Tel Aviv University

The Porter School of Environment and Earth Sciences

Hydrothermal Liquefaction:
Biomass and waste to biofuels

A thesis submitted toward the degree of

Master of Arts in Environmental Studies

by:

Siddaq Preet Singh

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This research was carried out in

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Abstract

The need for sustainable sources of energy and fuel has motivated the scientific research in the field of biofuels. Converting biomass to fuel reduces the use of fossil fuels which are currently the largest source of greenhouse gas emissions. Hydrothermal liquefaction(HTL) is a green and low-energy technique as it only uses water and thermal energy to transform any biomass to liquid fuel, usually known as bio-crude that is capable of being used directly or refined at existing fossil fuel refinery infrastructures to usable fuels and chemicals.

To test the potential of HTL to deal with diverse feedstocks, two completely different feedstocks were tested experimentally in an HTL batch system – rendered bovine waste fat from meat industry and marine macroalgae *Ulva* sp. Both these feedstocks have environmental advantages. The meat waste is being averted from landfills where it will contribute to greenhouse gas emissions and macroalgae has a high carbon capture capacity. The evaluation of parameters was different in both cases due to difference in composition of these feedstocks. As the focus in this study was generation of liquid biofuel, the bio-oil yields and calorific value of the products was studied in both cases.

HTL of animal fat demonstrated more promising results in comparison to macroalgae in terms of yield, HHV and composition of the bio-oil. The maximum bio-oil yield of around 27% with a high HHV of 38.56 MJ/kg was attained at conditions of 50% solid load at 330°C temperature for 20 min. The animal *fat* bio-oil had negligible nitrogen and sulphur content thereby ideal for use as fuel with least processing. Its fuel properties were compared with that of two grades of marine fuel, and it is compatible with permission limits of one of them, except the exceeding moisture content limit

The macroalgae *Ulva* sp. had the highest bio-oil yield of around 8% with HHV of 30 MJ/kg at conditions of 14% solid load at 290°C temp. for 10 min. This yield was lower than previously reported works which was attributed to difference in composition and use of different extraction solvent. This *Ulva* bio-oil has a lower potential due to its high nitrogen content and lower HHV, thus, it would require further refining and processing to be used as a fuel.

HTL has shown to be a promising biomass-to-biofuel technique in handling two diverse feedstocks however the quality of bio-oil is strongly dependent on the feedstock composition. The optimized

combination of parameters of HTL helps in achieving the maximum yield of bio-oil for a particular feedstock, which can be found experimentally.

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List of symbols

AF: animal fat

ABP: animal by-products

BTL: biomass-to-fuel

C,H,N,S & O : carbon, hydrogen, nitrogen, sulphur and oxygen percentage respectively when referring to elemental analysis

daf: dry ash free

DCM: Dichloromethane

DEE: Diethyl Ether

DFB: Distillate FAME grade B

DTG: Derivative thermo-gravimetric

dw: dry weight

DW: deionized water

FAME: Fatty acid methyl esters

FTIR: Fourier-transform Infrared

GCMS: Gas Chromatography Mass Spectrometry

GHG: Greenhouse gases

HHV: High heating value ($MJ.kg^{-1}$)

HTC: Hydrothermal carbonization

HTL: Hydrothermal liquefaction

MBM: meat and bone meal

NIST: National Institute of Standards and Technology

RMA 30: Residual Marine A30

RT: retention time

SEM: Scanning Electron Microscopy

SL: solid load

SW: seawater

TAN: total acid number

TGA: Thermogravimetric analysis

UL: *Ulva* sp

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1. Literature Review

1.1. **Need for Energy from biomass**

Our fast-developing world has made the requirement of energy a need rather than a want. With the increasing population, this need is increasing exponentially. Currently, most of the energy we consume comes from fossil fuels. Fossil fuels represent the hydrocarbons that have been stored over a period of millions of years deep within the Earth. When we combust these sources of carbon for our energy needs, we create an imbalance in carbon content in the atmosphere. Majority of the anthropogenic increase in carbon dioxide has been due to the burning of fossil fuels, which is claimed as one of the biggest drivers of global warming. According to US Energy Information Administration, 94% of total carbon emissions in 2016 in the US were due to fossil fuels with the major drivers being electricity generation and transportation.[1]

There is globally development for electrification of transportation, but it is estimated that all heavy-payload would be transported by fossil fuels to the extent that 90% of this demand would still be met by oil in 2040. [2] The increase in electrification would also mean more use of fossil fuels in the form of natural gas and coal as more than 50% of the demand is met by them. Therefore, there is a dire need for sustainable sources of energy which can work in the immense infrastructure we have developed for fossil fuels. Fuels developed from biomass can be a good solution to meet this need as there is so much biomass being generated everyday - naturally and through human intervention. To put it into perspective, the 2016 Billion-ton report by Department of Energy, US states that US is capable of sustainably producing 702 million dry tons per year of total biomass at $60 per dry ton out of which 205 million tons comes from waste resources.[3] Biomass includes everything we grow and the tons of waste like agricultural residues, food waste, sewage treatment sludge that we generate alongside. These are rich sources of carbon and hydrogen which can be concentrated to produce energy sources.

The immense infrastructure of more than 700 oil refineries (as reported in 2008) [4] works on fossil fuel crude oil which is extracted from inside the Earth. The latest report from BP reports that the oil reserves of the world would last close to 50 years at current consumption. [5] The bigger issue is that if we do manage to consume all the fossil fuel that is known, the carbon dioxide levels in the atmosphere will jump 5 times from currently at 408 ppm to 2000 ppm by the year 2300,

which means a catastrophic 8°C rise in global average temperature.[6] Use of biomass to compensate the energy demand can reduce the carbon emissions as biomass is a carbon neutral source as it absorbs carbon dioxide from the atmosphere to grow.[7] A simplistic comparison between fossil fuels and biomass as feedstock for fuel is shown in the Figure 1. However, the biomass as it exists cannot be used directly in the refineries, thus the role of a biomass-to-fuel (BTL) conversion process is to transform the biomass to energy dense fuel that can be used in the existing infrastructure. Through BTL processes, the biomass is converted to a highly viscous liquid energy source referred to as bio-crude or bio-oil, which has similarities to the fossil fuel crude oil visually and in composition. Bio-crude is a mixture of hydrocarbons, oxygenates and nitrogenates which can be refined to petroleum products and valuable chemicals in the existing petroleum refineries.[8], [9] Thus, production of biocrude from biomass can help in solving the global environmental issues mentioned above.

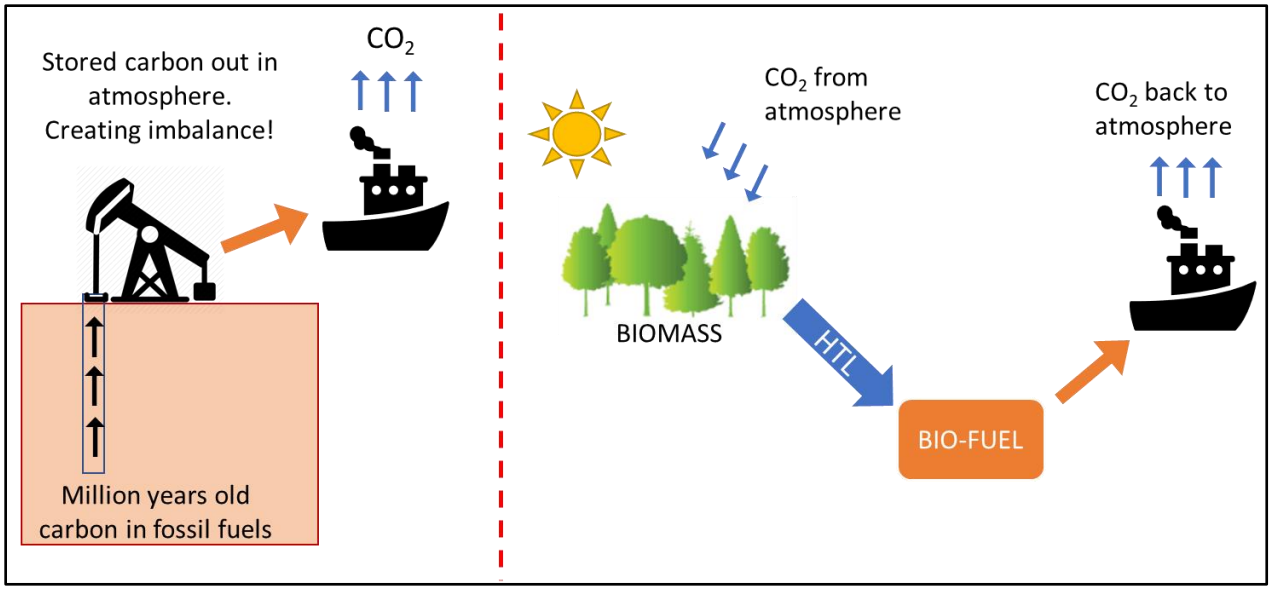


***Figure 1**: Advantage of using biomass to make fuel in comparison to fossil fuels.*

This literature review continues with explaining the unique properties of water and how they are crucial to the hydrothermal treatment process. Then hydrothermal liquefaction(HTL) is compared with other biomass to fuel processes. This is followed by the kinds of biomass that can be processed by HTL, parameters that are key to control the process and the products that can be generated from it. Finally, there is an introduction to the feedstocks that were tested in this work – meat waste from animal slaughter industry and marine macroalgae.

