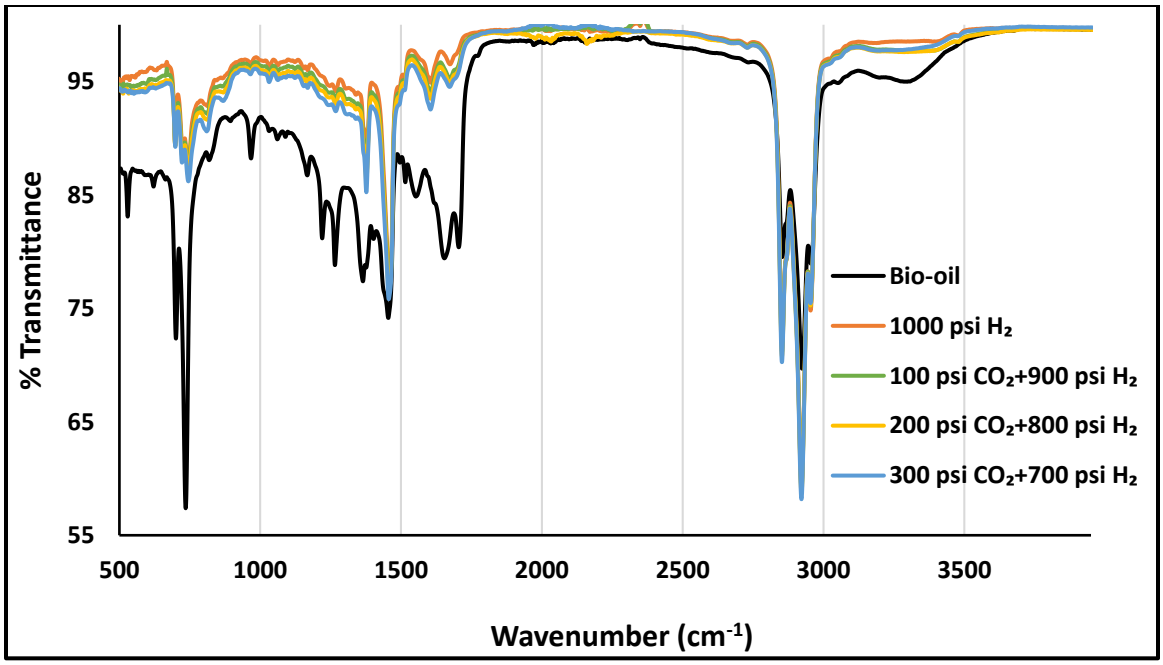

Figure 5.5. FTIR spectra of the original bio-oils and the upgraded bio-oils obtained using a binary mixture of CO_2 and H_2.

5.3.4. Effect of CO_2 and H_2 cold pressure on gas composition

Table 5.*3* illustrates the hydrogen consumption, carbon dioxide reacted or loss and gas composition of the gaseous product formed during upgrading of the bio-oil using binary mixtures of CO_2 and H_2. The hydrogen consumption significantly (p-value=0.0001) decreased with the increase in CO_2 pressure, and this decline may be due to the reduction in the initial hydrogen pressure. The lower amount of hydrogen consumption leads to a lower hydrogen content and heating values of the upgraded bio-oil. The carbon dioxide consumption was observed to be the highest for the reaction performed using the highest CO_2 cold pressure (300 psi). The carbon dioxide, being a C1 source might have undergone different types of reactions to form alcohols, alkanes, formates, carbon monoxide, etc.[15, 16, 45–48]. A significant increase (p-value=0.0001) in hydrogen sulfide (H_2S) in the gaseous product was observed with the increase in CO_2 cold pressure. It increased from

54.60±1.50 ppm to 87.31±1.22 ppm with the increase in CO_2 cold pressure from 0 to 300 psi. This increase in H_2S in the gaseous product suggested prominence of HDS reaction with the addition of CO_2. In a binary mixture of CO_2 and H_2, the major gases produced were not only the product of hydrocracking of the bio-oil but also hydrogenation of CO_2 occurring concurrently during the upgrading process. Initially, at the lower CO_2 cold pressure, a decrease in CO formation was observed when compared to the control experiment. With the increase of CO_2 cold pressure, CO formation increased which was expected. This increase in CO formation might be due to increase in reverse water gas shift reaction [47] as shown in Eq. 4. Still, the CO formation was equal to that observed in the control experiment. This indicates that the CO formation might be limited due to lower availability of hydrogen with decreased hydrogen pressure or also the CO might have undergone Fischer Tropsch synthesis to produce hydrocarbons [27, 45]. The decrease in selectivity of methane was also observed with the increase in CO_2 cold pressure; this decline may be due to the formation of methanol or CO at a higher pressure of CO_2 [27, 46, 47] as shown in Eq.7. Although the decrease in C_2 and C_3 hydrocarbons were observed with the increase in CO_2 cold pressure, there was a slight increase in C_4 and C_5 hydrocarbons.

$$CO_2 + H_2 \leftrightarrow CO + H_2O, \Delta H_{298K} = 41.2\ kJmol^{-1} \quad (4)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O, \Delta H_{298K} = -252.9\ kJmol^{-1} \quad (5)$$

$$CO_2 + 3H_2 \leftrightarrow CH_3OH + H_2O, \Delta H_{298K} = -49.4\ kJmol^{-1} \quad (6)$$

$$CH_4 + CO_2 \leftrightarrow 2CO + 2H_2, \Delta H_{298K} = 247.4\ kJmol^{-1} \quad (7)$$

Table 5.3. Gas composition of the gaseous product formed during upgrading of bio-oils using binary mixtures of CO_2 and H_2.

Pressure	Hydrogen consumed (mg/g feedstock)	Carbon dioxide reacted or loss (mg/g feedstock)	Gas composition of the product (Mol %)					
			H_2	CH_4	CO	CO_2	C_2H_6	C_3H_8
1000 H_2	34.61±0.29[a]	-	75.58±0.90[a]	9.67±0.19[a]	3.87±0.84[a]	0.06±0.04[a]	5.48±0.11[a]	4.50±0.08[a,b]
100 CO_2+900 H_2	33.79[a,¥]	7.26[a,¥]	58.56±1.61[b]	9.22±0.07[b]	2.94±0.18[b]	21.5±1.61[b]	4.72±0.17[b]	4.60±0.10[a]
200 CO_2+800 H_2	30.83±0.35[b]	44.02±5.60[b]	46.84±0.99[c]	8.01±0.25[c]	3.41±0.11[a,b]	33.76±0.67[c]	4.14±0.14[c]	4.30±0.07[b]
300 CO_2+700 H_2	25.71±0.10[c]	60.84±3.37[c]	32.44±2.62[d]	7.21±0.14[d]	3.82±0.09[a]	46.45±1.05[d]	3.86±0.05[c]	4.05±0.16[c]

Different alphabets in the superscripts of each group (column) denote the values are statistically significant at different pressure levels (rows) (α=0.05). numbers after ± symbol denotes standard deviation. [¥] standard deviation values are below one hundredth.

5.4. Conclusions

Influence of the mixture of CO_2 and H_2 on the catalytic upgrading of the algae bio-oils were studied. The upgraded bio-oil yields obtained using different binary mixture of CO_2 and H_2 at different pressure levels were observed to be similar with the control run. In the case of upgraded bio-oil properties, no improvement in the quality was observed with the use of the binary mixtures. Increase in the CO_2 cold pressure increased the upgraded bio-oil concentration in vacuum gas oil range. The results indicate that CO_2, being a C1 source not only, acted as reaction medium but also might have converted into the various product during upgrading. The use of CO_2 in the upgrading reaction increases the non-explosive regime which is an added benefit in terms of process safety. Although a significant effect of CO_2 on hydrogenation was not observed, further study with the increased CO_2 pressure is suggesssted.

