

**Characterization of Biofuels Produced from Hydrothermal Liquefaction of Algae, Its
Subsequent Upgrading and Fractional Distillation**

By

Nikhil Jain

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 15, 2018

Keywords: filamentous, algae, bio-oil, hydrothermal liquefaction, upgrading, distillation

Copyright 2018 by Nikhil Jain

Approved by

Sushil Adhikari, Chair, Professor of Biosystems Engineering
Carlos Carrero, Assistant Professor of Chemical Engineering
Brendan Higgins, Assistant Professor of Biosystems Engineering

Abstract

Crude petroleum is the major source of transportation fuels in the world whose reserves are declining day by day. It has become imperative to look for an alternative that can either replace or substitute the current energy demand and prolong its extinction. Biomass is the only source of renewable energy that can be converted into liquid hydrocarbon fuels. Algae, among various biomass, has clear advantage because of high productivity, high carbon dioxide sequestration rate and ability to grown in non-arable land. Algae being an aquatic biomass is well suited for Hydrothermal liquefaction (HTL), a thermochemical process that under hot compressed water conditions can convert wet biomass into biocrude with other by-products being solid char, aqueous phase, and gases and evade intensive energy required for drying in other processes.

Filamentous algae are cheaper to grow as compared to microalgae as it is easier to harvest them. Apart from that, algae require high amount of nitrogen nutrients for their growth which if substituted with cheap source could prove to be economical. The research objective was to compare the product yield and properties obtained from hydrothermal liquefaction of filamentous and microalgae. The filamentous algae were grown in five different nitrogen nutrient conditions that were: sufficient nitrate (A_NO₃), sufficient urea (A_Urea), 14- and 21-days starved nitrate (14_NO₃ and 21_NO₃) and 14 days starved urea (14_Urea). All HTL experiments were carried out at 320°C for 30 mins residence time and algae loading of 15 wt% with the rest being DI water.

Highest oil yield of 64.2 wt% was obtained from 14_Urea which had highest FAME of 53.2 wt% while 21_NO₃ having high carbohydrate content of 57.1 wt% produced highest char yield of 32.1 wt%. The oil yield for A_Urea, A_NO₃, and A_Micro were 44, 34.3, and 49.9 wt%, respectively. Highest heating value (HHV) of HTL oil from all algae ranged from 30.5 to 34.5 MJ/kg. Total acid number (TAN) was lowest for A_Micro (31.36 mg KOH/g) while for other non-stressed algae it was around 40 mg KOH/g. TAN for stressed algae oil was slightly above 100 mg KOH/g which was because of the presence of around 50% of Hexadecanoic acid as observed in GC-MS of oil. Aqueous phase was rich in nutrients with aqueous phase from nitrate algae having about 50% of its nitrogen in the form of ammonium ion.

The concentration of heteroatom, like nitrogen (2-5 wt%) and oxygen (11.5-21 wt%) prevents the bio-crude to be used as fuels. In order to reduce heteroatom concentration and TAN values and improve energy density upgrading of oil is necessary. The effect on upgraded oil from the use of polar or aromatic solvents, for product separation has not been accounted for after upgrading experiments. This study presents a detailed analysis on the yield and properties of oil obtained from upgrading with and without the presence of the catalyst (5% Ru/C and H₂ only) and effect of toluene and dichloromethane (DCM) as product separating solvent. Further atmospheric pressure fractional distillation of bio-oil and upgraded oils was carried out to estimate the yield and properties of distilled fractions. Hydrothermal liquefaction bio-crude from *Nannochloropsis* sp. microalgae was used in this study as other filamentous algae was not available. Mass yields, TAN, HHV, the elemental and chemical composition was evaluated for each oil.

Catalytic upgraded oil extracted from toluene had oxygen content of 0.75 wt% and HHV of 43.36 MJ/kg while DCM extracted oil had 5.93 wt% oxygen and HHV of 37.72 MJ/kg. Catalyst showed better activity for denitrogenation and nitrogen values were not all that different between

different solvents. Fractionation produced three distilled fractions (F1 <220°C, F2 220-350°C, and F3 >350°C). Light and middle fractions from toluene extracted upgraded oil had better fuel properties than DCM extracted upgraded oil. Middle and heavy fractions had higher heating value than starting oil for all the treatment conditions. Nitrogen was found to be distributed in heavy fraction in all cases and prominently as nitrile compounds. Highest heating value of 45.18 MJ/kg was obtained for F2_RuC_Tol which and had TAN value of 1.38 mg KOH/g.

Hydrothermal liquefaction of filamentous algae grown on cheap urea produced oil with similar yields and properties as oil from expensive microalgae. This could bring down the cost of production of bio-crude from algae. For upgrading experiments, different solvents during product separation effects the yield and quality of oil produced and is not solely depend on process parameters and activity of catalyst. Fractional distillation produced distilled fractions with different concentration of heteroatom distribution for which appropriate catalyst could be used for effective denitrogenation or deoxygenation and avoid catalyst poisoning because of presence of other heteroatoms.

Acknowledgements

I would like to express my deepest gratitude towards my major advisor Dr. Sushil Adhikari for giving me the opportunity to work with him as a graduate student and providing valuable mentorship. He showed great faith in me when he provided necessary funds and showed great patience when I was building a continuous high-pressure reactor. That project gave me confidence and a sense of accomplishment that I can never forget. I would like to thank Dr. Rajdeep Shakya who taught me almost all the instruments needed for my research and became a great friend during this process.

I thank my committee members Dr. Carlos Carrero and Dr. Brendan Higgins who provided valuable feedback during my research proposal presentation that ultimately formulated my research objectives. A special thank you to Dr. Thomas Dempster contributions from AzCATI, Mesa, Arizona who provided algae for my research and cleared my doubts whenever I approached.

I would like to thank all my past and present colleagues with whom I share memorable memories. Dr. Saravanan R. Shanmugam and Dr. Hyungseok Nam provided great help with my research and gave confidence to take ownership of the world class facility and instruments we have in our lab. I would also thank Sanjeev K.C., Vivek Patil, and Ritesh Karki for helping me with my research and being great friends.

At the end I would thank my parents and my whole family who sacrificed a lot and always imparted the feeling that I can achieve all I want. They unknowingly provide me with the strength to rise whenever I fail. I would acknowledge my friends who I made over the years that became family away from home.

Table of Contents

Abstract.....	ii
Acknowledgements	v
Table of contents	vii
List of Figures.....	ix
List of Tables	x
Chapter 1: Introduction	1
1.1 Background	1
1.2 Research Objectives	2
1.3 References	5
Chapter 2: Literature Review.....	6
2.1 Energy Scenario	6
2.2 Biomass and Biofuels.....	7
2.3 Algae as Biomass	8
2.4 Hydrothermal Technology	15
2.5 Properties of water	15
2.6 Hydrothermal Carbonization.....	17
2.7 Hydrothermal Liquefaction	17
2.7.1 Effect of Temperature	18
2.7.2 Effect of Residence Time	20
2.7.3 Effect of Catalyst.....	21
2.7.3.1 Homogenous Catalyst	21
2.7.3.2 Heterogenous Catalyst.....	22
2.7.4 Effect of Biochemical Composition of Algae	24
2.7.4.1 Conversion of Lipids	24
2.7.4.2 Conversion of Proteins	26
2.7.4.3 Conversion of Carbohydrates	27
2.8 Bio-oil Properties	29

2.9 Upgrading of Bio-oil	30
2.10 References	35
Chapter 3: Compare the Product Yield and Properties Obtained from Hydrothermal Liquefaction of Filamentous Algae and Microalgae.....	46
Abstract	46
3.1 Introduction	47
3.2 Materials and Methodology	49
3.2.1 Apparatus and Experimental Procedure	50
3.2.2 Product Separation	50
3.2.3 Product Analysis	51
3.3 Results and Discussion.....	54
3.3.1 Algae Characterization	54
3.3.2 Liquefaction Yields.....	56
3.3.3 Bio-oil Characterization	57
3.3.4 Char Characterization.....	64
3.3.5 Aqueous Phase Characterization	65
3.4 Conclusion.....	66
3.5 References	67
Chapter 4: Fractional Distillation and Characterization of Upgraded Oil from Hydrothermal Liquefaction of Nannochloropsis sp. Microalgae.....	71
Abstract	71
4.1 Introduction	72
4.2 Materials and Methodology	76
4.2.1 Apparatus and Experimental Procedure	77
4.2.2 Product Analysis	78
4.3 Results and Discussion.....	80
4.3.1 Yield and Properties of Oil.....	80
4.3.2 Chemical Composition of Oil	86
4.4 Conclusion.....	92
4.5 References	94
Chapter 5: Conclusions	97
5.1 Conclusions	97
Appendix.....	100

List of Figures

Figure 2.1 U.S. crude oil input qualities in refineries.....	7
Figure 2.2 Effect of rate of photosynthesis with varying light intensity regime	13
Figure 2.3 Yield of bio-oil (wt%) at different temperatures.....	19
Figure 2.4 Effect of residence time on the yield of HTL bio-oil of <i>Nannochloropsis</i> sp.	20
Figure 2.5 Conversion of lipids	25
Figure 2.6 Possible reaction pathway for amino acids found in CP and SP.....	27
Figure 2.7. Reaction pathway from hydrothermal liquefaction of cellulose	28
Figure 2.8. Boiling point distribution from TGA of upgraded oil with 9 different zeolite catalysts (A-J), pretreated oil (K), and crude bio-oil (L).....	32
Figure 3.1 Process flow diagram of product separation	51
Figure 3.2 FTIR spectra of all the bio-oils.....	63
Figure 3.3 Simulative distillation of all the bio-oils ASTM D2887	64
Figure 4.1 Distillation setup.....	78
Figure 4.2 Distribution of product fractions in wt% from fractional distillation (n = 2).....	83
Figure 4.3 From left to right F1_HTL, F2_HTL, F3_HTL, F1_H ₂ _Tol, F2_H ₂ _Tol, F3_H ₂ _Tol, F1_RuC_DCM, F2_RuC_DCM, F3_RuC_DCM, and RuC_DCM_UO	83
Figure 4.4 Simulative Distillation of HTL and upgraded oil.....	84
Figure 4.5 GC-MS data of HTL and upgraded oil. Average of 2 (n = 2*2).....	88
Figure 4.6 GC-MS of all fractions from fractional distillation of bio-oil and upgraded oil	89
Figure 4.7 Simulate Distillation of F2 and F3 Fractions	90
Figure 4.8 FTIR spectra of bio-oil and upgraded oil	91
Figure 4.9 FTIR spectra for F3 fraction from upgraded oils	92

List of Tables

Table 2.1 Effect of urea concentration after 6 days in batch culture mode	12
Table 2.2 Comparison of properties of microalgae biodiesel, diesel fuel and ASTM standard	14
Table 2.3 Comparison in properties of water at different conditions	16
Table 2.4 HHV and elemental composition of bio-oil obtained from HTL	29
Table 3.1 Biochemical composition of algae in wt% dry basis	55
Table 3.2 Properties of algae feedstock	55
Table 3.3 Ultimate analysis of algae biomass. O* obtained by difference.....	56
Table 3.4 Yield of different product fractions from HTL in dry basis. Alphabets in superscript shows the values are significantly different at $\alpha = 0.05$	57
Table 3.5 and 3.6 Properties of bio-oil and ultimate analysis. O* obtained by difference. Different alphabets in superscript denote the values are significantly different	59-60
Table 3.7 Chemical composition data of bio-oil from GC-MS (Average $n = 2 * 2$).....	61-62
Table 3.8 Properties of bio-char.....	65
Table 3.9 Aqueous phase characterization.....	66
Table 4.1 Yield and properties of oil obtained during upgrading experiments ($n = 2$)	81
Table 4.2 Ultimate analysis of products from upgrading experiments. O* is calculated by difference. Data are reported as an average of 2 ($n = 2$).....	82
Table 4.3 Ultimate analysis, high heating value and total acidic number of main oil and their distilled fractions. O* is calculated as difference	86

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Keeping up with energy demand with outburst of world population and standard of living is one of the major challenges of 21st century. According to International Energy Outlook for 2017, the average U.S petroleum consumption was about 19.96 million barrels per day and 1 million barrels per day was obtained from biofuels [1]. Transportation sector was the major consumer of these petroleum products with 71% of total energy consumed. The current infrastructure in transportation sector is designed to meet energy demands by consumption of liquid fuels. Biomass is the only source of renewable energy that is distributed worldwide and has the capability to produce liquid hydrocarbon fuels.

Biomass has been used as a source of fuel since early times and present a viable option for producing energy. Corn and sugarcane are being used to produce bioethanol by fermentation. Lignocellulosic biomass can also be converted into bioethanol or biobutanol by biochemical pathway or can employ thermochemical route like gasification or pyrolysis for conversion into biofuels [2]. This biomass, however, suffer from food vs fuel pressure and diversification of environment. To overcome these drawbacks aquatic biomass like algae can be used [3]. Algae can be grown in non-arable land and can also use waste or marine water for cultivation. Among various

biomass, algae has highest productivity of 40-60 dry ton/hectare-yr and high carbon sequestration rate of 1.8 kg CO₂/kg of dry algae [4].

Algae can be converted into bio-diesel by biochemical process of transesterification. This process utilizes only the lipid fraction of algae for conversion and cultivation of algal strain with high lipid productivity is an expensive venture. Thermochemical process like pyrolysis can be employed to convert algae into fuels but renders the process uneconomical because of the need of drying of algal feedstock. Hydrothermal liquefaction address both these issues of utilizing whole of algae for conversion and evading the need of drying the feedstock.

Hydrothermal liquefaction (HTL) is a wet process that operates at 280-380°C under autogenous high pressures [5]. The biocrude produced from HTL of algae has high energy content than pyrolysis oil but still contains 10-20% oxygen and 5-7% nitrogen and differs substantially from petroleum crude [6]. High oxygen content decreases the energy density of the fuel and imparts acidic nature to the oil which is represented by high total acid number (TAN) [7]. Aging of fuel is another serious problem which is caused by polymerization of aldehyde and hydroxyl groups that increases the viscosity of the oil and makes its handling and processability difficult [8]. High nitrogen content leads to release of NO_x emissions which is detrimental to the environment. Therefore, it becomes imperative to reduce TAN, viscosity, nitrogen and oxygen content of HTL biocrude from algae and achieve properties similar to that of petroleum crude. **1.2**

1.2 Research Objectives

Upstream processes such as algal strain selection, type of nutrient application for cultivation, harvesting of algae from the broth and downstream processes such as hydrothermal liquefaction and upgrading of fuel are all important factors that can influence the cost of biofuels obtained from algae. Numerous studies on hydrothermal liquefaction of algae has been done to

understand the influence of temperature, residence time, biomass loading, biochemical composition of algae feedstock, and have also explored both homogenous and heterogenous catalyst on the bio-oil yield and properties of oil obtained. This research addresses the gap in literature on hydrothermal liquefaction of filamentous type algae having different biochemical composition and compares it with microalgae and gains insight on the effectiveness of fractional distillation for upgrading. The first objective of the study was:

Objective 1. Compare the product yield and properties obtained from hydrothermal liquefaction of filamentous and microalgae

In this study, HTL of filamentous algae grown in five different nitrogen nutrient conditions were examined and compared with *Nannochloropsis* sp. microalgae on product yield and properties. The five different nitrogen nutrients conditions were filamentous algae grown on sufficient nitrate (A_NO₃), sufficient urea (A_Urea), nitrate starved algae for 14 and 21 days (14_NO₃ and 21_NO₃), and urea starved algae for 14 days (14_Urea). These conditions were chosen to alter the biochemical composition of filamentous algae and identify the best algae for HTL. Different by-products obtained from HTL were also analyzed for physical and chemical properties. Filamentous algae grown on cheaper urea produced oil with yield and properties similar to that from microalgae. These oil however, contained high concentration of nitrogen and oxygen rendering the oil undesirable for direct use and commanded upgrading which is discussed in second objective. Chapter 3 summarizes the details of experiments, results and discussion of this study.

Objective 2. Fractional distillation and characterization of upgraded oil from hydrothermal liquefaction of *Nannochloropsis* sp. microalgae

The biocrude from HTL of *Nannochloropsis* sp. microalgae produced oil with nitrogen content of 5.47 wt% and oxygen content of 11.54 wt%. In this study, the HTL biocrude from *Nannochloropsis* sp. microalgae was upgraded under the presence of heterogenous catalyst and the influence of polar and aromatic solvent during product separation on upgraded oil yield and properties was determined. Fractional distillation was used as an upgrading tool to understand the distribution of heteroatoms in different distilled fractions. Dichloromethane as polar solvent during product separation produced upgraded oil with high yield but better-quality oil with low concentration of oxygen content was produced when toluene was used as solvent. Distilled fractions from fractional distillation contained high concentration of nitrogen in the heavier fraction and high oxygen concentration in the lower fractions. The details of this study are summarized in chapter 4.

1.3 References

- [1] Use of Oil - Energy Explained, Your Guide To Understanding Energy - Energy Information Administration, (n.d.). https://www.eia.gov/energyexplained/index.php?page=oil_use (accessed October 14, 2018).
- [2] E.D. Larson, Biofuel Production Technologies: Status and Prospects, (2007). http://unctad.org/sections/wcmu/docs/ditc_tedb_ted0015_en.pdf (accessed June 29, 2018).
- [3] V. Patil, K.Q. Tran, H.R. Giselrød, Towards sustainable production of biofuels from microalgae, *Int. J. Mol. Sci.* 9 (2008) 1188–1195. doi:10.3390/ijms9071188.
- [4] J.P. Maity, J. Bundschuh, C.-Y. Chen, P. Bhattacharya, Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review, *Energy*. 78 (2014) 104–113. doi:10.1016/J.ENERGY.2014.04.003.
- [5] S.S. Toor, L. Rosendahl, A. Rudolf, Hydrothermal liquefaction of biomass: A review of subcritical water technologies, *Energy*. 36 (2011) 2328–2342. doi:10.1016/j.energy.2011.03.013.
- [6] D. López Barreiro, F. Ronsse, W. Brilman, Hydrothermal liquefaction (HTL) of microalgae for biofuel production: State of the art review and future prospects, *Biomass and Bioenergy*. 53 (2013) 113–127. doi:10.1016/J.BIOMBIOE.2012.12.029.
- [7] M. Saber, B. Nakhshiniev, K. Yoshikawa, A review of production and upgrading of algal bio-oil, *Renew. Sustain. Energy Rev.* 58 (2016) 918–930. doi:10.1016/J.RSER.2015.12.342.
- [8] Q. Lu, W.-Z. Li, X.-F. Zhu, Overview of fuel properties of biomass fast pyrolysis oils, *Energy Convers. Manag.* 50 (2009) 1376–1383. doi:10.1016/J.ENCONMAN.2009.01.001.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Energy Scenario

Energy needs exceeds all past demands with the increasing world population and their standard of living [1]. According to International Energy Outlook 2017 [2], the world energy consumption is expected to increase from 575 quadrillion British thermal units (Btu) in 2015 to 736 quadrillion Btu by 2040, provided the world gross domestic product (GDP) increases by 3.0%/year. In 2017, the energy consumption in the US was 97.71 quadrillion Btu of which 78.10 quadrillion Btu was derived from fossil fuels, 8.41 from nuclear power and 11.01 from renewable sources of energy [3]. According to BP statistical review of world energy [4], the crude oil reserves and natural gas reserve are expected to reach exhaustion by year 2066 and 2068, respectively. This exploitation of energy is not only causing questions about energy security but is also taking its toll on the environment by greenhouse gas emission causing global warming [5].

Transportation sector is the major consumer of liquid fuels which are dwindling reserve of declining quality. Figure 2.1 shows the weighted monthly average density and sulphur content of crude oil over a period of 30 years from 1985 to 2015 [6]. The graph clearly depicts that the crude oil is becoming heavier and that the sulphur content has doubled over the years. Processing of heavier crude oil increases greenhouse gas emissions and requirement of catalysts will increase because of higher sulphur content [7]. To counter this mismatch between high energy demand,

growing environmental concerns and depleting resources there is a need to replace the liquid fuels market with partly electric engines and partly by renewable feedstock that are carbon neutral. Liquid fuels can be obtained from biomass by thermochemical conversion processes such as pyrolysis, and hydrothermal liquefaction [8].

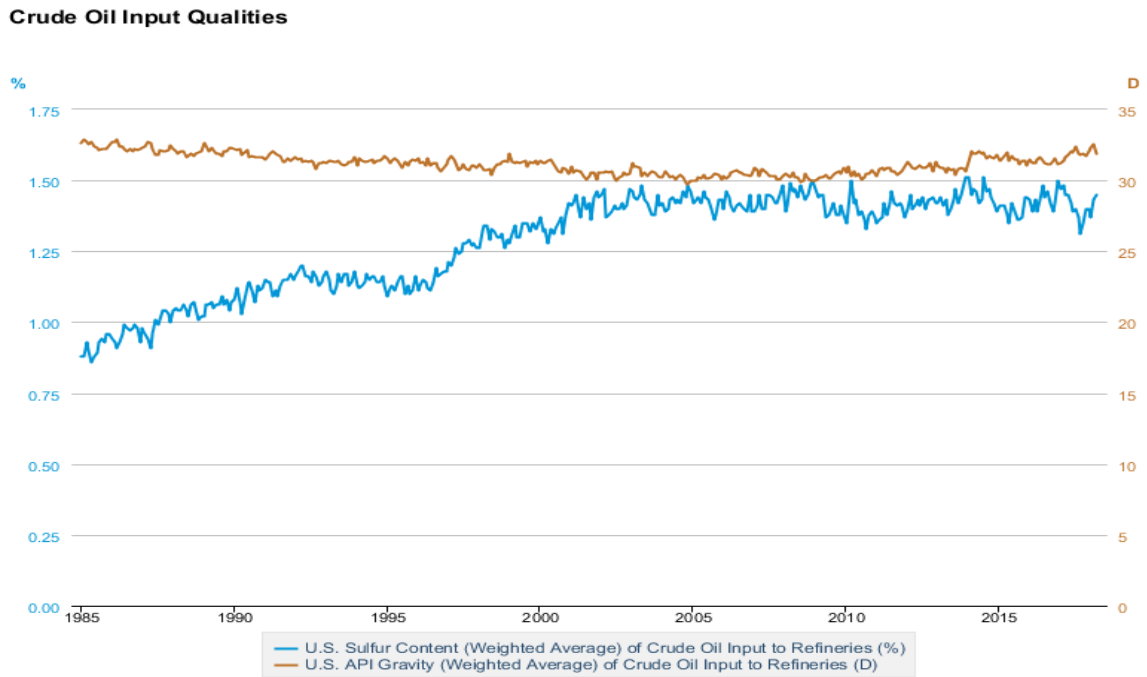


Fig. 2.1. U.S. crude oil input qualities in refineries [6]

Renewable fuel standard act of 2007 has set the target of 36 billion gallons of biofuels with cap of 15 billion gallons coming from starch based ethanol and the rest coming from cellulosic, biomass based diesel and advanced fuel [9].

2.2 Biomass and Biofuels

All organic matter that comes from plants and animals are termed as biomass and are renewable source of energy [10]. This includes agriculture crops and residues, forest reserves and wood wastes, municipal and animal wastes, aquatic plants such as algae. Fuels that can be derived

from biomass are termed as biofuels and are classified into two broad types. Primary fuels are unprocessed biomass like firewood, wood pellets that are combusted directly for energy and secondary fuels are processed primary fuels that are used in the form of solids (charcoal), liquid (bioethanol, biodiesel, bio-oil), and gases (synthesis gas, biogas, hydrogen) [11]. Secondary biofuels are further classified into first generation, second generation, and third generation fuels.

First generation biofuels are obtained from agriculture crops like corn and sugarcane to produce bioethanol by fermentation, and soybean and rapeseed are used for production of biodiesel by transesterification [12]. Although it is a great source of liquid fuels, it suffers from constant food vs fuel pressure and diversification of the environment [13]. Second generation biofuels are obtained from lignocellulosic biomass which are residues of agricultural crop or are non-edible plants and trees. They could employ biochemical process to produce bioethanol or biobutanol and could also use thermochemical pathway such as gasification and pyrolysis to produce biofuels. At present second generation biofuels are non-commercial until further advancements in energy output and economic viability is achieved [11,14]. Third generation biomass, like algae is probably the best alternative for renewable energy. They have the best growth rate amongst plants, have very high CO₂ sequestration rate of about 1.83 kg CO₂ per kg of dry algae cells, and they can be grown in non-arable land thereby mitigating food vs fuel pressure [15–18]. At present, biofuels production from algae is not commercial and is being researched.

2.3 Algae as Biomass

Algae are a broad range of organisms encompassing eukaryotes and prokaryotes (cyanobacteria – blue green algae) that can range from unicellular micro-organism having a dimension of 0.2-2 micrometers to multicellular macroalgae having a size of up to 60 meters [19–21]. The eukaryotic algae are further divided into nine divisions based on color with majority being

in Chlorophyceae (green algae), Phaeophyceae (brown algae), Pyrrophyceae (dinoflagellates), Rhodophyceae (red algae) and Chrysophyceae (yellow-green algae) [22]. Algae have very high photosynthetic efficiency as they grow in aqueous medium with easy access to water, CO₂ and other nutrients [23].

Photosynthesis in microalgae is carried out in two stages namely light reaction stage, and dark reaction stage. In the light reaction stage, the solar energy is trapped by the pigments in photosynthetic antenna of the algae cell and is used to produce NADPH and ATP by the splitting of water molecule into proton, electrons, and oxygen. The chemical energy thus produced is used to carry out the metabolic reactions and in the dark reaction stage, this energy is used in the Calvin cycle to reduce CO₂ into carbohydrates [24]. Storage compounds like lipids, proteins, and carbohydrates provide energy for algae growth and change according to changing environment [25].

Carbohydrates in algae are composed of monosaccharides and its polymers with most abundant carbohydrates sugars in the form of glucose, mannose, xylose, and rhamnose[26]. Protein is composed of different amino acids which contains most of nitrogen present in algae. Most of the figures reported on protein is crude protein which is obtained by hydrolysis of protein and estimation of total nitrogen. Nitrogen may also be present in other constituents like nucleic acids, glycosamides, amines, and cell wall which are not part of protein content of algae [27]. Some reported figures on the amount of non-protein nitrogen is 12% in *Scenedesmus obliquus*, 6% in *Dunaliella*, and 11.5% in *Spirulina* [27]. The other storage compound of importance is lipids which are synthesized during adverse growth conditions.

Lipids that are synthesized in normal conditions are membrane lipids which constitute about 5-20% of dry cell weight and are polar in nature. These lipids include glycosylglycerides

present in chloroplast and phosphoglycerides present in plasma membrane and endoplasmic reticulum [28,29]. In adverse condition certain oleaginous algae synthesizes storage lipids that are densely packed in the cytoplasm of the cell and are neutral [30]. These storage compounds are composed of triacylglycerides (TAGs) which can constitute about 80% of total algae lipids. These TAGs are characteristic fatty acids which may contain 16-18 carbons which are dominantly mono and di-unsaturated fatty acids [31]. Some of the unsaturated fatty acids that are found in different algae species include arachidonic acid, eicosapentanoic acid, docosahexaenoic acid, gamma-linolenic acid, and linoleic acid. These neutral lipids or TAGs can be used as a feedstock to produce biodiesel via transesterification [30].

Algaculture is a type of aquaculture that is used for the cultivation of algae. Algae can be cultivated in an open-air system like raceway ponds or in a closed system like photobioreactors. In raceway pond, a paddlewheel is used for continuous mixing and circulation. The culture and nutrient are fed in front of paddlewheel and broth is drawn for harvesting behind the paddlewheel. There is no control over temperature, illumination, and other environmental factors which effect the growth rate. There is significant loss of water due to evaporation which can affect the carbon dioxide conversion efficiency adversely [23]. Productivity is affected by contamination with other algae species, microorganisms that feed on the algae, and no growth during the night [23]. These shortcomings can be addressed in photobioreactors. Photobioreactors can be illuminated artificially or by sunlight but the carbon dioxide must be supplied as a source for carbon and at regular intervals along the tube to keep the pH at optimum range [32]. Sedimentation in the reactor is addressed by maintaining turbulent flow. Photosynthesis may result in high concentration of oxygen which may inhibit photosynthesis and it might also damage algal cells when combined

with intense sunlight irradiance [33]. Air is passed in degassing zone to remove oxygen. Cultures can tolerate 400% of air saturation value for dissolved oxygen which is highly undesirable.

In essence, harvesting means separating the broth from the culture media and dewatering or concentrating the biomass to form a slurry having a dry weight of 5-15%. Techniques such as filtration, froth floatation, centrifugation, and sedimentation are employed for harvesting. In general, harvesting is an expensive process especially in case of micro-algae cultivation (dimension <30 μ m) and, can amount to 20-30% of total biomass production cost [34,35]. To minimize the cost, culture media can be harvested when the biomass concentration is high, or when large size species is cultivated like filamentous algae or macroalgae.

The growth medium must supply inorganic nutrients like nitrogen (N), phosphorous (P), iron (Fe), and in some cases silicon (Si) that make essential cell components. Grobbellar determined a formula to estimate the minimal nutrient requirement, that is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ [23]. Phosphorous is supplied in excess as it forms complex with metal ions and is not bioavailable as nutrients. Depending on the carbon metabolism the algae can be divided into four types [36]

1. Photoautotrophic: Energy source is light and carbon source is CO_2
2. Heterotrophic: Organic compounds is source of energy and carbon
3. Photoheterotrophic: Energy source is light and carbon source is organic carbon.
4. Mixotrophic: Main energy source is light but both organic and CO_2 are essential.

Carbon dioxide concentration effects microalgae carbohydrates accumulation. At low CO_2 concentration carbon concentration mechanism is induced that enforces effective CO_2 utilization and increases carbohydrates accumulation [37]. On decreasing CO_2 concentration from 3 to 0.04%, Lzumo *et al.* obtained 2.5-fold increase in carbohydrates content [37]. Singh *et al.* [38] reviewed

the effect of CO₂ concentration on algal growth and found that increase in CO₂ concentration increases algal productivity and lipid accumulation but the optimum levels was not studied.

Nitrogen is an essential nutrient for algae growth. It participates in the synthesis of vital compounds such as DNA, proteins, pigments. Nitrogen can be utilized as NH₄⁺, NO₂⁻, NO₃⁻, Urea, and as N₂ in case of diazotrophs which can utilize elemental nitrogen and reduce it to NH₄⁺ [39]. In case of cyanobacteria the order of nitrogen utilization is NH₄⁺ > NO₃⁻ > N₂, if nitrate is only available nutrient then it is reduced to nitrite which in turn is reduced to ammonium [40,41].

Nitrogen starvation is a technique employed to increase lipid or carbohydrate accumulation and is species dependent. Majority of studies published on nitrogen starvation for green algae have concluded that lipid accumulation increases while there are some studies that showed increase in carbohydrates [42–44]. Effect of nutrient concentration in the culture medium effects the growth rate and storage compound accumulation greatly. Table 1, shows the change in growth rate and productivity with different nutrient concentration of urea for *Chlorella sp.* [42]

Table 2.1 Effect of urea concentration after 6 days in batch culture mode [42]

Urea Concentration (g/L)	Specific growth rate (h ⁻¹)	Biomass Concentration (g/L)	Lipid Content (g/g)	Lipid Productivity (g/d/L)
.025	.0358	.464	.661	.051
.050	.0498	.849	.602	.085
.100	.0576	1.422	.522	.124
.150	.0556	1.785	.365	.109
.200	.0590	2.027	.326	.110

Light intensity plays an important role in the photosynthetic efficiency of the algae cells. At low light intensities the rate of photosynthesis is proportional to light intensity, as the rate is governed by rate of photon capture [45]. Highest photosynthetic rate is achieved when the light intensity reaches saturation threshold and in this state the rate is governed by reaction of photon to produce energy [46]. On increasing the light intensity beyond this point the rate of photosynthesis is decreased due to the deactivation of key proteins in the photosynthesis unit [47]. Figure 2.2 [45] shows the rate of photosynthesis with vary light intensities.

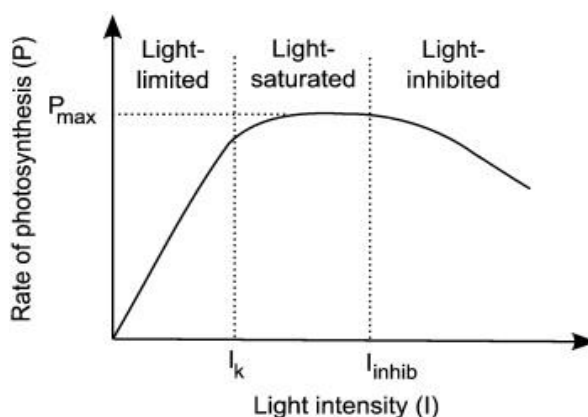


Fig. 2.2 Effect of rate of photosynthesis with varying light intensity regime

Extracting of oil from microalgae can be accomplished by various techniques such as mechanical pressing, homogenization, milling, solvent extraction, supercritical fluid extraction, enzymatic extraction, ultrasonic-assisted extractions, and osmotic shock [48]. Pressing and homogenization involve rupturing the cell wall with pressure. It is solvent free, easy to use process but needs high amount of sample [49]. Organic solvents such as cyclohexane, benzene, hexane is used with algae paste to extract the oil, in solvent extraction. The drawback with solvent extraction is that the solvents are highly flammable and toxic, and their recovery is expensive and energy intensive [50,51]. In supercritical fluid extraction, some chemicals like CO_2 , which behave both as liquid and gas beyond critical point is used for solvating the oil trapped in algae [48]. On

bringing back the system to room temperature the CO₂ is removed and thus it can also be used for food applications while, the drawback is drying of algae to overcome the barrier of diffusion of CO₂ and oil in and out of the algae cell wall [52,53].