1.2. **How HTL works: unique thermochemical properties of water.**

The critical point for pure water is 374°C temperature and 22.1MPa as shown in Figure 2, however it may vary with composition of feedstock in an aqueous mixture.[10] If the conditions are maintained above the vapor pressure curve, the water will remain in liquid phase and there will be no consumption of energy as the latent heat of vaporization of water. This makes the use of subcritical water a highly energy efficient process in comparison to thermal processes which require drying of biomass. Supercritical water is an advanced state of water when the conditions of temperature or pressure exceed the critical point. In this state, there is no distinction between liquid and vapor phase. We have focused on subcritical and near-critical conditions only in experiments in this work.

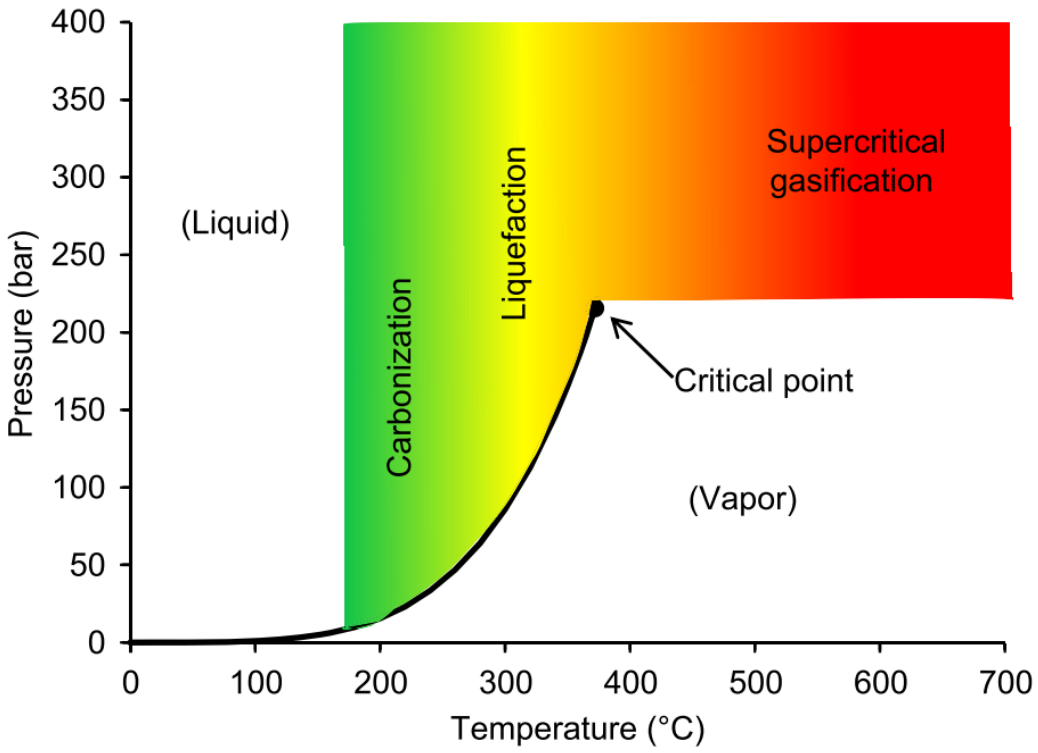


***Figure 2**: Different processes in hydrothermal treatment presented using water phase diagram.* [11]

One way to describe it is that water has tunable chemical properties as a reaction medium with temperature and pressure conditions as the radio buttons. As can be seen from Table 1, the properties of water in normal conditions change drastically when water is at supercritical conditions. Under near-critical conditions at 350°C, the density and viscosity are greatly reduced

to 0.573 g/cm^3 and 0.066 cP from 0.997 g/cm^3 and 0.89 cP at ambient conditions. This leads to enhanced rate of reaction. The ionic product decreases first and then increases by five orders of magnitude close to the critical point which support acid/base catalyzed reactions without the need to add any external catalysts.[11]

Table 1: *Properties of water at normal, subcritical and supercritical conditions in comparison with Diethyl Ether.*

Properties	Water at room temp.	Sub-critical Water		Super-critical Water	Diethyl Ether
Temperature (°C)	25	250	350	400	20
Pressure (MPa)	0.1	5	25	25	0.1
Density (g/cm^3)	.997	0.8	0.6	0.17	0.713
Dielectric constant (F/m)	78.5	27.1	14.07	5.9	4.33
Dynamic Viscosity (mPa s)	0.89	0.11	0.064	0.03	0.224
Heat capacity (KJ kg^{-1}K^{-1})	4.22	4.86	10.1	13.0	0.162

Another important change that happens to water at near-critical conditions is significant reduction in its dielectric constant. With increase in temperature, oxygen and hydrogen start to have a more shared distribution of electrons and thus reduced electronegativity of oxygen. The dielectric constant reduces from 78.5 at 25°C to 5.9 at 400°C which makes it closer to a non-polar solvent thereby more affinitive to organic hydrocarbons which is important for the HTL process.[12]

The combined effect of change in properties of subcritical water mentioned above like decrease in density and viscosity, variation in dielectric constant and ionic product aid in increased solubility of organic compounds and increased catalytic acid-base reactions which result in hydrolysis of the building components of the biomass into valuable compounds.[13] The changes of properties that occur in water with temperature also enable easier separation of products and by-products thereby reducing the energy consumption for their purification. [10] Thus, these unique properties of subcritical water allow it to depolymerize biomass(a combination of complex macromolecules) into smaller fragments and then these small molecules recombine with each other to form a mixture

new compounds including short carbon-chain compounds like hydrocarbons, thus producing the liquid fuel product known as bio-crude. When the product comes to STP, the formed oil products separate from water-soluble compounds due to difference in polarity. This conversion process is known as hydrothermal liquefaction or HTL. [14]

1.3. **Why HTL for processing of biomass?**

There are multiple avenues of converting biomass to energy: direct combustion, thermochemically and biochemically. [15], [16] All these methods serve different purposes as each of them have specific needs for the feedstock and the kind of output energy they can provide. Direct combustion of biomass, for example, can work in power plants to produce electricity but not in internal combustion engines where there is a need for liquid fuel. Fast pyrolysis, which is a thermochemical method, can produce liquid fuel but requires its feedstock to have less moisture content making it a less energy efficient and more expensive process . The fuel it produces has a relatively high oxygen content which cannot work for most applications without upgradation.[17] On the other hand, HTL , a process investigated in this work has more flexibility as it is capable of working with feedstock with higher moisture content, and the biofuel generated has a relatively lower oxygen content in comparison to pyrolysis. [10] This biofuel also requires further upgradation like hydrotreatment to improve the quality of bio-crude.[18]

Consequently, the focus in this work is on hydrothermal treatment of biomass which uses water as a reaction medium or heat transfer agent. This process tries to mimic in an accelerated way how fossil fuels were formed over a period of millions of years by processing the biomass in a reactor using water at moderate temperatures and high pressures for minutes to a couple of hours. Depending on the intensity of heat and pressure in the system, different processes occur - carbonization, liquefaction and gasification. There is no clear line of difference between these processes as can be seen in the diagram in Figure 2. It's the ratio of products that changes as we shift from lower to higher intensive conditions.

In addition, biomass with higher moisture content can cause issues in other biomass-to-fuel processes. In the case of transesterification of triglycerides, even low percentage of water content of can result in poor yields and high level of soap in biodiesel. [19] HTL on the other hand has been demonstrated in handling biomass with high moisture content like spent coffee grounds[20],

sewage wastes [21], [22], food wastes [7], [23] and agricultural residues [24] One of issues about working with wastes is the chances of contamination from pathogens and biologically active compounds. Another advantage of the HTL process is that the severe conditions of high temperatures are capable of obliterating any biological hazards and pathogens making the product streams sterile. [10] Another issue of such mixed wastes is the presence of inorganics like sulphates, nitrates and phosphates. Due to the varying properties of water with temperature, HTL enables in recovering these inorganics in ionic form which can be further used as fertilizers. [10]

The main motivation to use HTL for this work is its simplicity and versatility. If we consider the bare minimum for it, it requires biomass, water and thermal energy in a pressurized state. The capacity to convert any biomass into usable fuel and chemicals with such basic ingredients is worth mentioning. Using only water as a reaction medium makes it a green, environment friendly and cheap process [10] in comparison to other processes like transesterification where huge amounts of alcohol with molar ratios going up to 30:1 and catalysts are required in case of commercial production. [19]

1.4. **Types of biomass and waste that can be processed by HTL + Parameters of HTL**

Biomass is any organic material derived from plants or animals. However, with progression of biomass to fuel technologies, it has been realized that feedstocks like corn or soybean that compete with food resources, and even dedicated fuel crops that compete for land resources are not capable to suffice the immense demand for energy. Thus, in the new generation of biofuels, the research is currently focused on the industrial and agricultural wastes, and plants that do not compete for land like microalgae and macroalgae.