5.5. Reference

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Chapter 6

Conclusions and Future Recommendation

6.1. Conclusions

Production of biofuel from algae has been demonstrated in this study. Firstly, algae were converted to bio-oil via hydrothermal liquefaction, and the bio-oils were further catalytically upgraded to produce upgraded bio-oil. This research was conducted to 1) examine the influence of biochemical composition during hydrothermal liquefaction of algae on product yields and fuel properties, 2) evaluate the influence of different heterogeneous catalysts on the upgrading of algal bio-oil, 3) investigate the effect of residence time on the upgrading product yields and its properties, and 4) analyze the influence of binary mixture of CO_2 and H_2 on catalytic upgrading of bio-oil produced from HTL of algae. Each of these objectives was fulfilled, and the major findings and conclusions are summarized below:

Objective 1: HTL of nine different algae species (having a broad variation in biochemical composition) were performed to study the influence of its biochemical composition on product yields and properties at 280 and 320^{o}C. The bio-oil yields obtained were higher at 320^{o}C than at 280^{o}C. Comparing the biochemical composition, the maximum bio-oil yield was obtained from the HTL of high lipid containing algae (65.96 wt.% for N-2). The predictive relationship developed followed the trend of lipids > proteins > carbohydrates, which were in agreement with the previous studies. The bio-oil yields obtained were not

only the product of sole effect of individual biochemical composition but also the interaction effect between them. The heating values of the bio-oils ranged from 31-36 MJ/kg. TAN of the bio-oil followed the trend lipids > carbohydrates > proteins. The bio-oils obtained from high lipid containing algae had a significant fraction in the diesel range. The COD values (35-160 g/L) of the aqueous phase were higher than the permissible value (120 mg/L) set by EPA. Thus, it required further treatment before dumping in wastewater streams. The aqueous phase contained a varied proportion of phosphorus, magnesium, and ammonia, which can be recovered as a slow releasing fertilizer, struvite.

Objective 2: Upgrading of bio-oil obtained from HTL of *Nannochloropsis sp* was performed. Five different catalysts (Ni/C, ZSM-5, Ni/ZSM-5, Ru/C and Pt/C) at two reaction temperatures of 300 and 350^{o}C at a weight hourly space velocity (WHSV) of 0.51 g/g_{cat}.h were investigated. The upgraded bio-oil yields obtained were higher at 300^{o}C when compared to 350^{o}C. However, a better-quality fuel was obtained at 350^{o}C. The maximum yield (61.5 wt.%) at 350^{o}C was obtained using 10% Ni/C. Among the different catalysts, Ru/C and Pt/C showed a better hydrodenitrogenation and hydrodeoxygenation capacity, and gave lower acidity values. Around 35-40% of the upgraded bio-oils were in the diesel range. Overall, the catalytic upgrading of algae bio-oil was effective in improving the quality of the bio-oil.

Objective 3: The effect of residence time (2, 4, 6 and 10 h) on the upgrading product yields and its properties were investigated. The upgraded bio-oil yield decreased from 59.30 wt.% to 50.44 wt.% with the increase in the residence time from 2 to 10 h. The maximum yield (60.20 wt.%) was observed at 4 h residence time. The properties of the upgraded bio-oils improved with the increase in residence time. The maximum higher heating value (44.32

MJ/kg), the lowest TAN, viscosity, and nitrogen content was observed at 10 h. However, the maximum energy recovery was obtained at 4 h residence time. Comparing the properties, yields and energy recovery of the upgraded bio-oils, and also considering a high energy input penalty associated with a longer residence time, 4 h residence time was optimum for the upgrading of the algae bio-oil.

Objective 4: The effect of a binary mixture of CO_2 and H_2 cold pressure on the upgrading of the bio-oil was investigated. No significant change in the upgraded bio-oil yield was observed with the introduction of CO_2. In the case of upgraded bio-oil properties, no improvement was observed with the introduction of CO_2. The higher heating value decreased with the introduction of CO_2 cold pressure and was lowest at 300 psi of CO_2+700 psi of H_2. Similarly, TAN increased with the introduction of CO_2. Although a significant improvement in the upgraded bio-oil was not observed with the introduction of CO_2, its incorporation in the upgrading system added benefit regarding process safety as it expands the non-explosive regime.

6.2. Future Recommendation

In the last decade, several studies on production of bio-oil from HTL of algae and its techno-economic evaluation have been performed. From those studies, it has been shown that feedstock cost strongly affects the overall biofuel cost. Apart from the feedstock production, there are still a lot of challenges in downstream processing, HTL, upgrading and co-product processing. This research has tried to address some of the issues related to HTL and upgrading and attempted to fill the knowledge gap existing in the literature. With the knowledge gained from this study, the research on biofuels from algae can be further expanded. The following topics are recommended for the future study:

a. Fractionation of proteins via hydrothermal processing

Algae consist of proteins, which are converted to nitrogenous compounds during hydrothermal liquefaction. Due to the high amount of nitrogen content in the bio-oil produced from algae, it cannot be blended with the petroleum crude. Further, upgrading of high nitrogen-containing algae bio-oil causes catalyst deactivation and shortens catalyst life, thus affecting overall process economics. So, removal of proteins is required for reduction of high nitrogen in the bio-oil. In addition, the proteins can be used as food supplements, which has more value than fuel and can supplement the cost of the process. Some studies [1, 2] related to protein extraction using hydrothermal processing has been performed but the overall quantification of proteins needs to be performed and conversion of protein-extracted algae to biofuels via hydrothermal liquefaction and upgrading of the bio-oil can be investigated.

b. Effect of inorganics during upgrading process

After HTL, the bio-oil still has a small quantity of inorganics present. The presence of inorganics might have catalytic effect on the bio-oil, but on the other hand, it might also deactivate the catalysts. Thus, an investigation into the effect of inorganics during upgrading process is suggested.

c. Optimization of solvent used during upgrading of bio-oil.

Studies [3] using solvent during upgrading has been performed to test its applicability. The use of solvent during upgrading of bio-oil produced from hydrothermal liquefaction seems a favorable route as the results are promising. But, the use of solvent affects the process economics due to its cost. Therefore, optimization of solvent used during upgrading can be investigated.

d. Use of supercritical fluids during upgrading

In this study, the effect of supercritical carbon dioxide during upgrading of algae bio-oil was investigated. Although the use of supercritical carbon was not effective as hypothesized, the addition of solvents in the bio-oil while upgradinsg with supercritical carbon might help in hydrogenation and is suggested for further study. Other supercritical fluids such as propane can also be tested while upgrading of bio-oil.

6.3. Reference

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Appendix

A

<u>Discussion A</u>. Demonstration of model selection for bio-oil yields at 280^{o}C and 320^{o}C.

The predictive model to determine bio-oil yields obtained from HTL of algae having different biochemical composition of algae were developed. Multiple linear regression techniques were used to derive the equation for bio-oil yield. There were four independent variables and along with their interactions were tested for the model. Only double variable interactions were incorporated in the initial model as the interaction effect of more than two variables over-parameterized the model. The initial multiple linear regression model for both temperatures is given below:

$$\text{Bio-oil yield} = \beta_1*L + \beta_2*C + \beta_3*P + \beta_4*A + \beta_5*L*P + \beta_6*L*C + \beta_7*L*A + \beta_8*P*C + \beta_9*P*A + \beta_{10}*C*A \quad [A.1]$$

Where, L, P, C, and A are lipids, proteins, carbohydrates, and ash content, respectively.

In the case of bio-oil yields at 320^{o}C;

Using the equation A.1, the initial model obtained was as follows;

$$\text{Bio-oil yield} = -1.29*L + 1.33*C + 0.99*P + 5.57*A + 0.00*L*P + 0.02*L*C + 0.22*L*A - 0.02*P*C - 0.11*P*A - 0.22*C*A \quad [A.2]$$

The above given model [Eqn. A.2] was significant at 5% level and the adjusted R-square was 0.99. However, some of the variables were insignificant and were collinear with other

variables resulting in multicollinearity problems as shown by variable inflation factor in Table A.1.

Table A. 1. Parameter estimates of the initial multiple linear regression model obtained at 320°C.