The conversion of triglycerides into biodiesel can be achieved by transesterification. In transesterification, the oil or fat is reacted with alcohol in the presence of strong acid or base catalyst to form glycerol and ester [54]. Triglycerides to biodiesel conversion can achieve efficiency of 98% [55]. Table 2.2 [56] shows the properties of biodiesel obtained from acidic transesterification of *Chlorella Protothecoides* and compared with ASTM D6751 biodiesel standard.

Table 2.2 Comparison of properties of microalgae biodiesel, diesel fuel and ASTM standard [56]

Properties	Biodiesel	Diesel Fuel	ASTM Standard
Density (kg/L)	.864	.838	.86-.90
Viscosity (cSt at 40°C)	5.2	1.9-4.1	3.5-5.0
Flash Point (°C)	115	75	Min 100
Solidifying Point (°C)	-12	-50-10	-
Cold Filter Plugging Point (°C)	-11	-3.0 (Max -6.7)	Summer max 0 Winter max < -15
Acid Value (mg KOH/g)	.374	Max 0.5	Max 0.5
Heating Value MJ/kg	41	40-45	-

Algae can be used for conversion into fuel by thermochemical pathway. Hydrothermal liquefaction is the best possible route for conversion of algae into biofuel in comparison to pyrolysis and gasification as it obviates the need for drying the algae [57]. Hydrothermal

liquefaction can also convert fractions of proteins and carbohydrates into oil while conventional transesterification process only converts the lipid fraction of oil into biodiesel [58,59].

2.4 Hydrothermal Technology

Biomass valorization processes such as gasification and fast pyrolysis require dry biomass having moisture content below 20% to process or else suffer a great deal in terms of process economics [60,61]. Energy intensive drying has led to the development of hydrothermal processing technology which uses water as the reaction medium. Lignocellulosic biomass (10-60 % moisture) and aquatic biomass such as algae can be processed directly via hydrothermal technologies. Primary product such as bio-char (carbonization), bio-fuels (liquefaction), and syngas (gasification) can be obtained depending on temperature and pressure at which the reaction occurs[62]. Apart from processing wet feedstock it offers versatility of chemistry meaning it can convert ligno-cellulosic biomass, fatty acids, and proteins into products such as methane, hydrogen, biocrude that can be used commercially [60]. Another advantage is with enhanced reaction rates that might occur at sub and super-critical conditions because of elimination of inter-phase mass transfer resistance and efficient separation of products which can reduce energy consumption [60]. It is important to understand the changes in the property of hot compressed water which supports either free radical or polar and ionic reactions during hydrothermal processing.

2.5 Properties of water

The critical point of water is at 374 °C and 22.1 MPa and at this point the liquid phase and gaseous phase properties become identical. Below the critical point the water is referred to as sub-critical water and beyond critical point it is referred to as super-critical water. The physiochemical properties of water at these two regions are different and can be exploited based on the product

that is required. Table 2.3 [63,64] shows the comparison in the properties of water at sub and super-critical conditions.

Table 2.3. Comparison in properties of water at different conditions [63,64]

	Normal Water	Sub-critical Water		Super-critical Water	
Temperature (°C)	25	250	350	400	400
Pressure (MPa)	.1	5	25	25	50
Density, ρ (g/cm ³)	1	.8	.6	.17	.58
Dielectric Constant, ϵ (F/m)	78.5	27.1	14.07	5.9	10.5
Ionic Product (pK_w)	14.0	11.2	12.0	19.4	11.9
Heat Capacity, C_p (kJ/kg/K)	4.22	4.86	10.1	13.0	6.8
Dynamic Viscosity, η (mPa s)	.89	.11	.064	.03	.07

The dielectric constant of water decreases from 78.5 to around 10 in sub-critical condition which corresponds to dielectric constant of methylene chloride an organic solvent [65]. At this condition water has high solubility for hydrophobic organic compounds and gases such as free fatty acids and has poor solubility for salts [66,67]. Electron distributes more evenly between hydrogen and oxygen atoms in the water when thermal energy is increased resulting in higher affinity for non-polar organic compounds [68].

The ionic product in sub-critical water and super-critical water at high pressure is higher than that of ambient water which favors acid base catalyzed reactions, such as biomass hydrolysis, because of high concentration of H^+ and OH^- ions [69]. The relative high density combined with

ionic product favors ionic reactions. At supercritical condition, when the density is low, radical reactions are favored, resulting in gasification reactions [70,71].

2.6 Hydrothermal Carbonization

Hydrothermal carbonization (HTC) is a thermochemical process in which biomass under mild hydrothermal conditions such as, 180-250°C and under autogenous pressure, can be converted into carbonaceous material [72]. Lignocellulose substrates, sewage sludge, municipal solid wastes (MSW), as well as aquaculture and algal residues are the renewable feedstock for this process [73]. The advantages of using HTC over other biochemical process is that the reactor is compact, and it only takes few hours, instead of few days or months. High temperature can destroy pathogens, active pharmaceutical compounds, mitigate odor and greenhouse gases [74,75]. HTC produces higher solid char, water soluble organic compounds and fewer gases, comprising mainly of CO₂. The biochar obtained from dry pyrolysis and HTC differs substantially [76]. Both biochar has lower values of H/C and O/C than initial feedstock but, biochar resulting from HTC has higher H/C and O/C ratios similar to natural coal than resulting from dry pyrolysis. This implies that the ratio of reaction rates of decarboxylation to dehydration is higher in HTC as compared to dry pyrolysis [77,78].

2.7 Hydrothermal Liquefaction

Hydrothermal liquefaction (HTL) is carried out at temperatures of 280-380°C and pressure in the range 7-30 MPa, sufficient to keep water in liquid state in order to obtain liquid bio-crude as the primary product and by-products being solid char, water soluble organic phase, and gases [8]. The bio-crude oil produced from HTL has 10-20% oxygen, and 5-7% nitrogen (in case of algae) and differs substantially from petroleum crude [79]. This makes the oil undesirable as it has lower energy content, poor thermal stability, lower volatility, higher corrosivity, tendency to

polymerize, and high NO_x emission upon combustion [60]. The oil requires deoxygenation and denitrogenation in-order to be used alongside petroleum crude. Several studies on the HTL of lignocellulosic biomass have been published but HTL of algae draws more attention since the process is well suited for wet feedstock [80–82]. Operating parameters such as, temperature, residence time, biomass loading, and biochemical composition of algae plays an important role in the yield and properties of the oil obtained and are discussed below [83].

2.7.1 Effect of temperature

Temperature is critical parameter for the safety and economics of the HTL process. Many studies have been conducted to analyze the influence of temperature on the yield and quality of the oil produced [84–89]. It is evident from the studies that the maximum oil yield results in the temperature range from 250-375°C. As the temperature is increased from 250°C to 350°C hydrolysis reaction becomes dominant owing to thermal energy availability for bond cessation and two fold increase in the ionic product H₃O⁺ and OH⁻ from 10⁻¹⁴ at 25°C to 10⁻¹² in sub-critical region [70,90]. Isomerization, reforming, depolymerization, and repolymerization reactions are induced to produce bio-oil from carbohydrates, proteins, and lipids in the algae [91]. As the temperature is increased further in super-critical region the secondary reaction and Boudouard gas reaction become active leading to production of gases and radical reactions dominate which repolymerizes the intermediates formed into char thereby decreasing the bio-oil yield [90–92].

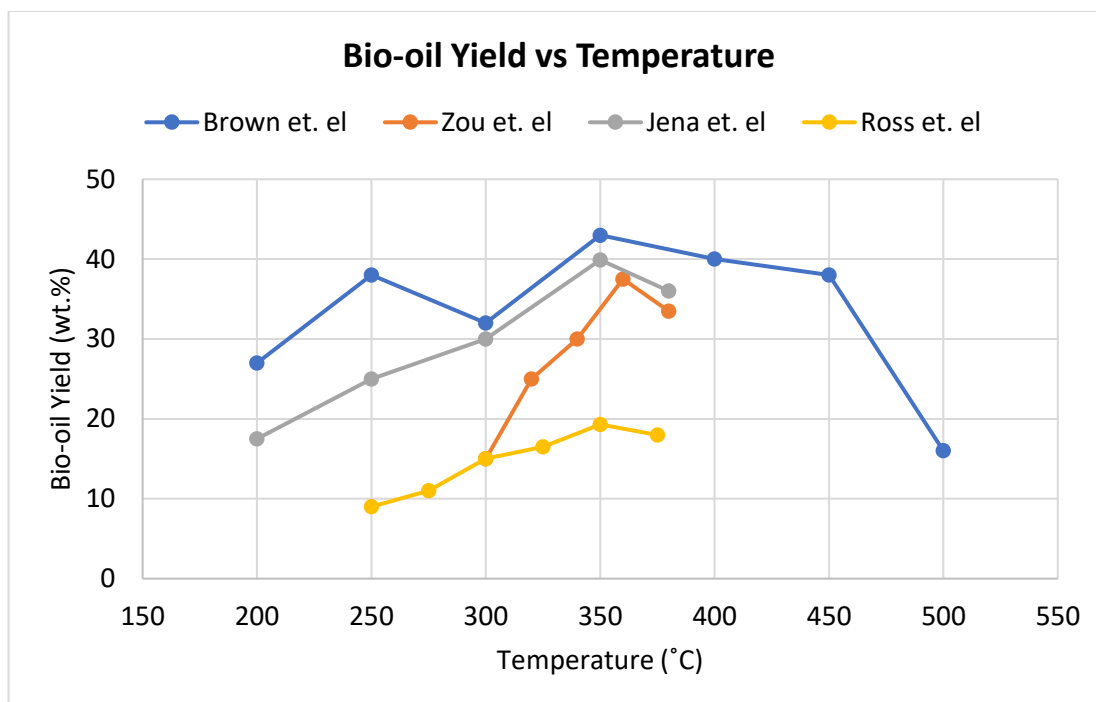


Figure 2.3 shows the yield of bio-oil (%) at different temperatures [59,84,85,89]

Studies show how the temperature increase has a synergistic effect on the oil yield till it reaches 350°C and then the yield begins to decline. In the study presented by Ross *et al.* [59] the HTL of *Laminaria Saccharina* at various temperature from 250-370°C and residence time of 15 mins showed that the yield of aqueous phase decreased and gaseous phase increased with increasing temperature. The solid char yield decreased with increasing bio-oil yield and at higher temperatures when the oil yield decreased the solid char content increased. The higher heating value of the oil increased from 34.5 MJ/kg at 275 °C to around 36 MJ/kg for all the oil obtained from 300 to 370°C. Shakya *et al.* [93] observed similar trend in all the product fractions for *Nannochloropsis* sp., *Palvova* sp., and *Isochrysis* sp. when the HTL was performed at 250 °C, 300°C, and 350°C. Jena *et al.* [84] reported the concentration of nitrogen to increase 5.48 wt% at 200°C to 6.53 wt% at 380°C and concentration of oxygen and sulphur to decrease from 28.85 wt% and 1.55 wt% at 200°C to 0.64 wt% and 0.87 wt% at 380°C. Increase in nitrogen content in the

bio-oil can be attributed to the fact that peptide bond is stable at lower temperature and can be broken at higher temperature [93].

2.7.2 Effect of Residence Time

Many studies have been performed to estimate the optimum residence time needed for maximum conversion of organics in microalgae into bio-oil [59,84,87,88,94]. Figure 2.4 shows the yield of bio-oil from HTL of *Nannochloropsis sp.* at different temperature and residence time

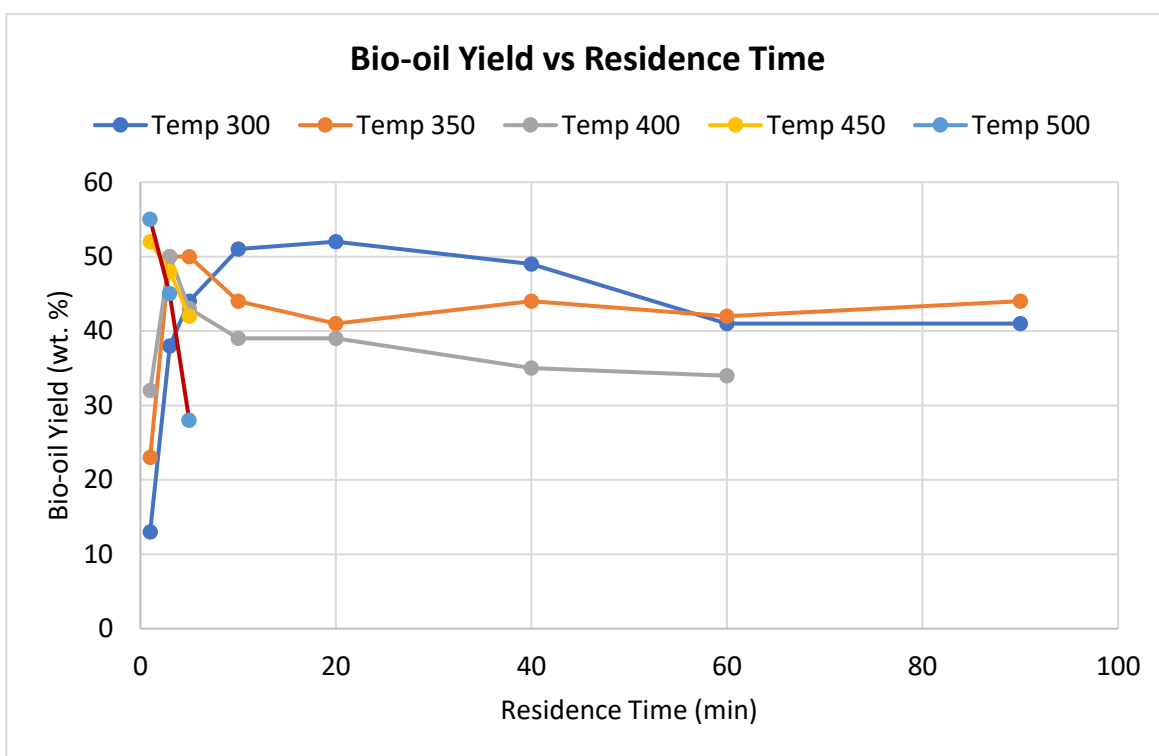


Figure 2.4. Effect of residence time on the yield of HTL bio-oil of *Nannochloropsis sp.* [94]

At supercritical conditions, since the rate of hydrolysis and decomposition of biomass is fast the yield of bio-oil is high. At temperatures around 300-350 bio-oil yield seems to increase with residence time and then reaches saturation point before decreasing. At 300°C maximum oil yield was obtained at 20 mins while at 350°C the optimum residence time was 5 mins. The quality of bio-oil is also influenced by residence time. At both 300°C and 400°C the elemental composition

of bio-oil changed with change in residence time [94]. To obtain high liquid yield it is important to inhibit the secondary and tertiary decomposition of light bio-crude which can be achieved by adding reducing agents like hydrogen gas, tetralin, and syngas [90].

2.7.3 Effect of Catalyst

To improve the yield and quality of bio-oil homogenous catalyst, which are inorganic compounds soluble in water and heterogenous catalyst such as, noble metal supported on carbon, transition metal catalyst, and zeolite catalyst have been studied [86,93,95–98]. They are discussed below.

2.7.3.1 Homogenous Catalyst

Alkali catalyst such as Na_2CO_3 , KOH and acid catalyst like formic acid, and acetic acid have been tested for *Spirulina* and *Chlorella sp.* by Ross *et al.* [99] and was found that the bio-oil yield followed the trend $\text{Na}_2\text{CO}_3 > \text{CH}_3\text{COOH} > \text{KOH} > \text{HCOOH}$ and there was considerable reduction in the heteroatoms present in bio-oil as compared to the feedstock. Temperature at which HTL is carried out under the presence of catalyst can influence the yield and quality of bio-oil obtained. In the study carried out by Shakya *et al.* [93], it was observed that for microalgae species of *Palvova* and *Isochrysis sp.*, the yield increased with increasing temperature when compared both in the presence and absence of 5% Na_2CO_3 catalyst but for *Nannochloropsis sp.* the yield decreased in the presence of catalyst at 300°C and 350°C. However, at 250°C the yield of bio-oil in the presence of 5% sodium carbonate catalyst was more than that obtained without it. Na_2CO_3 increased the hydrolysis of protein at lower temperature but at higher temperature secondary decomposition of nitrogenous compound into ammonia and water-soluble compounds is induced [93,100]. Although base catalysts are favorable option but excessive amounts of Na_2CO_3 or KOH can have adverse effect on the bio-oil yield. Ross *et al.* observed that with the increase in

KOH/biomass (*Laminaria Saccharina*) containing high carbohydrates the yield of bio-oil kept decreasing while the water soluble phase containing organic compounds kept increasing [86]. Biller *et al.* [59] investigated the effect of HTL with alkali catalyst (Na_2CO_3), organic acid (HCOOH), and just water on different biochemical composition of the feedstock. It was concluded that alkali catalyst increased the bio-oil yield for carbohydrates rich feedstock by selectively promoting the decarboxylation of carbohydrates, leading to lower fractions in aqueous phase. For model compounds rich in protein like soya protein, *Spirulina sp.* and *Nannochloropsis sp.* it was observed that catalyst decreased the bio-oil yield which was corroborated by Shakya *et al.* [93] at severe conditions of 350°C due to decomposition of nitrogenous compound into aqueous phase. For compounds rich in lipids the trend was water > HCOOH > Na_2CO_3 . When using HCOOH the yield of bio-oil was comparable to yield obtained with water, but the fraction of gas phase was the major by-product [59]. So, it can be concluded that use of homogenous catalyst can have positive impacts only when it is used with appropriate feedstock and operating parameters.

2.7.3.2 Heterogenous Catalyst

Several heterogenous catalyst have been tested based on their efficacy in facilitating hydrogenation of HTL oil. Savage *et al.* [96] tested six different heterogenous catalyst, three noble metal on carbon support (5% Pt/C, 5% Pd/C, and 5% Ru/C) that have selectivity for hydrodeoxygenation, one typical hydrotreatment catalyst (sulfide $\text{CoMo}/\gamma\text{-Al}_2\text{O}_3$) that is used to promote heteroatom removal, one transition metal catalyst supported on silica–alumina ($\text{Ni}/\text{SiO}_2\text{-Al}_2\text{O}_3$) as hydrogenating catalyst, and one zeolite (aluminum silicate) catalyst that facilitate cracking reactions as against HTL without any catalyst, both in inert helium environment and reduction gas (H_2) environment. In inert medium, bio-oil yield increased in all cases as compared to uncatalyzed HTL and was maximum for 50% loading of 5% Pd/C catalyst with

respect to dry microalgae weight. The elemental composition revealed with noble metal as catalyst the H/C ratio increased from 1.63 to 1.71-1.76 and O/C ratio was least for Pt/C and thus resulted in highest heating value (HHV) of 39.6 MJ/kg. As for HTL with catalyst in hydrogen gas environment, the yield of bio-oil decreased in all cases except uncatalyzed HTL and 5% Pt/C catalyst condition. Reasons such as catalyst poisoning by sulphur present in algae, loss of light fractions during removal of dichloromethane solvent, or insufficient metal loading could be reasonable explanation for low bio-oil yield, nevertheless, the yield was more than uncatalyzed HTL in inert medium [101]. Here also, Pt/C gave highest H/C ratio and lowest O/C ratio and thus resulted in maximum HHV of 40.1 MJ/kg. Ru/C had best selectivity for denitrogenation and desulphurization [101]. Jena *et al.* carried out HTL of *Spirulina Platensis* at 350°C with 5% loading of $\text{Ca}_3(\text{PO}_4)_2$ and NiO as catalyst was added. In both cases the bio-oil yield decreased, and gas formation increased. NiO catalyst HTL oil resulted in maximum HHV of 38.41 MJ/kg owing to lowest oxygen content but suffered with denitrogenation. This oil had viscosity less than that obtained by uncatalyzed HTL as nickel based catalyst is known to promote cracking of aliphatic and aromatic nitro compounds [102]. Ni based catalyst are known to promote hydrogen production during hydrothermal gasification and this behavior was also observed by Biller *et al.* [103] during HTL. It was observed that residual pressure in the reactor was continuously higher for Ni/Al catalyzed reaction and this reason could explain the lower yield in bio-oil reported by Jena *et al.* [98,103]. CoMo/Al₂O₃ and Pt/ Al₂O₃ favored species rich in carbohydrates and proteins for deoxygenation whereas Ni/Al₂O₃ favored high lipid feedstock like soya oil for deoxygenation [103].

2.7.4 Effect of Biochemical Composition of Algae

Hydrothermal liquefaction of different algae species at same reaction conditions give different product fractions in terms of bio-oil yield, solid char residue, water soluble compounds or aqueous phase, and gaseous fraction. This variability in the result is because of different biochemical composition namely, lipids, proteins, and carbohydrates in algae [83]. The reaction pathway of individual components is the subject of review here.

2.7.4.1 Conversion of Lipids

Storage lipids are manufactured in cytoplasm of algae cell that are neutral and mainly composed of triglycerides (TAGs), which contain 3 molecules of fatty acid attached to glycerol backbone. TAGs split to form free fatty acid and glycerol in hydrothermal media. This process is called Colgate-Emery process which employs hydrothermal condition of 250°C and 5MPa with TAGs to water ratio of 2:1 [104]. Glycerol obtained from splitting is water soluble rather than oil, don't contribute to bio-oil from HTL. Decomposition of glycerol has been studied by Buhler *et al.* in supercritical conditions with maximum yield of 31%. The main products of degradation were methanol, acetaldehyde, propionaldehyde, acrolein, allyl alcohol, ethanol, formaldehyde, and gaseous products like carbon monoxide, carbon dioxide, and hydrogen. Fatty acids are relatively stable in subcritical water but can partly decompose in hydrothermal media into long changed hydrocarbons through decarboxylation. Biller *et al.* observed during HTL of sunflower oil decomposition of TAGs into fatty acids such as, hexadecenoic acid, tetradecanoic acid, and oleic acid under water and formic acid as catalyst. When Na₂CO₃ was used, resulting oil produced alkenes, enols, and enones mainly in 6-7 carbon chain length [59]. Ross *et al.* [103] carried out HTL of soya oil in the presence of Ni/Al₂O₃ and without it in water. Alkanes ranging from pentadecane to nonadecane and fatty acids in the range of dodecanoic acid to hexadecenoic acid

was produced when Ni/Al₂O₃ as catalyst was used. Higher molecular weight fatty acid like hexadecenoic acid, and oleic acid was produced. The use of catalyst resulted in further deoxygenation of fatty acid into alkanes. Also, the fatty acids produced were hydrogenated as compared to unsaturated fatty acids in unprocessed soya oil [103]. Watanabe *et al.* studied the decomposition of stearic acid at 400°C and 25MPa and found only 2% of stearic acid conversion into major C₁₆ alkene. Conversion of 13% and 32% was achieved with the addition of NaOH and KOH, respectively. CO plus CO₂ yield were 0.4%, 0.6% and 1.9% without any catalyst, with NaOH, and with KOH. Although the gaseous fraction increased, it was not enough to account for the rise in conversion. Small peaks of carbonyl compounds were found in the chromatogram in case of NaOH. SCW alone can stabilize stearic acid and prevent degrading.

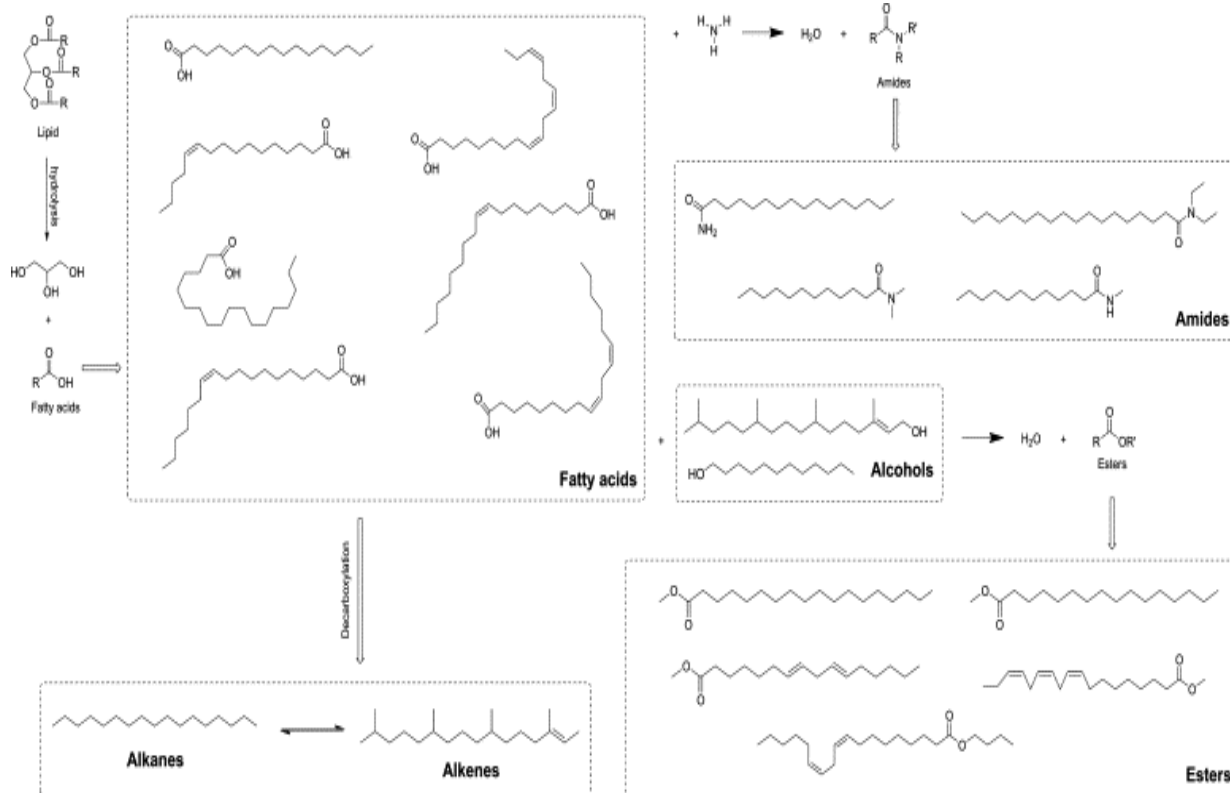


Figure 2.5. Conversion of lipids [105]

Figure 2.5 shows the conversion of lipids through various mechanism. First the TAGs are hydrolyzed into fatty acid (ex. Hexadecenoic acid) and glycerol. Fatty acid degrading to alkanes (Heptadecane) and alkenes (2-Hexadecene) because of decarboxylation. Hydroxyl group in long chain fatty acid reacting with ammonia obtained from deamination of amines to form aliphatic amine compounds like Hexadecanamide. Fatty acids can also react with alcohols produced from reduction of amino acids to form ester such as hexadecenoic acid, methyl ester.

2.7.4.2 Conversion of Proteins

Amino acids are the building blocks of protein. Amino acids are linked with a peptide bond (C-N) which is between carboxyl and amine groups. All amino acids have different chemical structure thereby react in different reaction pathway but undergo similar decarboxylation and deamination reaction [60]. Decarboxylation can remove oxygen by forming CO₂ and amines, and deamination is path for denitrogenation by formation of NH₃ and carbonic acids [106,107].

19 different amino acid compounds were chosen by Dote *et al.* to investigate the distribution of nitrogen compounds in the product fraction. It was found that 80% of nitrogen was dissolved in aqueous phase [108]. Biller *et al.* [59] used soya protein and albumin as model compounds for protein and glutamine and asparagine as amino acids model compound to understand the HTL pathway for proteins. The organic compounds produced from protein hydrolysis under severe condition and higher residence time tend to repolymerize by Fischer-Tropsch type reactions with water acting as hydrogen source and re-polymerize to longer chain hydrocarbons and aromatic ring type structures like phenols and nitrogen heterocycles such as, indoles, pyrroles that are soluble in bio-oil [59,60]. Figure 2.6 shows the estimation of reaction pathway by Dong *et al.* [105] for HTL of *Chlorella pyrenoidosa* (CP) and *Spirulina platensis* (SP)

[105]. Most of the nitrogenous compounds were distributed in the aqueous phase instead of the bio-oil phase.

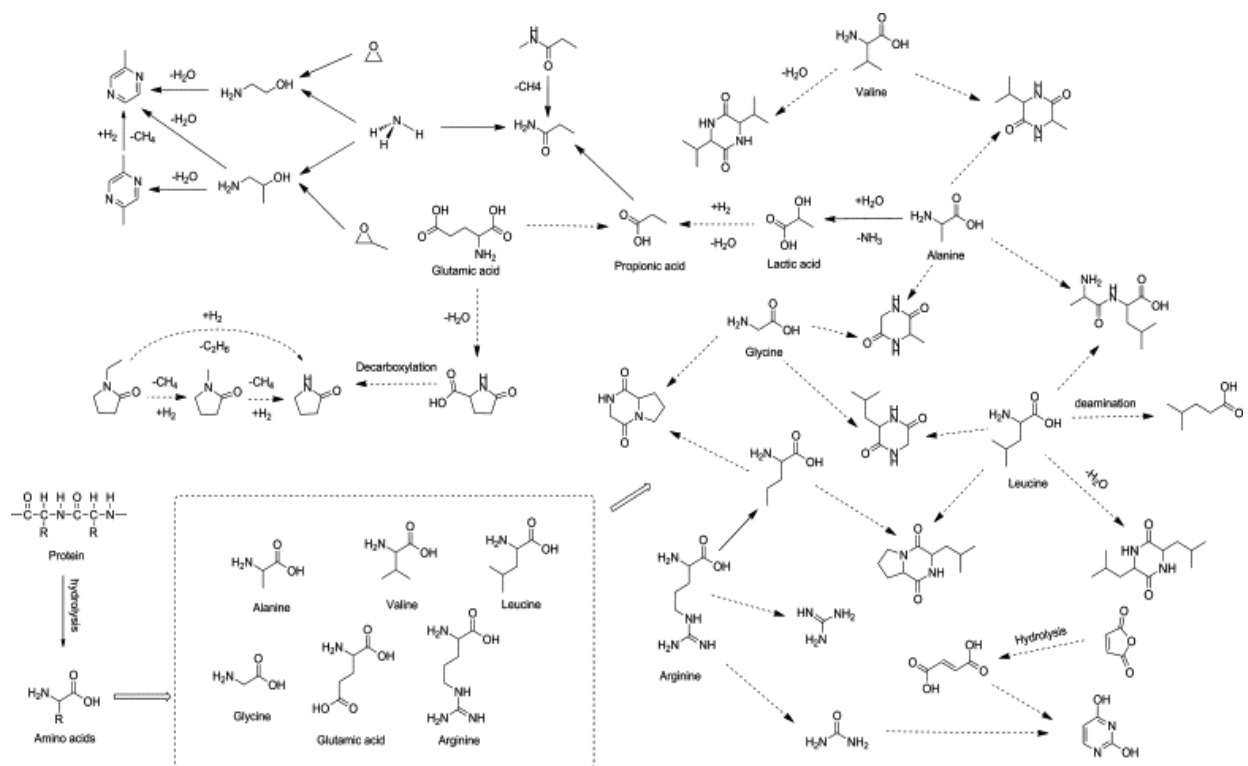


Figure 2.6. Possible reaction pathway for amino acids found in CP and SP [105]

2.7.4.3 Conversion of Carbohydrates

Microalgae carbohydrates are complex and consist of a mixture of neutral sugars, amino sugars and uronic acids and these compositions vary from species to species [109]. Cyanobacteria synthesize glycogen (α -1,4 linked glucan), red algae synthesize floridean starch (hybrid of starch and glycogen) and green algae synthesize amylopectin-like polysaccharides (starch) [110,111]. The most abundant carbohydrates sugars are glucose, rhamnose, mannose, galactose, and xylose [26]. Starch is a polysaccharide consisting of glucose monomers bound with α -(1/4) and α -(1/6) bonds. Cellulose are also polymers of glucose but are bound with β -(1-4)-glycosidic bonds, which forms strong intra and inter hydrogen bonds and resists swelling in water or attack by enzymes. At

elevated temperature in hydrothermal conditions both starch and cellulose can be hydrolyzed into glucose monomers. Hydrothermal liquefaction of three kinds of starches (rice, potato, and sweet potato) into reducing sugars was studied by Li *et al.* [112] in sub-critical water and maximum yield of 15% glucose, 30% fructose, and 33% maltose was observed. Glucose dissolves in water and exist in three forms: an open chain, a pyranose ring, and furanose ring like fructose [60]. The rate of degradation of glucose and fructose is relatively higher than the rate of isomerization of glucose [113]. Glucose and fructose degrades mostly to glycolaldehyde, pyruvaldehyde, glyceraldehyde and form 5-hydroxymethylfurfural (5-HMF) [113–115]. Figure 2.7 shows the reaction pathway for hydrothermal liquefaction of cellulose.