Irrespective of the source, all types of biomass are composed of a combination of biochemical components: lignin, cellulose, protein and lipids or fats.[14] However, the proportion of these components varies significantly for different biomass. The degradation reactions of each component require different activation energies and intensity of conditions for conversion which makes it tricky for a single process to handle all kinds of biomass. HTL with its wide range of temperature (200C to 600C) and pressure has the capacity to process each kind of biomass into products like biochar, biocrude or gases as fuels, and useful chemicals. A basic representation of

the HTL process and the products generated is shown in Figure 3. Water is shown separately in the figure; however wet biomass can be used directly.

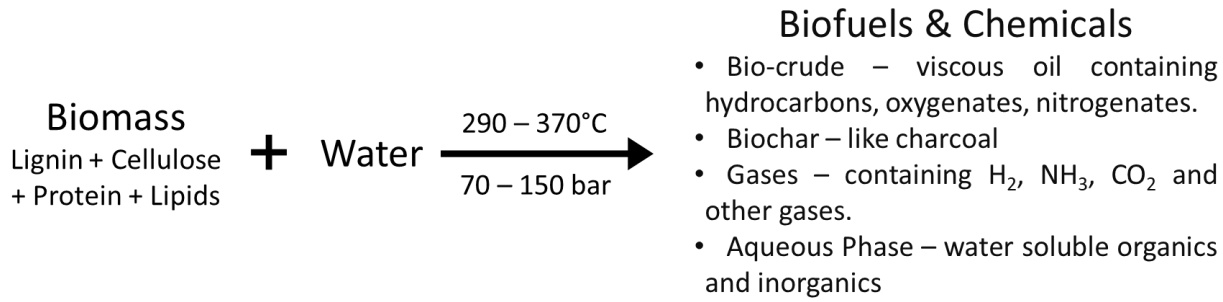


***Figure 3**: A basic representation for the HTL process.*

In addition to feedstock, the process conditions or parameters of HTL play an important role in the kind of products that can be obtained. They can be adjusted to the proportion of biochemical components and their combined structure in the feedstock to optimize the yield for specific products. The most commonly studied parameters include - temperature, retention time and solid load. Temperature controls the properties that water exhibits as shown in section 1.2 and is the key in determining the component degradation. Retention time (RT) is the time period for which the sample is subjected to specified temperature. Solid load (SL) refers to the percentage of feedstock in total mass of the sample which includes water.

The combination of temperature and RT are the deciding factors for the rate of reaction of the hydrolysis of the component and further degradation of products. Hemicellulose from wood biomass was shown to completely hydrolysed at 230°C and 2 min RT.[25] Starch from sweet potato was 100% hydrolysed at 180°C, 10 min but the maximum glucose yield was obtained at 200°C, 30 min and 220°C, 10 min. [14], [26] Hemicellulose and starch both begin to hydrolyse at 180°C nevertheless the optimized conversion requires the right combination of temperature and RT.

The variation in HTL parameters used for diverse feedstocks can be seen in the following examples. Yang et al conducted HTL of spent coffee grounds which are 15% lipid, 17.4% protein and 23% lignin. They generated a yield of 47.3% bio-oil mainly containing long chain aliphatic acids and esters at conditions of 275°C, 10 min RT and 4.7% SL.[20] HTL of lignocellulosic biomass - jack pine sawdust saw maximum yield of 51% oil products at 300°C and 50% RT where

the heavy oil contained mainly carboxylic acids, phenolic compounds and long-chain alkenes.[27] Shuping et al subjected microalgae *D. Tertiolecta* whose composition was 61.32% crude protein and 21.69% carbohydrate to HTL with 5% Na_2CO_3 as catalyst. The maximum bio-oil yield of 25.8% was observed at conditions 360°C and 50 min. RT, which was composed of fatty acids, fatty methyl esters, ketones and aldehydes.[28] Thus, it can be noticed how optimal process conditions can vary for different types of feedstocks, and the composition of bio-oil also varies significantly depending on the feedstock.

The versatility of HTL has been demonstrated by processing mixed feedstocks where multiple kinds of biomasses can be found together like food processing wastes & sewage sludge wastes. Qian et al [21] conducted HTL on sewage sludge from wastewater treatment plant and deduced that that sludge moisture content had a large effect on bio-oil yields. A biocrude yield of 27.5% and 26.8% mainly containing long-chain aliphatic hydrocarbons and aliphatic acids was obtained at 400° C, 60 min RT and 500°C,1 min RT.[21] Another study on sewage sludge was conducted by Malins et al [22] which also mentions the importance of high water content, where they achieved a bio-oil yield of 40% at 300°C, 40 min RT, 80% water. The highest yield of 47.79% was achieved using a catalyst $FeSO_4$ 5% wt. of feedstock at the same conditions. The bio oil contained cholestane, heterocyclic and carboxylic derivatives however no significant amount of hydrocarbons were detected.[22] The composition of sewage sludge would be different in different parts of the world but it can be processed through HTL by tuning the parameters accordingly, although it will generate diverse results as can be seen with the bio-oil compositions in the studies mentioned.

However, due to this variance of composition of biomass; different reaction energies for the components; secondary and tertiary reactions between components and their degradation products and different parameters to adjust in HTL, it is hard to understand the exact mechanism of reactions of HTL as so many variables are involved. Barreiro et al 2015 has beautifully shown the complexity of the amount of reactions that occur during HTL of macroalgae as shown in Figure 4 [29], however it does not include every reaction that takes place. The comprehensive review by Déniel et al in 2016 on valorization of food processing residues through HTL came to the conclusion that the exact mechanisms of HTL still remain unclear and require further investigation. [7]

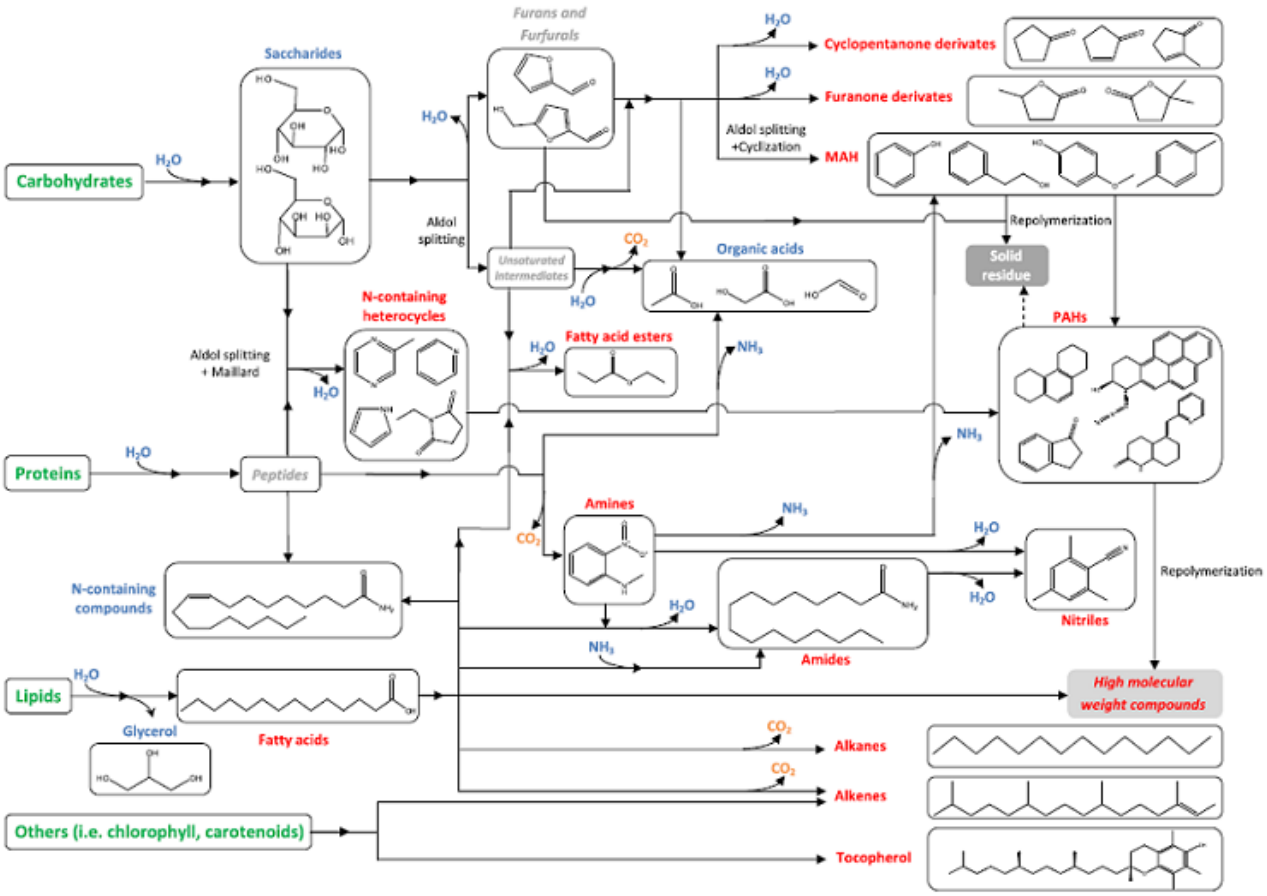


***Figure 4**: A detailed reaction pathway of HTL of macroalgae by Barreiro et al.*[29]

Text color: Green – components of biomass; Red – compounds found in bio-oil; Blue – aqueous phase compounds; Orange – gas phase.