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
L	**1**	-1.29089	1.24611	-1.04	0.3587	1462.49334
C	**1**	1.33412	0.44952	2.97	0.0412	218.02464
P	**1**	0.99144	0.70007	1.42	0.2297	861.03822
A	**1**	5.56972	7.33672	0.76	0.4900	1810.08064
L*P	**1**	0.00308	0.01990	0.15	0.8845	286.39783
L*C	**1**	0.01844	0.03129	0.59	0.5874	896.60031
L*A	**1**	0.21590	0.10778	2.00	0.1157	360.17062
P*C	**1**	-0.02190	0.00658	-3.33	0.0291	34.37878
P*A	**1**	-0.11421	0.14546	-0.79	0.4762	1194.66992
C*A	**1**	-0.22435	0.15714	-1.43	0.2266	274.78776

Thus, variable selection was performed using forward selection method. The forward selection method gave the following model;

Bio-oil yield = - 0.54*L + 1.54*C + 0.57*P + 0.01*L*P + 0.18* L*A - 0.02*P*C - 0.13*C*A [A.3]

The above given model [Eqn. A.3] was significant at 5% level and adjusted R-square was 0.99. The model showed significant cross interaction effect between different variables (L*A, P*C and C*A). However, high variance inflation factor was observed which showed multicollinearity in the model as shown in Table A.2. Thus, Pearson correlation coefficients were compared among the different variables, and the variables (only interaction variables) having high correlation were removed to complete the model.

Table A. 2. Parameter estimate obtained using forward elimination method at 320°C.

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation
L	**1**	-0.53684	0.27799	-1.93	0.0948	105.08027
C	**1**	1.54575	0.22329	6.92	0.0002	77.66682
P	**1**	0.57235	0.11955	4.79	0.0020	36.24813
L*P	**1**	0.01002	0.00799	1.25	0.2502	66.73404
L*A	**1**	0.18757	0.03733	5.02	0.0015	62.36441
P*C	**1**	-0.02329	0.00392	-5.94	0.0006	17.61857
C*A	**1**	-0.13451	0.04981	-2.70	0.0306	39.85968

The final model obtained is given below;

Bio-oil yield = 0.96*L + 0.30*C + 0.43*P [A.4]

The model [Eqn. A.4] was significant at 5% level and had adjusted R-square of 0.98. Analysis of variance of the model is shown in Table A.3. All the variables were significant, and the variance inflation factor was within the range (<4) as shown in Table A.4. Model adequacy check on the obtained model was performed. Studentized residual and cook's D were checked for outliers and influential points. No, outlier points were observed, but an influential point was found as shown in Figure A.1. The accuracy of the predicted values was observed by plotting the predicted values with the experimental values as shown in Figure A.2. When compared to other studies, prediction with this model [Eqn. A.4] was more accurate and the distribution was balanced. Residual distribution was also consistent for all the variables as shown in Figure A.3. In addition, model validation was also performed with the model using the results obtained in other literature irrespective of the process condition as shown in Table A.5.

Table A. 3. Analysis of Variance for model obtained at 320ºC.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	**3**	32835	10945	256.22	<.0001
Error	**11**	469.89124	42.71739		
Uncorrected Total	**14**	33305			

Table A. 4. Parameter estimates and confidence limits of variables at 320ºC.

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Variance Inflation	95% Confidence Limits	
L	**1**	0.96372	0.10279	9.38	<.0001	2.87326	0.73748	1.18996
C	**1**	0.29730	0.08934	3.33	0.0067	2.48675	0.10066	0.49395
P	**1**	0.43499	0.05980	7.27	<.0001	1.81377	0.30338	0.56660

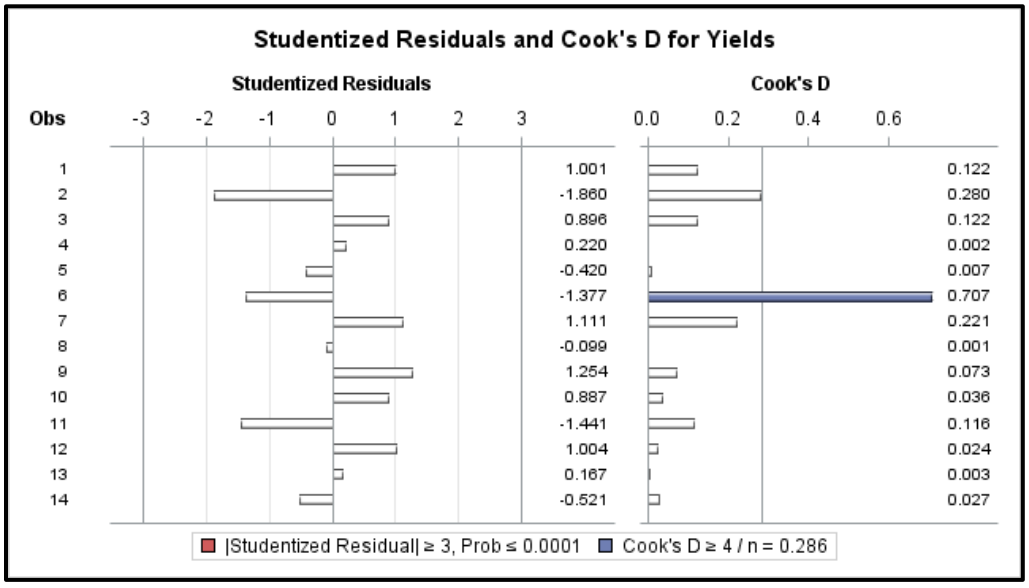


Figure A. 1. Studentized residual and cook's D of the model obtained at 320ºC.

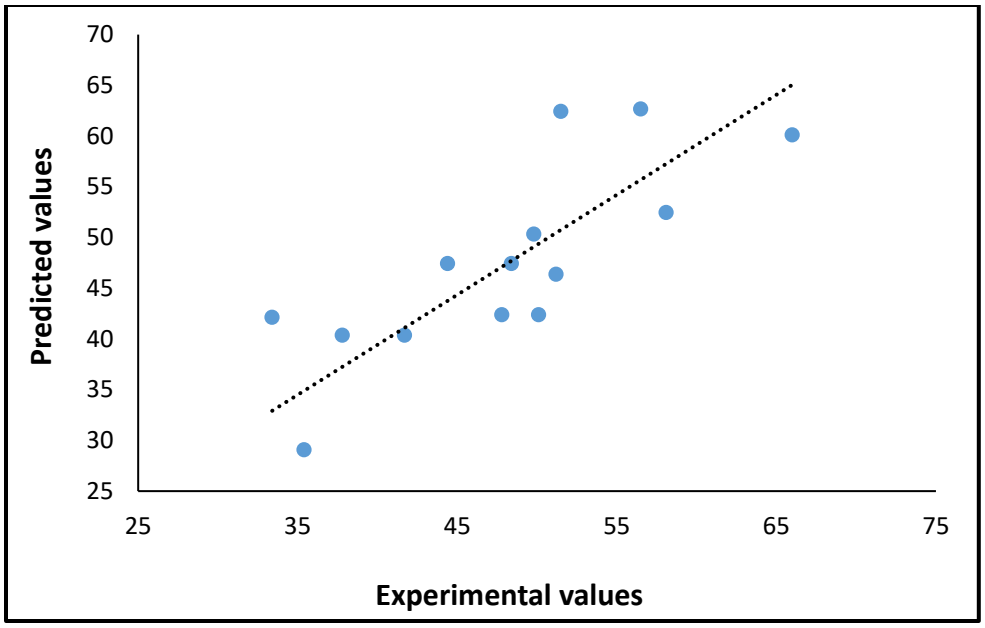


Figure A.2. Relationship between predicted yield with the experimental yield obtained at 320ºC.