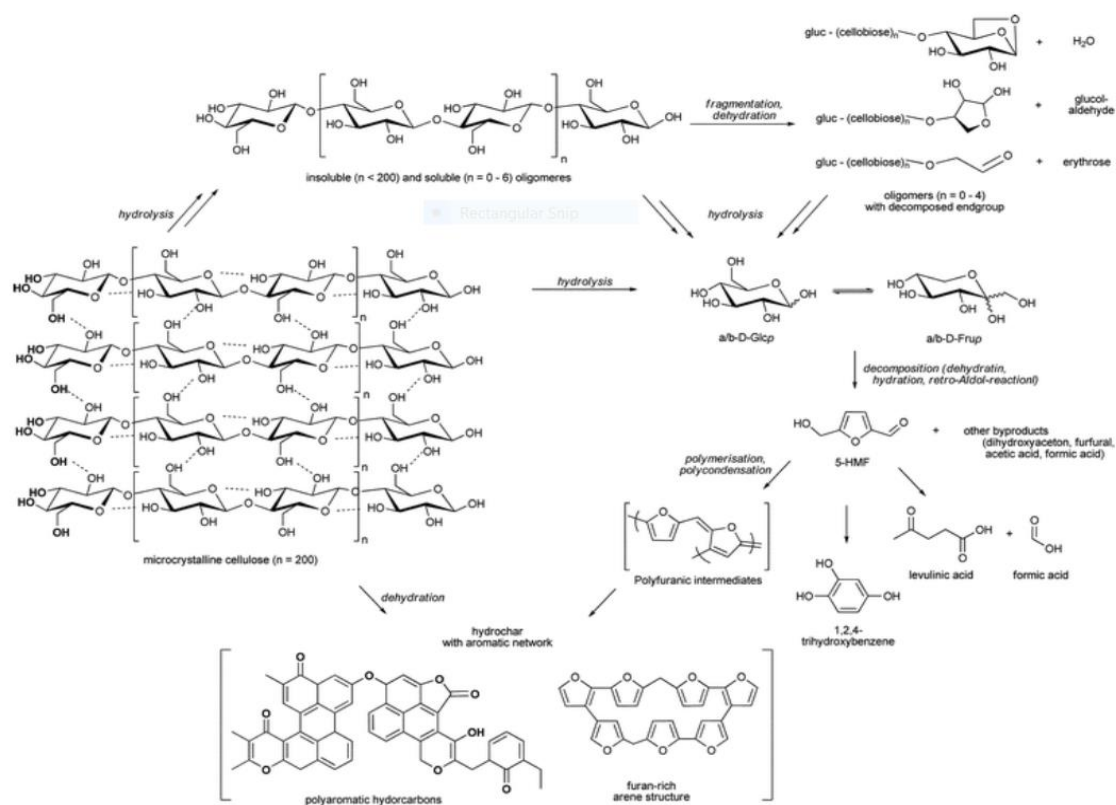


Figure 2.7. Reaction pathway from hydrothermal liquefaction of cellulose [116].

Dehydration and fragmentation of soluble oligomers products are possible. 5-HMF, further react to levulinic acid, formic acid or to trihydroxybenzene [117]. Polymerization and polycondensation of 5-HMF to polyfuranic intermediates may take place [118]. Finally, with enhanced reaction times hydro-char, insoluble carbonaceous material can be obtained. These hydro-chars can also be obtained by simultaneous degradation of cellulose and is common based on aromatic network [116]. Sakaki *et al.* [119] proposed degradation of cellulose followed saccharification and carbonization processes. Saccharification yielded water soluble sugars with minor decomposition and then followed carbonization. Decomposition of water soluble sugars through dehydration, decarboxylation and aromatization produced gases and water insoluble compounds which further polymerized to product like char [119].

2.8 Bio-oil Properties

The properties of bio-oil are strongly influenced by feedstock and process conditions.

Table 2.4. HHV (MJ/kg) and elemental composition of bio-oil obtained from HTL

Condition	350, 15min	350, 60min	350, 60min	350, 60min	350, 60 min	
Species	<i>Laminaria saccharina</i>	<i>Desmodemus</i> sp.	<i>Nannochloropsis</i> sp.	<i>Porphyridium</i> cruentum	<i>Nannochloropsis</i> oculata	Norther Sea Crude
Ref.	Ross <i>et al.</i> [86]	Garcia <i>et al.</i> [58]	Brown <i>et al.</i> [89]	Biller <i>et al.</i> [99]	Biller <i>et al.</i> [59]	Jensen <i>et al.</i> [120]
C wt%	82.00	75.80	76.00	72.80	68.10	86.6
H wt%	7.10	9.10	10.30	8.50	8.80	13.1
N wt%	4.90	6.00	3.90	5.40	4.10	
S wt%			0.90	0.30	0.00	
O wt%	5.40	9.10	9.00	0	18.90	0.30
H/C	1.04	1.44	1.63	1.40	1.55	1.80
O/C	0.05	0.09	0.09	0.14	0.21	
HHV	36.50	36.60	39.00	35.70	34.50	44.40

Table 2.4 shows the elemental composition and higher heating value reported on HTL of different algae at 350°C and 15-60 mins reaction time. The results are compared with fossil oil from northern sea crude as reported by Jensen *et al.* [120]. It is evident from the data that high percentage of heteroatom like nitrogen, oxygen, and sulphur is present in bio-oil that reduces its quality. The presence of nitrogen and sulfur in bio-oil will release NO_x and SO_x emission on burning the fuel which is detrimental to the environment. Oxygen in bio-oil decreases the overall energy density in the bio-oil and also contributes to aging of the fuel by the polymerization of aldehydes and hydroxyl group present in the oil thereby making it viscous overtime [121]. Bio-oils contain large portion of fatty acids from hydrolysis of lipids that imparts acidity and corrosive properties [122]. Bio-oil is a complex mixture of several undesirable organic compounds like ketones, aldehydes, fatty acids, phenols, esters, indole, pyrrole, amide that needs to be upgraded to be used as fuel [123].

2.9 Upgrading of Bio-oil

Many studies have been published to address the upgrading of bio-oil from HTL of biomass [122–127]. The effect of *in-situ* homogenous and heterogenous catalyst has already been discussed in section 2.7.3, and here we will discuss effect of heterogenous catalyst in ex-situ upgrading of HTL bio-oil from algae. Supercritical water with various heterogenous catalyst has been used and they are discussed below.

Pioneering work in upgrading algae bio-oil in supercritical water was performed by Duan and Savage [124] by using 25% loading of 5% Pt/C, 1:1 ratio of deionized water to bio-oil (*Nannochloropsis sp.*), 493 PSI initial pressure of hydrogen gas, supercritical temperature of 400°C for 4 hours reaction time. Dichloromethane was used as a solvent for product separation. The effect of SCW without catalyst rendered the bio-oil free of sulphur as was also observed in other studies

[124,128]. Alkanes ranging from C9 to C33 were detected in catalyzed SCW while alkanes smaller than C15 was almost negligible in crude bio-oil, which explains cracking during hydrotreatment. About 68% of the total compounds detected during Pt/C with H₂ upgrading were alkanes and no alkenes were detected, which explains hydrogenation. Fatty acids were decarboxylated to produce alkanes, but they also reacted with ammonia to produce amides like palmitamide and stearamide which was also observed at 430°C SCW with catalysts in Duan and Savage [128]. Oxygen content reduced by 32% to 4.46% and nitrogen content reduced by almost 50% to 2.24% [124]. Although the results obtained were encouraging it was not sufficient to meet fossil fuel requirements. Duan and Savage [128] further carried out upgrading experiments on algae bio-oil produced from HTL of *Nannochloropsis sp.* to deduce the effect of temperature (430, 480, and 530°C), catalyst loading (5%, 10%, and 20%), reaction time (2, 4, and 6h), and catalyst activity among Pt/C, Mo₂O, and HZSM-5 in supercritical water (4:5, water to bio-oil). Interestingly at higher temperatures of 480 and 530°C aromatics compounds dominated to having more than 90% composition in bio-oil which also included alkyl substituted phenol bearing oxygen atoms. At a given temperature, highest reaction time and highest catalyst loading resulted in hydrodeoxygenation, but the N/C ratio reduced to only half of bio-oil, invariably to the treatment condition. Duan *et al.* [129] tested nine different zeolite catalyst having different acidic sites and pore distribution on the upgraded bio-oil. The bio-oil was first pretreated in subcritical water to reduce heteroatom concentration and viscosity to limit catalyst poisoning and improving heat and mass transfer, respectively. MCM-41 and HZSM-5 provided highest bio-oil yield of around 54%. On two occasions it was found that high total acidic sites in zeolite catalyst because of Si/Al ratio seemed to have favored denitrogenation and desulphurization, but the theory failed when HY catalyst with low Na₂O (having high acidic sites) showed low activity for denitrogenation as compared to HY catalyst with

high Na_2O (low total acidic sites). MCM-41 catalyst was most effective with producing around 82% of hydrocarbons, as against 14.65% in crude bio-oil and 14.5% in pretreated oil. Figure 2.8 shows the boiling point distribution for upgraded bio-oil with 9 different catalyst, pretreated bio-oil and crude bio-oil [129]. It is evident from the figure how zeolite catalyst is an excellent catalyst to promote cracking and hydrogenation and decarboxylation of bio-oil.

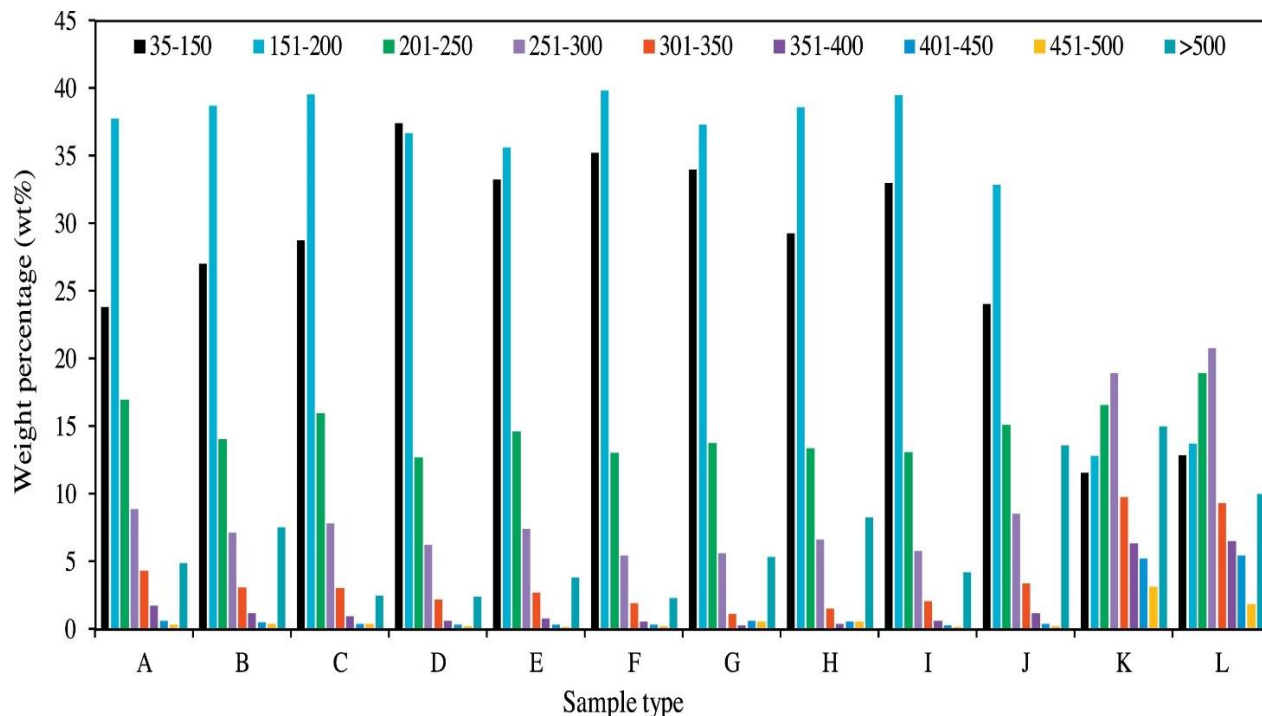


Figure 2.8. Boiling point distribution from TGA of upgraded oil with 9 different zeolite catalysts (A-J), pretreated oil (K), and crude bio-oil (L) as reported by Duan *et al.* [129]

Bai *et al.* also followed similar reaction setup to screen the activities of 5% Pt/C, 5% Pd/C, 5% Ru/C, 5% Pt/C (sulfided), Mo_2C , MoS_2 , alumina, $\text{CoMo}/\gamma\text{-Al}_2\text{O}_3$ (sulfided), $\text{Ni}/\text{SiO}_2\text{-Al}_2\text{O}_3$, HZSM-5, activated carbon, and Raney-Ni for hydrothermal hydrodeoxygenation and hydrodenitrogenation of the pretreated algal oil at 400 °C. Except HZSM-5 all the catalyst loaded experiments produced more oil than without catalyst, and the combination of Raney-Ni and Ru/C

together produced highest oil yield of 77.2% exceeding their individual action. Oil produced from action of all Pt/C catalyst had similar elemental composition, but the yield of oil followed the trend Pt/C (sulfided) > Pt/C in n-hexane (instead of water) > Pt/C > Pt/C in CO (instead of water). It was concluded that sulfided catalyst is not necessary for algae bio-oil upgrading under hydrothermal conditions [130]. Another important conclusion was that activated carbon might be responsible for most of the denitrogenation of bio-oil and that noble metal plays a smaller role in denitrogenation, as they gave similar nitrogen content in upgraded oil. Ru/C and Raney-Ni showed highest activity for deoxygenation (O wt%- 1.1) and denitrogenation (N wt%- 1.6), respectively while the combination of both the catalyst gave nitrogen and oxygen content of 2 wt% each [130]. Barreiro *et al.* [131] compared the effect of Pt/Al₂O₃, HZSM-5, and uncatalyzed upgrading at 400°C and 4 hours residence time on bio-crude obtained from *Scenedesmus almeriensis* and *Nannochloropsis gaditana* in wet (with water, initial H₂ pressure 4MPa) and dry (water less, initial H₂ pressure 8MPa). For *N. gaditana* dry upgrading gave more oil yield, was equally effective in denitrogenation, and produced at least 50% less oxygen content in the upgraded oil. Highest denitrogenation was observed without addition of any catalyst. For *S. almeriensis* wet process gave more upgraded oil yield except with HZSM-5 catalyst, dry process resulted in 50% more reduction in oxygen content while denitrogenation was ineffective when compared to crude bio-oil. Dry process resulted in better yield and deoxygenation as compared to wet process and the activity of catalyst is dependent on the species of algae used to produce crude bio-oil [131].

Shakya *et al.*[127] and Wang *et al.* [126] both studied the effect of catalyst activity on upgrading experiments at 350°C, initial hydrogen pressure of 1000 PSI, 4 hours residence time in water less environment. Shakya *et al.* used *Nannochloropsis* sp. algae and additionally upgraded at 300°C apart from 350°C, while Wang *et al.* used *Scenedesmus* species. In both the cases the

solvent for product separation was toluene which is an aromatic solvent. Both studies found 5% Ru/C for maximum TAN reduction in upgraded bio-oil at 350°C. Pt/C and Ru/C had maximum activity for nitrogen reduction and Ni/ZSM5 showed highest activity for hydrodeoxygenation. Upgrading at 350°C resulted in oil having better fuel characteristics than at 300°C. Separation of reaction mixture using a polar solvent like dichloromethane and an aromatic solvent like toluene will affect both the yield and properties of oil obtained. None of the studies in the literature compare the effect of using different solvents.

2.10 References

- [1] M. Crocker, R. Andrews, The Rationale for Biofuels, *Thermochem. Convers. Biomass to Liq. Fuels Chem.* (2010) 1–25. doi:10.1039/9781849732260-00001.
- [2] U.S. Energy Information Administration, *International Energy Outlook 2017*, *Int. Energy Outlook. IEO2017* (2017) 143. doi:www.eia.gov/forecasts/ieo/pdf/0484(2016).pdf.
- [3] U.S. Energy Information Administration (EIA) - Data, U.S. Energy Inf. Adm. (2018). <https://www.eia.gov/totalenergy/data/browser/?tbl=T01.01#/?f=A&start=1949&end=2017&charted=4-6-7-14> (accessed June 28, 2018).
- [4] BP: World Reserves of Fossil Fuels - knoema.com, (n.d.). <https://knoema.com/infographics/smsfgud/bp-world-reserves-of-fossil-fuels> (accessed June 28, 2018).
- [5] U. Soytaş, R. Sari, B.T. Ewing, Energy consumption, income, and carbon emissions in the United States, *Ecol. Econ.* 62 (2007) 482–489. doi:10.1016/J.ECOLECON.2006.07.009.
- [6] U.S. Refinery Crude Oil Input Qualities, (2018). https://www.eia.gov/dnav/pet/pet_pnp_crq_dcu_nus_m.htm (accessed June 28, 2018).
- [7] J. Chen, H. Farooqi, C. Fairbridge, Experimental Study on Co-hydroprocessing Canola Oil and Heavy Vacuum Gas Oil Blends, *Energy & Fuels.* 27 (2013) 3306–3315. doi:10.1021/ef4005835.
- [8] S.S. Toor, L. Rosendahl, A. Rudolf, Hydrothermal liquefaction of biomass: A review of subcritical water technologies, *Energy.* 36 (2011) 2328–2342. doi:10.1016/j.energy.2011.03.013.
- [9] Alternative Fuels Data Center, (n.d.). <https://www.afdc.energy.gov/laws/RFS.html> (accessed June 28, 2018).
- [10] P. McKendry, Energy production from biomass (part 1): overview of biomass, *Bioresour. Technol.* 83 (2002) 37–46. doi:10.1016/S0960-8524(01)00118-3.
- [11] P.S. Nigam, A. Singh, Production of liquid biofuels from renewable resources, *Prog. Energy Combust. Sci.* 37 (2011) 52–68. doi:10.1016/J.PECS.2010.01.003.
- [12] E.D. Larson, *Biofuel Production Technologies: Status and Prospects*, (2007). http://unctad.org/sections/wcmu/docs/ditc_tedb_ted0015_en.pdf (accessed June 29, 2018).
- [13] V. Patil, K.Q. Tran, H.R. Giselsrød, Towards sustainable production of biofuels from microalgae, *Int. J. Mol. Sci.* 9 (2008) 1188–1195. doi:10.3390/ijms9071188.
- [14] E N E R Gy a G E N C Y for for, (n.d.).

- [15] J.P. Maity, J. Bundschuh, C.-Y. Chen, P. Bhattacharya, Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review, *Energy*. 78 (2014) 104–113. doi:10.1016/J.ENERGY.2014.04.003.
- [16] T. Minowa, S. Yokoyama, M. Kishimoto, T. Okakura, Oil production from algal cells of *Dunaliella tertiolecta* by direct thermochemical liquefaction, *Fuel*. 74 (1995) 1735–1738. doi:10.1016/0016-2361(95)80001-X.
- [17] K. Kumar, C.N. Dasgupta, B. Nayak, P. Lindblad, D. Das, Development of suitable photobioreactors for CO₂ sequestration addressing global warming using green algae and cyanobacteria, *Bioresour. Technol.* 102 (2011) 4945–4953. doi:10.1016/J.BIORTECH.2011.01.054.
- [18] D.D.-W. Tsai, R. Ramaraj, P.H. Chen, Growth condition study of algae function in ecosystem for CO₂ bio-fixation, *J. Photochem. Photobiol. B Biol.* 107 (2012) 27–34. doi:10.1016/J.JPHOTOBIO.2011.11.005.
- [19] F.B. Metting, Biodiversity and application of microalgae, *J. Ind. Microbiol.* 17 (1996) 477–489. doi:10.1007/BF01574779.
- [20] R.A. Andersen, Diversity of eukaryotic algae, *Biodivers. Conserv.* 1 (1992) 267–292. doi:10.1007/BF00693765.
- [21] E. Molina Grima, E.-H. Belarbi, F. Ación Fernández, A. Robles Medina, Y. Chisti, Recovery of microalgal biomass and metabolites: process options and economics, *Biotechnol. Adv.* 20 (2003) 491–515. doi:10.1016/S0734-9750(02)00050-2.
- [22] M.D. Guiry, C. VAN DEN HOEK, D. G. MANN and H. M. JAHNS. *Algae. An Introduction to Phycology*. Cambridge University Press, Cambridge. 1995, pp. xiv+623. ISBN: 0 521 30419 9 (hardback); 0 051 31687 1 (paperback). Price: £70.00 (hard); £24.95 (soft)., *Eur. J. Phycol.* 32 (1997) S096702629621100X. doi:10.1017/S096702629621100X.
- [23] C. Yusuf, Biodiesel from microalgae, *Biotechnol. Adv.* 25 (2007) 294–306. doi:10.1016/j.biotechadv.2007.02.001.
- [24] L. Taiz, E. Zeiger, *Plant Physiology*, *Ann. Bot.* (2002) 690. doi:10.1104/pp.900074.
- [25] J. Kromkamp, Formation and functional significance of storage products in cyanobacteria, *New Zeal. J. Mar. Freshw. Res.* 21 (1987) 457–465. doi:10.1080/00288330.1987.9516241.
- [26] G. Markou, I. Angelidaki, D. Georgakakis, Microalgal carbohydrates: an overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels, *Appl. Microbiol. Biotechnol.* 96 (2012) 631–645. doi:10.1007/s00253-012-4398-0.
- [27] E.W. Becker, Micro-algae as a source of protein, *Biotechnol. Adv.* 25 (2007) 207–210.

doi:10.1016/J.BIOTECHADV.2006.11.002.

- [28] G.J. B., C.K. E., TRIGLYCERIDE ACCUMULATION AND FATTY ACID PROFILE CHANGES IN CHLORELLA (CHLOROPHYTA) DURING HIGH pH-INDUCED CELL CYCLE INHIBITION1, *J. Phycol.* 26 (2018) 72–79. doi:10.1111/j.0022-3646.1990.00072.x.
- [29] J.L. Harwood, Membrane Lipids in Algae BT - Lipids in Photosynthesis: Structure, Function and Genetics, in: S. Paul-André, M. Norio (Eds.), Springer Netherlands, Dordrecht, 1998: pp. 53–64. doi:10.1007/0-306-48087-5_3.
- [30] S. Samantray, S. Guruprasad, T. V Ramachandra, Diversity of lipids in algae, *Lake 2010 Wetl. Biodivers. Clim. Chang.* (2010) 1–9.
- [31] M.A. Borowitzka, 13 - Algae Oils for Biofuels: Chemistry, Physiology, and Production, in: Z. Cohen, C.B.T.-S.C.O. (Second E. Ratledge (Eds.), AOCS Press, 2010: pp. 271–289. doi:<https://doi.org/10.1016/B978-1-893997-73-8.50017-7>.
- [32] G.E. Molina, F.S.J. María, A.F.F. Gabriel, Microalgae, Mass Culture Methods, *Encycl. Ind. Biotechnol.* (2010). doi:doi:10.1002/9780470054581.eib418.
- [33] E. Molina, J. Fernández, F.G. Acién, Y. Chisti, Tubular photobioreactor design for algal cultures, *J. Biotechnol.* 92 (2001) 113–131. doi:10.1016/S0168-1656(01)00353-4.
- [34] A. Mizrahi, A.L. van. Wezel, *Advances in biotechnological processes.*, Alan R. Liss, 1983. <http://agris.fao.org/agris-search/search.do?recordID=US8723172> (accessed June 29, 2018).
- [35] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: A review, *Renew. Sustain. Energy Rev.* 14 (2010) 217–232. doi:10.1016/J.RSER.2009.07.020.
- [36] G. Markou, I. Angelidaki, D. Georgakakis, Microalgal carbohydrates: An overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels, *Appl. Microbiol. Biotechnol.* 96 (2012) 631–645. doi:10.1007/s00253-012-4398-0.
- [37] A. Izumo, S. Fujiwara, Y. Oyama, A. Satoh, N. Fujita, Y. Nakamura, M. Tsuzuki, Physicochemical properties of starch in Chlorella change depending on the CO₂ concentration during growth: Comparison of structure and properties of pyrenoid and stroma starch, *Plant Sci.* 172 (2007) 1138–1147. doi:10.1016/J.PLANTSCI.2007.03.001.
- [38] S.P. Singh, P. Singh, Effect of CO₂ concentration on algal growth: A review, *Renew. Sustain. Energy Rev.* 38 (2014) 172–179. doi:10.1016/J.RSER.2014.05.043.
- [39] J.R. Benemann, Production of nitrogen fertilizer with nitrogen-fixing blue - green algae, *Enzyme Microb. Technol.* 1 (1979) 83–90. doi:10.1016/0141-0229(79)90103-0.

- [40] S. Boussiba, J. Gibson, Ammonia translocation in cyanobacteria, *FEMS Microbiol. Lett.* 88 (1991) 1–14. doi:10.1016/0378-1097(91)90692-4.
- [41] R. Chaiklahan, N. Chirasuwan, W. Siangdung, K. Paithoonrangsarid, B. Bunnag, Cultivation of spirulina platensis using pig wastewater in a semi-continuous process, *J. Microbiol. Biotechnol.* 20 (2010) 609–614. doi:10.4014/jmb.0907.07026.
- [42] C.-H. Hsieh, W.-T. Wu, Cultivation of microalgae for oil production with a cultivation strategy of urea limitation, *Bioresour. Technol.* 100 (2009) 3921–3926. doi:10.1016/J.BIORTECH.2009.03.019.
- [43] O. Bernard, Hurdles and challenges for modelling and control of microalgae for CO₂ mitigation and biofuel production, *J. Process Control.* 21 (2011) 1378–1389. doi:10.1016/j.jprocont.2011.07.012.
- [44] G. Dragone, B.D. Fernandes, A.P. Abreu, A.A. Vicente, J.A. Teixeira, Nutrient limitation as a strategy for increasing starch accumulation in microalgae, *Appl. Energy.* 88 (2011) 3331–3335. doi:10.1016/j.apenergy.2011.03.012.
- [45] Q. Béchet, A. Shilton, B. Guieysse, Modeling the effects of light and temperature on algae growth: State of the art and critical assessment for productivity prediction during outdoor cultivation, *Biotechnol. Adv.* 31 (2013) 1648–1663. doi:10.1016/J.BIOTECHADV.2013.08.014.
- [46] P.A. Crill, The photosynthesis-light curve: A simple analog model, *J. Theor. Biol.* 64 (1977) 503–516. doi:10.1016/0022-5193(77)90284-3.
- [47] R.F. Camacho, C.F. García, S.J.M. Fernández, C. Y., G.E. Molina, A mechanistic model of photosynthesis in microalgae, *Biotechnol. Bioeng.* 81 (2002) 459–473. doi:10.1002/bit.10492.
- [48] P. Mercer, R.E. Armenta, Developments in oil extraction from microalgae, *Eur. J. Lipid Sci. Technol.* 113 (2011) 539–547. doi:10.1002/ejlt.201000455.
- [49] extraction cassava oil.pdf, (n.d.).
- [50] J.A. Galloway, K.J. Koester, B.J. Paasch, C.W. Macosko, Effect of sample size on solvent extraction for detecting cocontinuity in polymer blends, *Polymer (Guildf).* 45 (2004) 423–428. doi:10.1016/J.POLYMER.2003.10.098.
- [51] M. Herrero, E. Ibáñez, J. Señoráns, A. Cifuentes, Pressurized liquid extracts from *Spirulina platensis* microalga: Determination of their antioxidant activity and preliminary analysis by micellar electrokinetic chromatography, *J. Chromatogr. A.* 1047 (2004) 195–203. doi:10.1016/J.CHROMA.2004.07.001.
- [52] F. Sahena, I.S.M. Zaidul, S. Jinap, A.A. Karim, K.A. Abbas, N.A.N. Norulaini, A.K.M. Omar, Application of supercritical CO₂ in lipid extraction – A review, *J. Food Eng.* 95

- (2009) 240–253. doi:10.1016/J.JFOODENG.2009.06.026.
- [53] M.D. Macías-Sánchez, C. Mantell, M. Rodríguez, E. Martínez de la Ossa, L.M. Lubián, O. Montero, Supercritical fluid extraction of carotenoids and chlorophyll a from *Nannochloropsis gaditana*, *J. Food Eng.* 66 (2005) 245–251. doi:10.1016/J.JFOODENG.2004.03.021.
- [54] A.K. Agarwal, Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines, *Prog. Energy Combust. Sci.* 33 (2007) 233–271. doi:10.1016/J.PECS.2006.08.003.
- [55] H. Nouredini, D. Harkey, V. Medikonduru, A continuous process for the conversion of vegetable oils into methyl esters of fatty acids, *J. Am. Oil Chem. Soc.* 75 (1998) 1775–1783. doi:10.1007/s11746-998-0331-1.
- [56] H. Xu, X. Miao, Q. Wu, High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters, *J. Biotechnol.* 126 (2006) 499–507. doi:10.1016/J.JBIOTEC.2006.05.002.
- [57] A.V. Bridgwater, D. Meier, D. Radlein, An overview of fast pyrolysis of biomass, *Org. Geochem.* 30 (1999) 1479–1493. doi:10.1016/S0146-6380(99)00120-5.
- [58] L. Garcia Alba, C. Torri, C. Samorì, J. Van Der Spek, D. Fabbri, S.R.A. Kersten, D.W.F. Brilman, Hydrothermal treatment (HTT) of microalgae: Evaluation of the process as conversion method in an algae biorefinery concept, *Energy and Fuels.* 26 (2012) 642–657. doi:10.1021/ef201415s.
- [59] P. Biller, A.B. Ross, Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content, *Bioresour. Technol.* 102 (2011) 215–225. doi:10.1016/J.BIORTECH.2010.06.028.
- [60] A.A. Peterson, F. Vogel, R.P. Lachance, M. Fröling, M.J. Antal, J.W. Tester, Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies, *Energy Environ. Sci.* 1 (2008) 32–65. doi:10.1039/b810100k.
- [61] Thermochemical Conversion of Biomass to Liquid Fuels and Chemicals - Google Books, (n.d.). [https://books.google.com/books?hl=en&lr=&id=bnIoDwAAQBAJ&oi=fnd&pg=PA192&dq=hydrothermal+processing+of+biomass&ots=nq5ZlJunj1&sig=DdX3UEOkef_YU7CNrztZe6_ZRCK#v=onepage&q=hydrothermal processing of biomass&f=false](https://books.google.com/books?hl=en&lr=&id=bnIoDwAAQBAJ&oi=fnd&pg=PA192&dq=hydrothermal+processing+of+biomass&ots=nq5ZlJunj1&sig=DdX3UEOkef_YU7CNrztZe6_ZRCK#v=onepage&q=hydrothermal+processing+of+biomass&f=false) (accessed July 1, 2018).
- [62] M. Kröger, F. Müller-Langer, Review on possible algal-biofuel production processes, *Biofuels.* 3 (2012) 333–349. doi:10.4155/bfs.12.14.
- [63] A. Kruse, E. Dinjus, Hot compressed water as reaction medium and reactant: Properties and