1.5. Types of reactors (batch, continuous)

There are two types of reactors generally used in research of HTL processes - batch and continuous reactors. Batch reactors are mostly used in labs due to simplicity and versatility in handling the feedstock, especially with high solid load. [10] Particle size does not play much role. In case of a continuous HTL system, the sample is directly subjected to the desired temperature and for the required time. The retention time is controlled by the length of the reactor and flowrate of the feedstock slurry. Continuous systems have limitations on the solid load of the feedstock as if it exceeds a certain percentage, it becomes hard to pump the slurry in the system.[30]

Most of the research on HTL is carried out on batch reactors. [14] In most of the studies, the reactor is heated with the sample inside from room temperature to the desired temperature. This

forces the sample to go through all the temperature range until the desired temperature is achieved instead of subjecting the sample directly to the desired temperature. The same issue is encountered during the cooling part of the process as the reactor is shut down after the desired retention time is completed and it is left to cool down to room temperature without any intervention resulting in additional time of heat energy than the selected retention time. This makes it hard to separate the effects of temperature and retention time. [17] Overend and Chornet's severity index can help to combine the effect of these two parameters into one. [31] Faeth et al demonstrated a method they called as Fast HTL which might be closer to conditions in the continuous system that used faster heating rates and lower retention time in the batch experiment and produced significantly higher yields in comparison to conventional method. [32]

Another limitation of the batch reactors is scalability. The progressive goal for HTL systems is to produce biofuel and chemicals commercially on a large scale. It might be possible to use large-scale batch reactors for high-value compounds of limited quantity but for huge production of fuels, continuous systems are needed to achieve industrial scale economical and energy efficient systems.[17] Batch experiments give a good idea of products and conditions of the HTL process, thus providing a good starting point for understanding HTL mechanism. However, for progression to commercial scale, it is important to move to testing in continuous systems to experience the issues that are specific to them. [30] The construction of continuous systems is faced with some technical problems like systems need to be resistant to high temperatures (<400°C) and high pressures (up to 200 bar). Berglin et al did identified high pressure pumping systems that might be suited for near-critical and supercritical water processing, but they need to be vendor-tested.[33]

In this work, all the experiments were conducted in a batch reactor.

1.6. **Products generated from HTC and HTL**

As mentioned previously, process conditions of HTL and composition of the feedstock, both play a significant role in the products that are generated from the hydrothermal treatment process. When any feedstock is subjected to HTL, there are generally four components formed - bio-crude or the oil phase, biochar or the solid phase, aqueous phase and gas phase. The proportion of these components depends on the severity of the operating conditions. Hydrothermal treatment can be divided into three parts based on temperature ranges: hydrothermal carbonization(HTC),

hydrothermal liquefaction (HTL) and hydrothermal gasification (HTG). Although the composition of various biomass is very diverse, the common reactions that happen during the hydrothermal treatment are summarized by various authors [14], [33] as:

1. Depolymerization of biomass macromolecules like (carbohydrates, lipids and proteins)
2. Further breakdown by cleavage, dehydration, decarboxylation and deamination.
3. Recombination of these components via condensation, cyclization and polymerization into new compounds.

HTC occurs between the temperatures of 180°C and 250°C with pressures ranging from 12-40 bar where the major product is solid biochar with aqueous phase containing soluble organics and gaseous phase containing mostly CO_2. The biochar formed has properties similar to low-grade coal. [11] Depending on the intensity of temperature, the aqueous phase can contain sugars or degradation products of sugars like hydroxymethylfurfural (HMF). The temperatures above the critical point of water tend to favor gasification reactions, thus the process is known as HTG or supercritical water gasification(SCWG). The main product here is syngas which has high contents of hydrogen and methane with some carbon dioxide.

The process conducted between intermediate temperature range of 250°C and 375°C is known as HTL in which the major product is the liquid fuel called bio-oil or biocrude. Bio-oil usually has the highest concentration of energy in terms of calorific value in the products generated. Another product which can be used for energy is the biochar which also has significantly higher calorific value than original feedstock. The aqueous phase contains minerals like nitrates and phosphates in ionic form which can be used as a fertilizer.

The focus in this work is on producing bio-oil from two different feedstocks using HTL process at temperatures between 250 – 370°C. The yield and quality of the bio-oil generated at varying parameters of HTL like solid load, retention time and temperature are evaluated in this work. There are co-products generated alongside bio-oil – biochar and aqueous phase whose composition and uses are also discussed for some cases. There are two feedstocks studied in this work where the differentiation of the products can be clearly noticed. The next section explains why these specific feedstocks were selected.

1.7. **Why HTL of animal fat and seaweed**

The amount of energy required by the current population of the world is so immense that not one source can suffice it all. Thereby, it is necessary to diversify the sources from which the energy is extracted. This makes it essential to identify sustainable sources of energy which can be used in existing infrastructure. A biofuel that would be generated from a biomass with least effect on the environment can be considered as a sustainable one. Thus, this work is focusing on such biomass as the feedstock for HTL.

Animal fat waste is a by-product of the meat industry, thus it will be always be produced if meat is produced. Thus, converting it into biofuel is a form of resource recovery. Utilizing residues from any organic source helps to divert it from landfills and consequently diminishes the greenhouse gas emissions that would have been caused by degradation of this waste.[34] Due to the issues that arise from dealing with biological wastes like animal carcasses and other by-products of meat slaughter industry, a number of countries in EU provide credits from biofuels from animal fats. Some countries even include it from double counting credits.[35] Netherlands is a good example where the organization receives double amount of HBEs or renewable energy units if they make biofuels from a certain feedstock (which includes animal fat waste.[36] Feed costs account for majority of the cost in production of biofuels. Animal fat is a cheap and energy dense source which causes environmental problems if not dealt well, thus making it an ideal source for conversion to biofuels.

Waste or residues from any industry is still limited by the number of products produced by such industries. This can satisfy a certain demand of the energy but to compensate the humungous energy demand, it is important to explore sources which can be developed independently for making biofuels and simultaneously have the least effect on our environment. Algae have been considered as one such source due to their capacity to grow in aquatic ecosystems thus not compete with food for land resources and high photosynthetic efficiency. There has been extensive research on HTL of microalgae, however, macroalgae has been investigated less for this process. Thus, we chose to examine a local mediterranean algae, *Ulva* Sp. for production of biofuel using HTL process.

1.8. **Case study 1: Animal fat from meat slaughter waste**

The amount of meat production in the world reached 315.14 million tons in 2013 with beef and buffalo accounting for 1/5th of it.[37], [38] According to FAO, there is a projected 1.5% production growth per annum from 2015-2030.[37] While creating edible meat from animals, a large percentage of waste is generated per live weight of the animal – approx. 47% for cattle, 47% for sheep and lambs, 44% for pigs and 37% for poultry. [39] These wastes include fat trim, meat, viscera, bone, blood and feathers from the animals [40] known as animal by-products(ABP) but the percentage can vary depending on the efficiency of the meat plant.

A standardized process of rendering is used to manage this waste wherever meat industry is established well. In this process, ABP are collected, grinded and heated together at temperatures between 115 – 145°C for enough time to reduce the moisture content, kill bacteria and viruses, and facilitate fat separation.[40], [41] The products generated from rendering of cattle are meat and bone meal(MBM), animal fats and effluent water. The animal fats of cattle from the rendering process, commonly known as tallow, is about 10-15% of ABP. It is important to note that animal fats are a by-product or co-product of the animal meat industry, and will increase with the increase in meat production,[42] thus it is by-product readily available in huge amounts to be converted.