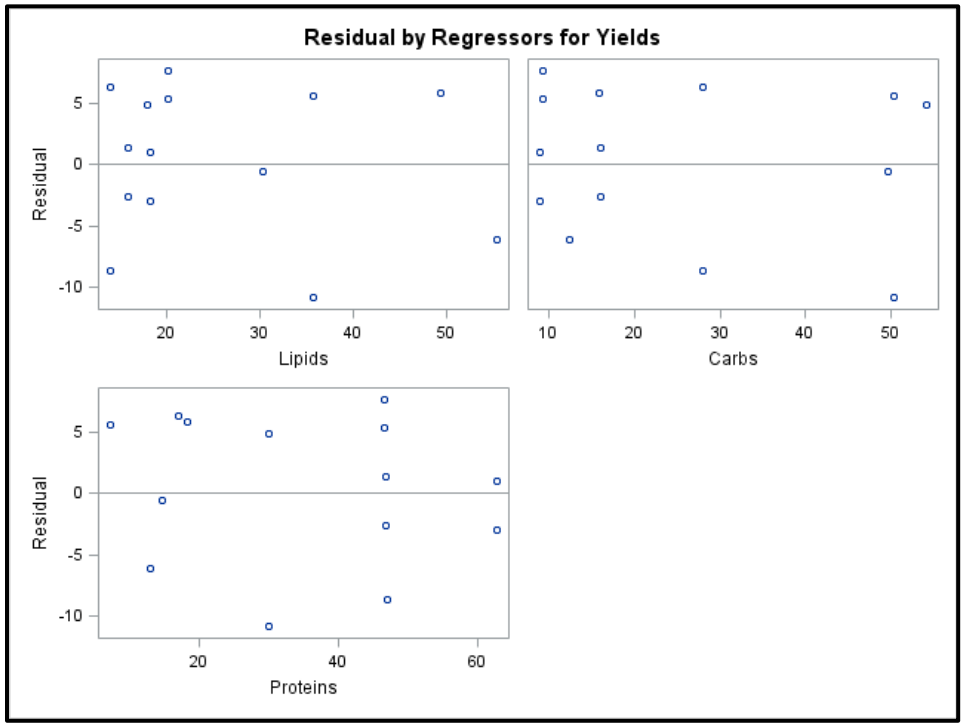


Figure A.3. Residual by regressors for bio-oil yields at 320ºC.

Table A.5. Biochemical composition of algae and HTL data for model validation for the model obtained at 320ºC.

Algae	Biochemical Composition				HTL Process Conditions			Bio-oil Yields (wt.%)		Citation
	Lipids	Carbohydrate	Protein	Ash	Temp (ºC)	Time (min)	Solid %	Experimental	Prediction*	
Chlorella pyrenoidosa	0.2	23.3	71.5	5	300	60	25	41	37.92	Gai et al. (2015)
Chlorella pyrenoidosa	0.2	23.3	71.5	5.7	320	60	25	38	37.92	Gai et al. (2015)
Spirullina platensis	4.8	21.2	64.7	9.6	300	60	25	41.9	38.78	Gai et al. (2015)
Spirullina platensis	4.8	21.2	64.7	9.6	320	60	25	36	38.78	Gai et al. (2015)
Desmodesmus sp.	14	33	44	8	300	5	10	40.5	42.26	Alba et al. (2012)
Desmodesmus sp.	14	33	44	8	300	15	10	41.9	42.26	Alba et al. (2012)
Desmodesmus sp.	14	33	44	8	300	30	10	43.8	42.26	Alba et al. (2012)
Desmodesmus sp.	14	33	44	8	300	60	10	46.6	42.26	Alba et al. (2012)
Desmodesmus sp.	14	33	44	8	325	5	10	40.7	42.26	Alba et al. (2012)
Desmodesmus sp.	14	33	44	8	325	60	10	41.2	42.26	Alba et al. (2012)
Tetraselmis sp.	14	22	58	6	310	5	16	40	44.98	Eboibi et al. (2014)
Tetraselmis sp.	14	22	58	6	310	15	16	40	44.98	Eboibi et al. (2014)
Tetraselmis sp.	14	22	58	6	310	30	16	41	44.98	Eboibi et al. (2014)
Tetraselmis sp.	14	22	58	6	310	45	16	46	44.98	Eboibi et al. (2014)
Tetraselmis sp.	14	22	58	6	310	60	16	43	44.98	Eboibi et al. (2014)
Nannochloropsis sp.	14	20	59	3	300	20	15	51	44.81	Valdez et al. (2012)
Nannochloropsis sp.	14	20	59	3	300	40	15	47	44.81	Valdez et al. (2012)
Nannochloropsis sp.	14	20	59	3	300	60	15	42	44.81	Valdez et al. (2012)
Nannochloropsis sp.	14	20	59	3	300	90	15	42	44.81	Valdez et al. (2012)
Scenedesmus sp.	8	31	50	11	300	10	15	39	38.48	Valdez et al. (2014)
Scenedesmus sp.	8	31	50	11	300	20	15	38	38.48	Valdez et al. (2014)
Scenedesmus sp.	8	31	50	11	300	40	15	41	38.48	Valdez et al. (2014)
Scenedesmus sp.	8	31	50	11	300	60	15	34	38.48	Valdez et al. (2014)
Chlorella sp.	60	26	9	5	300	30	25	63	69.27	Li et al (2014)
Chlorella sp.	60	26	9	5	300	60	15	61	69.27	Li et al (2014)
Chlorella sp.	60	26	9	5	300	90	20	66	69.27	Li et al (2014)
Chlorella (C-1)	15.78	16.1	46.8	7.42	320	30	15	39.73	40.10	This study
Chlorella (C-2)	30.28	49.7	14.63	2.99	320	30	15	49.75	50.26	This study
Nannochloropsis (N-1)	55.36	12.39	12.92	6.65	320	30	15	56.87	62.41	This study
Nannochloropsis (N-2)	49.26	15.94	18.15	7.37	320	30	15	65.96	59.87	This study
Nannochloropsis (N-3)	20.09	9.21	46.62	8.28	320	30	15	48.93	42.09	This study
Nannochloropsis (N-4)	18.12	8.92	62.78	3.42	320	30	15	46.40	47.06	This study

Pavlova (P-1)	13.88	28	46.94	3.47	320	30	15	36.35	41.91	This study
Scenedesmus (S-1)	35.66	50.4	7.15	2.02	320	30	15	54.79	52.43	This study
Scenedesmus (S-2)	17.83	54.17	30.06	2.1	320	30	15	51.22	46.29	This study

*Mathematical regression model developed in this study was used for prediction.

In the case of bio-oil yields at 280^{o}C similar method (forward selection) used for 320^{o}C was followed. The final model obtained at 280^{o}C is given below;

Bio-oil yield = 0.90*L + 0.22*C + 0.32*P [A.7]

The model [Eqn. A.7] was significant at 5% level and had adjusted R-square of 0.97. Analysis of variance of the model is shown in Table A.6. All the variables were significant, and the variance inflation factor was within the range (<4) as shown in Table A.4. Model adequacy check on the obtained model was performed. No correlation among the independent variables were found as the variance inflation factor were <4. Studentized residual and cook's D were checked for outliers and influential points. No, outlier points were observed, but an influential point was found as shown in Figure A.4. Comparison between predicted values and experimental values were made by plotting them as shown in Figure A.5. Residual distribution was also consistent for all the variables as shown in Figure A.6.

Table A. 6. Analysis of variance of the model obtained at 280^{o}C.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	3	21959	7319.63013	160.40	<.0001
Error	10	456.32471	45.63247		
Uncorrected Total	13	22415			

Table A. 7. Parameter estimates of the model obtained at 280^{o}C.