- synthesis reactions, *J. Supercrit. Fluids.* 39 (2007) 362–380. doi:10.1016/J.SUPFLU.2006.03.016.
- [64] P. Krammer, H. Vogel, Hydrolysis of esters in subcritical and supercritical water, *J. Supercrit. Fluids.* 16 (2000) 189–206. doi:10.1016/S0896-8446(99)00032-7.
- [65] F.E. U., R. S., C. M., Calculation of the Dielectric Constant of Water to 1000°C and Very High Pressures, *Berichte Der Bunsengesellschaft Für Phys. Chemie.* 94 (2018) 199–203. doi:10.1002/bbpc.19900940219.
- [66] J. King, R. Holliday, G. List, Hydrolysis of soybean oil. in a subcritical water flow reactor, *Green Chem.* 1 (1999) 261–264.
- [67] R.L. Holliday, J.W. King, G.R. List, Hydrolysis of Vegetable Oils in Sub- and Supercritical Water, *Ind. Eng. Chem. Res.* 36 (1997) 932–935. doi:10.1021/ie960668f.
- [68] H.P. Blaschek, T.C. Ezeji, J. Scheffran, Wiley InterScience (Online service), *Biofuels from agricultural wastes and byproducts*, Wiley-Blackwell, 2010. https://books.google.com/books?hl=en&lr=&id=TBhfRUt6kcEC&oi=fnd&pg=PA201&dq=Hydrothermal+Liquefaction+to+Convert+Biomass+into+Crude+Oil&ots=YClggwIzGv&sig=b3klbG0_b8uT2TN_x7iWnv7FCI4#v=onepage&q=Hydrothermal+Liquefaction+to+Convert+Biomass+into+Crude+Oil&f=false (accessed July 1, 2018).
- [69] S.E. Hunter, P.E. Savage, Recent advances in acid- and base-catalyzed organic synthesis in high-temperature liquid water, *Chem. Eng. Sci.* 59 (2004) 4903–4909. doi:10.1016/J.CES.2004.09.009.
- [70] A. Kruse, E. Dinjus, Hot compressed water as reaction medium and reactant: 2. Degradation reactions, *J. Supercrit. Fluids.* 41 (2007) 361–379. doi:10.1016/J.SUPFLU.2006.12.006.
- [71] M. OSADA, T. SATO, M. WATANABE, M. SHIRAI, K. ARAI, CATALYTIC GASIFICATION OF WOOD BIOMASS IN SUBCRITICAL AND SUPERCRITICAL WATER, *Combust. Sci. Technol.* 178 (2006) 537–552. doi:10.1080/00102200500290807.
- [72] M.M. Titirici, M. Antonietti, Chemistry and materials options of sustainable carbon materials made by hydrothermal carbonization, *Chem. Soc. Rev.* 39 (2010) 103–116. doi:10.1039/b819318p.
- [73] J.A. Libra, K.S. Ro, C. Kammann, A. Funke, N.D. Berge, Y. Neubauer, M.M. Titirici, C. Fühner, O. Bens, J. Kern, K.H. Emmerich, Hydrothermal carbonization of biomass residuals: A comparative review of the chemistry, processes and applications of wet and dry pyrolysis, *Biofuels.* 2 (2011) 71–106. doi:10.4155/bfs.10.81.
- [74] M.J. Antal, M. Grønli, *The Art, Science, and Technology of Charcoal Production*, *Ind. Eng. Chem. Res.* 42 (2003) 1619–1640. doi:10.1021/ie0207919.
- [75] I. Ferrer, E.T. Furlong, Accelerated Solvent Extraction Followed by On-Line Solid-Phase

- Extraction Coupled to Ion Trap LC/MS/MS for Analysis of Benzalkonium Chlorides in Sediment Samples, *Anal. Chem.* 74 (2002) 1275–1280. doi:10.1021/ac010969l.
- [76] S. O., Die Anwendung hoher Drucke bei chemischen Vorgängen und eine Nachbildung des Entstehungsprozesses der Steinkohle. Von Friedrich Bergius. 58 S. und 4 Abbildungen. Verlag von Wilhelm Knapp, Halle a. S. 1913. Preis geb. 2,80 Mk, *Zeitschrift Für Elektrochemie Und Angew. Phys. Chemie.* 20 (1913) 260. doi:10.1002/bbpc.19140200813.
- [77] H.P. Ruyter, Coalification model, *Fuel.* 61 (1982) 1182–1187. doi:10.1016/0016-2361(82)90017-5.
- [78] L. Axelsson, M. Franzén, M. Ostwald, G. Berndes, G. Lakshmi, N.H. Ravindranath, Perspective: *Jatropha* cultivation in southern India: Assessing farmers' experiences, *Biofuels, Bioprod. Biorefining.* 6 (2012) 246–256. doi:10.1002/bbb.
- [79] D. López Barreiro, W. Prins, F. Ronsse, W. Brilman, Hydrothermal liquefaction (HTL) of microalgae for biofuel production: State of the art review and future prospects, *Biomass and Bioenergy.* 53 (2013) 113–127. doi:10.1016/j.biombioe.2012.12.029.
- [80] C. Xu, T. Etcheverry, Hydro-liquefaction of woody biomass in sub- and super-critical ethanol with iron-based catalysts, *Fuel.* 87 (2008) 335–345. doi:10.1016/J.FUEL.2007.05.013.
- [81] B. Wang, Y. Huang, J. Zhang, Hydrothermal liquefaction of lignite, wheat straw and plastic waste in sub-critical water for oil: Product distribution, *J. Anal. Appl. Pyrolysis.* 110 (2014) 382–389. doi:10.1016/J.JAAP.2014.10.004.
- [82] D.C. Elliott, P. Biller, A.B. Ross, A.J. Schmidt, S.B. Jones, Hydrothermal liquefaction of biomass: Developments from batch to continuous process, *Bioresour. Technol.* 178 (2015) 147–156. doi:10.1016/J.BIORTECH.2014.09.132.
- [83] R. Shakya, S. Adhikari, R. Mahadevan, S.R. Shanmugam, H. Nam, E.B. Hassan, T.A. Dempster, Influence of biochemical composition during hydrothermal liquefaction of algae on product yields and fuel properties, *Bioresour. Technol.* 243 (2017) 1112–1120. doi:10.1016/j.biortech.2017.07.046.
- [84] U. Jena, K.C. Das, J.R. Kastner, Effect of operating conditions of thermochemical liquefaction on biocrude production from *Spirulina platensis*, *Bioresour. Technol.* 102 (2011) 6221–6229. doi:10.1016/J.BIORTECH.2011.02.057.
- [85] S. Zou, Y. Wu, M. Yang, C. Li, J. Tong, Bio-oil production from sub- and supercritical water liquefaction of microalgae *Dunaliella tertiolecta* and related properties, *Energy Environ. Sci.* 3 (2010) 1073–1078. doi:10.1039/C002550J.
- [86] K. Anastakis, A.B. Ross, Hydrothermal liquefaction of the brown macro-alga *Laminaria Saccharina*: Effect of reaction conditions on product distribution and composition, *Bioresour. Technol.* 102 (2011) 4876–4883. doi:10.1016/J.BIORTECH.2011.01.031.

- [87] Z. Shuping, W. Yulong, Y. Mingde, I. Kaleem, L. Chun, J. Tong, Production and characterization of bio-oil from hydrothermal liquefaction of microalgae *Dunaliella tertiolecta* cake, *Energy*. 35 (2010) 5406–5411. doi:10.1016/J.ENERGY.2010.07.013.
- [88] P.J. Valdez, M.C. Nelson, H.Y. Wang, X.N. Lin, P.E. Savage, Hydrothermal liquefaction of *Nannochloropsis* sp.: Systematic study of process variables and analysis of the product fractions, *Biomass and Bioenergy*. 46 (2012) 317–331. doi:10.1016/J.BIOMBIOE.2012.08.009.
- [89] T.M. Brown, P. Duan, P.E. Savage, Hydrothermal Liquefaction and Gasification of *Nannochloropsis* sp., *Energy & Fuels*. 24 (2010) 3639–3646. doi:10.1021/ef100203u.
- [90] J. Akhtar, N.A.S. Amin, A review on process conditions for optimum bio-oil yield in hydrothermal liquefaction of biomass, *Renew. Sustain. Energy Rev.* 15 (2011) 1615–1624. doi:10.1016/j.rser.2010.11.054.
- [91] Y. Guo, T. Yeh, W. Song, D. Xu, S. Wang, A review of bio-oil production from hydrothermal liquefaction of algae, *Renew. Sustain. Energy Rev.* 48 (2015) 776–790. doi:10.1016/j.rser.2015.04.049.
- [92] Z. Abu El-Rub, E.A. Bramer, G. Brem, Review of Catalysts for Tar Elimination in Biomass Gasification Processes, *Ind. Eng. Chem. Res.* 43 (2004) 6911–6919. doi:10.1021/ie0498403.
- [93] R. Shakya, *Hydrothermal Liquefaction of Algae for Bio-oil Production*, (2014).
- [94] J.L. Faeth, P.J. Valdez, P.E. Savage, Fast Hydrothermal Liquefaction of *Nannochloropsis* sp. To Produce Biocrude, *Energy & Fuels*. 27 (2013) 1391–1398. doi:10.1021/ef301925d.
- [95] J. Zhang, W.-T. Chen, P. Zhang, Z. Luo, Y. Zhang, Hydrothermal liquefaction of *Chlorella pyrenoidosa* in sub- and supercritical ethanol with heterogeneous catalysts, *Bioresour. Technol.* 133 (2013) 389–397. doi:10.1016/J.BIORTECH.2013.01.076.
- [96] P.E. Savage, P. Duan, P.E. Savage, Hydrothermal Liquefaction of a Microalga with Heterogeneous Catalysts Hydrothermal Liquefaction of a Microalga with Heterogeneous Catalysts, *Ind. Eng. Chem. Res.* (2011) 52–61. doi:10.1021/ie100758s.
- [97] D. Zhou, L. Zhang, S. Zhang, H. Fu, J. Chen, Hydrothermal Liquefaction of Macroalgae *Enteromorpha prolifera* to Bio-oil, *Energy & Fuels*. 24 (2010) 4054–4061. doi:10.1021/ef100151h.
- [98] U. Jena, K. C. Das, Production of Biocrude Oil from Microalgae via Thermochemical Liquefaction Process, *An ASABE Conf. Present.* 0300 (2009) 1–10.
- [99] A.B. Ross, P. Biller, M.L. Kubacki, H. Li, A. Lea-Langton, J.M. Jones, Hydrothermal processing of microalgae using alkali and organic acids, *Fuel*. 89 (2010) 2234–2243. doi:10.1016/J.FUEL.2010.01.025.

- [100] N. Sato, A.T. Quitain, K. Kang, H. Daimon, K. Fujie, Reaction Kinetics of Amino Acid Decomposition in High-Temperature and High-Pressure Water, *Ind. Eng. Chem. Res.* 43 (2004) 3217–3222. doi:10.1021/ie020733n.
- [101] P. Duan, P.E. Savage, Hydrothermal Liquefaction of a Microalga with Heterogeneous Catalysts, *Ind. Eng. Chem. Res.* 50 (2011) 52–61. doi:10.1021/ie100758s.
- [102] D.C. Gowda, A.S. Prakasha Gowda, A.R. Baba, S. Gowda, Nickel-Catalyzed Formic Acid Reductions. A Selective Method for the Reduction of Nitro Compounds, *Synth. Commun.* 30 (2000) 2889–2895. doi:10.1080/00397910008087439.
- [103] P. Biller, R. Riley, A.B. Ross, Catalytic hydrothermal processing of microalgae: Decomposition and upgrading of lipids, *Bioresour. Technol.* 102 (2011) 4841–4848. doi:10.1016/J.BIORTECH.2010.12.113.
- [104] B.H. L., B.A. C., Continuous fat splitting plants using the colgate-emery process, *J. Am. Oil Chem. Soc.* 25 (1948) 95–99. doi:10.1007/BF02579733.
- [105] C. Gai, Y. Zhang, W.-T. Chen, P. Zhang, Y. Dong, An investigation of reaction pathways of hydrothermal liquefaction using *Chlorella pyrenoidosa* and *Spirulina platensis*, *Energy Convers. Manag.* 96 (2015) 330–339. doi:10.1016/J.ENCONMAN.2015.02.056.
- [106] D. Klingler, J. Berg, H. Vogel, Hydrothermal reactions of alanine and glycine in sub- and supercritical water, *J. Supercrit. Fluids.* 43 (2007) 112–119. doi:10.1016/J.SUPFLU.2007.04.008.
- [107] A. Demirbaş, Mechanisms of liquefaction and pyrolysis reactions of biomass, *Energy Convers. Manag.* 41 (2000) 633–646. doi:10.1016/S0196-8904(99)00130-2.
- [108] Y. Dote, S. Inoue, T. Ogi, S. Yokoyama, Distribution of nitrogen to oil products from liquefaction of amino acids, *Bioresour. Technol.* 64 (1998) 157–160. doi:10.1016/S0960-8524(97)00079-5.
- [109] S. Pieper, I. Unterrieser, F. Mann, P. Mischnick, A new arabinomannan from the cell wall of the chlorococcal algae *Chlorella vulgaris*, *Carbohydr. Res.* 352 (2012) 166–176. doi:10.1016/J.CARRES.2012.02.007.
- [110] Y. Nakamura, J. Takahashi, A. Sakurai, Y. Inaba, E. Suzuki, S. Nihei, S. Fujiwara, M. Tsuzuki, H. Miyashita, H. Ikemoto, M. Kawachi, H. Sekiguchi, N. Kurano, Some Cyanobacteria Synthesize Semi-amylopectin Type α -Polyglucans Instead of Glycogen, *Plant Cell Physiol.* 46 (2005) 539–545. <http://dx.doi.org/10.1093/pcp/pci045>.
- [111] K.M. Sekharam, L.V. Venkataraman, P.V. Salimath, Structural studies of a glucan isolated from blue-green alga *Spirulina platensis*, *Food Chem.* 31 (1989) 85–91. doi:10.1016/0308-8146(89)90019-8.
- [112] F. Li, L. Liu, Y. An, W. He, N.J. Themelis, G. Li, Hydrothermal liquefaction of three kinds

- of starches into reducing sugars, *J. Clean. Prod.* 112 (2016) 1049–1054. doi:10.1016/J.JCLEPRO.2015.08.008.
- [113] M.J. Antal, W.S.L. Mok, G.N. Richards, Mechanism of formation of 5-(hydroxymethyl)-2-furaldehyde from d-fructose and sucrose, *Carbohydr. Res.* 199 (1990) 91–109. doi:10.1016/0008-6215(90)84096-D.
- [114] G. Bonn, O. Bobleter, Determination of the hydrothermal degradation products of D-(U-14C) glucose and D-(U-14C) fructose by TLC, *J. Radioanal. Chem.* 79 (1983) 171–177. doi:10.1007/BF02518929.
- [115] Z. Srokol, A.-G. Bouche, A. van Estrik, R.C. Strik, T. Maschmeyer, J.A. Peters, Hydrothermal upgrading of biomass to biofuel; studies on some monosaccharide model compounds, *Carbohydr. Res.* 339 (2004) 1717–1726. doi:10.1016/J.CARRES.2004.04.018.
- [116] M. Mo, RSC Advances water — the role of crystallinity on the cellulose, (2013) 11035–11044. doi:10.1039/c3ra41582a.
- [117] K. Ehara, S. Saka, A comparative study on chemical conversion of cellulose between the batch-type and flow-type systems in supercritical water, *Cellulose.* 9 (2002) 301–311. doi:10.1023/A:1021192711007.
- [118] C. Falco, N. Baccile, M.M. Titirici, Morphological and structural differences between glucose, cellulose and lignocellulosic biomass derived hydrothermal carbons, *Green Chem.* 13 (2011) 3273–3281. doi:10.1039/c1gc15742f.
- [119] T. Sakaki, M. Shibata, T. Miki, H. Hirose, N. Hayashi, Decomposition of cellulose in near-critical water and fermentability of the products, *Energy and Fuels.* 10 (1996) 684–688. doi:10.1021/ef950160+.
- [120] C.U. Jensen, J. Hoffmann, L.A. Rosendahl, Co-processing potential of HTL bio-crude at petroleum refineries. Part 2: A parametric hydrotreating study, *Fuel.* 165 (2015) 536–543. doi:10.1016/j.fuel.2015.08.047.
- [121] Q. Lu, W.-Z. Li, X.-F. Zhu, Overview of fuel properties of biomass fast pyrolysis oils, *Energy Convers. Manag.* 50 (2009) 1376–1383. doi:10.1016/J.ENCONMAN.2009.01.001.
- [122] M. Saber, B. Nakhshiniev, K. Yoshikawa, A review of production and upgrading of algal bio-oil, *Renew. Sustain. Energy Rev.* 58 (2016) 918–930. doi:10.1016/J.RSER.2015.12.342.
- [123] S. Xiu, A. Shahbazi, Bio-oil production and upgrading research: A review, *Renew. Sustain. Energy Rev.* 16 (2012) 4406–4414. doi:10.1016/J.RSER.2012.04.028.
- [124] P.E. Savage, P. Duan, P.E. Savage, Upgrading of crude algal bio-oil in supercritical water.pdf, *Bioresour. Technol.* 102 (2013) 1899–1906. doi:10.1016/j.biortech.2010.08.013.

- [125] A. Galadima, O. Muraza, Hydrothermal liquefaction of algae and bio-oil upgrading into liquid fuels: Role of heterogeneous catalysts, *Renew. Sustain. Energy Rev.* 81 (2018) 1037–1048. doi:10.1016/J.RSER.2017.07.034.
- [126] Z. Wang, S. Adhikari, P. Valdez, R. Shakya, Upgrading of Hydrothermal Liquefaction Biocrude from Algae Grown in Municipal Wastewater, 2015 ASABE Int. Meet. 7004 (2015). doi:10.13031/aim.20152191148.
- [127] R. Shakya, S. Adhikari, R. Mahadevan, E.B. Hassan, T.A. Dempster, Catalytic upgrading of bio-oil produced from hydrothermal liquefaction of *Nannochloropsis* sp., *Bioresour. Technol.* 252 (2018) 28–36. doi:10.1016/J.BIORTECH.2017.12.067.
- [128] P. Duan, P.E. Savage, Catalytic treatment of crude algal bio-oil in supercritical water: Optimization studies, *Energy Environ. Sci.* 4 (2011) 1447–1456. doi:10.1039/c0ee00343c.
- [129] P. Duan, Y. Xu, F. Wang, B. Wang, W. Yan, Catalytic upgrading of pretreated algal bio-oil over zeolite catalysts in supercritical water, *Biochem. Eng. J.* 116 (2016) 105–112. doi:10.1016/J.BEJ.2015.12.006.
- [130] X. Bai, P. Duan, Y. Xu, A. Zhang, P.E. Savage, Hydrothermal catalytic processing of pretreated algal oil: A catalyst screening study, *Fuel.* 120 (2014) 141–149. doi:10.1016/J.FUEL.2013.12.012.
- [131] D. López Barreiro, B.R. Gómez, F. Ronsse, U. Hornung, A. Kruse, W. Prins, Heterogeneous catalytic upgrading of biocrude oil produced by hydrothermal liquefaction of microalgae: State of the art and own experiments, *Fuel Process. Technol.* 148 (2016) 117–127. doi:10.1016/J.FUPROC.2016.02.034.

CHAPTER 3

Compare the Product Yield and Properties Obtained from Hydrothermal Liquefaction of Filamentous Algae and Microalgae

Abstract

This study presents the comparison on product yield and properties obtained from hydrothermal liquefaction (HTL) of microalgae of species *Nannochloropsis* sp. (A_Micro), and filamentous algae grown on five different nitrogen nutrient conditions namely: sufficient nitrate (A_NO₃), sufficient urea (A_Urea), nitrate starved algae for 14 days and 21 days (14_NO₃ and 21_NO₃), and urea starved algae for 14 days (14_Urea). HTL was carried out at 320°C, with reaction time of 30 mins, algae loading of 15%, and with nitrogen as inert atmosphere. Highest oil yield of 64.2 wt% was observed for 14_Urea, while 21_NO₃ produced highest char yield of 32.7 wt%. The oil yield for A_Urea, A_NO₃, and A_Micro were 44, 34.3, and 49.9 wt%, respectively. Highest heating value (HHV) of HTL oil from all algae were around 30.5 to 34.5 MJ/kg. Total acid number (TAN) was lowest for A_Micro (31.36 mg KOH/g) while for other non-stressed algae it was around 40 mg KOH/g. TAN for stressed algae oil was slightly above 100 mg KOH/g which was because of the presence of around 50% of hexadecanoic acid as observed in GC-MS of oil. 77-89% of the bio-oil fraction were in boiling point range of vacuum gas oil. Char obtained had high energy content of around 30 MJ/kg. Aqueous phase was rich in nutrients with aqueous phase from A_NO₃ having about 50% of its nitrogen in the form of ammonium ions.

3.1 Introduction

Transportation fuels are obtained from fossil fuel which are dwindling reserves of declining quality [1]. There is a need to find an alternative source of energy that can replace or try substituting the existing liquid fuel demand. Biomass is the only abundant source of renewable energy that has the capability of producing liquid hydrocarbon fuels [2]. Among various biomass, algae a wet feedstock have gained considerable attention because of its high productivity, flexibility in growing conditions, and high carbon sequestration rate relative to terrestrial biomass [3]. Transesterification is the conventional process employed to produce bio-diesel, but the drawback of the process is that, it can utilize only the lipid fraction for conversion into bio-diesel [4]. Hydrothermal liquefaction has advantages over transesterification because it can process wet feedstock and utilizes the whole algae (lipids, proteins, and carbohydrates) for conversion into bio-oil. Hydrothermal liquefaction (HTL) operates at temperature of 280-380°C and pressure of 7-30 MPa, sufficient to keep water in liquid state, that acts both as reaction medium and catalyses the valorisation of biomass by producing H⁺ and OH⁻ ions [5,6]. Crude bio-oil is the primary product of HTL and the by-products are solid char, water soluble organic compounds, and gases.

To date, numerous study on hydrothermal liquefaction of algae on product yield and properties has been carried out to investigate the effect of different biochemical composition of algae feedstock [7,8], temperature [9,10], biomass loading [11,12], residence time [13,14], and effect of homogenous [7,15] and heterogenous catalyst [16,17]. Barreiro *et al.* [18] studied HTL of 8 different alga strains grown in non-axenic photobioreactor at different environment (marine and freshwater) and found at mild conditions of 250°C, the bio-oil yield varied from 17.6 wt% to 44.8 wt% because of the influence of cell structure (cell wall) and biochemical composition. At 375°C, the variation in bio-oil yield from different algae was less (45.6 to 58.1 wt%) and was a

result of harsh hydrothermal environment. Shakya *et al.* [8] predicted a model on bio-oil yield based on linear regression of data obtained from HTL of 9 different microalgae at 320°C and found 96% of lipids, 30% of carbohydrates, and 43% of protein contribute towards bio-oil yield. Thus, high bio-oil yield can be obtained with algae having high lipid content and the lipid accumulation can be enhanced by depriving the algae of nutrients. High lipid content in algae can be accumulated by nitrogen nutrient starvation [19,20] but in some cases this deprivation can lead to increase in carbohydrates, as seen in the study published by Chen *et al.* [21]. Nitrogen is an important nutrient for algae growth and can be supplied in the form of NH_4^+ , NO_2^- , NO_3^- , urea. Arumugam *et al.* [22] investigated the effect of different nitrogen sources on productivity of *Scenedesmus bijugatus* at lower concentration of 5mM and 10mM. At 10 mM nitrogen concentration, potassium nitrate showed best biomass growth then came sodium nitrate. Urea, an affordable agriculture fertilizer performed at par with the sodium nitrate, with growth around 0.4 to 0.5 and slightly below 0.5 for sodium nitrate as measured with OD 540 nm.

Harvesting of microalgae (cell dimension < 30 μm) can contribute towards 20-30% of cultivation cost and can be minimized by the use of algae having filament like structure whose dimension can range from 200 μm to 60m [23]. Harvesting of filamentous algae can be achieved by simple filtration, sedimentation, or flotation [24–26]. In addition, mass culture of microalgae is susceptible to crash as it is more palatable to grazers that proliferate during cultivation as compared to filamentous algae [27,28]. Combining the benefits of harvesting of filamentous algae grown at cheap nitrogen nutrient source, major hurdle in economic viability of biofuel from hydrothermal liquefaction can be tackled.

Hence, the main objective of this study is to compare the performance of hydrothermal liquefaction of microalgae and filamentous algae at subcritical temperature of 320°C on bio-oil

yields and its properties. Additionally, characteristic of solid char and aqueous phase will be investigated.

3.2 Materials and Methodology

Nannochloropsis sp. (A_Micro) microalgae was obtained from Reed Mariculture Inc. (Campbell, CA) and filamentous algae was obtained from Arizona Centre for Algae Technology and Innovation (AzCATI), Mesa, Arizona. The filamentous algae were grown at different nitrogen nutrient conditions namely: sufficient nitrate (NO_3), sufficient urea (Urea), 14 days and 21 days starved nitrate (14_ NO_3 and 21_ NO_3), and 14 days starved urea (14_Urea). Nitrate and urea filamentous algae were grown in 1000 litres open pond and fed with 0.764 g/L of sodium nitrate (NaNO_3) and 0.191 g/L of urea during inoculation, respectively and was harvested at the end of 15 days. To stress the algae, the broth was inoculated in vertical flat panels having the dimension 4' * 8' * 4" and fed with same concentration of nutrients as fed in open pond. The starvation period was counted from the day when nitrate went deplete and same day was taken to be the date when urea went deplete. Table 3.1 in section 3.3.1, lists the biochemical composition of algae that was obtained from the supplier. In case of microalgae biochemical composition comprised of lipids, proteins, and carbohydrates and for filamentous algae FAME, proteins, and carbohydrates was supplied as biochemical composition. All the algae samples were obtained in the form of slurry except 14_Urea, which was obtained as freeze-dried sample. The moisture content for all the samples was measured by calculating the weight loss of the samples by heating at 105°C for 24 h. Ash content was determined according to ASTM E1755 standard. Heating value of algae was determined by oxygen bomb calorimeter (IKA model C2000) and elemental composition of algae samples was determined using vario MICRO cube from Elementar. Ultrapure (Type 1) water was used for all experiments and was obtained from Synergy Ultrapure Water Systems. High purity

nitrogen was obtained from Airgas Inc. Hardened ashless Whatman Filter papers (CAT NO. 1541-150) was obtained from VWR (Atlanta, GA.).

3.2.1 Apparatus and Experimental Procedure

HTL experiments were performed in 1000 ml high pressure reactor from Parr Instrument Company. The reactor was equipped with controllable stirrer, and PID controlled heating furnace, pressure gauge to measure pressure inside the reactor, J type thermocouple to measure temperature. HTL experiments were performed at 320°C, residence time of 30 mins and biomass loading of 15%. The reactor was purged with nitrogen to remove any air trapped inside the reactor and was pressurized to initial pressure of 50 PSI. The reactor was then heated to 320°C at which the maximum pressure reached was around 2020 PSI and held for 30 mins. Heating was suspended, and ice water was used to bring the temperature of the reactor back to 45 to 50°C and gas was released and not analysed in this study.

3.2.2 Product Separation

The content of the reactor was poured in a beaker which consisted of crude bio-oil, solids char, and aqueous phase. The reactor and stirrer were rinsed with dichloromethane (DCM) and poured in the beaker. Figure 3.1 shows the process flow diagram of the process. The content of the beaker shown in was vacuum filtered using Whatman No. 541 filter paper. The char was recovered and dried in an oven at 105°C for 24 hours to obtain the solids yield. The remaining liquid mixture containing aqueous phase and organic phase composed of bio-oil and dichloromethane was separated in a separatory funnel and collected in different containers. The dichloromethane in the bio-oil was separated using IKA rotary evaporator maintained at 50°C and 720mbar.

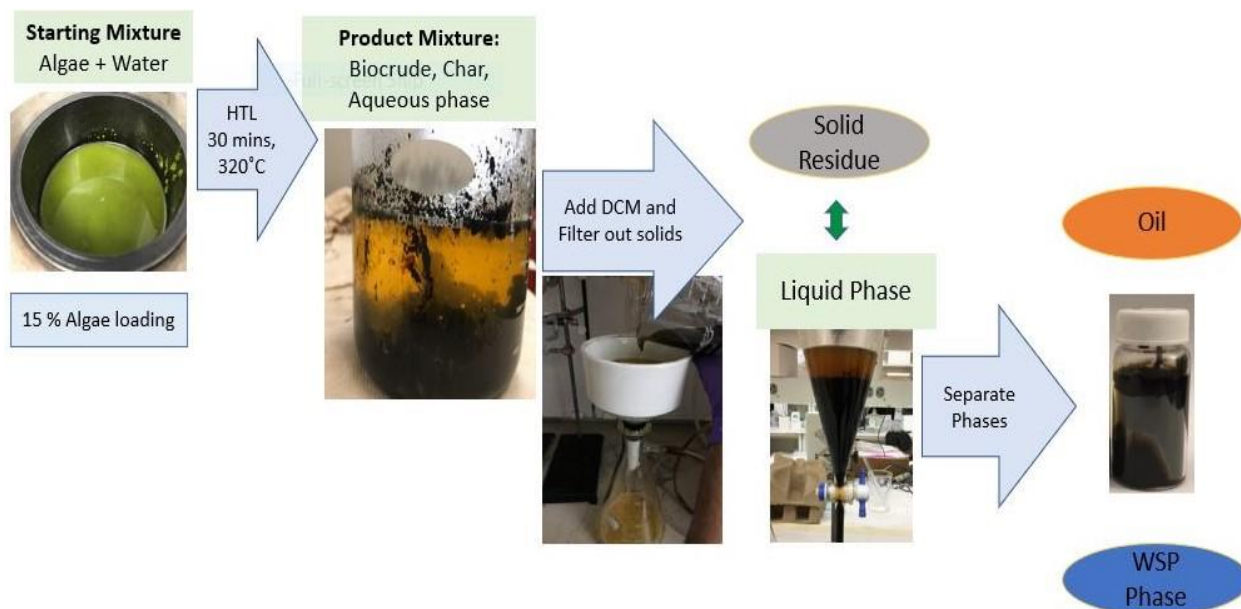


Figure 3.1. Process flow diagram of product separation

A mass balance for each experiment was performed to calculate the yield of each fraction in the product from HTL.

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

$$\text{Solid Char yield (wt. \%)} = \frac{\text{Mass of solid char in dry basis}}{\text{Mass of dry algae}} \times 100$$

$$(\text{Water soluble product} + \text{Gas}) \text{ wt. \%} = 100 - (\text{Bio - oil} + \text{Solid char}) \text{ yield wt. \%}$$

3.2.3 Product Analysis

Physical and chemical properties of the bio-oil produced from HTL of different algae was determined. Properties of the bio-oil included higher heating value (HHV MJ/kg), total acid number (TAN mg KOH/g), moisture content, and ash content. Ash content was measured using ASTM E1755 standard method. Moisture content was determined using Karl Fisher (KF) analysis using V20 volumetric KF titrator from Mettler Toledo. Total acid number was determined by

ASTM D664 using 0.3-0.5 g crude bio-oil in T50 titrator from Mettler Toledo and R2 data was reported. Heating value was measured as HHV in MJ/kg and was determined in an IKA C2000 model oxygen bomb calorimeter. For chemical composition, the bio-oil was analysed with an Agilent 7890 GC/5975MS using a DB-1701 column. A typical gas chromatography (GC) sample consisted of 2-5 wt% crude bio-oil in dichloromethane. This diluted sample was injected into the column and each sample was injected twice. The initial temperature of the column, 40°C was maintained for 6 mins, then heated to 280°C at 6°C/min and held for 10 min. Helium (99.999%) was used as a carrier gas with flow rate set at 1.76 ml/min. Compounds were identified using NIST (National Institute of Standards and Technology) mass spectral library.

We ran each oil twice and the average of single oil was averaged with its duplicate run. The compounds obtained were divided into alkane, alkene, aromatics, only nitrogen containing compounds (N Compounds), only oxygen containing compounds (O Compounds), and compounds containing both nitrogen and oxygen (N-O Compounds). N compounds were subdivided into pyrrole, pyridine, pyrazine, indole, amine, nitrile, and other N compounds. O compounds were subdivided into ketone, alcohol, aldehyde, organic acids, phenolics, ester, ether, and other O compounds. N-O compounds were subdivided into amides, other N-O compounds, and 1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide (C₁₃H₁₆N₂O₂S). These subdivisions were based on the parent compound and its derivatives that includes alkyl or other group substituents, saturated or unsaturated compounds.

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 following ASTM D2887 on an Agilent 7890A GC, equipped with split/splitless inlet, Agilent DB-2887 column (10 m × 0.53 mm, 3.00 μm), and FID detector. Bio-oil was diluted with carbon disulfide to 1-2 wt%. The inlet was set at 350 °C with a split ratio of 1:2. The heating program started from 40 °C and then ramped to 350 °C with a heating rate of 20 °C/min under 14 mL/min of helium carrier gas. FID detector was set at 350 °C. The instrument was calibrated by calibration standard mixtures purchased from Ultra Scientific (ASTM-120-1) and Restek (31222 D2887 Calibration Mix). Calibration mix D2887 containing 17 alkanes ranging from C6 to C44 was injected twice and standard curve was developed for time at which compounds were detected vs the boiling point of these compounds. Baseline area was obtained by injecting only carbon disulfide before running each sample and was deducted from sample area. The time obtained from the sample injection were translated into temperature using the ASTM D2887 method calculations. The boiling points were categorized into five different sections namely: <193°C (Gasoline and Heavy Naptha), 193-271°C (Kerosene), 271-343°C (Diesel), 343-538°C (Vacuum Gas Oil), and >538°C (Vacuum Residue).