Among the top uses of rendered animal fat in EU are energy, animal feed and soap & oleochemical industry sharing 33%, 28% and 22% respectively in 2011 with about 2/3rd of the energy portion comprising of biodiesel.[42] Rendered animal fat is used to make biodiesel along with plant oils for quite some time now and the process of transesterification is used industrially to convert oils and fats to biodiesel. This biodiesel is mainly composed of fatty acid methyl esters(FAME) and has similar ignition properties to conventional diesel.[42] This method has high yields of biodiesel but uses humungous amounts of ethanol or methanol, and requires catalysts for effective conversion.[19] A major challenge transesterification faces while dealing with animal fats having free fatty acids and water content is that they can affect catalyst efficiency and even promote soap formation which prevents phase separation of esters and glycerol. [19] Even low content of water (0.1%) causes reduction of FAME yield. [43]

In contrast to transesterification, HTL is not affected by moisture content as the reaction medium is water which makes it a relevant method for converting animal fat to liquid fuel. Hydrolysis of

animal fat has been around for two centuries now. It has been an industrial process since late 1940s when Colgate-Emery patented it for splitting fat into fatty acids and glycerine. This fat splitting process is conducted at 250°C temperature and 50 bar pressure.[44] There have been many studies and patents to optimize this process. The mechanism of hydrolysis of animal fat or fat splitting in water is well studied [44]–[48] however these studies are focused on complete conversion to fatty acids and glycerine, which are backbone of the oleochemical industry. One of the major conclusions was that hydrolysis of triglycerides and esterification are both sides of a reversible reaction in equilibrium.[47], [48]

The focus at the time of these studies was not to produce energy or fuel from tallow. Recently, one study was conducted to convert ABP(meat waste before the rendering process) which was mixture of bovine and porcine origin to liquid fuel through HTL. Their optimized yield of 61% bio-crude was obtained at 225°C which mainly contained rendering products like triglycerides and fatty acids, however the biocrude obtained at higher temperatures had amides and heterocyclic compounds. [49] Another recent work studied effects of HTL on pork meat and generated a maximum bio-crude yield of 55.6% at 320°C, 10%SL and 60 min RT. This bio-oil contained fatty acids, hydrocarbons, amides, esters and N-heterocyclic compounds,[50] which is similar to previous study at higher temperatures. However, in both the studies, the bio-oil obtained is high in nitrogen (2% to 5%) which can be attributed to the N present in raw material. Biofuels with high N require additional processing like denitrification to make the fuel usable as per emission standards.

Consequently, it would be a good idea to use a raw material which has very low or no nitrogen like rendered fat. It is known that lipids have a larger contribution to production of bio-oil whereas proteins can be valorized for various other more valuable applications. [7] For example, the second product from rendering process – meat and bone meal (MBM) is used as pet feed which is a better valorization of this product than fuel. There was no study found which uses subcritical water to transform rendered fat of bovine or porcine origin to liquid fuel without any catalyst. Thus, in this work, we conducted HTL on rendered bovine fat and studied effects of retention time and solid load on yield and HHV of bio-oil generated. Also, fuel properties like HHV, density, viscosity, total acid number and elemental analysis of the bio-oil were compared to marine fuel.

1.9. **Case study 2: Macroalgae *Ulva* sp.**

The second case study is based on using macroalgae *Ulva* sp. as a feedstock for HTL process. Algae are considered sustainable sources for biofuel production due to their high photosynthetic efficiency, faster growth rate and capability to grow in fresh or marine water bodies. [51] Macroalgae and microalgae are collectively known as the third generation of feedstocks for biofuels. One of the main advantages is that they are not main food sources and they do not compete with food crops for the land resource, which is becoming a scarce resource as the population and its demands increase.[52], [53]

Another major benefit of macroalgae is their high carbon capturing capacity which was shown by Chung et al to be close to a billion tons of CO_2 per year according to current productivity.[54] In addition to a potential source for biofuels, macroalgae is currently being used to produce high-value products like alginates, animal feed, chemicals, pharmaceuticals, health and body care products. [52] Extraction of these products from macroalgae leads to generation of waste biomass in the biorefinery which can be a good source for making biofuels. [29]

There are three major groups of species of macroalgae – brown, red and green seaweeds. [53]The HTL of microalgae has been researched extensively, however, recently there has been an increase in studies on converting various types of macroalgae to energy resources using HTL.[29], [55]–[57] Table 2 presents the type of macroalgae used, and the conditions that generated maximum bio-oil yield from most of the studies on HTL of macroalgae conducted till now. Their focus has been to study combinations of HTL parameters on different genus of macroalgae and their effects on the products like bio-crude and biochar.

Entomorpha prolifera in green macroalgae [55], [58]–[60] and *Laminaria saccharina* [29], [56], [57] in brown macroalgae have been studied in multiple works as can be seen from Table 2. Although similar conditions of HTL are used for the studies on *Entomorpha prolifera*, the max. bio-oil yields in [55] and [59] are 20.4% and 28.4%, which can be attributed to higher carbon content in the later. However, for studies [58] and [60], the bio-oil yields are much lower – 12%. The only major difference in HTL process is the solvent used for extraction of bio-oil which is DCM for the first two and DEE for the latter ones. It is interesting to note that the extraction solvent can have a significant effect on the bio-oil yield. When we consider *Laminaria saccharina*, the

conditions used are exactly the same and elemental composition of biomass is similar in all the studies mentioned, although the bio-oil yield is significantly lower in [57] with 13% in comparison to 19.3% and 20.9% in [56] and [29]. This variance is hard to explain.

The works mentioned in Table 2 were conducted using one type of algae and using deionized water(DW) only. In addition, studies have been performed with solvents and catalysts to optimize the yield of bio-oil. [60], [61] Co-liquefaction of seaweed with lignocellulosic biomass to increase the yields has also been tested.[62], [63] The optimized conditions for co-liquefaction showed a 71.7% increase in bio-oil yield in comparison to only green seaweed (*Enteromorpha clathrata*), however there was insignificant increase over only lignocellulosic feedstock (rice husk). [63]

Table 2: *Results from most of the studies on HTL of macroalgae conducted till now. [RT – retention time, SL – Solid load]*

Ref.	Macroalgae	C	H	N	O	Ash	Conditions Temp.; RT; SL; extraction solvent; catalyst	Max. Bio-oil yield(%)	Biochar yield(%)
[55]	*Enteromorpha prolifera* (green)	28.7	5.2	3.6	32.3	30.1	300°C; 30 min.; 11.8%; DCM	20.4	16.2
	Enteromorpha prolifera (green)	28.7	5.2	3.6	32.3	30.1	300°C; 30 min.; 11.8%; DCM; 5% Na_2CO_3;	23	14.4
[56]	*Laminaria saccharina* (brown)	31.3	3.7	2.4	26.3	24.2	350°C; 15 min.; 9.1%; DCM	19.3[a]	10.7
[64]	*Derbesia tenuissima* (green)	29.2	4.8	4.5	27.4	34.7	330-341°C; 8 min.; 6.6%; DCM	19.7	8.1
	Ulva ohnoi (green)	27.7	5.5	3.5	41.1	30.7	330-341°C; 8 min.; 6.6%; DCM	18.7	12.1
	Chaetomorpha linum (green)	26.5	4.1	3.4	31	36.6	330-341°C; 8 min.; 6.6%; DCM	9.7	8.4
	Cladophora coelothrix (green)	30.9	5	5.2	34.9	25.5	330-341°C; 8 min.; 6.6%; DCM	13.5	10.4
[59]	*Enteromorpha prolifera* (green)	35.2	5.2	2.1	32.9		290°C; 20 min.; 25%; DCM	28.4	

[57]	*Laminaria digitate* (brown)	33.1	4.7	1.8	33.9	23.9	350°C; 15 min.; 9.1%; DCM	17.6 [a]	10.9
	Laminaria hyperborean (brown)	35.8	5.1	1.5	39.1	16.6	350°C; 15 min.; 9.1% ; DCM	9.8 [a]	16.7
	Laminaria saccharina (brown)	32.5	4.5	1.1	37.9	21.8	350°C; 15 min.; 9.1% ; DCM	13 [a]	18.6
	Alaria esculenta (brown)	34.6	4.7	1.9	31.1	25.2	350°C; 15 min.; 9.1%; DCM	17.8 [a]	17.9
[29]	Fucus vesiculosus (brown)	34.3	5.2	2	19.7	36	350°C; 15 min.; 9.1%; DCM	22 [a]	24.5
	Laminaria saccharina (brown)	32.5	5.3	2	37.3	22.2	350°C; 15 min.; 9.1%; DCM	20.9 [a]	7.7
	Alaria esculenta (brown)	33	4.4	2.5	36.6	33.6	350°C; 15 min.; 9.1%; DCM	28.1 [a]	13.4
[58]	*Ulva fasciata* (green)	14.3% protein; 46.7% carb.; 1.8% lipid				25.4	280°C; 15 min.; 14.3%; DEE + Acetone [b]	4+3 [c]	33
	Enteromorpha sp. (green)	7.9% protein; 39.9% carb.; 5.6% lipid				23.2	280°C; 15 min.; 14.3%; DEE + Acetone [b]	6+6 [c]	19
	Sargassum tenerrimum (brown)	10.75% protein; 30.3% carb.; 2.03% lipid				32	280°C; 15 min.; 14.3%; DEE + Acetone [b]	4+5 [c]	23
[65]	*Gracilaria gracilis* (green)	36.7	5.9	2.9	17.5	36	350°C; 15 min.; 9.1%; DCM	15.7	15.1
	Cladophora glomerate (green)	31.3	4.9	4.9	30.7	26.1	350°C; 15 min.; 9.1%; DCM	16.9	15
[60]	*Ulva prolifera* (green)	46.2	7.4	3.0	43.2	7.3	290°C; 10 min; 14.3%; DEE	12	42

[a] – dry ash free (daf) basis; [b] – two solvents were used to extract bio-oil; [c] – bio-oil1 + bio-oil; DCM – Dichloromethane; DEE – Diethyl ether

All the studies mentioned above use deionized water for their work or in combination with solvents or catalysts. Preparing the marine macroalgae for the HTL processes requires a lot of washing for

removing the salts on the seaweed. To our knowledge, no study could be found that used seawater(SW) as the reaction medium for the HTL process. In this study, we decided to use SW instead of DW so that seaweed can be harvested directly along with seawater. This can make HTL an even greener process as the use of fresh water and the energy required for washing seaweed can be greatly reduced in the process.