Parameter Estimates						
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**Variance Inflation**
Lipids	**1**	0.89823	0.10651	8.43	<.0001	2.78761
Carbs	**1**	0.21812	0.09343	2.33	0.0417	2.52919

Parameter Estimates						
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**Variance Inflation**
Proteins	**1**	0.31540	0.06448	4.89	0.0006	1.77601

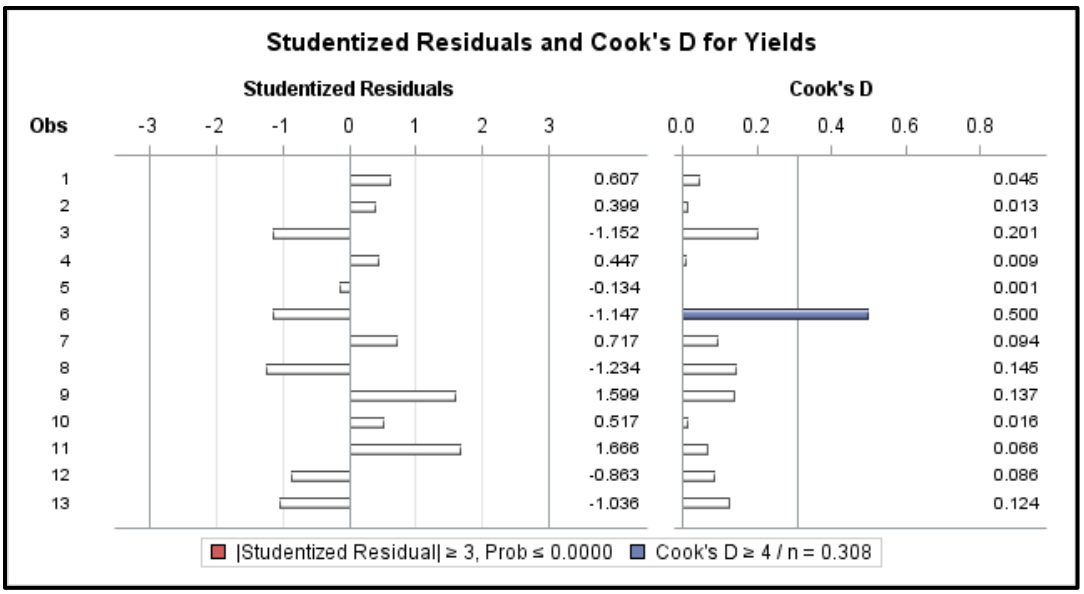


Figure A. 4. Studentized residual and cook's D of the model obtained at 280ºC.

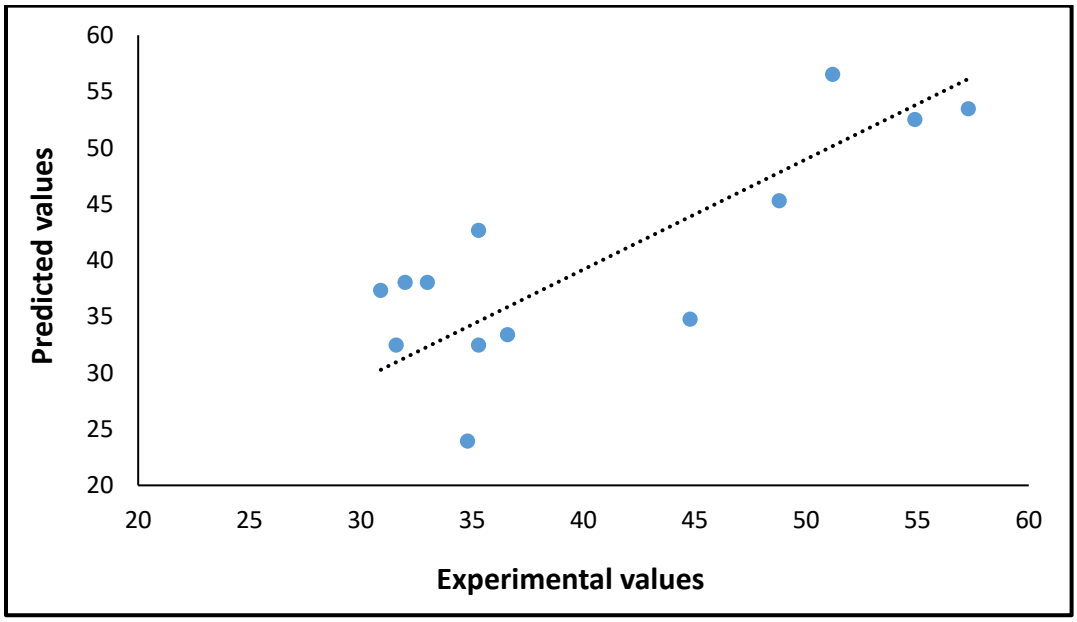


Figure A. 5. Relationship between predicted yield with the experimental yield at 280ºC.

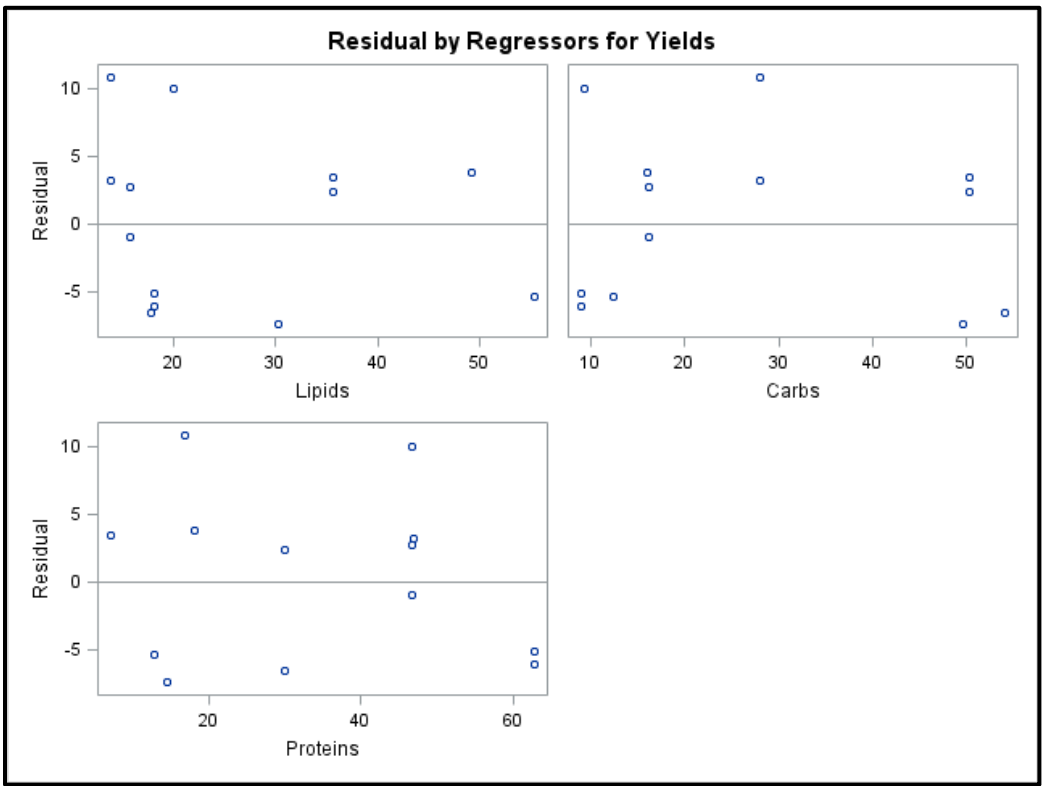


Figure A. 6. Residual by regressors for bio-oil yields at 280ºC.

Table A. 8. Fatty acid profile (as FAMEs) present in different algae strains.