The aqueous phase characterization consisted of total organic carbon (TOC), total nitrogen (TN), chemical oxygen demand (COD), phosphate (PO_4^{3-}), magnesium (Mg), and ammonium (NH_4^+). The aqueous phase was filtered with 0.2 μm filter paper and centrifuged for 5 mins at 10000 rpm. The TOC and TN were analysed in a TOC-L analyzer attached with TNM-L unit (Shimadzu Corp., Japan). Samples were diluted 100 times in ultra-high purity water and kept for measurement in autosampler. COD was determined according to 5220 Chemical Oxygen Demand standard [29]. YSI reagent kit was used to determine the concentration of ammonium ion (YPM152), phosphate ion (YPM177), and magnesium ion (YPM193) in aqueous phase.

The char from the HTL of algae was measured for its HHV, ash content and elemental composition by using the methods as described earlier. 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°C for 10 h.

3.3 Results and Discussion

3.3.1 Algae Characterization

Table 3.1 shows the biochemical composition of all the algae samples. Highest FAME was observed for 14_Urea which is the algae obtained by starving the filamentous algae grown on urea for 14 days. When the nitrate grown filamentous algae was starved of nitrate for 14 days and 21 days, the productivity of carbohydrates increased to more than twice. The FAME which is the oil content of the algae increased by six times under urea starvation while for nitrate starvation, FAME increased by a factor of only two. d. A given microalga will accumulate either starch or neutral lipids (FAME) which depend not only on the species but also on environmental conditions at which they are grown. *Chlorella vulgaris* P12 responded to limitation in urea by accumulating starch [30] while *Chlorella minutissima* responded with lipid production when it was starved with sodium nitrate as the nitrogen source [31]. This explains how same genus *Chlorella* could accumulate different storage compound when subjected to nitrogen starvation from different sources. *Nannochloropsis* sp. microalgae (A_Micro) had highest protein content of 62.79 wt%. Lipids in algae are in the form of phospholipids (10-20 % of total lipids), glycolipids and non-polar glycerolipids (mono, di and tri saturated and unsaturated) that are neutral and which appear in FAME. Other lipids may be present which may be characteristic of particular genus or species.

Table 3.1 Biochemical composition of algae in wt% dry basis

Algae	FAME	Lipids	Protein	Carbohydrates
NO₃	8.75 ± 0.12	Na.	28.35 ± 0.27	29.99 ± 1.98
Urea	8.79 ± 0.33	Na.	31.29 ± 1.45	36.82 ± 0.74
14_NO₃	16.89 ± 0.43	Na.	14.87 ± 1.22	59.47 ± 3.71
14_Urea	53.22	Na.	13.94	22.95
21_NO₃	16.39 ± 0.09	Na.	13.43 ± 0.14	57.14 ± 5.22
A_Micro	Na.	18.12	62.79	8.92

Na. represents data that were not reported by the supplier.

Table 3.2 shows heating value, ash content and moisture content of the algae and Table 3.3 shows the ultimate analysis of the algae feedstock. High lipid containing 14_Urea algae gave highest C and H wt% which translated into highest HHV of 25.8 MJ/kg. A_Micro also had HHV of 24 MJ/kg comparable to 14_Urea as it had low oxygen content. Highest protein containing A_Micro gave highest nitrogen content of 7.6 wt%. C and H wt% in other filamentous algae were not significantly different. Stressed filamentous algae 14_NO₃ and 21_NO₃ had low nitrogen content and high oxygen content owing to low protein and high carbohydrates, respectively. The increase in ash content from sufficient nitrate to 14_NO₃, and to 21_NO₃ reflects the loss in biomass because of nitrogen starvation.

Table 3.2 Properties of algae feedstock

Algae	HHV (MJ/kg)	Ash (wt%)	Water Content (wt%)
A_NO₃	20.4 ^a ± 0.11	8.23 ^a ± 0.25	78.82 ± 0.25
A_Urea	21.5 ^a ± 0.12	5.43 ^b ± 0.04	83.44 ± 0.06
14_NO₃	20.9 ^a ± 0.10	4.05 ^c ± 0.08	85.54 ± 0.12
14_Urea	25.8 ^b ± 0.13	4.21 ^c ± 0.06	02.14 ± 0.35
21_NO₃	20.4 ^a ± 0.03	5.81 ^c ± 0.20	85.39 ± 0.29
A_Micro	24.0 ^c ± 0.43	3.42 ^c ± 0.20	72.20 ± 0.08

Different alphabets in the superscript of each column denote that the values are significantly different for each algae type. Values after ± denote standard deviation

Table 3.3 Ultimate analysis of algae biomass. O* obtained by difference

Algae	C (wt. %)	H (wt. %)	N (wt. %)	S (wt. %)	O* (wt. %)
NO3	46.2 ^a ± 1.27	7.2 ^a ± 0.14	5.6 ^a ± 0.34	0.9 ^a ± 0.30	40.1 ^a ± 2.05
Urea	46.8 ^a ± 0.34	7.1 ^a ± 0.11	5.7 ^a ± 0.13	0.7 ^a ± 0.00	39.7 ^{a,b} ± 0.57
14_NO3	45.7 ^a ± 1.42	7.4 ^a ± 0.39	1.8 ^c ± 0.29	0.3 ^a ± 0.03	42.3 ^a ± 1.17
14_Urea	54.2 ^b ± 0.69	8.5 ^a ± 0.27	3.3 ^d ± 0.31	0.3 ^a ± 0.06	33.6 ^b ± 0.48
21_NO3	45.8 ^a ± 0.15	7.5 ^a ± 0.01	1.2 ^c ± 0.05	0.4 ^a ± 0.01	45.2 ^a ± 0.20
A_Micro	53.4 ^b ± 0.36	10.3 ^b ± 0.59	7.6 ^e ± 0.07	0.6 ^a ± 0.13	28.0 ^{b,c} ± 1.00

Different alphabets in the superscript of each column denote that the values are significantly different for each algae type. Values after ± denote standard deviation

3.3.2 Liquefaction Yields

Table 3.4 shows the yield of different fractions obtained from HTL of individual algae feedstock at 320°C in dry basis. Bio-oil yields from all the algae exceeds the total lipids (FAME) fraction which confirms that some fraction of proteins and carbohydrates contribute towards oil yield because of hydrolysis, depolymerization, cracking, condensation and repolymerization during HTL. Highest bio-oil yield of 64.23 wt% was achieved with HTL of 14_Urea owing to highest FAME in the algae feedstock. Other stressed algae 14_NO₃ and 21_NO₃ produced high char yield of 31.18 and 32.49 wt%, respectively. This high yield of char can be attributed to the fact that these algae had high carbohydrate content that may have been hydrolysed to 5-HMF and because of severe conditions may have undergone polymerization and polycondensation to produce polyfuranic intermediates as proposed by Falco *et al.* [32] and by simultaneous degradation of cellulose following saccharification and carbonization, that eventually polymerized to char [33]. Among the non-stressed filamentous algae and A_Micro, the oil yield obtained from A_Micro was highest at 49.86 wt%. Shakya *et al.* [8] reported oil yield of 46 wt% for HTL of

Nannochloropsis sp. algae under the same operating conditions as used in this study. The difference in result could be transpired by the use of dichloromethane in this study as against acetone in the other study. The yield of oil from sufficient urea grown filamentous algae was 44.67 wt% but was not significantly different than A_Micro ($p = 0.0531 > 0.05$). Garcia *et al.* reported 43.8 wt% of bio-oil yield for *Desmodesmus sp.* microalgae at 300°C and 30 mins residence time which had similar biochemical composition to that of sufficient urea filamentous algae. Significant portion of protein and carbohydrate of sufficient urea and A_Micro were converted into bio-oil as compared to sufficient nitrate algae. Biller *et al.* compared HTL of different model compounds for protein (soya and albumin) and amino acids (asparagine and glutamine) and found different yields of bio-oil in each case. This explains how different kind of protein and amino acids could result in different conversion into bio-oil.

Table 3.4 Yield of different product fractions from HTL of different algae in dry basis

Algae type	Oil (wt%)	Char (wt%)	Gas + WSP (wt%)
NO ₃	32.99 ^a ± 1.02	15.77 ^a ± 0.06	51.24 ^a ± 1.04
Urea	44.67 ^b ± 2.78	13.25 ^{a,d} ± 0.74	42.08 ^b ± 2.58
14_NO ₃	29.61 ^a ± 1.29	31.18 ^b ± 0.87	39.21 ^b ± 1.61
14_Urea	64.23 ^c ± 1.71	11.55 ^{d,e} ± 0.05	24.22 ^c ± 1.78
21_NO ₃	29.26 ^a ± 0.80	32.49 ^b ± 1.78	38.24 ^b ± 1.29
A_Micro	49.86 ^b ± 1.56	09.43 ^c ± 0.38	40.70 ^b ± 1.18

Different alphabets in the superscript of each column denote that the values are significantly different for each algae type. Values after ± denote standard deviation

3.3.3 Bio-oil Characterization

Table 3.5 and 3.6 represent physical properties and ultimate analysis of bio-oil obtained from HTL of individual algae feedstock. All the bio-oil obtained were black viscous liquid that

were hydrophobic in nature. The oil obtained from nitrate starved filamentous algae were semisolid at room temperature. Moisture content in bio-oil ranged from 0.5 wt% for 14_Urea to 7 wt% for NO₃ algae. Total acidic number indicated if the oil is corrosive or not and is a product of the amount of organic acids and phenolic groups present in oil. Lowest TAN of 31.36 mg KOH/g was reported for *Nannochloropsis* sp. algae (A_Micro). Other observed values for HTL of *Nannochloropsis* are 32.68 mg KOH/g at 300°C and 32.45 mg KOH/g at 350°C by Shakya *et al.* [9] which is comparable to the value obtained in this study. For the nutrient starved filamentous algae, the TAN values were above 100 mg KOH/g. This is because of the fact that these bio-oil contained a high amount of organic acids from lipid fraction and phenolics from carbohydrate fraction of the algae [8,34]. FAME which is also referred to as acyl lipids is the amount of oil in algae. In A_Micro the lipid content signifies both acyl (FAME) and non-acyl lipids. In A_Micro the fatty acids coming from FAME might have decomposed into saturated or unsaturated hydrocarbons by decarboxylation during HTL [35].

The carbon and hydrogen content in the bio-oil ranged from 66.77 to 71.16 and 9.97 to 11.36 wt%, respectively. The H/C ratio ranged from 1.75 to 1.91 which was in the range of petroleum crude. Oxygen content was highest for 14_NO₃ which had highest carbohydrate content, similar to that of 21_NO₃. The bio-oil obtained from 14_Urea also had high oxygen content which could be partly drawn from carbohydrate part of the algae and the rest from FAME fraction which hydrolysed to fatty acid having hydroxy group and water-soluble glycerol in subcritical environment [36]. Overall, the bio-oil had high carbon and hydrogen content and lower oxygen content which resulted in bio-oil having higher heating value as compared to algae feedstock. The heating value was highest for A_Micro (33.19 MJ/kg) because it had highest H/C ratio and lowest O/C ratio, but was lower than 34.82 MJ/kg as reported by Shakya *et al.* [8]. In that study, acetone

(dielectric constant, $\epsilon = 20.7$) was used as the solvent which could not have dissolved all the organics present in aqueous phase as compared to dichloromethane ($\epsilon = 8.93$). Bio-oil from A_Micro had high nitrogen content of 5.47 wt% and there was no significant difference in nitrogen content from other non-stressed filamentous algae ($p = 0.636 > 0.05$). The peptide bond hydrolyses to form amino acids which undergo deamination and decarboxylation [37]. Deamination should have been dominant in *Nannochloropsis sp.* as there was considerable reduction in its nitrogen content producing ammonia gas and carbonic acids. All nutrient starved filamentous algae produced bio-oil with low nitrogen and sulphur content. All the bio-oil obtained needs considerable upgrading to remove nitrogen and oxygen atoms to be used as liquid fuels. Highest energy recovery was obtained for 14_Urea at 77.14% followed by Urea at 65.90%. Urea being the cheapest source of nitrogen nutrient is economical viable for mass cultivation of algae and also produced highest energy recovery of 77.14% in the case of 14_Urea followed by 65.90% for filamentous algae grown on sufficient urea.

Table 3.5 and 3.6 Properties and ultimate analysis of bio-oil. O* obtained by difference.

Algae	HHV (MJ/kg)	Moisture Content (wt. %)	TAN (mgKOH/g)	ASH (wt. %)	E.R. %
NO ₃	33.54 ^a ± 0.30	7.0 ± 4.64	41.3 ^a ± 2.14	0.60 ± 0.40	56.39
Urea	33.91 ^a ± 1.11	6.1 ± 3.86	37.5 ^{a,c} ± 2.08	0.31 ± 0.06	69.40
14_NO ₃	30.68 ^{a,c} ± 0.33	1.0 ± 0.06	104.5 ^b ± 2.27	0.14 ± 0.06	42.86
14_Urea	30.96 ^{a,c} ± 0.15	0.5 ± 0.16	111.9 ^b ± 1.37	0.17 ± 0.08	77.14
21_NO ₃	31.10 ^{a,c} ± 0.80	1.9 ± 0.32	116.1 ^b ± 6.22	0.18 ± 0.06	44.06
A_Micro	34.52 ^{a,b} ± 0.07	0.82 ± 0.13	31.4 ^c ± 0.53	0.16 ± 0.23	73.30

Different alphabets in the superscript of each column denote that the values are significantly different for each algae type. Values after ± denote standard deviation

	Ultimate analysis of bio-oil (wt.% in dry basis)					
Oil	C (wt. %)	H (wt. %)	N (wt. %)	S (wt. %)	O* (wt. %)	H/C
NO3	70.84 ^a ± 1.96	9.41 ^a ± 1.14	5.28 ^a ± 0.52	0.35 ^a ± 0.02	14.12 ^a ± 2.65	1.88
Urea	70.22 ^a ± 3.66	9.27 ^a ± 1.21	5.90 ^a ± 0.25	0.46 ^a ± 0.07	14.13 ^a ± 5.11	1.84
14_NO3	66.77 ^a ± 2.84	9.69 ^a ± 0.33	1.64 ^b ± 0.42	0.07 ^b ± 0.04	21.81 ^b ± 3.42	1.82
14_Urea	68.45 ^a ± 0.17	9.86 ^a ± 0.09	1.83 ^b ± 0.17	0.06 ^b ± 0.06	19.79 ^b ± 0.19	1.75
21_NO3	67.43 ^a ± 1.86	10.02 ^a ± 0.30	1.42 ^b ± 0.12	0.02 ^b ± 0.02	21.11 ^b ± 2.10	1.82
A_Micro	71.16 ^a ± 0.26	11.36 ± 0.58	5.47 ^a ± 0.16	0.45 ^a ± 0.04	11.54 ^a ± 0.94	1.91

Different alphabets in the superscript of each column denote that the values are significantly different for each algae type. Values after ± denote standard deviation

Table 3.7 shows the common compounds and their mean peak area %. Least common peak area of 36.65% was determined for Urea bio-oil and maximum common peak area of 75.14% was obtained for 21_NO₃ bio-oil. It is clear from the table that organic acids constituted major portion of bio-oil from nutrient deficient filamentous algae. The triglycerides present in these algae may have undergone hydrolysis into fatty acids and glycerol. The major fatty acids were n-hexadecanoic acid, 9-hexadecenoic acid, and tetradecanoic acid. 9-hexadecenoic acid was also the major constituent of bio-oil from sufficient nitrate and urea algae. Fatty acids have high thermal stability but can partly be decomposed into saturated or unsaturated hydrocarbons [35] which can be observed in bio-oil from NO₃, Urea and A_Micro. 1-Pentadecene (4.8%) and heptadecane (1.21%) in bio-oil from A_Micro bio-oil could be a result of decarboxylation of fatty acid. High protein containing A_Micro bio-oil had high amount of nitrogen compounds like pyrrole, pyrazine, and pyridine which is obtained by re-polymerization of organic compounds from protein fractions following Fisher-Tropsch type reaction pathway [38]. After hydrolysis of peptide bond in proteins, it can follow decarboxylation or deamination releasing carbon dioxide and amines, or ammonia and carbonic acids [39]. The ammonia can react with fatty acids to produce amides as observed in

all the bio-oil except for nitrate starved filamentous algae bio-oil which produced high amount of 1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide (C₁₃H₁₆N₂O₂S). Biller *et al.* [7] observed no hexadecamide and pyrrole in bio-oil from HTL of *Nannochloropsis sp.* which was the major constituent from the protein fraction in this study. Glucose and starch as model compounds for carbohydrate was also studied by Biller *et al.* where ketones and phenols were the major compounds [7]. However, in this study ketones were not observed as major constituents. The difference might be due to the presence of 13 to 16 wt% FAME in these compounds which had major contribution in bio-oil yield and carbohydrates yielded high solid char.

Table 3.7. Chemical composition data of bio-oil from GC-MS (Average n = 2 * 2)

Compounds	Area % of compounds from GC-MS					
	NO3	Urea	14_NO3	14_Urea	21_NO3	A_Micro
Alkane						
Heptadecane						1.21
Thiophene, tetrahydro-						0.93
Other Alkane	0.73	0.46				
Alkene						
Tetrachloroethylene	2.28	1.47	1.13	1.10	1.41	
C ₂₀ H ₄₀	2.80	1.18	0.27		0.15	1.38
Cholest-4-ene	1.90	1.68	0.62			
Cyclohexene						1.80
1-Pentadecene						4.60
5-Eicosene, (E)-		0.66				
Other Alkene	0.99	0.11				
Aromatics						
Naphthalene	0.57	0.50	0.07		0.06	
Toluene						1.24
Ethylbenzene						2.55
Styrene						0.63
N-Compounds						
Pyrrole	0.61	0.27			0.23	4.54
Pyridine	0.36					1.65
Pyrazine	0.43	0.07				3.08
Indole	1.29	0.94	0.18			0.99
Amine	0.71					
Nitrile	0.28	0.89		0.40		
9H-Pyrido[3,4-b]indole	0.48	0.66				0.42
Benz[c]acridine		0.54			0.34	

Other N Compounds		0.51				
O Compounds						
Ketone	1.34	0.35	0.59		0.58	
Alcohol	4.94	1.22	0.74			
Aldehyde	0.00	0.27				0.32
Organic Acids	12.03	10.83	50.90	58.62	58.16	
Phenol	0.19	0.22				3.80
Ether		0.15				
Ester	0.16					
Other O Compounds	0.50			0.21	0.73	
N-O Compounds						
Amide	12.09	10.05	1.96	10.50		13.80
C ₁₃ H ₁₆ N ₂ O ₂ S		0.45	6.98	0.92	13.27	
Other N-O Compounds	2.42	3.17	0.17	1.21	0.20	1.77
Total	47.09	36.65	63.61	72.96	75.14	44.71

Figure 3.2 shows the FTIR spectra of bio-oil from different algae. Spectral bands assignment and interpretation were based on the literature [8,40]. All bio-oil had spectral bands stretching in 3130-3560 cm⁻¹ range, which represents O-H and N-H stretching. The N-H stretching is obtained from amides and amines which can be supported by stretching in 1600-1680 cm⁻¹ bandwidth. This was most prominent in A_Micro which is high protein containing *Nannochloropsis sp.* microalgae. O-H stretching is supported by peak in 1370 cm⁻¹, which represents presence of phenolics and alcohol. Bandwidth 2800-3000 cm⁻¹ is obtained because of prominent C-H supported with CH₃ (2930 cm⁻¹), CH₂ (1460 cm⁻¹), and methyl in methoxy (2850 cm⁻¹). Fatty acids constituted major portion of bio-oil from 14_NO₃, 14_Urea, and 21_NO₃. This is supported sharp peak in 1710 cm⁻¹ region for these bio-oils, which signifies presence of C=O bonds. Besides bending in 700-950 cm⁻¹ can be attributed to bending from C-H in aromatics and its derivative compounds like phenol.

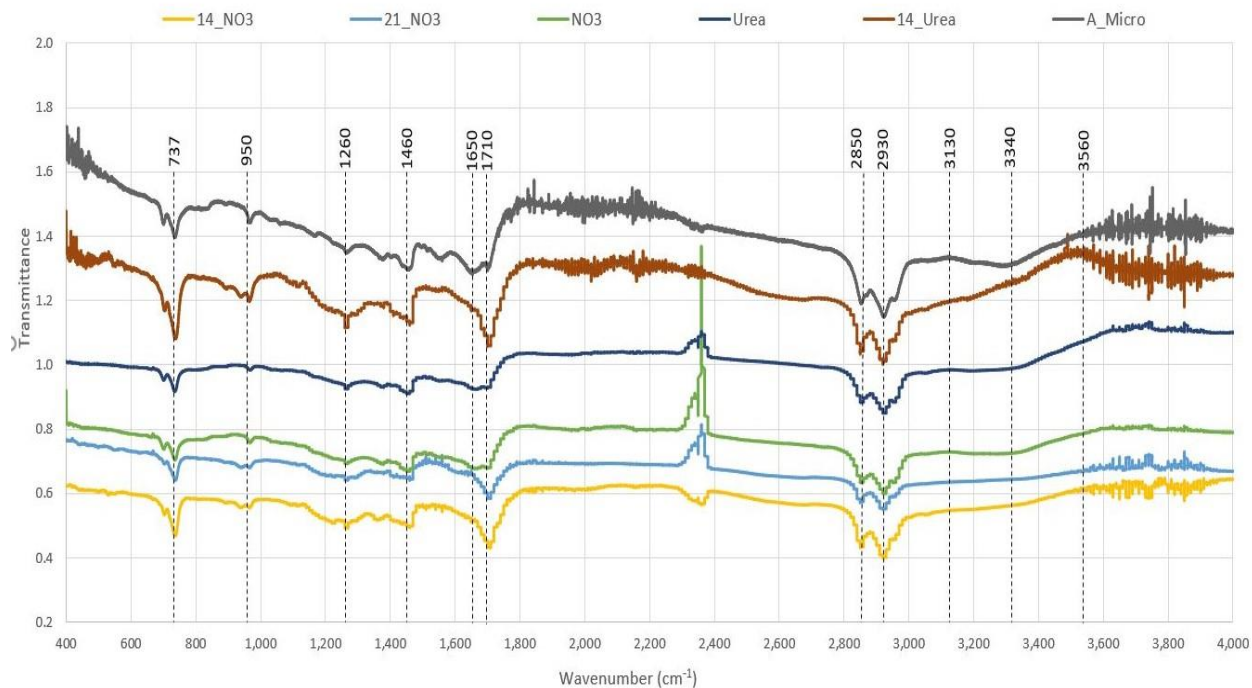


Figure 3.2. FTIR spectra of all the bio-oils

Figure 3.3 shows the boiling point distribution obtained from simulative distillation. Boiling point ranges were grouped [41]. Stressed algae oil had higher fraction of crude bio-oil in vacuum gas oil as compared to non-stressed algae oil. Of the peaks identified 75-80% of the stressed bio-oil was palmitic acid having a boiling point of 352°C [42]. Bio-oil from A_Micro had highest lighter fractions (<343 °C) at 19.2% while other non-stressed had around 16% as light fractions. Shakya *et al.* obtained about 30% of bio-oil fraction in vacuum residue for N-4 which is the same *Nannochloropsis* sp. algae used in this study [8]. Overall, the light fraction in bio-oil is very low and needs further upgrading to be used as transportation fuel and needs further upgrading.

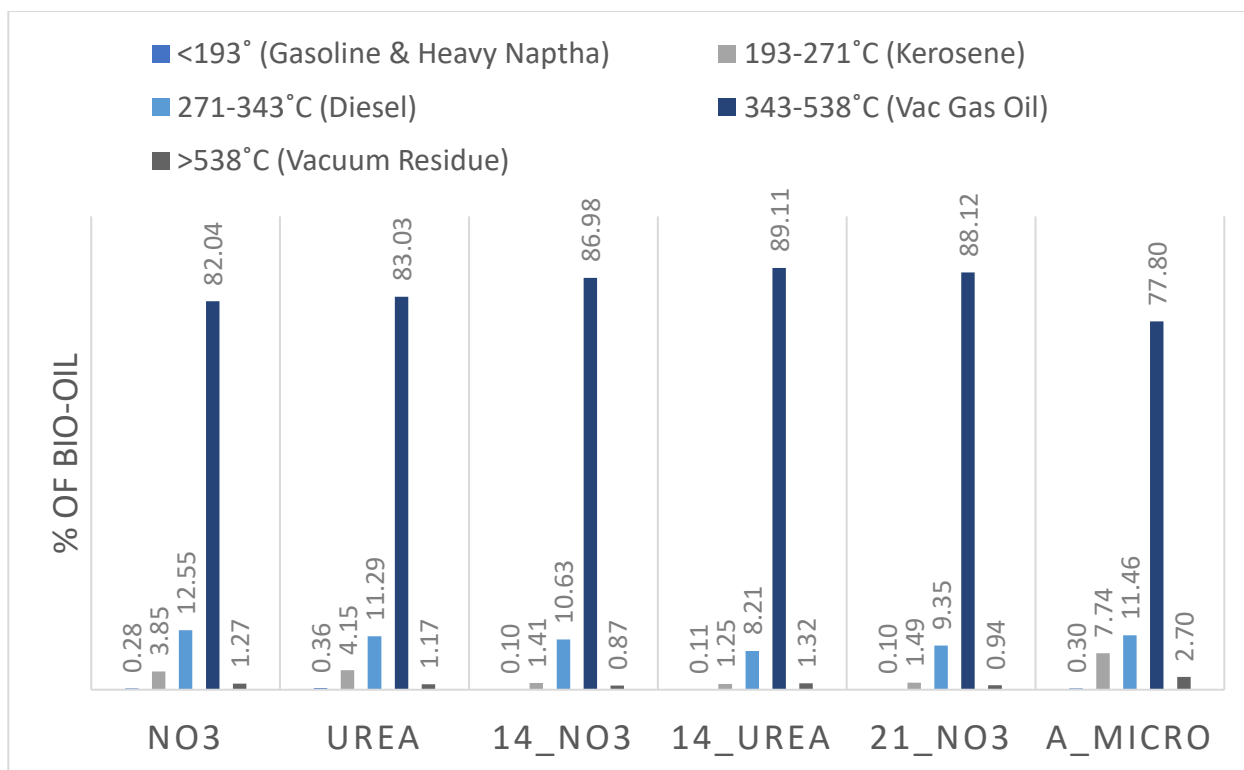


Figure 3.3. Simulative distillation of all the bio-oils ASTM D2887

3.3.4 Char Characterization

The char obtained in the HTL of all the algae were characterized for HHV, ash content, and ultimate analysis which is listed in Table 3.9. High C and H content in the char resulted in high HHV of around 30 MJ/kg comparable to the heating value of the crude bio-oil obtained. Heating value of these obtained bio-char was similar to heating value of Anthracite coal and can be used as a solid fuel [43]. Heating value coupled with the yield for 14_NO₃ and 21_NO₃ had higher energy recovery at around 46% in the form of char as compared to bio-crude oil (44%). The surface area of char obtained from NO₃ was 28.25 m²g⁻¹ and that for urea was 12.56 m²g⁻¹. These bio-char had high oxygen content and can be used to reduce contaminants from soil and water with

the help of oxygenates present in the form of carboxyl, phenolic, and hydroxyl that act as excellent adsorption sites [44].

Table 3.9. Properties and ultimate analysis in dry basis of bio-char. O* calculated by difference

Char	HHV (MJ/kg)	ASH (wt%)	C (wt%)	H (wt%)	N (wt%)	S (wt%)	O* wt%
NO ₃	29.66 ± 0.49	13.6 ± 0.27	68.05	5.80	5.59	0.48	20.08
Urea	29.27 ± 0.60	15.6 ± 1.04	66.06	5.61	5.82	0.41	22.10
14_NO ₃	30.88 ± 0.19	06.2 ± 2.61	69.64	5.17	3.47	0.07	21.66
14_Urea	29.13 ± 0.28	17.8 ± 4.29	64.71	3.90	4.60	0.09	26.71
21_NO ₃	30.35 ± 0.05	13.2 ± 2.34	70.61	5.18	2.72	0.08	21.41

3.3.5 Aqueous Phase Characterization

Table 3.10 illustrates the aqueous phase characterization containing total organic carbon (TOC), total nitrogen (TN), chemical oxygen demand (COD), phosphate ion (PO₄⁻), magnesium (Mg) and ammonium ion (NH₄⁺). High protein containing microalgae produced water phase with highest TOC, TN and COD values. Among filamentous algae urea had highest protein content and produced similar levels of TOC, TN and COD values as did microalgae. Phosphate, ammonia and potassium pose major economic constraints in algal cultivation and were significantly high for aqueous phase from nitrate and urea algae [46]. Significant amount of total nitrogen in the aqueous phase was in the form of ammonia. Rest of the nitrogen can be nitrate and nitrite, and organic compounds like pyrroles, indoles, and phenols. Chen *et al.* [21] studied the effect of nutrients on the growth of *Dunaliella tertiolecta* and found both nitrate and ammonia source to have similar efficiency in algal growth. About 75-200 times dilution of aqueous phase would be necessary for effectively growing algae from it. In order to reduce water consumption some nutrients can be extracted prior to dilution. Shanmugam *et al.* [47] presented a technique of extracting ammonium,

phosphate, and magnesium in the form of struvite and achieved around 99% and 44-100% of phosphate and ammonia recovery, respectively.

Table 3.10. Aqueous phase characterization

Aqueous	TOC (g/L)	TN (g/L)	COD (g/L)	PO₄⁻ (g/L)	Mg (g/L)	NH₄⁺ (g/L)
NO₃	21.95 ± 0.73	07.95 ± 0.19	55.2 ± 8.00	13.78 ± 2.14	5.50 ± 0.50	5.10 ± 0.90
Urea	32.43 ± 0.51	12.08 ± 0.30	65.7 ± 30.5	08.57 ± 0.90	5.05 ± 0.45	6.60 ± 0.50
14_NO₃	21.96 ± 2.56	01.82 ± 0.31	39.7 ± 2.50	06.77 ± 0.04	1.85 ± 0.15	0.81 ± 0.12
14_Urea	12.88 ± 0.17	03.25 ± 0.17	07.7 ± 5.00	05.32 ± 0.36	1.90 ± 0.30	1.51 ± 0.09
21_NO₃	14.32 ± 0.13	01.05 ± 0.11	27.2 ± 0.50	05.93 ± 0.11	1.65 ± 0.35	0.44 ± 0.00
A_Micro	31.92 ± 1.15	12.37 ± 0.05	59.5 ± 8.30	07.29 ± 1.49	1.20 ± 0.10	5.80 ± 0.20

Numbers after ± represent standard deviation

3.4 Conclusions

Hydrothermal liquefaction of filamentous algae grown on five different nitrogen nutrients was studied alongside microalgae *Nannochloropsis sp.* for product yield and properties. Highest bio-oil yield (64.24 wt%) was obtained for 14_Urea having highest FAME (53.22 wt%). Sufficient urea and nitrate grown filamentous algae having similar FAME content produced significantly different bio-oil yields of 43.98 and 34.31 wt%, respectively. Nitrate starved filamentous algae having high carbohydrate content produced highest char yield of around 30 wt%. The HHV of bio-oil ranged from 30.5 to 34.5 MJ/kg. TAN for bio-oil from nutrient starved algae were high above 105 mg KOH/g. Considering the savings in harvesting of filamentous type algae when grown on cheap urea the cost of biocrude production of algae can be brought down. Aqueous phase was rich in nutrients that could possibly be used for nutrient recycling. Char had high energy content of around 30 MJ/kg comparable to that of anthracite coal.