There have been studies on green macroalgae but there is not much work on the specific genus of green macroalgae which is native to Mediterranean Sea – *Ulva sp.* This case study examines how severity of the HTL conditions affects the products of HTL of *Ulva* sp. while using seawater(SW) as a reaction medium. The bio-oil and biochar are examined using various analytical methods.

2. Goal of this Study

As mentioned in section 1.8, the waste generated by meat slaughter is humungous and a large proportion of it is used for production of biodiesel. Transesterification is used for making biodiesel which is a chemical intensive process as it requires high amounts of alcohol and catalysts. The aim is to provide a greener alternative to convert animal fat waste to fuel. Thus, the objective of case study 1 is to evaluate the capability of HTL as a technique to produce liquid fuel from rendered bovine fat. This was investigated by assessing the maximum yield and quality of bio-oil generated. Applicability of the bio-oil was examined by comparing it with two currently used grades of marine fuel.

Multiple works have been conducted on HTL of different species of macroalgae which all use deionized water as shown in Table 2. However, the macroalgae native to Mediterranean Sea – *Ulva* sp. has not been examined before as a feedstock for HTL, and also no study was found which used seawater instead of deionized water. The objective of case study 2 is to find the optimal parameters of HTL for *Ulva* sp. while using seawater as a reaction medium. A set of HTL parameter combinations(which give highest bio-oil yield for macroalgae) were selected from previous works to check which one works best for our marine macroalgae – *Ulva* sp. The yield, quality and composition of the bio-oil produced was evaluated through various analytical methods to see its applicability as biofuel. Also, the comparison between yields of experiments using DW and SW keeping other conditions constant was conducted to see the effect of SW on bio-oil yield.

Through the two case studies conducted in the same HTL reactor, the final objective is to evaluate the capacity of HTL to deal with two diverse feedstocks and examine how the composition of the feedstock affects the composition of bio-oil.

3. Experimental

3.1. **Experimental Batch Setup**

Hydrothermal treatment of the feedstock was conducted in a batch reactor experimental setup. The schematic is presented in Figure 5 and actual picture of the system is shown in Figure 6. The experimental system consists of a 0.25 liter "Zhengzhou Keda Machinery and Instrument Equipment CJF-0.25" batch reactor heated by an electric heater (Keda Machinery, China). The temperature is controlled and measured with an MRC TM-5005 digital temperature gauge using "Watlow" 1/16" thermocouple type K. The pressure is constantly measured using "MRC PS-9302" pressure gauge with "MRC PS100-50BAR" sensor. A magnetic coupling drive was used to mix the slurry inside the pressure reactor with the stirrer set at 70 RPM in all the experiments. The magnet coupling is cooled using a chiller (Guangzhou Teyu Electromechanical Co., Ltd Cw-5200ai, China). The reactor has 2 gas sampling ports and 1 liquid sampling line. The liquid line goes through a condenser (also cooled by the chiller) and a cold trap, before entering the sampling tube. The gas is collected with a 1-liter sampling bag. The gas line goes through a condenser and a cold trap before reaching the gas sampling bag. An MRC ST-85 vacuum pump (0.13 mbar) is used to drain air in the system before each experiment. The reactor was filled with 100 grams mixture of feedstock and water, with different proportions depending on the experiment. The reactor was then heated to the selected temperature and after the desired retention time, the reactor was switched off. The sample was collected once the reactor reached room temperature.

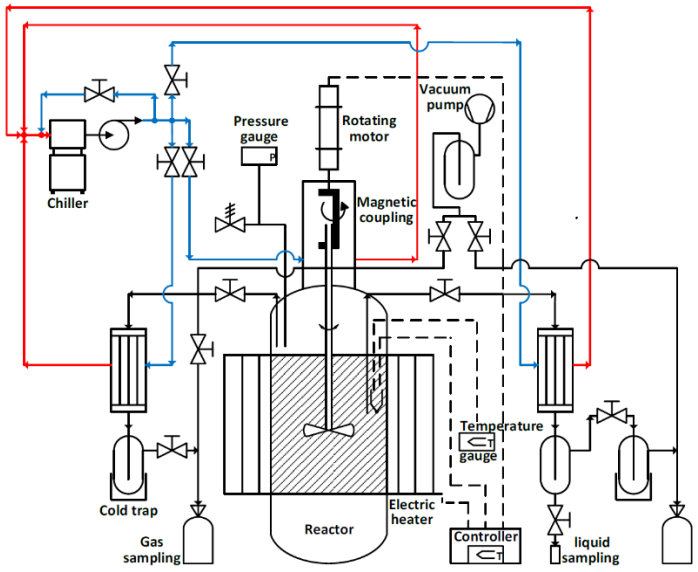


Figure 5: Schematic diagram of the HTL reactor.

Figure 6: Actual picture of the HTL batch reactor used in this study.
1.Electric heater 2. Reactor 3. Stirrer motor 4. Heat exchanger 5. Pressure sensor 6. Thermocouple 7. Temperature gauge 8. Pressure gauge 9. Controller

3.2. **Material & Methods**

3.2.1. Case Study 1: Using animal fat waste

The bovine waste fat was provided by Celitron which produced it from animal by-products of the meat industry using their version of the rendering process by Integrated Sterilizer and Shredder (ISS). In the ISS, the meat waste which includes waste fat, skin, tendons and bones is subjected to low temperatures(121 - 134 C) for a short duration(34 min). [66] This is followed by a decanter which separates the particulate matter(bone and protein fraction) from the liquid fat fraction. Finally, the fat is separated from the liquid. This whole process is depicted in Figure 7. This fat fraction (whose elemental analysis is provided in Table 4) was used with deionized water(DW) in different proportions as the feedstock for the HTL process. The animal fat(AF) was stored in refrigerated conditions between experiments.

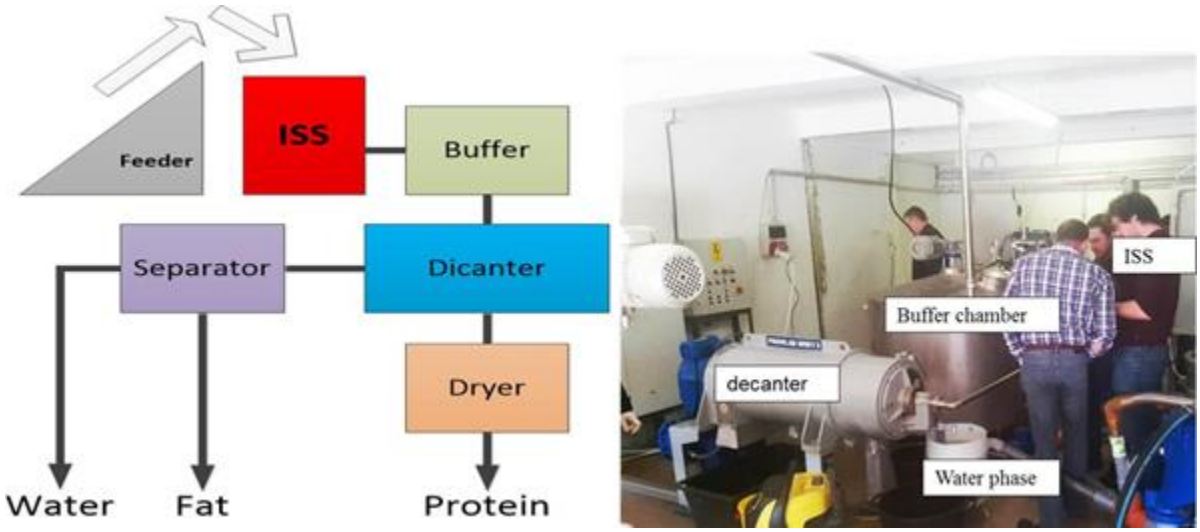


Figure 7: Celitron's process of rendering animal meat waste.