Group	Name	Percentage Lipids						
		C-1	C-2	N-1	N-2	N-3	S-1	S-2
C6:0	Caproic acid methyl ester	1.39	0.69	0.21	0.20	-	-	1.50
C8:0	Caprylic acid methyl ester	1.20	0.54	0.18	0.17	0.71	0.36	1.10
C10:0	Capric acid methyl ester	0.88	0.40	0.16	0.16	0.65	0.30	0.87
C11:0	Undecanoic acid methyl ester	10.56	4.25	1.99	2.03	7.63	2.46	8.98
C12:0	Lauric acid methyl ester	-	-	0.25	0.29	-	-	-
C14:0	Myristic acid methyl ester	0.78	0.52	3.13	4.18	3.83	0.41	0.85
C15:0	Pentadecanoic acid methyl ester	0.65	0.27	0.21	0.23	0.42	0.18	0.47
C16:1	Palmitoleic acid methyl ester	9.02	3.25	31.70	34.34	27.28	3.59	2.49
C16:0	Palmitic acid methyl ester	30.30	45.83	38.97	41.99	21.84	25.46	35.34
C17:1	cis-10-Pentadecenoic acid methyl ester	3.79	0.39	0.24	0.22	0.76	0.28	1.66
C17:0	Heptadecanoic acid methyl ester	17.60	0.73	0.39	0.31	4.90	0.28	4.60
C18:2	Linoleic acid methyl ester	1.13	18.89	11.00	7.68	2.86	42.56	9.43
C18:1	Oleic acid methyl ester	3.39	10.54	4.20	1.58	2.56	6.36	5.72
C18:0	Stearic acid methyl ester	0.57	1.56	0.81	0.55	-	3.30	0.77
C18:3	Linolenic acid methyl ester	18.76	11.63	1.60	0.65	0.80	13.37	26.23
C20:4	Methyl eicosatetraenoic acid metyl ester	-	-	1.69	2.08	7.34	-	-
C20:3	Ecosaterienoic acid methyl ester	-	-	0.13	0.19	0.44	0.75	-
C20:2	Eicosadienoic acid methyl ester	-	-	3.14	3.18	17.98	-	-
C20:0	Arachidic acid methyl ester	-	0.50	-	-	-	0.33	-
C20:1	Eicosenoic acid methyl ester	-	-	-	0.01	-	-	-
	Sum	100	100	100	100	100	100	100

Note: N-4 and P-1 were not analyzed for FAME determination

Table A. 9. List of compounds present in aqueous phase obtained from HTL of algae at 320ºC.

Compounds	Retention Time
Pyrazine, 2,6-dimethyl-	6.983
2-Cyclopenten-1-one, 2-methyl-	7.7898
4-Pyridinamine, N,N-dimethyl-	9.1116
Pyrazine, 2-ethyl-5-methyl-	9.1688
2-Cyclopenten-1-one, 2,3-dimethyl-	11.8524
2-Cyclopenten-1-one, 2,3,4-trimethyl-	13.1513
Phenol	13.2657
2,2-Dimethyl-N-ethylpyrrolidine	14.6104
Piperidine, 1,2-dimethyl-	14.6562
1-Ethyl-2-pyrrolidinone	14.8736
Phenol, 4-methyl-	15.5202
2,5-Pyrrolidinedione, 1-ethyl-	16.1439
1-Piperidinecarboxaldehyde	16.4986
N-(3-Methylbutyl)acetamide	16.7847
5,6-Dihydro-6-methyluracil	16.9736
2,3,4-Trimethyl-isoxazol-5(2H)-one	18.1122
2,5-Pyrrolidinedione, 1-propyl-	18.4098
Caprolactam	20.1378
2-Hydroxy-4-hydroxylaminopyrimidine	20.4983
2-Methoxy-3-propyl-phenylamine	26.4377
Acetamide, N-(2-phenylethyl)-	26.7639
2-Formylbenzeneboronic acid	27.6679
4-Chromanol	28.1543
2-Pyrrolidinone, 1-(phenylmethyl)-	28.6578
Benzene, (4-methyl-4-pentenyl)-	31.3471
1,2,3,4-Tetrahydro-7-isoquinolinol	33.5558
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	36.537
Proline, N-methyl-, butyl ester	38.6484
Pyrrolidine, 2-butyl-1-methyl-	38.8887
2,5-Piperazinedione, 3-benzyl-6-isopropyl-	41.681
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-	42.1846
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	43.6894
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	43.9298

Table A. 10. Elements found in aqueous phase of different algae strains.[¥]

Elements	C-1 (mg/kg)	N-1 (mg/kg)	N-3 (mg/kg)	N-4 (mg/kg)	S-1 (mg/kg)
Ca	758.36±1.54	252.42±7.02	562.69±1.40	24.72±4.79	346.23±8.05
Fe	78.59±2.12	0.66±0.34	8.23±0.48	5.12±2.21	9.40±1.84
Cu	2.96±0.22	2.76±0.23	1.19±0.15	7.03±0.28	10.71±0.37
Zn	9.47±0.22	8.63±0.27	4.67±0.09	21.59±0.28	16.90±0.21
Mn	0.73±0.09	0.46±0.06	nd	1.20±0.09	2.09±0.07
Mg	15.30±0.17	147.16±2.42	2.28±0.10	32.53±0.61	185.84±1.82
Ni	4.13±0.99	2.22±0.49	0.55±0.86	1.74±1.06	1.51±1.50
K	1799.92±56.88	807.96±81.58	2288.51±72.36	4151.15±73.76	903.35±40.70
Na	209.85±8.16	371.06±6.28	1062.06±11.63	1841.88±41.01	242.65±5.69

[¥] Reported values are the average of two (n=2). nd: Not detectible

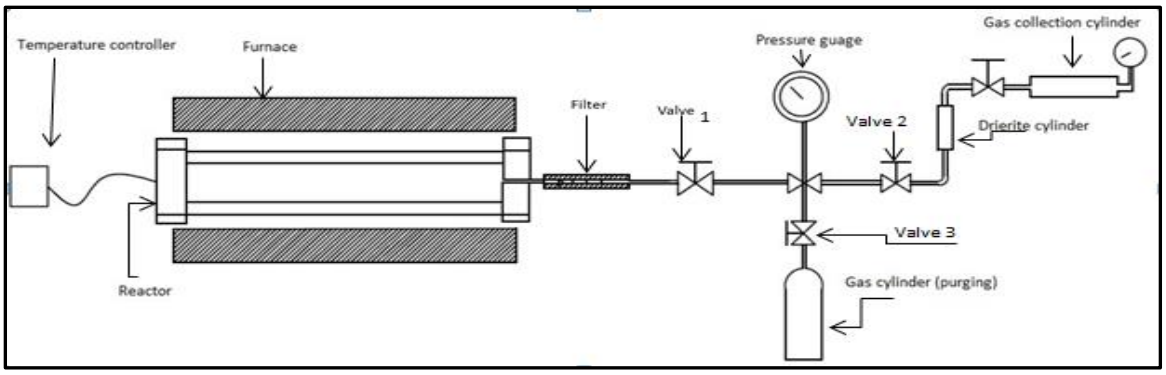


Figure A. 7. Schematic representation of a high-pressure experimental unit for hydrothermal liquefaction

Appendix B

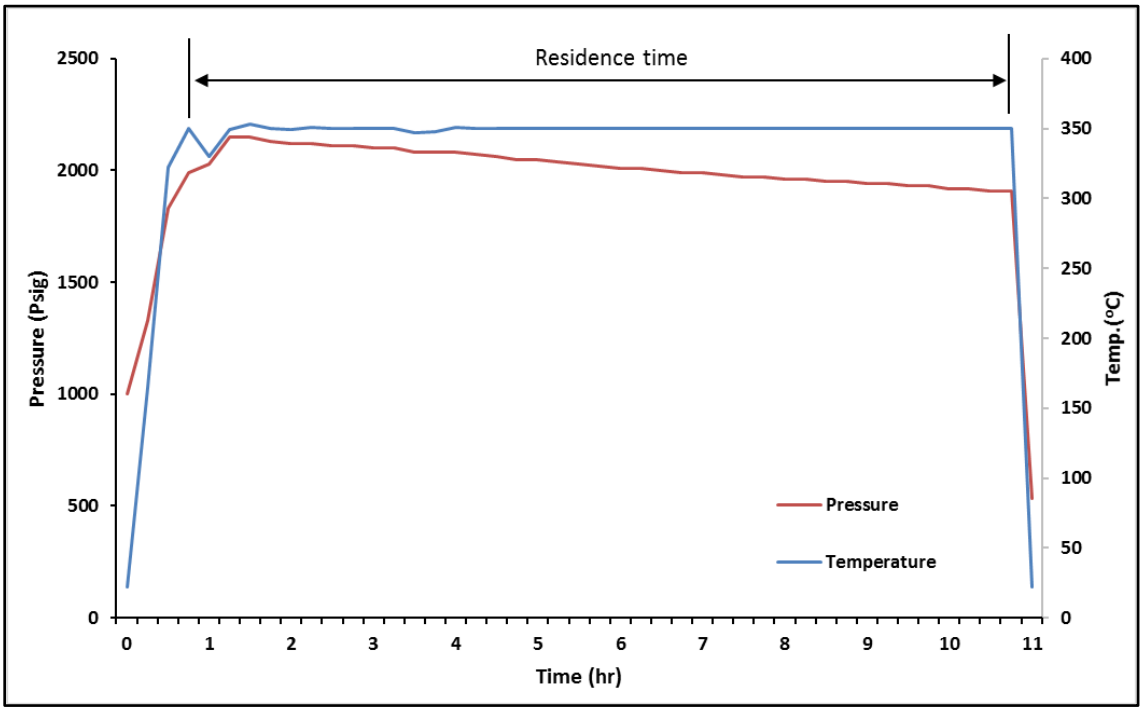


Figure B.1. Pressure and temperature profile of a typical upgrading process.