3.5 References

- [1] U.S. Refinery Crude Oil Input Qualities, (2018). https://www.eia.gov/dnav/pet/pet_pnp_crq_dcu_nus_m.htm (accessed June 28, 2018).
- [2] M. Crocker, R. Andrews, The Rationale for Biofuels, *Thermochem. Convers. Biomass to Liq. Fuels Chem.* (2010) 1–25. doi:10.1039/9781849732260-00001.
- [3] C. Yusuf, Biodiesel from microalgae, *Biotechnol. Adv.* 25 (2007) 294–306. doi:10.1016/j.biotechadv.2007.02.001.
- [4] A.K. Agarwal, Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines, *Prog. Energy Combust. Sci.* 33 (2007) 233–271. doi:10.1016/J.PECS.2006.08.003.
- [5] S.S. Toor, L. Rosendahl, A. Rudolf, Hydrothermal liquefaction of biomass: A review of subcritical water technologies, *Energy.* 36 (2011) 2328–2342. doi:10.1016/j.energy.2011.03.013.
- [6] S.E. Hunter, P.E. Savage, Recent advances in acid- and base-catalyzed organic synthesis in high-temperature liquid water, *Chem. Eng. Sci.* 59 (2004) 4903–4909. doi:10.1016/J.CES.2004.09.009.
- [7] P. Biller, A.B. Ross, Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content, *Bioresour. Technol.* 102 (2011) 215–225. doi:10.1016/J.BIORTECH.2010.06.028.
- [8] R. Shakya, S. Adhikari, R. Mahadevan, S.R. Shanmugam, H. Nam, E.B. Hassan, T.A. Dempster, Influence of biochemical composition during hydrothermal liquefaction of algae on product yields and fuel properties, *Bioresour. Technol.* 243 (2017) 1112–1120. doi:10.1016/j.biortech.2017.07.046.
- [9] R. Shakya, *Hydrothermal Liquefaction of Algae for Bio-oil Production*, (2014).
- [10] K. Anastasakis, A.B. Ross, Hydrothermal liquefaction of the brown macro-alga *Laminaria Saccharina*: Effect of reaction conditions on product distribution and composition, *Bioresour. Technol.* 102 (2011) 4876–4883. doi:10.1016/J.BIORTECH.2011.01.031.
- [11] U. Jena, K.C. Das, J.R. Kastner, Effect of operating conditions of thermochemical liquefaction on biocrude production from *Spirulina platensis*, *Bioresour. Technol.* 102 (2011) 6221–6229. doi:10.1016/J.BIORTECH.2011.02.057.
- [12] P.J. Valdez, J.G. Dickinson, P.E. Savage, Characterization of Product Fractions from Hydrothermal Liquefaction of *Nannochloropsis* sp. and the Influence of Solvents, *Energy & Fuels.* 25 (2011) 3235–3243. doi:10.1021/ef2004046.
- [13] Z. Shuping, W. Yulong, Y. Mingde, I. Kaleem, L. Chun, J. Tong, Production and

- characterization of bio-oil from hydrothermal liquefaction of microalgae *Dunaliella tertiolecta* cake, *Energy*. 35 (2010) 5406–5411. doi:10.1016/J.ENERGY.2010.07.013.
- [14] J.L. Faeth, P.J. Valdez, P.E. Savage, Fast Hydrothermal Liquefaction of *Nannochloropsis* sp. To Produce Biocrude, *Energy & Fuels*. 27 (2013) 1391–1398. doi:10.1021/ef301925d.
- [15] A.B. Ross, P. Biller, M.L. Kubacki, H. Li, A. Lea-Langton, J.M. Jones, Hydrothermal processing of microalgae using alkali and organic acids, *Fuel*. 89 (2010) 2234–2243. doi:10.1016/J.FUEL.2010.01.025.
- [16] P. Duan, P.E. Savage, Hydrothermal Liquefaction of a Microalga with Heterogeneous Catalysts, *Ind. Eng. Chem. Res.* 50 (2011) 52–61. doi:10.1021/ie100758s.
- [17] J. Zhang, W.-T. Chen, P. Zhang, Z. Luo, Y. Zhang, Hydrothermal liquefaction of *Chlorella pyrenoidosa* in sub- and supercritical ethanol with heterogeneous catalysts, *Bioresour. Technol.* 133 (2013) 389–397. doi:10.1016/J.BIORTECH.2013.01.076.
- [18] D. López Barreiro, C. Zamalloa, N. Boon, W. Vyverman, F. Ronsse, W. Brilman, W. Prins, Influence of strain-specific parameters on hydrothermal liquefaction of microalgae, *Bioresour. Technol.* 146 (2013) 463–471. doi:10.1016/J.BIORTECH.2013.07.123.
- [19] A.. Illman, A.. Scragg, S.. Shales, Increase in *Chlorella* strains calorific values when grown in low nitrogen medium, *Enzyme Microb. Technol.* 27 (2000) 631–635. doi:10.1016/S0141-0229(00)00266-0.
- [20] C.-H. Hsieh, W.-T. Wu, Cultivation of microalgae for oil production with a cultivation strategy of urea limitation, *Bioresour. Technol.* 100 (2009) 3921–3926. doi:10.1016/J.BIORTECH.2009.03.019.
- [21] M. Chen, H. Tang, H. Ma, T.C. Holland, K.Y.S. Ng, S.O. Salley, Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*, *Bioresour. Technol.* 102 (2011) 1649–1655. doi:10.1016/J.BIORTECH.2010.09.062.
- [22] M. Arumugam, A. Agarwal, M.C. Arya, Z. Ahmed, Influence of nitrogen sources on biomass productivity of microalgae *Scenedesmus bijugatus*, *Bioresour. Technol.* 131 (2013) 246–249. doi:10.1016/J.BIORTECH.2012.12.159.
- [23] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: A review, *Renew. Sustain. Energy Rev.* 14 (2010) 217–232. doi:10.1016/J.RSER.2009.07.020.
- [24] G. Markou, D. Georgakakis, Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review, *Appl. Energy*. 88 (2011) 3389–3401. doi:10.1016/j.apenergy.2010.12.042.
- [25] J. de la Noüe, A. Bassères, Biotreatment of anaerobically digested swine manure with microalgae, *Biol. Wastes*. 29 (1989) 17–31. doi:10.1016/0269-7483(89)90100-6.

- [26] Y. Pouliot, G. Buelna, C. Racine, J. de la Noüe, Culture of cyanobacteria for tertiary wastewater treatment and biomass production, *Biol. Wastes.* 29 (1989) 81–91. doi:10.1016/0269-7483(89)90089-X.
- [27] H. Wang, L. Gao, L. Chen, F. Guo, T. Liu, Integration process of biodiesel production from filamentous oleaginous microalgae *Tribonema minus*, *Bioresour. Technol.* 142 (2013) 39–44. doi:10.1016/J.BIORTECH.2013.05.058.
- [28] H. Wang, W. Zhang, L. Chen, J. Wang, T. Liu, The contamination and control of biological pollutants in mass cultivation of microalgae, *Bioresour. Technol.* 128 (2013) 745–750. doi:10.1016/J.BIORTECH.2012.10.158.
- [29] Standard Methods Committee, 5220 CHEMICAL OXYGEN DEMAND (COD) * 5220 B . Open Reflux Method, *Compute.* (1997) 14–19.
- [30] G. Dragone, B.D. Fernandes, A.P. Abreu, A.A. Vicente, J.A. Teixeira, Nutrient limitation as a strategy for increasing starch accumulation in microalgae, *Appl. Energy.* 88 (2011) 3331–3335. doi:10.1016/j.apenergy.2011.03.012.
- [31] T. Haiying, C. Meng, G. M.E.D., A. Nadia, N.K.Y. Simon, S.S. O., Culture of microalgae *Chlorella minutissima* for biodiesel feedstock production, *Biotechnol. Bioeng.* 108 (2011) 2280–2287. doi:10.1002/bit.23160.
- [32] C. Falco, N. Baccile, M.M. Titirici, Morphological and structural differences between glucose, cellulose and lignocellulosic biomass derived hydrothermal carbons, *Green Chem.* 13 (2011) 3273–3281. doi:10.1039/c1gc15742f.
- [33] T. Sakaki, M. Shibata, T. Miki, H. Hirosue, N. Hayashi, Decomposition of cellulose in near-critical water and fermentability of the products, *Energy and Fuels.* 10 (1996) 684–688. doi:10.1021/ef950160+.
- [34] Z. Wang, S. Adhikari, P. Valdez, R. Shakya, Upgrading of Hydrothermal Liquefaction Biocrude from Algae Grown in Municipal Wastewater, 2015 ASABE Int. Meet. 7004 (2015). doi:10.13031/aim.20152191148.
- [35] C. Gai, Y. Zhang, W.-T. Chen, P. Zhang, Y. Dong, An investigation of reaction pathways of hydrothermal liquefaction using *Chlorella pyrenoidosa* and *Spirulina platensis*, *Energy Convers. Manag.* 96 (2015) 330–339. doi:10.1016/J.ENCONMAN.2015.02.056.
- [36] B.H. L., B.A. C., Continuous fat splitting plants using the colgate-emery process, *J. Am. Oil Chem. Soc.* 25 (1948) 95–99. doi:10.1007/BF02579733.
- [37] D. Klingler, J. Berg, H. Vogel, Hydrothermal reactions of alanine and glycine in sub- and supercritical water, *J. Supercrit. Fluids.* 43 (2007) 112–119. doi:10.1016/J.SUPFLU.2007.04.008.
- [38] A.A. Peterson, F. Vogel, R.P. Lachance, M. Fröling, M.J. Antal, J.W. Tester,

- Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies, *Energy Environ. Sci.* 1 (2008) 32–65. doi:10.1039/b810100k.
- [39] A. Demirbaş, Mechanisms of liquefaction and pyrolysis reactions of biomass, *Energy Convers. Manag.* 41 (2000) 633–646. doi:10.1016/S0196-8904(99)00130-2.
- [40] R. Mahadevan, S. Adhikari, R. Shakya, K. Wang, D.C. Dayton, M. Li, Y. Pu, A.J. Ragauskas, Effect of torrefaction temperature on lignin macromolecule and product distribution from HZSM-5 catalytic pyrolysis, *J. Anal. Appl. Pyrolysis.* 122 (2016) 95–105. doi:10.1016/J.JAAP.2016.10.011.
- [41] D.R. Vardon, B.K. Sharma, J. Scott, G. Yu, Z. Wang, L. Schideman, Y. Zhang, T.J. Strathmann, Chemical properties of biocrude oil from the hydrothermal liquefaction of *Spirulina* algae, swine manure, and digested anaerobic sludge, *Bioresour. Technol.* 102 (2011) 8295–8303. doi:10.1016/J.BIORTECH.2011.06.041.
- [42] Hexadecanoic acid(57-10-3) MSDS Melting Point Boiling Point Density Storage Transport, (n.d.). https://www.chemicalbook.com/ProductMSDSDetailCB9388222_EN.htm#2 (accessed August 5, 2018).
- [43] Standard Grade Coal - Heating Values, (n.d.). https://www.engineeringtoolbox.com/coal-heating-values-d_1675.html (accessed August 5, 2018).
- [44] M. Uchimiya, L.H. Wartelle, K.T. Klasson, C.A. Fortier, I.M. Lima, Influence of Pyrolysis Temperature on Biochar Property and Function as a Heavy Metal Sorbent in Soil, *J. Agric. Food Chem.* 59 (2011) 2501–2510. doi:10.1021/jf104206c.
- [45] D. Mohan, C.U. Pittman, M. Bricka, F. Smith, B. Yancey, J. Mohammad, P.H. Steele, M.F. Alexandre-Franco, V. Gómez-Serrano, H. Gong, Sorption of arsenic, cadmium, and lead by chars produced from fast pyrolysis of wood and bark during bio-oil production, *J. Colloid Interface Sci.* 310 (2007) 57–73. doi:10.1016/J.JCIS.2007.01.020.
- [46] P. Biller, A.B. Ross, S.C. Skill, A. Lea-Langton, B. Balasundaram, C. Hall, R. Riley, C.A. Llewellyn, Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process, *Algal Res.* 1 (2012) 70–76. doi:10.1016/J.ALGAL.2012.02.002.
- [47] S.R. Shanmugam, S. Adhikari, R. Shakya, Nutrient removal and energy production from aqueous phase of bio-oil generated via hydrothermal liquefaction of algae, *Bioresour. Technol.* 230 (2017) 43–48. doi:10.1016/J.BIORTECH.2017.01.031.
- [48] L. Garcia Alba, C. Torri, C. Samorì, J. Van Der Spek, D. Fabbri, S.R.A. Kersten, D.W.F. Brilman, Hydrothermal treatment (HTT) of microalgae: Evaluation of the process as conversion method in an algae biorefinery concept, *Energy and Fuels.* 26 (2012) 642–657. doi:10.1021/ef201415s.

CHAPTER FOUR

Fractional Distillation and Characterization of Upgraded Oil from Hydrothermal Liquefaction of *Nannochloropsis sp.*

Abstract

Catalytic upgrading of crude bio-oil from HTL of algae has received great attention in the past. The effect on bio-oil from use of different solvents for product separation has not been accounted. This study presents a detailed analysis on the yield and properties of oil obtained from upgrading with and without the presence of catalyst (5% Ru/C and H₂ only) and effect of toluene and dichloromethane (DCM) as product separating solvent. Further fractional distillation at atmospheric pressure of bio-oil and upgraded oils was carried out to estimate the yield and properties of distilled fractions. The bio-oil used in current study was produced from hydrothermal liquefaction of *Nannochloropsis sp.* microalgae. Mass yields, TAN, HHV, elemental and chemical composition was evaluated for each oil.

Catalytic upgraded oil extracted from toluene had oxygen content of 1.01 wt% and HHV of 43.36 MJ/kg while DCM extracted oil had 6.13 wt% oxygen and HHV of 37.72 MJ/kg. Catalyst showed better activity for denitrogenation and nitrogen values were not all that different between different solvents. Fractionation produced three distilled fractions (F1 <220°C, F2 220-350°C, and F3 >350°C). Light and middle fractions from toluene extracted upgraded oil had better fuel properties than DCM extracted upgraded oil. Middle and heavy fractions had higher heating value

than starting oil for all the treatment conditions. Nitrogen was found to be distributed in heavy fraction in all cases and prominently as nitrile compounds. Highest heating value of 45.18 MJ/kg was obtained for F2_RuC_Tol which with TAN value of 1.38 mg KOH/g.

Keywords: Hydrothermal liquefaction, catalytic upgrading, solvent, fractional distillation, algae

4.1 Introduction

Liquid transportation fuels are obtained from crude oil whose reserves are suspected to reach exhaustion by year 2066 [1]. It has become imperative to replace the existing energy demand in transportation sector by renewable source of energy. Biomass is the only source of renewable energy that can produce liquid hydrocarbon fuels, and algae has clear advantages among various biomass. Algae have very high productivity, high CO₂ sequestration rate, and can be grown in non-arable land thereby mitigating food vs fuel pressure [2]. Thermochemical conversion such as hydrothermal liquefaction is best suited for conversion of algae into fuel because it obviates the need for drying of algae feedstock which is energy intensive and utilizes the whole of algae for conversion into fuels unlike transesterification which utilizes only the lipid portion of algae for biodiesel production. Hydrothermal liquefaction is carried out at subcritical water conditions of 280-380°C and autogenous pressure to obtain liquid bio-oil with other byproducts being aqueous phase having dissolved organics, solid char, and gases.

Extensive studies on hydrothermal liquefaction of algae has been carried out to understand the influence of temperature [3,4], biomass loading [5,6], residence time [7,8], biochemical composition of algae feedstock [9,10], and both homogenous [9,11] and heterogenous catalyst [12,13] on the bio-oil obtained. The yield and quality of bio-oil was shown to be influenced by all

above process variables. Brown *et al.* [14] reported HHV of 39 MJ/kg for bio-oil from HTL of *Nannochloropsis sp.* at 350°C and 60 mins residence time however, the bio-oil had nitrogen and oxygen content of 3.9 % and 9 %, respectively. It is known that high concentration of heteroatom like oxygen, nitrogen, sulphur reduces the quality of bio-oil. High oxygen content imparts acidic nature, reduces energy density, and can cause aging by polymerization of aldehyde groups. Combustion of bio-oil with high nitrogen and sulphur content can release NO_x and SO_x emission which is detrimental to the environment [15]. Hence, upgrading of bio-oil is a necessary step to overcome these drawbacks and improve its quality.

Pioneering work in hydrothermal upgrading of algae bio-oil was carried by Duan and Savage [16]. Equal amounts of bio-oil and water at supercritical conditions of 400°C and 4 h residence time with 25% loading of 5% Pt/C catalyst was used for upgrading work. About 32% reduction in oxygen content and almost 50% reduction in nitrogen content was observed but was not enough to meet fossil fuel requirements. Duan and Savage [17] further tested effect of temperature (430, 480, and 530°C), catalyst loading (5, 10, and 20%), reaction time (2, 4, and 6h) and catalyst activity among Pt/C, Mo₂O, and HZSM-5 in supercritical water (4:5, water to bio-oil). At a given temperature, highest reaction time and catalyst loading resulted in hydrodeoxygenation, but nitrogen content reduced by only 50%. In both studies, complete desulphurization was achieved and was influenced by temperature alone. Three step process involving liquefaction of algae to produce bio-oil, pretreatment of bio-oil at 350°C with water and upgrading in supercritical environment under the presence of nine different zeolite catalyst was carried out by Duan *et al.* [18]. Pretreatment of bio-oil at moderate temperature of 350°C with 5:3 bio-oil to water mass ratio and under 6 MPa initial pressure of reducing hydrogen gas was carried out to reduce heteroatom concentration and decrease the viscosity of bio-oil to improve heat and

mass transfer and limit catalyst poisoning during upgrading. Pretreatment reduced the nitrogen content from 5.95 to 4.58% and oxygen content from 7.37 to 5.04%. Barreiro *et al.* [19] upgraded bio-oil from *Nannochloropsis gaditana* and *Scenedesmus almeriensis* at 400°C with and without water and 4h residence time. Uncatalyzed reaction resulted in highest denitrogenation for *N. gaditana* while highest deoxygenation was observed with dry Pt/Al₂O₃ catalyzed reaction. Overall dry upgrading process was effective for deoxygenation. Bai *et al.* [20] extensively studied the activity of catalyst on pretreated bio-oil at 4h and 400°C. Ru/C showed highest activity for deoxygenation (2.1 to 1.1%) and Raney-Ni had highest activity for denitrogenation (8 to 1.6%). In all the studies discussed above dichloromethane was the solvent used for product separation. Shakya *et al.* [21] carried out upgrading experiments at 300 and 350°C and 10-hour residence time to screen the activity of different heterogenous catalyst. High yields of upgraded bio-oil were obtained at 300°C while upgrading experiments at 350°C produced oil having better quality. Maximum denitrogenation (5.37 to 1.39%) was achieved with Pt/C catalyst. Treatment under hydrogen only resulted in 50% reduction in nitrogen concentration and about 75% reduction in TAN value at 350°C. Toluene was used as the solvent for product separation in this study. Solvent used during product separation can play an important role in influencing the yield and quality of bio-oil produced which is not addressed in any study.

Distillation has also been employed as a treatment step to improve the quality of crude bio-oil. Eboibi *et al.* [22] studied the properties of distilled bio-crude obtained from vacuum distillation of crude bio-oil from HTL of *Spirulina sp.* and *Tetraselmis sp.* at 300 and 350°C and 5 mins residence time. At 350°C vacuum distillation yielded 67% and 73% of distilled biocrude for *Spirulina sp.* and *Tetraselmis sp.*, respectively. Distilled biocrudes obtained had improved quality over crude bio-oil from HTL. High heating value increased from 32 and 36 MJ/kg for different

algal oil to around 40 MJ/kg for distilled fractions. There were significant reductions in oxygen content, metallic content, and boiling point distribution. Hoffmann *et al.* [23] used fractional distillation of bio-oil obtained from HTL of hardwood following ASTM D1160 to understand the properties like heating value, density, elemental composition and distribution of oxygen-containing functional groups on the fractions obtained. 11 fractions at 25°C temperature intervals were collected till atmospheric equivalent temperature of 375°C. These fractions had heating values (41.6-43.89 MJ/kg) higher than HTL hardwood bio-oil (40.43MJ/kg) owing to less oxygen content. The residual fraction having yield of 43 wt% had lowest heating value of 38.5 MJ/kg and had about 9.89 wt% oxygen which was significantly higher than 5.3 wt% in crude bio-oil. Another study published by Chang *et al.* [24] compared the fractions obtained from atmospheric distillation of bio-oil from glycerol assisted HTL of swine manure. Bio-oil was heated up to 500°C to recover 90% bio-oil as fractions. The remaining 10% was mainly char as a result of thermal cracking at high temperature. Thermal cracking improved the quality of oil by increasing the heating value from 37.16 to 45.38 MJ/kg with increasing distillation temperature and was higher than 36.41 MJ/kg for crude bio-oil. The remaining 10% of the residue was mainly char that was obtained by polymerization at higher temperatures. Distillation of pyrolytic oil has been reported in many studies [25–28]. Nam *et al.* [26] carried out comparative study of distilled fractions obtained from fractional and vacuum distillation of pyrolytic bio-oil from microalgae *Nannochloropsis species*. Vapor temperature <120°C constituted light fraction which contained higher percent of oxygen atom than crude bio-oil while both middle fraction (120-200°C vapor temperature) and heavy fraction (residue) had lower concentration of oxygen than crude bio-oil. Nam *et al.* [29] also upgraded the distilled fractions under Pd/C catalyst and found that oil obtained from upgraded

distilled fraction had better quality in terms heteroatom concentration than oil produced from direct upgrading of crude bio-oil.

Even though many individual studies on upgrading of algae bio-oil from HTL and distillation of bio-oil has been carried out but there is no study that compares the influence of solvents during product separation of upgrading experiments and distillation of upgraded bio-oil from HTL of algae. In this study this gap in literature will be addressed. Specifically,

1. Influence of catalytic and non-catalytic upgrading along with effects of dichloromethane and toluene as solvents during product separation will be addressed, and
2. Fractional distillation at atmospheric pressure of bio-oil and upgraded will be carried out to estimate the yield and properties of distilled fractions.

4.2 Material and Methodology

Microalgae *Nannochloropsis sp.* was purchased from Reed Mariculture Inc., CA. Hydrothermal liquefaction was carried out in 1000 ml high pressure reactor from Parr Instruments company. Ultra-high purity hydrogen and nitrogen were obtained from Air Gas Inc. (Charlotte, NC). Each HTL experiment was carried out at 320°C and 30 mins reaction time with dry algae loading of 100 g. After the reaction the contents of the reactor were poured in a beaker and the reactor was rinsed with dichloromethane (DCM). The products were separated by filtration under vacuum. The liquid mixture containing aqueous phase and organic phase (bio-oil and DCM) were separated in separatory funnel. DCM was evaporated in rotary evaporator (IKA) at 60°C and 720 mbar.

4.2.1 Apparatus and Experimental Procedure

Upgrading experiments were carried out in 450 ml reactor from Parr Instruments Company. The catalyst used in this study 5% Ru/C (206180-100G) was purchased from Sigma-Aldrich.

Uncatalyzed upgrading run were carried out under the presence of hydrogen gas only. In catalyzed upgrading experiments, 8 grams of 5% Ru/C (Ru/C) was first reduced at 300°C for 1-hour with initial hydrogen pressure of 1000 PSI. After catalyst reduction 40 grams of bio-oil from HTL of *Nannochloropsis sp.* was added. Reactor was repeatedly purged with hydrogen to remove atmospheric air and charged with 1000 PSI of hydrogen gas. Upgrading work was carried out at 350°C and 2 h residence time. Same reaction condition was used for non-catalytic upgrading.

After the reaction the product mixture was mixed with either toluene (Tol) or dichloromethane (DCM) to study the influence of solvents. The slurry was vacuum filtered and the liquid mixture containing upgraded oil and solvent was rotary evaporated to remove solvent and obtain upgraded bio-oil. In case of toluene the rotary evaporator was operated at 60°C and 76 mbar while for dichloromethane 60°C and 720 mbar was used. Char trapped on filter during vacuum filtration was dried at 105°C for 24 h to estimate the char yield.

$$\text{Product yield (wt. \%)} = \frac{\text{Mass of product obtained in dry basis}}{\text{Mass of dry feedstock}} \times 100$$

Fractional distillation was carried out for HTL bio-oil, toluene extracted upgraded oil from Ru/C and hydrogen only (H₂) and DCM extracted upgraded oil from Ru/C and H₂ only. Figure 4.1 shows the apparatus for distillation which was assembled in-house. The parts included Vigreux column consisting of west condenser, vacuum adapter, bent delivery tube (14208-732), rotating cow receiver and distributor (60002-132), 100 ml rotary flask for main oil and 25 ml rotary flasks to collect the distillate. Cold tap water ran through condenser and 25 ml rotary flask were kept immersed in ice cold water to avoid any loss. The oil was heated using a heater and temperature

was measured on the surface of 100 ml rotary flask. The vapor temperature was measured but distillation was relied on main oil temperature. In typical run about 12 grams of oil was distilled. Light fraction (F1) was collected after heating the main oil to 220°C, middle fraction (F2) was collected from 220-350°C, and the residue was regarded as heavy fraction (F3). The distillation was carried out at atmospheric pressure.

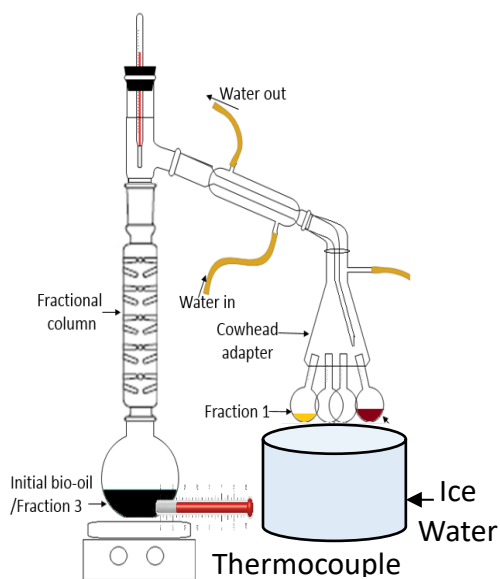


Figure 4.1 Distillation setup

4.2.1 Product analysis

High heating value (HHV MJ/kg) of the oils were measured using oxygen bomb calorimeter from IKA, moisture content was measured using V20 volumetric Karl-Fischer titrator from Mettler Toledo, and total acidic number was determined using ASTM D664 T50 titrator from Mettler Toledo. Elemental composition was determined by vario MICRO cube from Elementar in CHNS mode.

Chemical composition of the oil was analyzed with an Agilent 7890 GC/5975MS using a DB-1701 column that had Polyarc installed in it. Diluted samples contained about 2% oil in dichloromethane. Each sample was injected twice in the column. The initial temperature of the column was maintained at 40°C for 6 mins which increased to 280°C at heating rate of 6°C/min and maintained there for 10 mins. Split ratio of 1:10 was maintained and ultra-high purity helium gas was carrier gas. The GC results were analyzed for individual oil. FID areas were converted into area % and any area % less than 0.25 was ignored. NIST library was used to assign individual area % and these areas are reported. The compounds were grouped as alkane, alkene, aromatics (hydrocarbons), compounds having nitrogen only (N-Only) like pyrrole, indole, nitrile, compounds consisting oxygen only (O-Only) were majorly phenolics and compounds having both nitrogen and oxygen (N-O compounds) were majorly amides.

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 according to ASTM D2887 on an Agilent 7890A GC, equipped with split/splitless inlet, Agilent DB-2887 column (10 m × 0.53 mm, 3.00 μm), and an FID detector. Bio-oil was diluted with carbon disulfide to 1-2 wt%. The inlet was set at 350 °C with a split ratio of 1:2. The heating program started from 40 °C and then ramped to 350 °C with a heating rate of 20 °C/min under 14 mL/min of helium carrier gas. FID detector was set at 350 °C. The instrument was calibrated by calibration standard mixtures purchased from Ultra Scientific and Restek. Each sample run was followed by blank carbon disulfide and was analyzed twice.

4.3 Results and Discussion

4.3.1 Yield and Physical Characterization

Table 4.1 shows the yield and physical properties of products and Table 4.2 shows the elemental analysis of products obtained during upgrading experiments. The solvent was used only during product separation and did not take any part during reaction. The highest oil yield of 86.7 wt% was obtained during non-catalytic H₂ run with DCM as solvent and was significantly higher than 46.1 wt% with toluene as solvent. Comparing the elemental composition of upgraded oil in uncatalyzed reaction it can be seen that DCM was effective in dissolving certain compounds in char that toluene failed to dissolve which ultimately appeared in the upgraded oil. The yield of oil reported by Shakya *et al.* [21] in their upgrading study for non-catalytic reaction with toluene as solvent was around 65 wt%. The difference in result could be because of difference in separation process and because of high residence time of 10-hour as against 2-hour in this study. The highest heating value and lowest TAN was obtained from catalyzed reaction with RuC_Tol. 5% Ru/C showed better activity as against no catalyst in producing oil with lower TAN value and slightly higher heating value. HTL oil from algae has high nitrogen content because of algae having high protein content. Upgrading experiments resulted in oil having lower final nitrogen content. Bai *et al.* concluded activated carbon to show activity for denitrogenation of algae bio-oil. Pretreated oil in their study had 4.1% nitrogen which decreased to 2.9% with activated carbon as catalyst and to 2.6% with 5% Ru/C as catalyst. Oil from RuC_Tol experiment had lower yield of 56.2 wt% but higher heating value of 43.4 MJ/kg than oil from RuC_DCM experiment (66.3 wt% and 37.59 MJ/kg). RuC_Tol > H₂_Tol > RuC_DCM > H₂_DCM was the trend for quality of oil produced in terms of high heating value and low TAN value and the trend reversed when considering yield of oil.

Toluene could not dissolve certain compounds that could have transferred to upgraded oil and evaporation of toluene at 76mbar must have removed certain volatile compounds that were rich in oxygen atom. Nam *et al.* [26] found the lighter fraction (<120°C) to have more oxygen content during fractional distillation at atmospheric pressure of pyrolytic bio-oil from *Nannochloropsis* species. Overall DCM extracted upgraded oil had higher oxygen content that resulted in lower heating value and higher TAN value. Char of non-catalytic upgrading experiments can be compared on elemental composition to see that DCM was able to strip the char of almost all the carbon. The char from DCM extraction was sandy in color while with toluene extraction the char was pitch black.

Table 4.1 Yield and properties of oil obtained during upgrading experiments

Oil Type	Upgrading Yields in wt%		Properties of Oil			
	Oil Yield %	Char Yield %	Moisture %	HHV _{Dry} MJ/kg	TAN (mg KOH/g)	E.R. %
HTL	Feedstock	na	0.81 ± 0.17	34.5 ^a ± 0.09	31.4 ^a ± 0.53	
RuC_Tol	56.2 ^a ± 0.41	17.5 ^a ± 2.50	1.12 ± 0.06	43.4 ^b ± 0.09	11.5 ^b ± 0.82	70.69
H ₂ _Tol	46.1 ^b ± 1.60	25.2 ^{a,b} ± 1.21	1.43 ± 0.34	42.6 ^b ± 0.10	16.4 ^c ± 1.10	54.20
RuC_DCM	66.2 ^c ± 0.48	18.9 ^{a,b} ± 1.13	0.90 ± 0.02	37.7 ^c ± 0.77	23.0 ^d ± 1.04	72.34
H ₂ _DCM	86.7 ^d ± 0.02	9.80 ^{a,c} ± 0.60	1.02 ± 0.12	37.1 ^c ± 0.38	31.5 ^a ± 0.50	93.23

Different alphabets in the superscript of each column denote that the values are significantly different from each other. Values after ± denote standard deviation

Table 4.2 Ultimate analysis of products from upgrading experiments. O* calculated by difference

Ultimate Analysis of Oil in wt% dry basis					
Oil	C	H	N	S	O*
HTL_Oil	71.2 ^a ± 0.26	11.4 ^a ± 0.58	5.5 ^a ± 0.16	0.45 ^a ± 0.04	11.5 ^a ± 0.94
RuC_Tol	85.2 ^b ± 0.24	12.0 ^a ± 0.35	1.73 ^b ± 0.02	0.07 ^b ± 0.07	1.01 ^{b,c} ± 0.20
H ₂ _Tol	85.2 ^b ± 0.56	11.6 ^a ± 0.84	2.32 ^b ± 0.05	0.11 ^b ± 0.06	2.15 ^{b,c} ± 0.17
RuC_DCM	79.5 ^c ± 1.95	12.2 ^a ± 0.36	1.96 ^b ± 0.03	0.20 ^{a,b} ± 0.04	6.13 ^{a,c} ± 2.23
H ₂ _DCM	76.6 ^{a,c} ± 0.62	11.0 ^a ± 0.15	3.35 ^c ± .04	0.23 ^{a,b} ± 0.07	8.77 ^a ± 0.89
Ultimate Analysis of Char in wt% dry ash free basis					
	C	H	N	S	O
HTL_Char	5.73 ^a ± 0.04	1.02 ^a ± 0.05	0.63 ^a ± 0.01	0.99 ^a ± 0.41	64.2 ^a ± 1.48
RuC_Tol	56.7 ^b ± 4.22	3.28 ^a ± 0.23	4.64 ^b ± 0.70	0.31 ^a ± 0.07	5.29 ^b ± 5.74
H ₂ _Tol	42.8 ^b ± 0.94	6.95 ^b ± 0.10	7.03 ^b ± 1.15	0.31 ^a ± 0.03	23.4 ^c ± 3.04
RuC_DCM	56.0 ^b ± 0.32	3.81 ^b ± 0.12	6.11 ^b ± 0.06	0.37 ^a ± 0.02	18.1 ^{b,c} ± 2.38
H ₂ _DCM	7.59 ^a ± 3.44	6.21 ^b ± 0.97	14.2 ^c ± 0.43	0.81 ^a ± 0.21	45.7 ^d ± 3.11

Different alphabets in the superscript of each column denote that the values are significantly different from each other. Values after ± denote standard deviation

Figure 4.2 shows the yield of distilled fractions from bio-oil and upgraded oil. Highest yield in light and middle fractions was produced from RuC_Tol oil. High yield of light and middle fractions was obtained during catalytic upgrading as compared to upgrading under hydrogen only which shows the effectiveness of 5% Ru/C in cracking the heavier fractions of oil. Yields of distilled fractions were similar for HTL bio-oil and H₂_DCM upgraded oil.