Separation of products after HTL: All the samples collected from HTL reactor were subjected to centrifugation for separation of phases. They were subjected to two rounds of 30 minutes each at 4000 rpm (TGL18, Yingtai Instrument Co. Ltd, China) using a swing-bucket rotor. The phase separation was favored by a swing rotor in comparison to a fixed angle rotor. Figure 9(a) illustrates how the tube looked after centrifugation. The bio-oil was collected from the top after each round.

Even after the second round, there was some oil remaining in the solid phase which was hard to extract through further centrifugation. The bio-oil yield was calculated using equation 1.

$$bio - oil\ yield(\%) = \frac{w_{bio-oil}}{w_{AF}} \quad (1)$$

where $w_{bio\text{-}oil}$ = wt. of animal fat bio-oil collected after centrifugation, and w_{AF} = wt. of animal fat feedstock used.

The parameters that were studied is this case study are retention time and solid load and their variations are presented in Table 3. The impact of these parameters was inspected on bio-crude yield and its calorific value.

Table 3: The HTL parameters that were studied for case study 1 and their variation.

Constant condition	Varying condition	No. of Experiments
330C temp. , 50% SL	20, 40, 60, 80 & 120 min RT	2 each; 10 experiments in total
330C temp. , 60 min RT	25%, 50% & 75% SL	2 each; 6 experiments in total

3.2.2. Case study 2: Using marine biomass – macroalgae (*Ulva* sp.)

Macroalgae (*Ulva* sp.): Green marine seaweed *Ulva* sp. was cultivated at the Israel Oceanographic and Limnological Research (IOLR Ltd., Haifa, Israel), under controlled conditions using 40 L outdoor tanks. After 2 weeks of cultivation, *Ulva* sp. biomass was transferred to larger outdoor tanks having volume of 1,000 L. In both tanks, the biomass was constantly aerated and supplied with running natural Mediterranean seawater pumped from the nearby seashore. During the cultivation in the 40 L tanks, the seaweeds were fertilized once a week, with 0.06 mM NaH_2PO_4 and 0.59 mM NH_4Cl (both chemicals from Haifa Chemicals Ltd., Israel). After 4 weeks of cultivation, the biomass was harvested, washed with sea water (SW) and drained using a spinner. For the HTL process, the biomass is used as dry weight (dw). dw was obtained by drying the fresh biomass at 40ºC until constant weight.

All experiments except two for the *Ulva* case study were conducted using artificial SW with 3.8% salinity. It was prepared by dissolving dried Red Sea salt (Red Sea Inc., IS) in deionized water

(DW) according to 3.8% salinity proportion. The other two experiments were conducted using DW.

The description of the HTL reactor for most of the experiments is given in section 3.1. There was another bigger reactor of 1 liter used for two conditions. The bigger reactor was Lab autoclave non-stirred 1L reactor, model no.: P2315 from Amar Equipments, India.

Separation of HTL products: Figure 8 describes the process of preparation and separation of HTL products in this case study. After conducting the HTL experiments, the hydrolysate was collected from HTL reactor and subjected to centrifugation of 10,000 RPM for 3 min (Hettich Rotanta 46 RSC, Switzerland) for separation of liquid phase and solid residue. The liquid phase was poured out of the tube with the solid phase remaining in the tube. The solid residue collected after centrifugation was dried in the oven at 40°C until constant weight. This residue is termed as *biochar*. The liquid phase was subjected to bio-oil extraction process using diethyl ether (DEE). Liquid phase was subjected to three consequent extractions using equal amount of DEE in each one. DEE soluble phase was filtered and then the solvent was evaporated using rotary evaporator at 37°C with reduced pressure. The remaining oil phase after evaporation is termed as *bio-oil*. The water-soluble phase is termed as the *aqueous phase.*

Mass balance:

The weight of biochar was measured after drying it in the oven at 40°C until constant weight. For aqueous phase, 5ml was taken from the it and dried in the oven at 40°C until constant weight so that all water content is removed. The weight of solids obtained was multiplied by the volume of total aqueous phase collected to calculate the total water-soluble solids. The presence of gas content was confirmed by residual pressure of 3 bar after the reactor was cooled. The gas content was estimated from previous literature with similar conditions of HTL on macroalgae.

The bio-oil and biochar yield reported in this work is calculated using the following equations:

$$bio-oil\ yield(\%) = \frac{w_{bio-oil}}{daf w_{Ulva}} \quad (2)$$

$$biochar\ yield(\%) = \frac{w_{biochar}}{dw_{Ulva}} \quad (3)$$

In case of experiments with seawater(SW), the biochar yield was calculated using:

$$biochar\ yield(\%) = \frac{w_{biochar}}{dw_{Ulva} + w_{SWsalt}} \quad (4)$$

where dw_{Ulva} = wt. of *Ulva* feedstock used – moisture content, daf_{Ulva} (dry ash free weight) = dw_{Ulva} – ash content, $w_{bio\text{-}oil}$ = wt. of extracted bio-oil, $w_{biochar}$ = wt. of dried biochar, and w_{SWsalt} = wt. of salt in the seawater used.

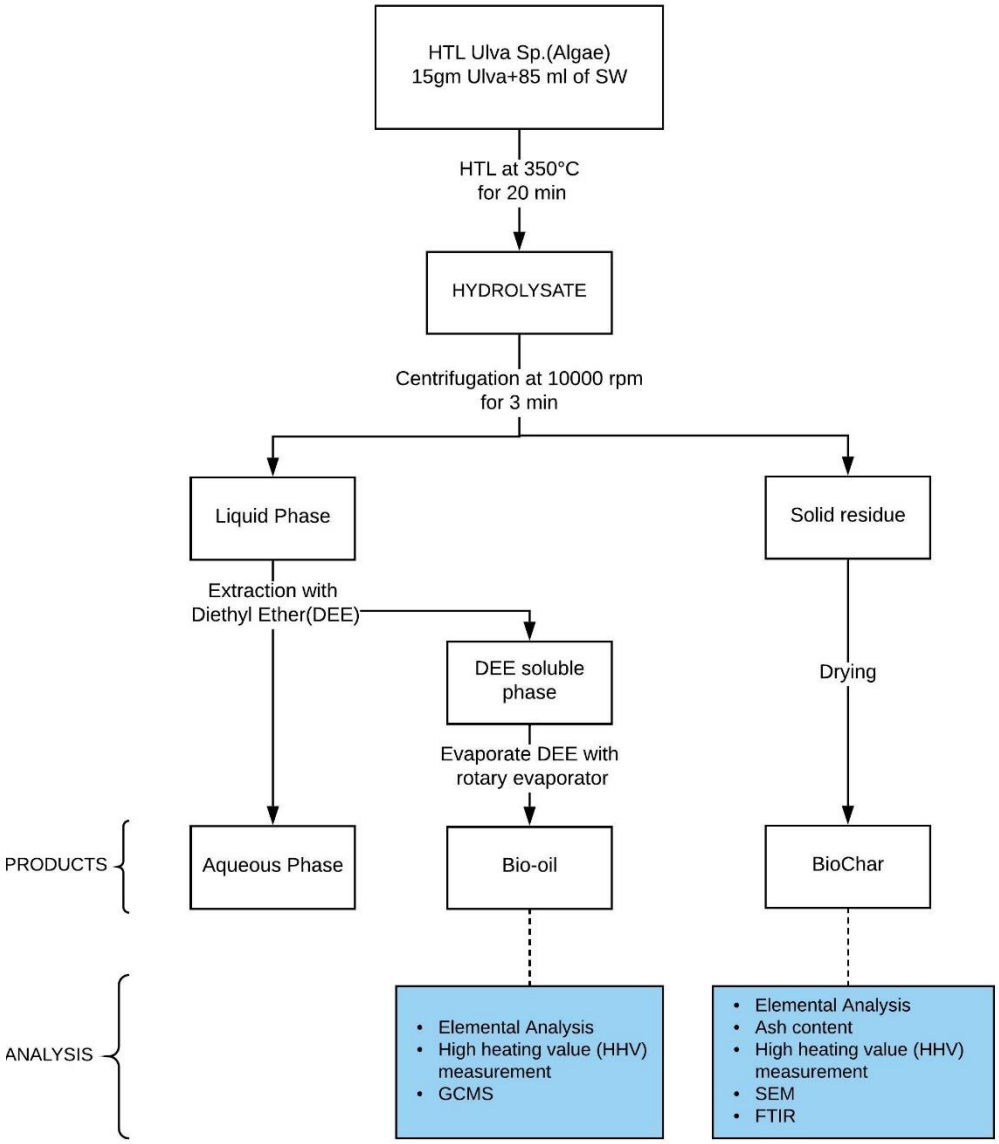


Figure 8: Flowchart of HTL products preparation, separation and analysis conducted in this case study.