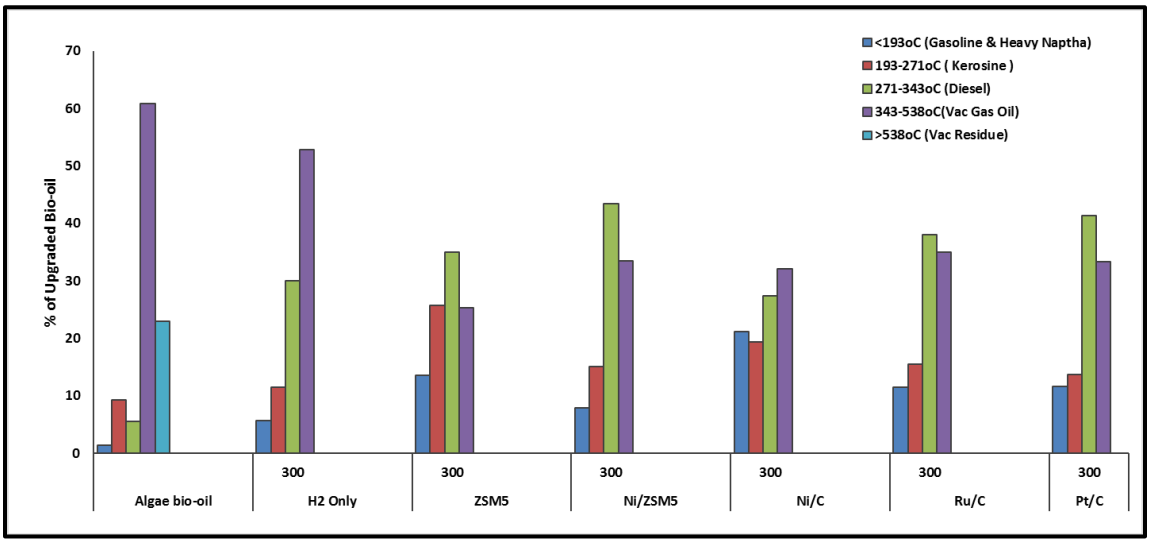
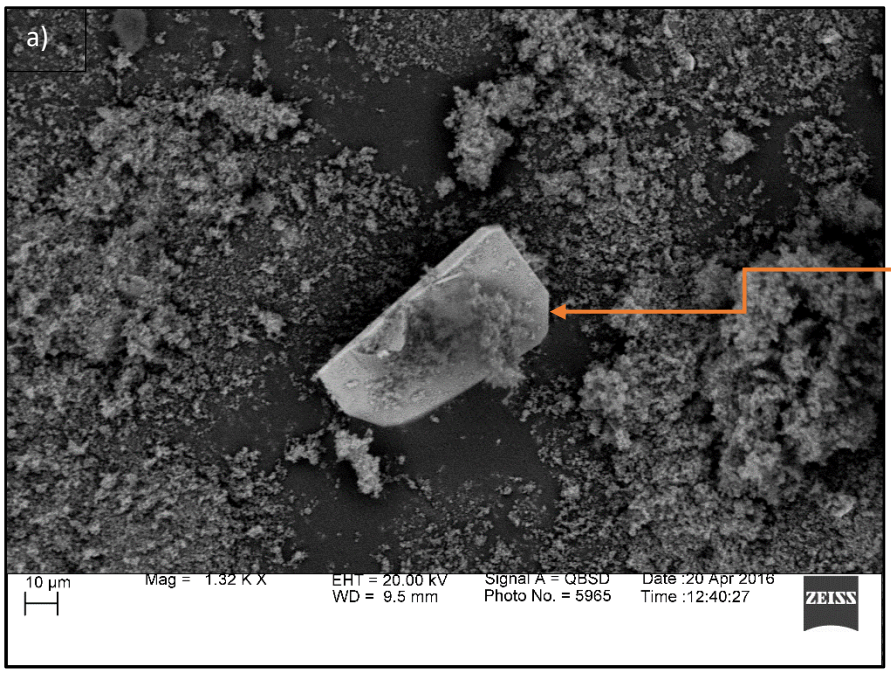
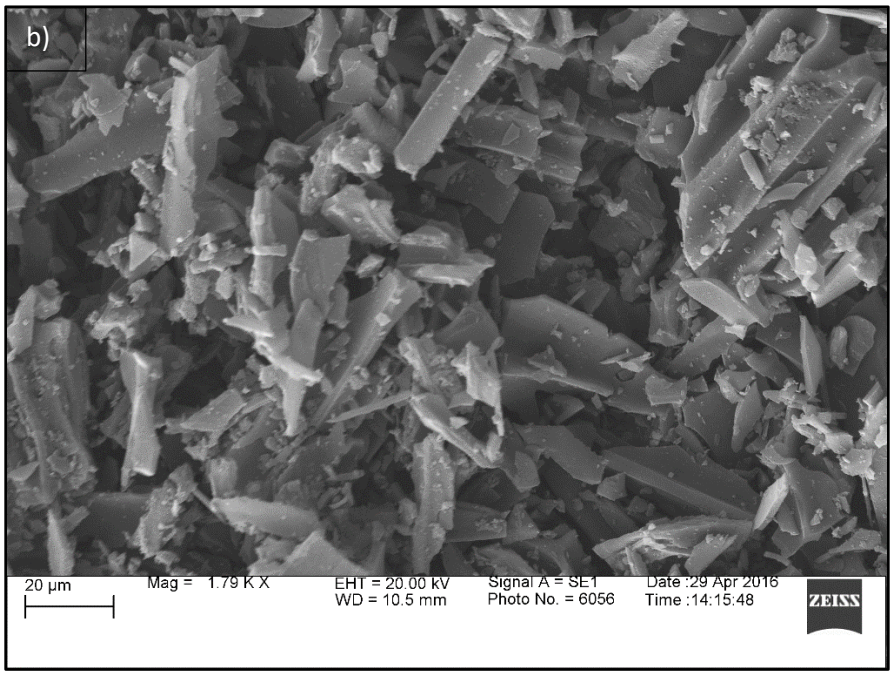