Figure 4.3 shows the difference in color of the different fractions obtained in distillation of HTL, H₂_Tol, and RuC_DCM. F1 resulting from H₂_Tol had dark yellow color while F1 resulting from distillation of HTL oil was almost transparent. F2_H₂_Tol was dark red while F2 from both RuC_DCM and HTL were light yellow in color.

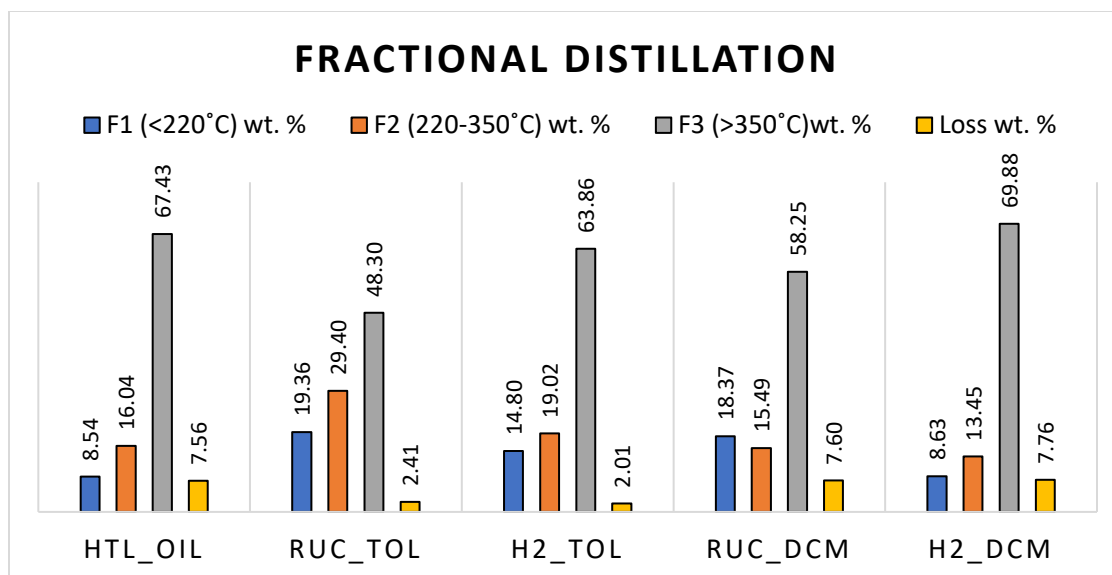


Figure 4.2 Distribution of product fractions in wt% from fractional distillation (n = 2)



Figure 4.3 From left to right F1_HTL, F2_HTL, F3_HTL, F1_H₂Tol, F2_H₂Tol, F3_H₂Tol, F1_Ru_C_DCM, F2_Ru_C_DCM, F3_Ru_C_DCM, and Ru_C_DCM_UO

Figure 4.4 gives the boiling point distribution of bio-oil and upgraded oil. Boiling points were grouped as reported by Vardon *et al.* [30]. Similar results as obtained in fractional distillation can be seen in simulative distillation. High yield of light and middle fraction in toluene extracted upgraded oil is also seen in sim distillation. Activity of 5% Ru/C catalyst in upgrading oil is such that it results in cracking of heavier fractions resulting in higher yield of lighter fractions.

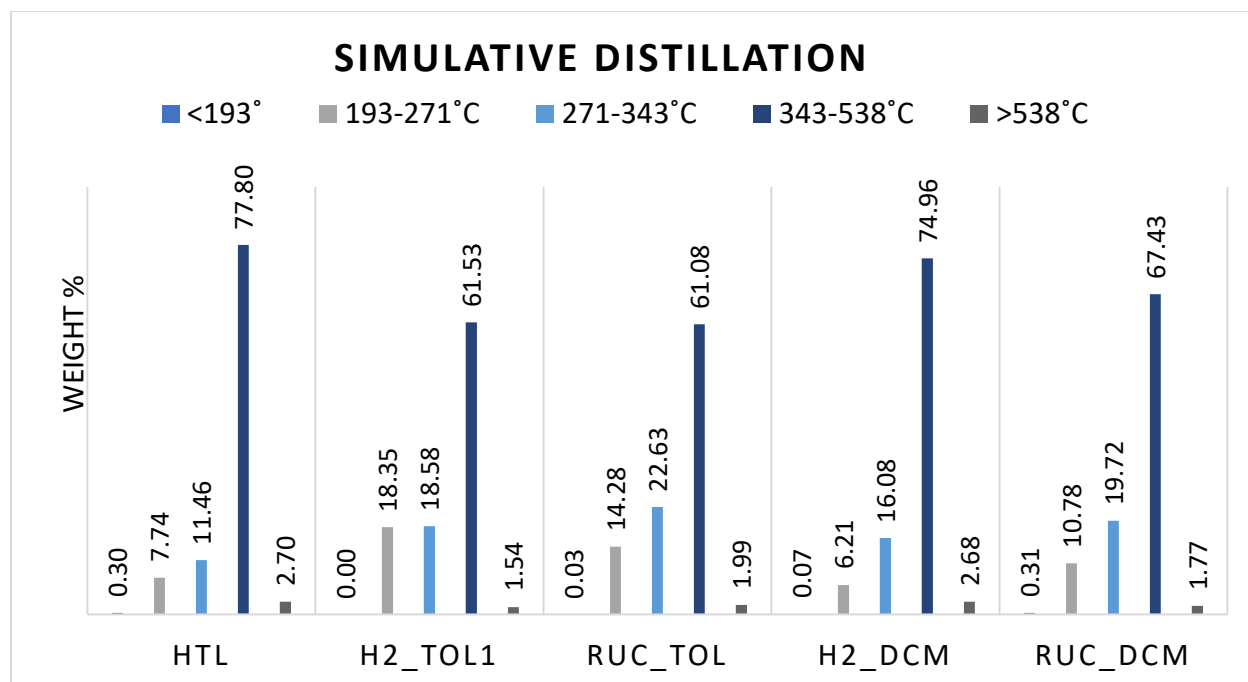


Figure 4.4 Simulative Distillation of HTL and upgraded oil

Table 4.3 lists the ultimate analysis, heating value, and TAN of main oils and their distillates. na represents the data that could not be found because of shortage of sample. F2_HTL showed significant improvement in heating value from 34.52 to 37.55 MJ/kg owing to reduction in oxygen content but had high TAN value of 60.48 mg KOH/g. Hoffmann *et al.* [23] observed that the heavier fractions had lower H/C ratio and lower O/C ratio. Nam *et al.* [26] also found a decreasing H/C and O/C ratio and increasing N/C ratio from lighter to heavier fractions. In this study, as such the carbon content did not increase at the expense of hydrogen but oxygen content decreased at the expense of increase in nitrogen content. This trend is visible in data set of H₂Tol and RuC_DCM. Nitrogen content in the different fractions followed low to high concentration from light to heavy fractions. This trend is also shown in petroleum distillates, presented by Kasztelan *et al.*, where they found nitrogen content to be distributed in heavier fractions obtained from various sources. Toluene extracted catalytic and non-catalytic upgraded oils had higher

energy density as compared to DCM extracted upgraded oils. Light fraction F1 from DCM extracted catalytic and non-catalytic oil had lower energy density in terms of heating value in 16-18 MJ/kg range. Although the elemental composition is unknown for F1_H₂_DCM it can be suspected that the oxygen content in light fractions was significantly high and had most of the moisture content present in main oil. The highest heating value of 45.18 MJ/kg was achieved for middle fraction from RuC_Tol which had TAN of 1.38 mg KOH/g slightly higher than TAN of diesel fuel (<1 mg KOH/g) from crude petroleum. In all, significant increase in quality of middle fraction and heavier fraction can be observed in terms of heating value and oxygen content. Further upgrading of different fractions using appropriate catalyst such as Ru/C for deoxygenation of lighter fractions, zeolite for heavier fractions can be employed to further increase the quality of fuel produced.

No trend in TAN was observed from lighter to heavier fractions. TAN values are not reported in literature of distillation studies except from Nam *et al.* [26]. They reported TAN values of light fraction at .12 mg KOH/g and middle fraction at 17.2 mg KOH/g which was significantly lower than and higher than TAN of bio-oil (12.2 mg KOH/g), respectively. Highest TAN value was found to be 147.03 mg KOH/g for F1_H₂_DCM. Heavier fractions had TAN's significantly lower than TAN of corresponding bio-oil or upgraded oil.

Table 4.3 Ultimate analysis in dry basis, high heating value in dry basis and total acid number of main oil and their distilled fractions. O* calculated as difference.

Oil Type	Ultimate Analysis of Oil in wt% dry basis. O* calculated by difference						HHVDry	TAN
	C	H	N	S	O*	H/C	MJ/kg	mg KOH/g
HTL	71.16	11.36	5.47	0.45	11.54	1.92	34.52	31.36
F1_HTL	13.22	1.88	0.45	0.55	83.89	1.71	8.68	na
F2_HTL	77.42	11.57	4.82	0.66	5.53	1.79	37.55	60.48
F3_HTL	79.84	10.67	5.50	0.19	3.80	1.60	38.64	25.17
RuC_Tol	85.15	12.04	1.73	0.07	1.01	1.70	43.36	11.54
F1_RuC_Tol	86.05	10.32	1.10	0.07	2.47	1.44	41.26	6.05
F2_RuC_Tol	85.83	12.79	1.20	0.11	0.22	1.79	45.18	1.38
F3_RuC_Tol	84.74	12.15	2.19	0.17	0.74	1.72	43.89	1.57
H ₂ _Tol	85.20	11.59	2.32	0.11	2.15	1.63	42.55	16.43
F1_H ₂ _Tol	84.63	11.57	0.68	0.07	3.05	1.64	41.66	39.83
F2_H ₂ _Tol	81.56	12.07	1.93	0.06	4.38	1.78	43.10	4.23
F3_H ₂ _Tol	84.40	11.86	2.38	0.05	1.31	1.69	42.67	9.58
RuC_DCM	79.49	12.22	1.96	0.20	6.13	1.84	37.72	23.04
F1_RuC_DCM	34.08	5.81	0.50	0.04	59.57	2.05	19.28	na
F2_RuC_DCM	81.65	11.83	1.70	0.21	4.61	1.74	43.55	15.58
F3_RuC_DCM	84.54	12.49	2.06	0.07	0.84	1.77	43.78	3.57
H ₂ _DCM	76.63	11.01	3.35	0.23	8.77	1.72	37.11	31.50
F1_H ₂ _DCM	na	na	na	na	na	na	17.73	147.03
F2_H ₂ _DCM	82.21	11.75	2.99	0.32	2.73	1.72	39.60	74.97
F3_H ₂ _DCM	83.58	11.84	3.58	0.30	0.71	1.70	41.22	4.52

Ultimate analysis and Heating values are in dry basis and are average of 2*2 data points. na represents not available

4.3.2 Chemical Composition of Oil

Figure 4.5 represents the GC-FID data for HTL and upgraded oil and supplementary data on each chemical compound identified in GC is presented in Table S.1 to S.6 of the appendix. Substantial increase in hydrocarbons was detected in upgraded oil as a result of hydrogenation,

decarboxylation and deamination of bio-oil. HTL oil resulting from algae had high concentration of nitrogen atoms that were in the form of pyridines, pyrroles, pyrazines and indoles and N-O compounds containing about 13.7 % of total amides. N-only compounds were produced by re-polymerization of organic compounds obtained from protein fractions following Fisher-Tropsch reaction pathway [31]. Lipids hydrolyze to produce fatty acids which interact with amines from protein hydrolysis to produce amides. Significant denitrogenation (HDN) was observed in all the upgrading cases with maximum HDN occurring with catalyst. In all the upgrading cases nitriles were the only nitrogen (N-only) containing compounds that were detected which was absent in bio-oil. Interestingly, fatty acid amides were detected only in upgraded oil from DCM extraction which in turn increased the oxygen and nitrogen content as can be seen from ultimate analysis.

Oxygen another nuisance in the bio-oil was mainly in the form of phenolics. 3.8 % of the total 4.12% O-only were phenolics in bio-oil. Among various oxygen compounds phenols have the lowest reactivity for hydrodeoxygenation according to Furimsky *et al.* [32]. Phenols were the only oxygenated compound that was detected in upgraded oil. Across all treatment conditions the percentage of phenols remained in 0.54-1.35 % range. However, DCM extracted upgraded oil shows higher oxygen content in elemental analysis as compared to toluene extracted oil. This difference in result is primarily because of fatty acid amides that contains oxygen and other chemicals that cannot be detected by GC [33]. TAN values were higher for DCM extracts which hints that these chemicals were acidic in nature.

Of the total 45% peak area of HTL bio-oil, 5.1% was aromatics of which toluene contributed 1.2%. In DCM extract upgraded oil toluene was around 1.5 to 2% of the total peak area. While for toluene extract upgraded oil the concentration of toluene was high at around 37%

for H₂_Tol and 26.5% for RuC_Tol. This shows that toluene was not fully extracted at 60°C and 76mbar. Toluene having heating value around 42 MJ/kg additional improved the quality of the oil.

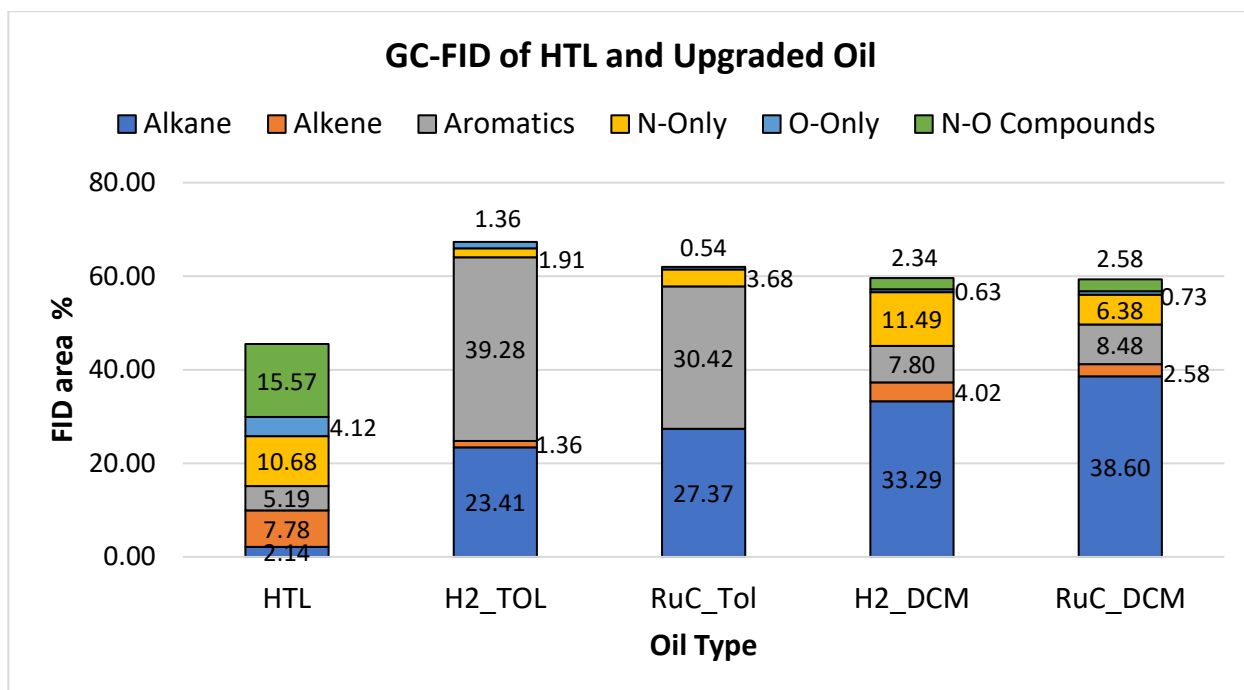


Figure 4.5 GC-MS data of HTL and upgraded oil. Average of 2 (n = 2)

Figure 4.6 shows the GC-MS data for light fraction (F1), middle fraction (F2), and heavy fraction (F3) for all the treatment conditions and supplementary data from S.7 to S.18 in Appendix lists each chemical compound identified in the GC. DCM extracted bio-oil and upgraded oil had decent amount of DCM in it while toluene extracted upgraded oil had high amount of toluene in the first fraction. Of the total 33% alkane in F1_HTL 2% was dimethyl disulfide and 2% was thiophene tetrahydro. 1, 8 Nonadien-3-ol was detected in F1_H₂_DCM which was not detected in H₂_DCM. Similarly, 1,3-Cyclohexanedione and 1-H pyrrolo (2, 3- b) pyridine was detected in F1_H₂_Tol which remained undetected in H₂_Tol.

F2_HTL was dominated by nitrogenates that comprised of pyridines and pyrazines which were also main forms of nitrogenates in HTL oil. These nitrogen heterocyclic compounds did not

undergo decomposition under 350°C distillation temperature. Pyrrolidone and its derivatives were major constituents apart from low molecular weight amides like propenamide in N-O compounds of F2_HTL. Low molecular weight organic acids like acetic acid and heptanoic acid were also detected while these compounds escaped detection in bio-oil. F2 from upgraded oil was dominated by alkanes which were tridecane, pentadecane, hexadecane, and heptadecane.

High nitrogen content in heavier fractions was observed from ultimate analysis. Half of the compounds identified in F3_HTL were N-only. 76% of this was in the form of pentadecanitrile, 5% was oleanitrile, and other 14% was hexadecanitrile. Nitriles were also major contributor for nitrogenates (N-only) for F3 fraction from upgraded oil. Hexadecane 2,6,10,14-tetramethyl was present in all heavy fractions. C14:0 to C20:0 were other major alkane present in F3 from upgraded oil.

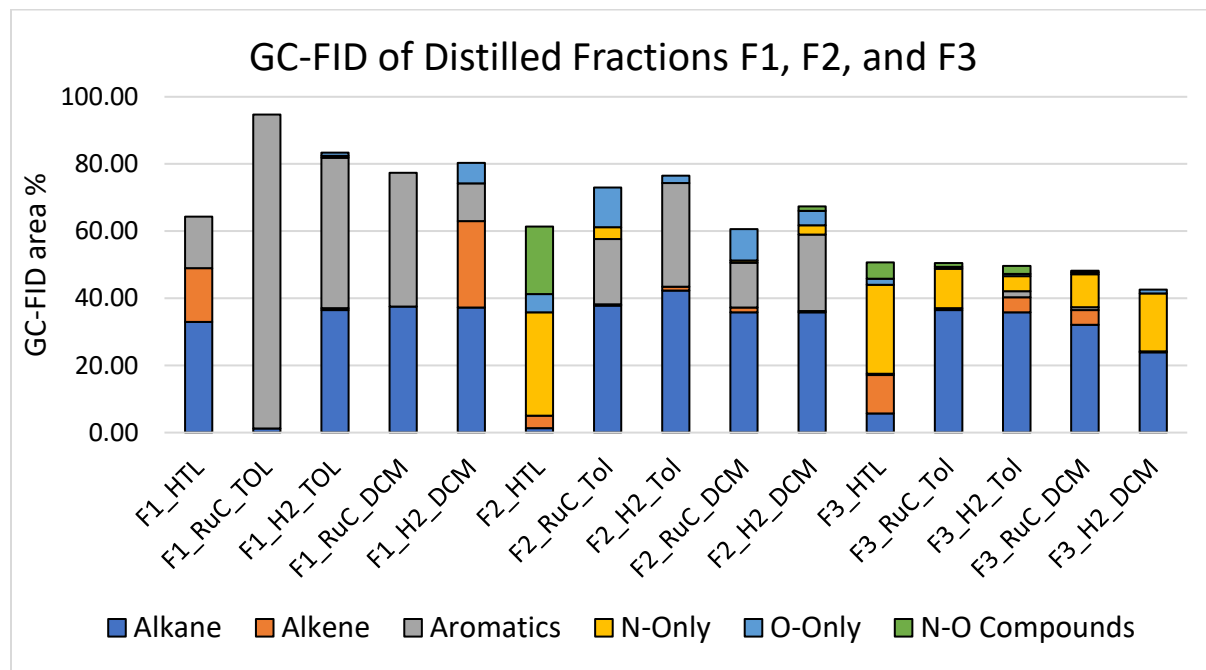


Figure 4.6 GC-MS of all fractions from fractional distillation of bio-oil and upgraded oil

Figure 4.7 shows the simulated distillation of middle and heavy fractions of HTL oil and upgraded oil. Although F2 is the distilled cut from 220-350°C there was some portion still below 193°C. This shows that relying on the temperature of main oil for distillation is not an ideal approach for distillation. Nevertheless, the aim of fractional distillation was to identify the distribution of heteroatoms in different fractions. F2_HTL contained both highest amount of gasoline and kerosene range, boiling point distribution in diesel range was observed to be maximum in F2_RuC_Tol, and F3_RuC_Tol contained highest amount of vacuum gas oil range.

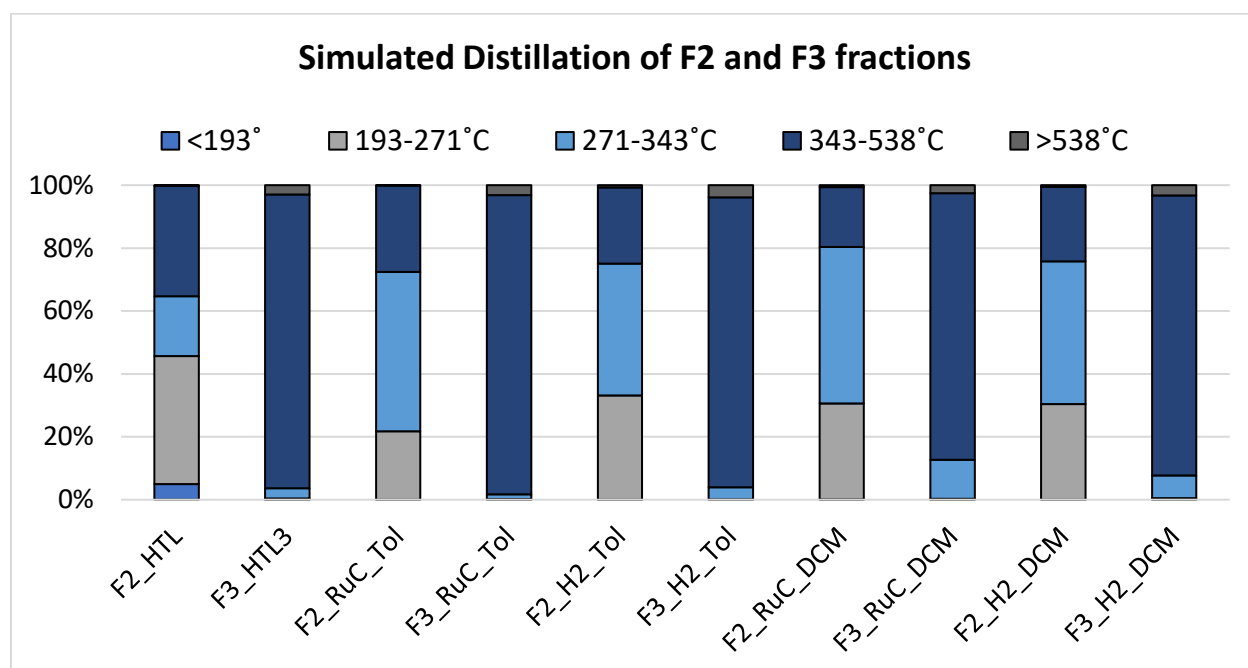


Figure 4.7 Simulated distillation of F2 and F3 fractions

Figure 4.8 shows the FTIR spectra of bio-oil and upgraded oils. Interpretation of spectral bands were based on literature [25,34]. Two strong peaks at 2860 and 2930 cm^{-1} indicate C-H stretching in aliphatic and paraffinic hydrocarbons respectively. Stretching in bandwidth 3100-3500 cm^{-1} was observed as a result of C=O bonds and N-H bonds which was intense in HTL oil and H₂_DCM upgraded oil. This can be seen in stretching near 1650-1850 cm^{-1} and dominantly

for HTL oil. The peak from 1650-1700 cm^{-1} indicate the present of amide groups. High intensity peak at 1270 cm^{-1} for H₂_DCM and RuC_DCM shows C-O single bond stretch in carboxylic acids and ester. C=C stretching in aromatic group is visible in RuC_Tol and H₂_Tol by three small peaks near 1600, 1500, and slight less than 1500 cm^{-1} . This C=C bond stretching can also be seen with peak at 730 cm^{-1} , which was intense in upgraded oil. High intensity peak at 1460 cm^{-1} was observed for upgraded oils that signifies C-H stretching as a result of alkanes.

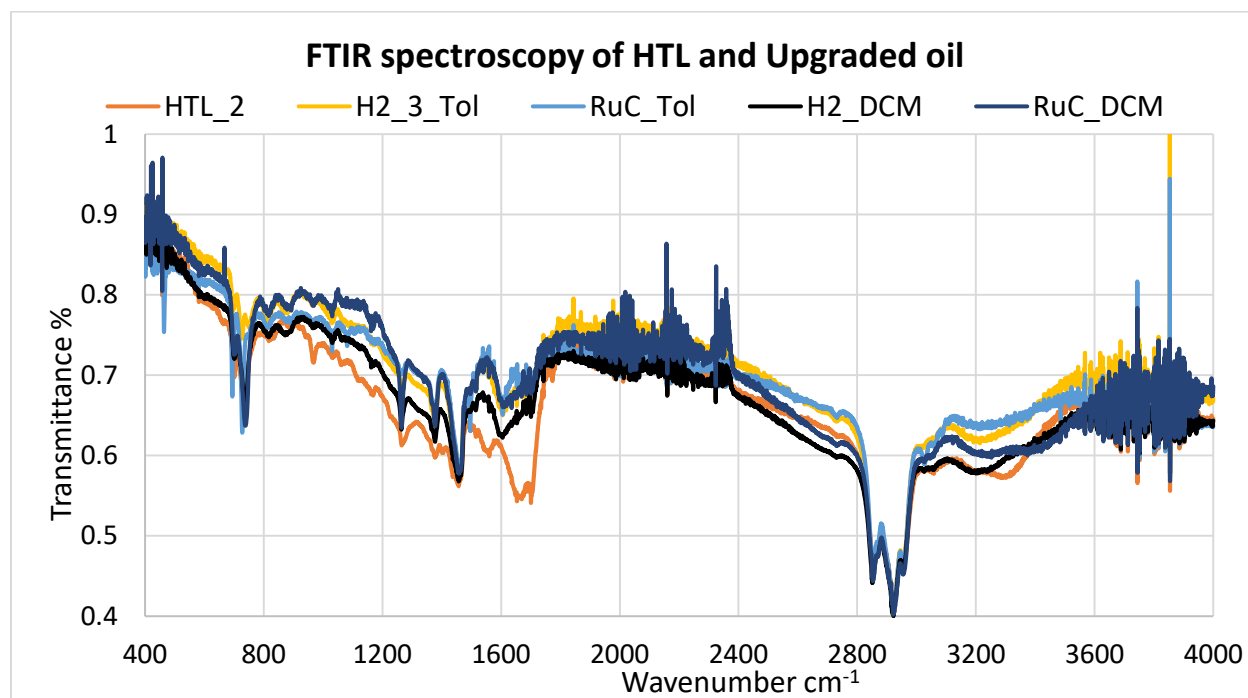


Figure 4.7 FTIR spectra of bio-oil and upgraded oil

Figure 4.10 shows the FTIR spectra of F3 fraction from upgraded oil. Similar spectra peaks were observed in all the fractions except 3100-3500 cm^{-1} bandwidth. Low frequency broad stretching can be seen in all the heavy fractions. This 3100-3500 cm^{-1} peak represents stretching due to carboxylic acids and is intense in the case of F3_H₂_DCM. Compounds in F3 had C-H bond absorption at 2850 and 2930 cm^{-1} indicating the presence of alkane structure. Peak at 1375 cm^{-1}

indicates methylene group CH_2 scissoring and peak at 1460 cm^{-1} indicates methyl group in alkanes. Peak at 1615 cm^{-1} signifies $\text{C}=\text{C}$ stretching in unsaturated ketone. Weak stretching from 2200 to 2260 indicates $\text{C}\equiv\text{N}$ bond in nitrile groups which were present as major nitrogen containing compound obtained from GC-MS analysis.

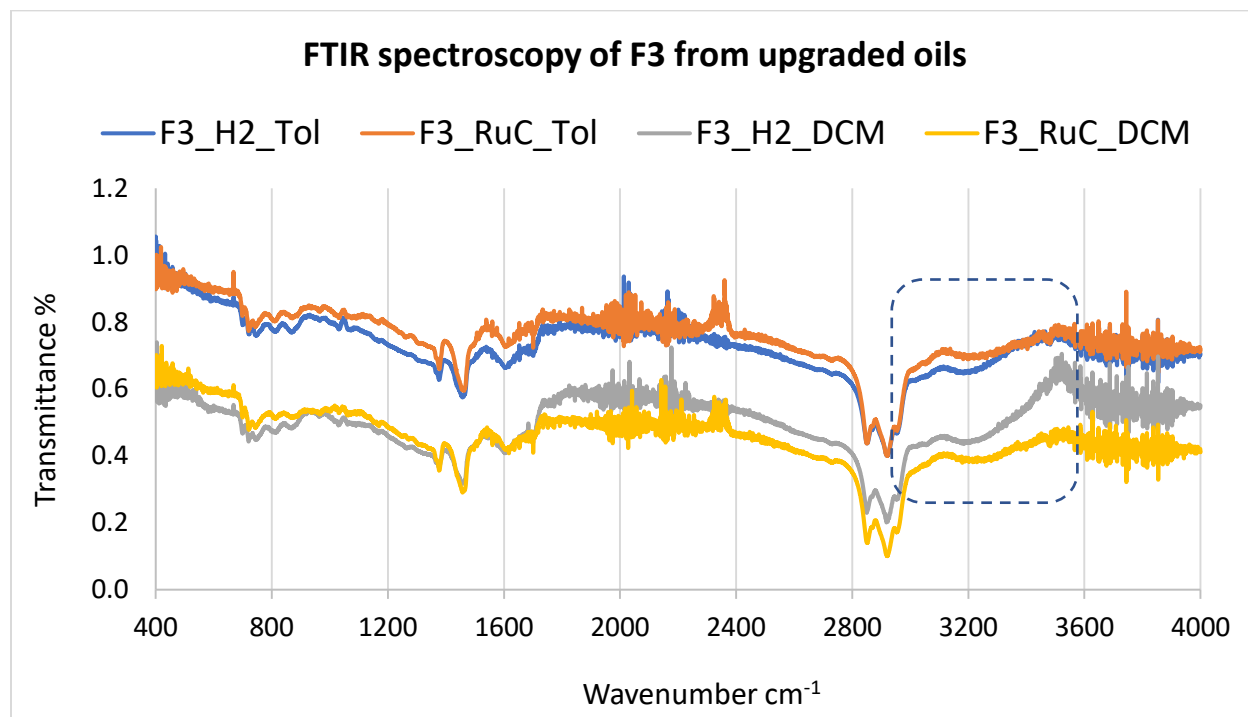


Figure 4.9 FTIR spectra for F3 fraction from upgraded oils.

4.4 Conclusion

The effect of dichloromethane and toluene as solvent during product separation from upgrading experiments was studied. Use of DCM as solvent resulted in higher yield of oil but better-quality oil was produced with toluene as solvent. 5% Ru/C with toluene as solvent resulted in oil having least nitrogen (1.73 wt%), oxygen (1.01 wt%) content, TAN (11.5 mg KOH/g), and highest HHV of 43.25 MJ/kg.

Fractional distillation at atmospheric pressure was carried out successfully for HTL bio-oil and upgraded oils. The yield of distilled fractions was comparable with boiling point distribution in simulative distillation. Highest yield of light and middle fraction having heating value of 41.26 and 45.18 MJ/kg, respectively was obtained for catalytic upgraded oil that used toluene as the product separating solvent. Trend of nitrogen distribution from low to high was observed for light to heavy fractions, respectively. Heating value of middle and heavy fractions were higher than the heating value of the oil undergoing distillation. High yield of heavy fraction containing having boiling point distribution in vacuum gas oil was obtained which had high nitrogen concentration. These nitrogenates as identified by GC-MS were dominantly nitriles. An effective denitrogenation route needs to be employed to make the oil useful. Previous work on fractional distillation at atmospheric pressure of HTL bio-oil and upgraded oil and their characterization could not be found in the literature.