Figure B.2. Boiling point distribution of upgraded oils obtained at 300 °C



Iron sinters



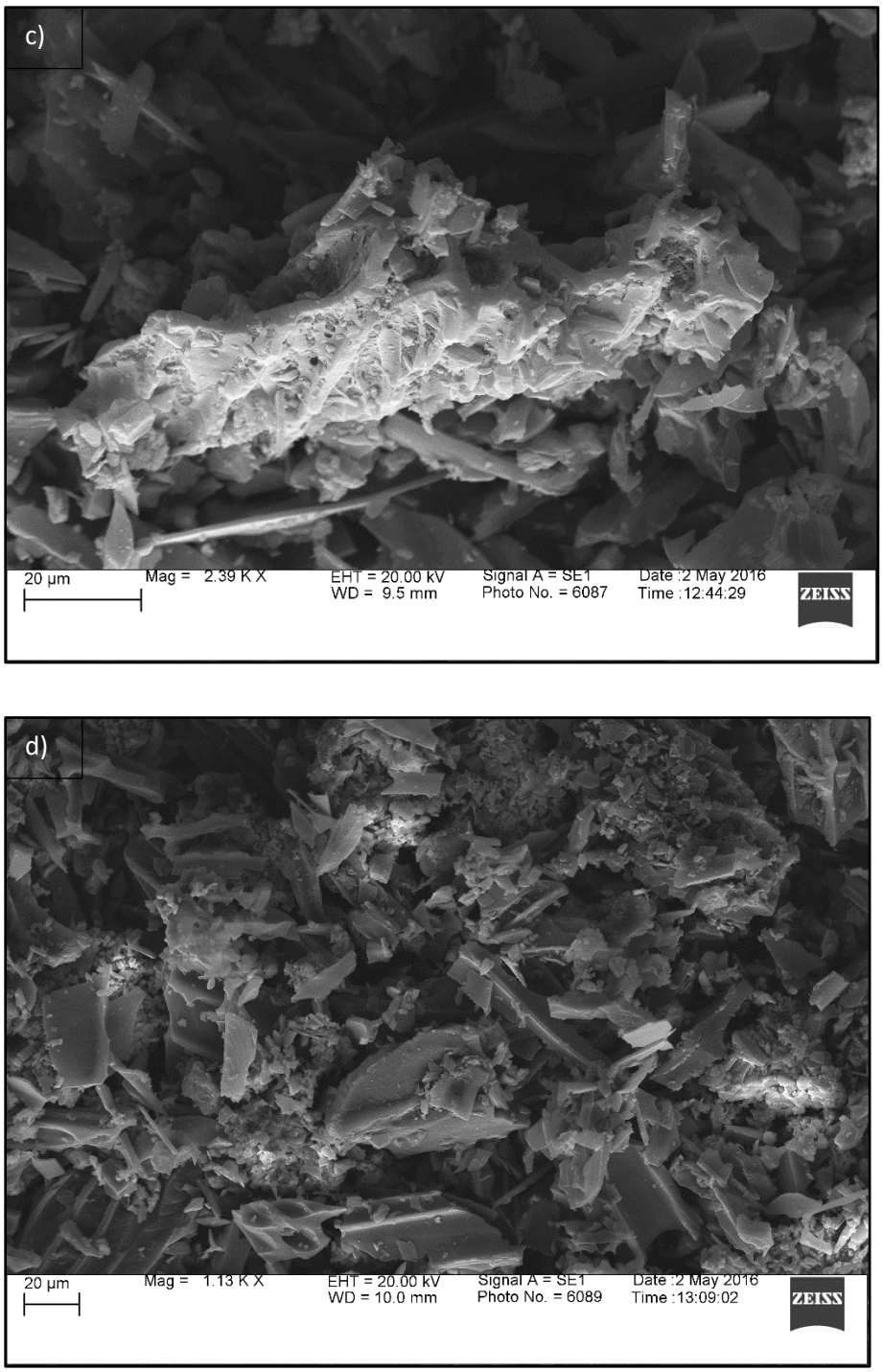


Figure B.3. SEM pictures a) iron sinter in the spent catalyst b) fresh Ni/C catalyst c) spent catalyst Ni/C at 300 ºC and d) spent catalyst Ni/C at 350 ºC

Table B.1. List of bio-oil compounds under each group detected by GCMS.

Groups	Retention Time (min)
Hydrocarbons	
Aromatics	
Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	18.18
Naphthalene, 1,2-dihydro-1,5,8-trimethyl-	18.34
Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	44.97
Cholest-4-ene	45.73
Aliphatics	
Pentadecane	20.16
Hexadecane	22.34
Heptadecane	24.69
1-Eicosene	27.35
Nonadecane	35.96
Esters	
Hexadecanoic acid, methyl ester	30.74
Hexadecanoic acid, ethyl ester	31.95
9-Octadecenoic acid, ethyl ester	35.24
Oxygenates	
2-Pentadecanone, 6,10,14-trimethyl-	29.68
9,17-Octadecadienal, (Z)-	39.48
7,11-Hexadecadienal	41.59
Nitrogenates	
Amides	
Nonadecanamide	38.10
Octadecanamide	38.20
Tetradecanamide	38.36
Cyclic nitrogenates	
1H-Pyrrole, 2-ethyl-3,4,5-trimethyl-	15.86
Indole	21.23
Pyridine, 2-phenyl-	22.91
1H-Indole, 3-methyl-	23.03
Carbazole,1-4 dimethyl-	36.98
Phenols*	
Phenol	13.23
Phenol, 4-methyl-	15.51
Phenol, 3-(ethylamino)-4-methyl-	16.18

*observed only in upgraded oil obtained at 300 ^{o}C

Table B.2. SEM-EDS study of the fresh and spent catalysts.

Element	ZSM5 Fresh Catalyst Weight%	ZSM5 Fresh Catalyst Atomic%	ZSM5 Spent Catalyst Weight %	ZSM5 Spent Catalyst Atomic %
C	nd	nd	29.78±2.24	41.96±2.37
O	54.33	67.6	39.05±0.19	41.34±0.94
Al	0.82	0.61	0.51±0.06	0.32±0.04
Si	44.85	31.79	21.20±1.44	12.79±1.09
Cl	nd	nd	3.96±0.50	1.89±0.27
Cr	nd	nd	0.88±0.08	0.28±0.02
Fe	nd	nd	4.60±0.42	1.39±0.15

Element	Ni/ZSM5 Fresh Catalyst Weight %	Ni/ZSM5 Fresh Catalyst Atomic %	Ni/ZSM5 Spent Catalyst Weight %	Ni/ZSM5 Spent Catalyst Atomic %
C	nd	nd	20.92±2.29	31.77±2.87
O	49.22±0.19	65.25±0.45	41.27±0.99	47.14±1.91
Al	0.94±0.04	0.74±0.03	0.54±0.07	0.37±0.04
Si	40.50±1.38	30.57±0.96	24.04±1.05	15.64±0.74
Cl	nd	nd	4.82±0.96	2.49±0.55
Cr	nd	nd	1.74±1.97	0.83±1.07
Fe	nd	nd	3.19±0.37	1.04±0.14
Ni	9.03±0.81	3.28±0.32	4.64±0.05	1.44±0.04

Element	Ni/C Fresh Catalyst Weight%	Ni/C Fresh Catalyst Atomic%	Ni/C Spent Catalyst Weight%	Ni/C Spent Catalyst Atomic%
C K	90.21±3.97	97.81±0.95	90.66±0.21	97.86±0.39
Cr K	nd	nd	3.00±0.70	0.70±2.43
Fe K	nd	nd	4.64±0.14	1.05±0.53
Ni k	9.07±4.96	2.18±0.95	1.68±0.44	0.37±1.81

Element	Ru/C Fresh Catalyst Weight %	Ru/C Fresh Catalyst Atomic %	Ru/C Spent Catalyst Weight %	Ru/C Spent Catalyst Atomic %
C	93.90±1.53	99.23±0.20	86.97±1.53	96.91±0.36
Si	nd	nd	0.67±0.06	0.32±0.03
Cr	nd	nd	1.23±0.00	0.32±0.00
Fe	nd	nd	8.66±0.10	2.07±0.05
Ni	nd	nd	1.24±0.15	0.28±0.03
Ru	6.09±1.53	0.76±0.20	1.84±0.49	0.24±0.06

Element	Pt/C Fresh Catalyst Weight %	Pt/C Fresh Catalyst Atomic %	Pt/C Spent Catalyst Weight %	Pt/C Spent Catalyst Atomic %
C	93.43	99.57	76.43±8.14	89.90±7.96
O	nd	nd	15.23±0.44	13.67±0.27
Cr	nd	nd	1.04±0.18	0.29±0.06
Fe	nd	nd	10.83±0.83	2.74±0.19
Pt	6.57	0.43	4.49±0.85	0.32±0.05