4.5 References

- [1] BP: World Reserves of Fossil Fuels - knoema.com, (n.d.). <https://knoema.com/infographics/smsfgud/bp-world-reserves-of-fossil-fuels> (accessed June 28, 2018).
- [2] C. Yusuf, Biodiesel from microalgae, *Biotechnol. Adv.* 25 (2007) 294–306. doi:10.1016/j.biotechadv.2007.02.001.
- [3] R. Shakya, Hydrothermal Liquefaction of Algae for Bio-oil Production, (2014).
- [4] K. Anastakis, A.B. Ross, Hydrothermal liquefaction of the brown macro-alga *Laminaria Saccharina*: Effect of reaction conditions on product distribution and composition, *Bioresour. Technol.* 102 (2011) 4876–4883. doi:10.1016/J.BIORTECH.2011.01.031.
- [5] P.J. Valdez, J.G. Dickinson, P.E. Savage, Characterization of Product Fractions from Hydrothermal Liquefaction of *Nannochloropsis* sp. and the Influence of Solvents, *Energy & Fuels.* 25 (2011) 3235–3243. doi:10.1021/ef2004046.
- [6] U. Jena, K.C. Das, J.R. Kastner, Effect of operating conditions of thermochemical liquefaction on biocrude production from *Spirulina platensis*, *Bioresour. Technol.* 102 (2011) 6221–6229. doi:10.1016/J.BIORTECH.2011.02.057.
- [7] Z. Shuping, W. Yulong, Y. Mingde, I. Kaleem, L. Chun, J. Tong, Production and characterization of bio-oil from hydrothermal liquefaction of microalgae *Dunaliella tertiolecta* cake, *Energy.* 35 (2010) 5406–5411. doi:10.1016/J.ENERGY.2010.07.013.
- [8] J.L. Faeth, P.J. Valdez, P.E. Savage, Fast Hydrothermal Liquefaction of *Nannochloropsis* sp. To Produce Biocrude, *Energy & Fuels.* 27 (2013) 1391–1398. doi:10.1021/ef301925d.
- [9] P. Biller, A.B. Ross, Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content, *Bioresour. Technol.* 102 (2011) 215–225. doi:10.1016/J.BIORTECH.2010.06.028.
- [10] R. Shakya, S. Adhikari, R. Mahadevan, S.R. Shanmugam, H. Nam, E.B. Hassan, T.A. Dempster, Influence of biochemical composition during hydrothermal liquefaction of algae on product yields and fuel properties, *Bioresour. Technol.* 243 (2017) 1112–1120. doi:10.1016/j.biortech.2017.07.046.
- [11] A.B. Ross, P. Biller, M.L. Kubacki, H. Li, A. Lea-Langton, J.M. Jones, Hydrothermal processing of microalgae using alkali and organic acids, *Fuel.* 89 (2010) 2234–2243. doi:10.1016/J.FUEL.2010.01.025.
- [12] P. Duan, P.E. Savage, Hydrothermal Liquefaction of a Microalga with Heterogeneous Catalysts, *Ind. Eng. Chem. Res.* 50 (2011) 52–61. doi:10.1021/ie100758s.

- [13] J. Zhang, W.-T. Chen, P. Zhang, Z. Luo, Y. Zhang, Hydrothermal liquefaction of *Chlorella pyrenoidosa* in sub- and supercritical ethanol with heterogeneous catalysts, *Bioresour. Technol.* 133 (2013) 389–397. doi:10.1016/J.BIORTECH.2013.01.076.
- [14] T.M. Brown, P. Duan, P.E. Savage, Hydrothermal Liquefaction and Gasification of *Nannochloropsis* sp., *Energy & Fuels*. 24 (2010) 3639–3646. doi:10.1021/ef100203u.
- [15] M. Saber, B. Nakhshiniev, K. Yoshikawa, A review of production and upgrading of algal bio-oil, *Renew. Sustain. Energy Rev.* 58 (2016) 918–930. doi:10.1016/J.RSER.2015.12.342.
- [16] P.E. Savage, P. Duan, P.E. Savage, Upgrading of crude algal bio-oil in supercritical water.pdf, *Bioresour. Technol.* 102 (2013) 1899–1906. doi:10.1016/j.biortech.2010.08.013.
- [17] P. Duan, P.E. Savage, Catalytic treatment of crude algal bio-oil in supercritical water: Optimization studies, *Energy Environ. Sci.* 4 (2011) 1447–1456. doi:10.1039/c0ee00343c.
- [18] P. Duan, Y. Xu, F. Wang, B. Wang, W. Yan, Catalytic upgrading of pretreated algal bio-oil over zeolite catalysts in supercritical water, *Biochem. Eng. J.* 116 (2016) 105–112. doi:10.1016/J.BEJ.2015.12.006.
- [19] D. López Barreiro, B.R. Gómez, F. Ronsse, U. Hornung, A. Kruse, W. Prins, Heterogeneous catalytic upgrading of biocrude oil produced by hydrothermal liquefaction of microalgae: State of the art and own experiments, *Fuel Process. Technol.* 148 (2016) 117–127. doi:10.1016/J.FUPROC.2016.02.034.
- [20] X. Bai, P. Duan, Y. Xu, A. Zhang, P.E. Savage, Hydrothermal catalytic processing of pretreated algal oil: A catalyst screening study, *Fuel*. 120 (2014) 141–149. doi:10.1016/J.FUEL.2013.12.012.
- [21] R. Shakya, S. Adhikari, R. Mahadevan, E.B. Hassan, T.A. Dempster, Catalytic upgrading of bio-oil produced from hydrothermal liquefaction of *Nannochloropsis* sp., *Bioresour. Technol.* 252 (2018) 28–36. doi:10.1016/J.BIORTECH.2017.12.067.
- [22] B.E.O. Eboibi, D.M. Lewis, P.J. Ashman, S. Chinnasamy, Hydrothermal liquefaction of microalgae for biocrude production: Improving the biocrude properties with vacuum distillation, *Bioresour. Technol.* 174 (2014) 212–221. doi:10.1016/j.biortech.2014.10.029.
- [23] C.U. Jensen, J. Hoffmann, L.A. Rosendahl, Co-processing potential of HTL bio-crude at petroleum refineries. Part 2: A parametric hydrotreating study, *Fuel*. 165 (2015) 536–543. doi:10.1016/j.fuel.2015.08.047.
- [24] D. Cheng, L. Wang, A. Shahbazi, S. Xiu, B. Zhang, Characterization of the physical and chemical properties of the distillate fractions of crude bio-oil produced by the glycerol-

- assisted liquefaction of swine manure, *Fuel*. 130 (2014) 251–256. doi:10.1016/J.FUEL.2014.04.022.
- [25] J.A. Capunitan, S.C. Capareda, Characterization and separation of corn stover bio-oil by fractional distillation, *Fuel*. 112 (2013) 60–73. doi:10.1016/J.FUEL.2013.04.079.
- [26] H. Nam, J. Choi, S.C. Capareda, Comparative study of vacuum and fractional distillation using pyrolytic microalgae (*Nannochloropsis oculata*) bio-oil, *Algal Res.* 17 (2016) 87–96. doi:10.1016/j.algal.2016.04.020.
- [27] A.A. Lappas, S. Bezergianni, I.A. Vasalos, Production of biofuels via co-processing in conventional refining processes, *Catal. Today*. 145 (2009) 55–62. doi:10.1016/J.CATTOD.2008.07.001.
- [28] S. Wang, Y. Gu, Q. Liu, Y. Yao, Z. Guo, Z. Luo, K. Cen, Separation of bio-oil by molecular distillation, *Fuel Process. Technol.* 90 (2009) 738–745. doi:10.1016/J.FUPROC.2009.02.005.
- [29] H. Nam, C. Kim, S.C. Capareda, S. Adhikari, Catalytic upgrading of fractionated microalgae bio-oil (*Nannochloropsis oculata*) using a noble metal (Pd/C) catalyst, *Algal Res.* 24 (2017) 188–198. doi:10.1016/j.algal.2017.03.021.
- [30] D.R. Vardon, B.K. Sharma, J. Scott, G. Yu, Z. Wang, L. Schideman, Y. Zhang, T.J. Strathmann, Chemical properties of biocrude oil from the hydrothermal liquefaction of *Spirulina* algae, swine manure, and digested anaerobic sludge, *Bioresour. Technol.* 102 (2011) 8295–8303. doi:10.1016/J.BIORTECH.2011.06.041.
- [31] A.A. Peterson, F. Vogel, R.P. Lachance, M. Fröling, M.J. Antal, J.W. Tester, Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies, *Energy Environ. Sci.* 1 (2008) 32–65. doi:10.1039/b810100k.
- [32] E. Furimsky, Catalytic hydrodeoxygenation, *Appl. Catal. A Gen.* 199 (2000) 147–190. doi:10.1016/S0926-860X(99)00555-4.
- [33] A. Oasmaa, D.C. Elliott, J. Korhonen, Acidity of Biomass Fast Pyrolysis Bio-oils, *Energy & Fuels*. 24 (2010) 6548–6554. doi:10.1021/ef100935r.
- [34] R. Mahadevan, S. Adhikari, R. Shakya, K. Wang, D.C. Dayton, M. Li, Y. Pu, A.J. Ragauskas, Effect of torrefaction temperature on lignin macromolecule and product distribution from HZSM-5 catalytic pyrolysis, *J. Anal. Appl. Pyrolysis*. 122 (2016) 95–105. doi:10.1016/J.JAAP.2016.10.011.

CHAPTER FIVE

CONCLUSIONS

5.1 Conclusions

Biofuels from algae although an interesting and viable option is still in premature state. Reduction of production cost both in upstream and downstream stage are the need of the hour. To address this issue an attempt was made to 1) study product yield and properties from hydrothermal liquefaction of filamentous algae and compare it with microalgae. Filamentous type algae are easy to harvest and can save up to 30 % of cultivation cost and if grown with a cheap source of nitrogen nutrient can add to those savings. Subsequently fractional distillation was also explored on upgraded oil and HTL oil to understand the distribution of heteroatoms in different fractions so that appropriate catalyst can be used in further downstream processing. In doing so the role of polar and aromatic solvent during product separation after upgrading experiments were also examined. Specifically, 2) fractional distillation and characterization of upgraded oil from hydrothermal liquefaction of microalgae was carried out. Conclusions from each of these objectives are summarized below:

Objective 1: HTL of filamentous algae grown on five different nitrogen nutrient conditions was carried out and compared with HTL of *Nannochloropsis* sp. microalgae at 320°C and 30 mins of residence time. Five different nitrogen nutrients were, filamentous algae grown on sufficient nitrate (A_NO₃), sufficient urea (A_Urea), 14- and 21-days starved nitrate algae (14_NO₃ and

21_NO₃), and 14 days starved urea algae (14_Urea). Biochemical composition of these algae varied as a result of varying nitrogen source and conditions.

Highest bio-oil yield of 64.2 wt% was obtained for highest lipid containing 14_Urea algae (FAME 53.2 wt%). Starving the filamentous algae of nitrate increased the carbohydrate content that led to highest char yield (32.7 wt%) for 21_NO₃. Algae grown on cheaper urea have bio-oil yield of 44 wt% and was not significantly different from expensive microalgae at 49.9 wt%. Highest heating value (HHV) ranged from 30.5 to 34.5 MJ/kg. Total acid number (TAN) was lowest for microalgae bio-oil at 31.3 mg KOH/kg while for other non-stressed algae it was around 40 mg KOH/g. Chemical composition data of HTL oil from starved algae contained around 50 % of hexadecanoic acid which translated to oil having high TAN of above 100 mg KOH/g. About 77-89 % of the bio-oil was in boiling point range of vacuum gas oil. Aqueous phase was rich in nutrients which contained significant amounts of ammonia and phosphate. Char obtained during HTL as by-product had high energy density of around 30 MJ/kg.

These findings suggest that filamentous algae can be an excellent feedstock for production of algal biocrude from HTL. The advantage of using filamentous algae is reduction in harvesting cost and when grown under cheap urea as nitrogen source the economics of the process can be managed. Future research on filamentous algae should focus on its flowability in a continuous system and compare the results with batch experiments.

Objective 2: Upgrading of bio-oil from HTL of *Nannochloropsis* sp. obtained in first study was performed in the presence of 5 % Ru/C heterogenous catalyst obtained commercially at 350°C and 2 hours of residence time. The effect of catalyst on bio-oil yield and properties was studied along with polar and aromatic solvent used during product separation after upgrading runs.

Subsequently, each upgraded oil and HTL oil was fractionally distilled at atmospheric pressure to understand the distribution of heteroatoms in different fractions.

Highest oil yield of 86.7 wt% was obtained when control upgrading experiments was performed and polar dichloromethane solvent was used for product separation while the yield was merely 46.1 wt% under control and toluene as solvent. Better quality upgraded oil was produced when using toluene as solvent. Catalytic upgraded oil extracted from toluene had oxygen content of 1.01 wt% and HHV of 43.36 MJ/kg while DCM extracted oil had oxygen content of 6.13 wt% and HHV of 37.72 MJ/kg. 5 % Ru/C showed better activity for denitrogenation and the nitrogen content was not much different between different solvents. No study in the literature could be found on the influence of different solvents for product separation after upgrading experiments. This study presents first ever comparison on the same and provides insights on the significance of methodology of the experiment.

Fractionation produced three distilled fractions (F1 <220°C, F2 220-350°C), and F3 > 350°C). Middle and heavy fractions had higher heating value than starting oil on account of lower oxygen content. Nitrogen content in the fractions followed the trend low to high from light to heavy fractions with F3 having prominently nitrile compounds. Highest heating value of 45.18 MJ/kg was obtained for F2_RuC_Tol having a TAN of 1.38 mg KOH/g.

Fractional distillation can be used for effectively changing distribution of heteroatoms in different fractions which can avoid interference of other heteroatoms during catalytic upgrading. Further down streaming process of hydrogenation and hydrodenitrogenation or hydrodeoxygenation can be effective by use of appropriate catalyst.

Appendix

Supplementary data associated with GC-FID of different oils and their distilled fractions.

Table S.1 to S.6 for Figure 4.5: GC-FID data of upgraded oil

Table S.1 List of alkane compounds and their area % found in GC-FID of upgraded oil

R.T.	Alkane	RuC_Tol	H2_Tol	RuC_DCM	H2_DCM
4.21	Heptane, 2-methyl-			0.87	
4.593	Cyclohexane, 1,3-dimethyl-, cis-			0.29	
5.492	Heptane, 2,5-dimethyl-			0.37	
6.163	Octane, 4-methyl-			0.55	
6.355	Octane, 2,3-dimethyl-			0.44	
7.075	Nonane	0.28			
7.98	Octane, 2,6-dimethyl-			0.50	
9.97	Decane	0.43	0.34	0.62	0.40
13.10	Undecane	0.78	0.62	0.60	0.34
16.20	Dodecane	0.86	0.72	0.90	0.51
19.20	Tridecane	1.77	1.57	2.15	1.73
21.29	Dodecane, 2,6,10-trimethyl-	0.57	0.57	0.31	0.29
22.02	Tetradecane	1.74	1.68	1.74	1.57
23.90	Tetradecane, 3-methyl-		0.38		
24.82	Pentadecane	8.84	9.02	11.39	10.28

27.27	Hexadecane	3.47	2.12	4.29	4.20
28.31	Pentadecane, 2,6,10-trimethyl-			0.84	
28.90	Cyclotetradecane	0.58		0.58	
28.98	Cyclohexane, decyl-			0.50	
29.67	Heptadecane	3.55	4.38	4.35	4.55
31.92	Octadecane	1.44		1.65	1.63
32.10	Hexadecane, 2,6,10,14-tetramethyl-			2.65	5.09
33.82	Eicosane, 2-methyl-				0.38
34.06	Nonadecane	1.21	0.90	1.40	1.18
36.12	Eicosane	0.74	0.46	0.64	0.46
38.09	Hexadecane	0.67			
39.98	Docosane	0.45		0.42	
41.84	Tricosane		0.64	0.54	
		27.37	23.41	38.60	32.63

Table S.2 List of alkene compounds and their area % found in GC-FID of upgraded oil

R.T.	Alkene	RuC_Tol	H2_Tol	RuC_DCM	H2_DCM
3.43	Ethylidenecyclobutane		1.36	1.84	4.02
5.74	2-Pentene, 3-ethyl-2-methyl-			0.74	
		0.00	1.36	2.58	4.02

Table S.3 List of aromatics compounds and their area % found in GC-FID of upgraded oil

R.T.	Aromatics	RuC_Tol	H2_Tol	RuC_DCM	H2_DCM
5.27	Toluene	26.58	37.14	1.57	2.16
7.47	Ethylbenzene	0.68	0.59	3.05	2.99
7.72	o-Xylene		0.30	0.55	0.56
10.19	Benzene, propyl-	0.38		0.44	0.53
10.48	Benzene, 1-ethyl-2-methyl-			0.29	0.30
11.63	Benzene, 1,3,5-trimethyl-			0.26	
13.46	Benzene, (2-methylpropyl)-		0.37	0.58	
14.71	1-Phenyl-1-butene	0.42		0.44	
16.56	Benzene, pentyl-	0.45		0.40	
16.90	Benzene, 1-ethyl-2,3-dimethyl-	0.41			
17.41	Naphthalene, 1,2,3,4-tetrahydro-				0.93
17.76	1H-Indene, 2,3-dihydro-1,6-dimethyl-	0.42			
18.06	Benzene, (1-ethyl-1-propenyl)-	0.39			
20.95	Naphthalene, 1,2,3,4-tetrahydro-1,4-dimethyl-			0.27	
22.41	Naphthalene, 1-methyl-	0.36	0.31		
22.83	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-			0.27	0.93

25.86	Naphthalene, 2,3-dimethyl-		0.57		
28.70	Naphthalene, 1,6,7-trimethyl-	0.36			
36.64	Anthracene, 1,2,3,4,5,6,7,8-octahydro-			0.35	
		30.43	39.28	8.48	8.40

Table S.4 List of N-Compounds and their area % found in GC-FID of upgraded oil

R.T.	N-Compounds	RuC_Tol	H2_Tol	RuC_DCM	H2_DCM
31.03	1H-Indole, 2,3-dimethyl-			0.28	
32.79	2,3,7-Trimethylindole				0.29
33.11	Tetradecanenitrile			0.71	1.07
37.50	Pentadecanenitrile	2.84	1.41		
37.56	Hexadecanenitrile			4.44	8.41
41.44	Heptadecanenitrile	0.84	0.50	0.95	1.73
		3.68	1.91	6.38	11.49

Table S.5 List of O-Compounds and their area % found in GC-FID of upgraded oil

R.T.	O-Compounds	RuC_Tol	H2_Tol	RuC_DCM	H2_DCM
17.18	Phenol	0.54	0.31	0.45	
18.53	Phenol, 3-methyl-		0.29		
19.64	Phenol, 3-methyl-		0.49		0.33
20.79	Phenol, 3-ethyl-		0.26	0.29	0.29
		0.54	1.36	0.73	0.63

Table S.6 List of N-O-Compounds and their area % found in GC-FID of upgraded oil

R.T.	N-O-Compounds	RuC_Tol	H2_Tol	RuC_DCM	H2_DCM
44.90	Octadecanamide			0.71	2.34
		0.00	0.00	0.71	2.34

Table S.7 to S.18 for Figure 4.6: GC-FID area % of distilled fractions

Table S.7 List of alkane compounds and their area % found in GC-FID of F2 fractions

R.T.	Alkane	F2_HTL	F2_RuC_Tol	F2_H2_Tol	F2_RuC_DCM	F2_H2_DCM
3.78	Cyclohexane, methyl-					0.26
4.20	Heptane, 2-methyl-					0.41
4.81	Heptane, 2,4-dimethyl-					0.92
6.17	Octane, 4-methyl-					0.46
6.45	Thiophene, tetrahydro-	1.37				
7.08	Nonane		0.30	0.28	0.55	1.00
8.01	Octane, 2,6-dimethyl-				0.46	0.61
8.86	Nonane, 2-methyl-		0.37		0.46	
9.73	Cycloheptane, methyl-				0.36	
9.99	Decane		0.68	0.63	0.96	0.91
10.64	Decane, 4-methyl-		0.34		0.47	
11.30	Cyclohexane, (2-methylpropyl)-				0.25	
11.93	Decane, 2-methyl-				0.28	
12.91	Cycloheptane, methyl-		0.38			
13.15	Undecane		1.33	1.48	1.83	1.36
14.68	Indan, 1-methyl-		0.78	0.81		
16.26	Dodecane		1.71	2.38	2.28	1.58
16.61	Undecane, 2,6-dimethyl-			0.88		
19.30	Tridecane		3.54	4.20	4.47	3.62
21.35	Dodecane, 2,6,10-trimethyl-			1.42	0.98	1.31

22.07	Tetradecane		4.60	3.33	3.20	3.17
23.97	Tetradecane, 3-methyl-			1.32		
25.07	Pentadecane		13.27	16.13	11.25	12.52
26.30	Tetradecane, 3-methyl-			0.50		
27.37	Hexadecane		4.26	2.39	3.15	3.80
28.28	Pentadecane, 2,6,10-trimethyl-				0.81	0.74
28.94	Cyclohexane, decyl-		0.99	0.78	0.79	
29.73	Heptadecane		3.02	3.61	1.80	2.78
31.87	Octadecane		0.92		0.40	
32.09	Hexadecane, 2,6,10,14-tetramethyl-		1.31	1.71	0.82	
34.07	Nonadecane			0.41	0.28	0.35
		1.37	37.78	42.26	35.84	35.80

Table S.8 List of alkene compounds and their area % found in GC-FID of F2 fractions

R.T.	Alkene	F2_HT L	F2_RuC_T ol	F2_H2_T ol	F2_RuC_DC M	F2_H2_DC M
3.43	Cyclohexene	3.65	0.45	0.60	0.64	
18.80	4,4-Dimethyl-1,1a,3a,4,5,6-hexahydrocyclopropa[c]pentalene			0.55		
22.59	cis-8-tert-Butyl-bicyclo[4.3.0]non-3,7-diene				0.74	
27.57	3-Hexadecene, (Z)-					0.37
		3.65	0.45	1.15	1.38	0.37

Table S.9 List of aromatic compounds and their area % found in GC-FID of F2 fractions

R.T.	Aromatics	F2_HTL	F2_RuC_T ol	F2_H2_To l	F2_RuC_DC M	F2_H2_DC M
5.23	Toluene		9.31	16.92		3.03
7.48	Ethylbenzene		0.74	0.71	1.18	6.38
7.71	p-Xylene		0.29	0.36	0.37	0.90
9.18	Benzene, (1-methylethyl)-					0.73
10.18	Benzene, propyl-		0.51	0.39	0.67	1.05
10.46	Benzene, 1-ethyl-2-methyl-		0.40	0.44	0.49	0.50
11.17	Benzene, 1,2,4-trimethyl-				0.47	0.38
11.59	Benzene, 1,2,3-trimethyl-		0.36	0.39	0.55	0.51
11.83	Benzene, (1-methylpropyl)-		0.76			0.65
12.70	Benzene, 1,3,5-trimethyl-		0.42	0.37	0.61	0.63
13.43	Benzene, butyl-		0.80	1.05	1.42	1.04
13.90	Benzene, 1-methyl-4-propyl-			0.45		0.45
14.17	Benzene, 1-methyl-2-(1-methylethyl)-			0.47	0.78	0.41
14.48	Benzene, 1-butenyl-, (E)-		0.48	0.47	0.89	0.89
15.47	Benzene, (2-methylbutyl)-				0.27	0.38

15.56	Benzene, 1,2,4,5-tetramethyl-		0.51	0.71	0.30	
16.42	Benzene, 2-ethenyl-1,4-dimethyl-		0.37		0.45	0.28
16.60	Benzene, pentyl-		0.84		1.20	0.88
16.93	Benzene, 1-methyl-2-(1-methylethyl)-			1.11		0.95
17.19	Benzene, 1-methyl-4-(1-methylpropyl)-			0.34		
17.35	Naphthalene, 1,2,3,4-tetrahydro-		0.67	0.57		0.92
17.78	1H-Indene, 2,3-dihydro-4,7-dimethyl-			0.94	0.93	0.90
20.30	Naphthalene, 1,2,3,4-tetrahydro-5,7-dimethyl-			0.83	0.38	
20.39	Hexahydrobenzocyclooctene		0.86			
22.63	Benzene, heptyl-			0.53		
22.87	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-				1.04	0.94
22.94	Naphthalene, 2-methyl-		1.02	1.12		
23.89	Silane, dimethylphenyl-				0.90	
25.51	Naphthalene, 2,7-dimethyl-		1.11	1.93	0.43	
26.51	Tetralin, 1,4-diethyl-			0.79		
		0.00	19.43	30.89	13.32	22.79

Table S.10 List of N-Compounds and their area % found in GC-FID of F2 fractions

R.T.	N-Compounds	F2_HTL	F2_RuC_Tol	F2_H2_Tol	F2_RuC_DCM	F2_H2_DCM
5.36	Pyrazine	5.16				
5.69	Pyridine	5.66				
7.17	Pyridine, 2-methyl-	3.16				
8.73	Pyridine, 2,6-dimethyl-	2.31				
8.97	Pyridine, 3-methyl-	2.63				
9.91	Pyrazine, 2,5-dimethyl-	6.59				
12.72	4-Pyridinamine, 2,6-dimethyl-	3.85				
15.00	Pyrazine, 3-ethyl-2,5-dimethyl-	0.89				
15.06	Pyridine, 2,3,5-trimethyl-		0.75			
18.98	Piperidine, 1-ethyl-	0.57				
19.52	2-Methyl-1-ethylpyrrolidine					0.77
19.88	Pyridine, 2-ethyl-5-methyl-		0.80			
20.18	1H-Pyrrole, 2-ethyl-3,4,5-trimethyl-					0.36

32.78	1H-Indole, 1,2,3-trimethyl-		0.40			
33.09	Tetradecanenitrile				0.26	0.39
34.91	4H-Pyrrolo[3,2,1-ij]quinoline, 1,2,5,6-tetrahydro-6-methyl-		0.38			
35.31	1,2,3,7-Tetramethylindole		0.27			
37.49	Hexadecanenitrile		0.95		0.49	1.28
		30.82	3.55	0.00	0.75	2.80

Table S.11 List of O-Compounds and their area % found in GC-FID of F2 fractions

R.T.	O-Compounds	F2_HTL	F2_RuC_Tol	F2_H2_Tol	F2_RuC_DCM	F2_H2_DCM
3.29	Propanal, 2-methyl-	1.85				
4.88	Acetic acid	2.77				
9.56	Cyclotetrasiloxane, octamethyl-					0.54
11.84	2-Tolyloxirane			0.72		
17.04	Phenol		2.38		3.08	
18.46	Phenol, 2-methyl-		1.07		1.27	1.00
18.79	Phenol, 2,6-dimethyl-		0.58			

19.57	Phenol, 4-methyl-		1.97		3.02	1.06
21.18	Phenol, 2,5-dimethyl-		3.02		1.08	0.34
21.34	2,3-Dimethylanisole		1.24			
21.93	Heptanoic acid, anhydride	0.78				
22.47	Phenol, 3-ethyl-					0.67
23.33	Benzene, 1-ethyl-4-methoxy-			1.53		
23.90	Benzaldehyde, 3-methoxy-					0.30
24.05	Naphthalene, 1,2,3,4-tetrahydro-5-methoxy-		1.49			
25.24	4-Hydroxy-2-methylacetophenone				0.51	
25.46	2-Methoxy-5-methylbenzaldehyde					0.39
26.45	5-Methyl-2-phenyl-2-hexenal				0.31	
		5.41	11.75	2.25	9.28	4.30

Table S.12 List of N-O-Compounds and their area % found in GC-FID of F2 fractions

R.T.	N-O-Compounds	F2_HTL	F2_RuC_Tol	F2_H2_Tol	F2_RuC_DCM	F2_H2_DCM
11.48	Acetamide, N,N-dimethyl-	1.10				
12.10	Acetamide, N-methyl-	3.05				

14.23	Propanamide, N-methyl-	2.33				
15.52	Propanamide, N,N-dimethyl-	1.09				
17.45	2-Pyrrolidinone, 1-methyl-	4.42				
19.44	Quinuclidine-3-ol	1.58				
20.16	2-Pyrrolidinone	3.09				
20.50	2,5-Pyrrolidinedione, 1-ethyl-	1.78				
23.09	Azacyclohexan-3-one, 1,5,6-trimethyl-					0.55
23.53	2-Piperidinone	1.71				
25.99	2-Pyrrolidinone, 1-butyl-					0.71
		20.14	0.00	0.00	0.00	1.25

Table S.13 List of alkane compounds and their area % found in GC-FID of F3 fractions

R.T.	Alkane	F3_HT L	F3_RuC_T ol	F3_H2_T ol	F3_RuC_DC M	F3_H2_DC M
19.21	Tridecane			0.32	0.36	
22.06	Tetradecane		0.51	0.69	0.88	0.37
23.58	3-Ethyl-3-methylheptane			0.48	0.29	
24.74	Pentadecane	1.66	7.22	11.71	10.45	
27.24	Hexadecane		4.59	3.38		3.65
28.88	Cyclopentane, decyl-		1.64		1.47	

29.69	Heptadecane		6.26	9.61	5.99	5.64
31.92	Octadecane		3.07		2.55	2.33
32.01	Hexadecane, 2,6,10,14-tetramethyl-	3.23	4.44	5.67	4.20	6.33
34.07	Nonadecane		3.06	2.63	2.46	2.11
34.66	4-Methyl-exo-tricyclo[6.2.1.0(2.7)]undecane	0.85				
36.13	Eicosane		1.91	1.36	1.10	0.91
38.09	Heneicosane		1.91		1.59	1.46
41.81	Tricosane					1.11
43.67	Heneicosane		1.91			
51.57	Heptacosane				0.73	
		5.74	36.52	35.85	32.06	23.92

Table S.14 List of alkene compounds and their area % found in GC-FID of F3 fractions

R.T.	Alkene	F3_HTL	F3_RuC_Tol	F3_H2_Tol	F3_RuC_DCM	F3_H2_DCM
3.43	Ethylidenecyclobutane	9.64		4.41	4.43	
32.66	1-Eicosene	1.73				
		11.37	0.00	4.41	4.43	0.00

Table S.15 List of aromatic compounds and their area % found in GC-FID of F3 fractions

R.T.	Aromatics	F3_HTL	F3_RuC_Tol	F3_H2_Tol	F3_RuC_DCM	F3_H2_DCM
22.82	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	0.46				
26.36	Naphthalene, 2,3-dimethyl-					0.29
27.98	Naphthalene, 1,4,5-trimethyl-		0.57			
28.58	Azulene, 4,6,8-trimethyl-			0.50		
34.92	Naphthalene, 2-hexyl-			0.92		
36.64	Phenanthrene, 1,2,3,4,5,6,7,8-octahydro-			0.46		
38.71	Phenanthrene, 1,2,3,4,5,6,7,8-octahydro-				0.81	
		0.46	0.57	1.89	0.81	0.29

Table S.16 List of N-Compounds and their area % found in GC-FID of F3 fractions

R.T.	N-Compounds	F3_HTL	F3_RuC_Tol	F3_H2_Tol	F3_RuC_DCM	F3_H2_DCM
5.22	Benzo[h]quinoline, 2,4-dimethyl-		1.09			
22.00	Thiazole, 4,5-dimethyl-	0.61				
30.99	Indolizine, 2,3-dimethyl-				0.50	
32.76	1H-Indole, 5,6,7-trimethyl-					0.71
33.09	Pentadecanenitrile		0.84		1.04	
34.49	1,2,3,7-Tetramethylindole			0.95		

37.52	Hexadecanenitrile	20.30	7.30	3.47	6.70	13.67
41.22	Oleanitrile	1.39				
41.44	Heptadecanenitrile	3.89	2.44		1.59	2.90
44.47	9H-Pyrido[3,4-b]indole, 1-methyl-	0.26				
		26.45	11.67	4.42	9.82	17.28

Table S.17 List of O-Compounds and their area % found in GC-FID of F3 fractions

R.T.	O-Compounds	F3_HTL	F3_RuC_Tol	F3_H2_Tol	F3_RuC_DCM	F3_H2_DCM
17.28	Phenol	0.79				
19.81	Phenol, 3-methyl-	0.98				
26.47	5-Methyl-2-phenyl-2-hexenal			0.28		
37.24	2-t-Butyl-6-styryl-[1,3]dioxin-4-one					0.78
40.33	9H-Fluoren-9-one, 1,2,3,4,4a,9a-hexahydro-			0.40		0.35
42.91	Octadecanoic acid, 2-hydroxy-, methyl ester		0.55			
45.29	Ruthenium, tricarbonylnorbornadiene				0.61	
		1.77	0.55	0.68	0.61	1.13

Table S.18 List of N-Compounds and their area % found in GC-FID of F3 fractions

R.T.	N-O-Compounds	F3_HTL	F3_RuC_Tol	F3_H2_Tol	F3_RuC_DCM	F3_H2_DCM
27.08	2,5-Pyrrolidinedione, 1-propyl-	1.07				
35.68	1-Ethyl-3-formylindole			0.75		
36.75	N-Methoxy-2-carbomethoxy-2-carbethoxyaziridine		1.23	0.39		
39.27	Ethyl 6-formamidohexanoate			1.23		
42.15	3,4-Dihydrocoumarin, 4,4,5,7,8-pentamethyl-6-cyano-				0.44	
49.05	Octadecanamide, N-butyl-	3.83				
		4.90	1.23	2.37	0.44	0.00