Severity factor : Severity factor was introduced by Overend et al to combine the effect of temperature and retention time into one value thereby making comparison of severity of conditions easier. [31] It was calculated for all the experiments of *Ulva* case study using the following equation:

$$\log R_0 = \log \int_0^t \exp\left(\frac{T-100}{14.75}\right) dt \quad (5)$$

where T is the temperature of the reactor in °C and t is retention time in minutes.

3.3. **Analytical**

These analyses were conducted in both the case studies:

3.3.1. Elemental Analysis

Elemental analysis (CHNS) was done at the Technion, Chemical, and Surface Analysis Laboratory using Thermo Scientific CHNS Analyzer (Flash2000)**.** The oxygen content was determined by difference using the following equation:

$$O = 100 - (C + H + N + S + A) \quad (6)$$

where C, H, N, S, O, A are the weight percentage of carbon, hydrogen, nitrogen, sulfur, oxygen and ash respectably.

3.3.2. Fourier-transform infrared spectroscopy (FTIR)

FT-IR spectroscopy analyses were performed on a Bruker Tensor 27 FT-IR spectrophotometer, equipped with standard Pike ATR attachment. FT-IR spectra of the samples were measured in the spectral range of 4000-400 cm^{-1} (at 4 cm^{-1} resolution).

3.3.3. Higher heating value

Higher heating values of the bio-oil and biochar samples were obtained using a Parr 6200 bomb calorimeter with a 1104 oxygen bomb under oxygen (30 atm) environment; the samples were ignited using an electric fuse wire. The analysis was conducted by a certified laboratory of TAU.

Following analyses were conducted only for case-study 1: animal fat waste:

3.3.4. Density of bio-oil

The density was measured using DMA 35 Portable density meter. Each sample was measured 2 times and mean of the values was calculated. The value reported here is the mean of the duplicates.

3.3.5. Thermogravimetric analysis (TGA)

TGA measurements were performed on a Netzsch Simultaneous Thermal Analyzer (STA) 449 F5 Jupiter, under nitrogen flow, at a heating rate of 10 °C/min, in aluminum crucibles with a pinhole. Analyzed sample mass was 2-3 mg.

3.3.6. Fuel properties tested.

The bio-oil produced by HTL of animal fat was sent to Intertek (Schweiz) AG in Switzerland to check fuel properties like viscosity, total acid number (TAN), moisture content, caloric value and trace elements. They were measured by standard test methods – ASTM664 for TAN, ASTM D7042 mod. for viscosity, DIN 51777/1 for water (TF) content, DIN 51900 for Calorific Value and trace elements through ICP-OES.

Following analyses were conducted only for case-study 2: macroalgae:

3.3.7. Ash and moisture analysis

For ash and moisture analysis, the samples - untreated feedstock and biochar were dried at 40ºC to constant weight and analyzed. Ash and moisture content were analyzed according to D5142 standard. The analysis was conducted by a certified laboratory of TAU.

3.3.8. Scanning Electron Microscopy(SEM)

Dry *Ulva sp.* biomass and biochar were studied for their morphology by using light microscope and transmission electron microscopy. Surface morphology was observed using scanning electron microscopy (SEM). SEM image was obtained using Quanta 200 FEG ESEM, Oregon, USA after fixing the sample on silicon tape and then coating with gold using sputter coater.

3.3.9. Gas Chromatography Mass Spectrometry (GCMS)

The bio-oil products from HTL of macro-algae case study were analyzed by GCMS using Agilent 5975 Electron Ionization Gas Chromatography Mass Spectrometry (Agilent Technologies, Santa Clara CA). The column used for separation was 122-5532UI: 1DB-5ms Ultra Inert with dimensions 30 m length, 250 μm inner diameter and 0.25 μm film thickness. Helium was supplied at a flow rate of 1.2 ml/min. Samples of 1 μL were injected at 220ºC injector temperature with a split ratio of 9:1. Initially, the samples were at 80C for 1 min in GC oven, and then the temperature was increased to 310C at 10C/min and held there for 3 min. The mass spectral range used was 50-500 amu at normal scanning frequency. The compounds presented in this work had a match quality above 80% in accordance with the NIST (National Institute of Standards and Technology) library of mass spectra.

3.3.10. Values and graphs reported in this work

As each HTL experiment requires a time period of at least two days, only duplicates were conducted for each condition. The yields are reported for all the experiments conducted. Wherever a comparison is done in the study, mean value of the duplicates is reported with standard error.

All the graphs presented in the study were prepared using Microsoft Excel 365Pro.

4. Results & Discussion

4.1. **Case Study 1: Using animal fat (AF) waste**

4.1.1. Description of products of HTL

Figure 9(a) illustrates how the tube looked after centrifugation. Three phases can be distinctly observed in Figure 9 (b), (c) & (d) – bio-oil, aqueous phase and semi-solid phase. The bio-oil is a viscous light to dark brown colored oil. Semi-solid phase may be partially converted or unconverted animal fat as its texture is similar to the untreated feedstock. Aqueous phase contains water-soluble organics, thus is visually clear transparent liquid. Table 4 shows the elemental composition of the untreated AF feedstock.

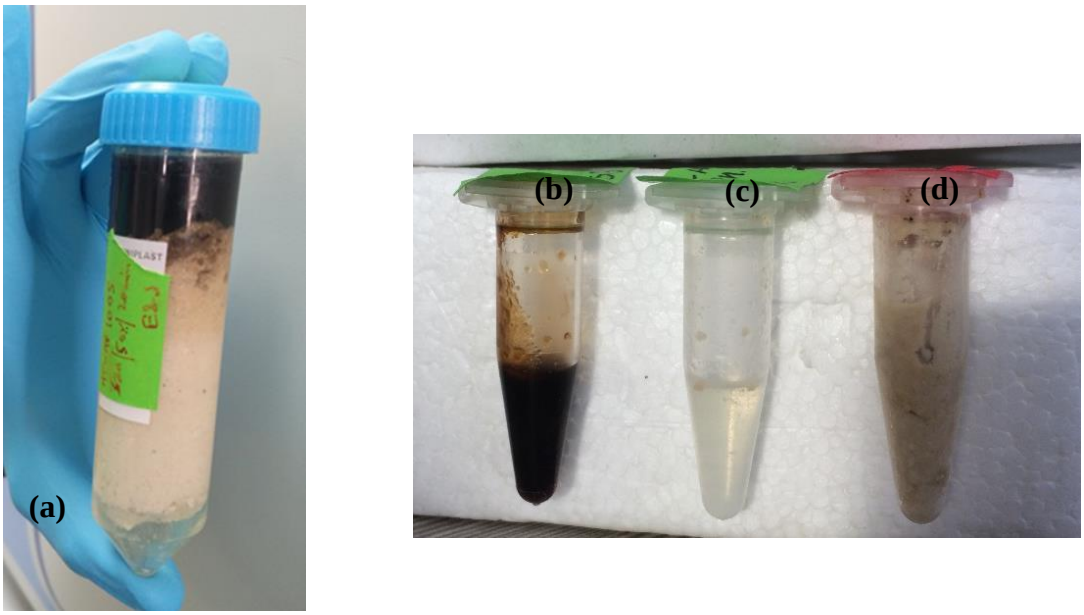


Figure 9: Products from HTL of animal fat: (a) Tube of HTL AF after centrifugation with three layers (b) bio-oil (c) aqueous phase (d) semi-solid phase

Table 4: Elemental analysis and measured HHV value of untreated AF feedstock.

Sample Name	N	C	H	S	O[1]	HHV (MJ/kg)
AF Feedstock	0	75.75	11.99	0	12.25	39.58 ± 0.23

1 – determined by difference

4.1.2. **Effect of HTL process parameters on bio-oil**

The experiments shown in Table 5 were conducted with the 50% SL at 330°C temperature by using different RT of 20, 40, 60, 80 and 120 min. It can be seen that the highest bio-oil yield of 26.5±1.5% was obtained at shortest RT of 20 minutes and lowest yield of 9±1% was observed at longest RT of 120 minutes. A decreasing trend of bio-oil yield can be observed as longer retention time is used. This decreasing trend is shown in Figure 10 where bio-oil yield is plotted against RT. In a recent work on HTL of animal by-products, Leon et al observed a similar trend of reduction in bio-crude yield after 5 min at 290°C.[49] Qu et al also observed a similar trend of the drop after 10 min RT at all temperatures tested during HTL of *Cunning* lanceolata. [67] This trend has also been observed by different studies on HTL of other biomass however the fall in the bio-oil yield happens at higher RT and varies with biomass and conditions.[50], [60], [68], [69] The decrease in bio-oil yield after reaching a maximum is attributed to further degradation to other fractions at longer RT.[50][70]

Table 5: HHV, yield% and density of bio-oils generated at varying RT with temperature at 330°C and 50% SL.

Experiment	Temperature (°C)	Solid Load(SL) (%)	Retention time(RT) (min.)	High heating value (MJ/kg)	Bio-oil yield (%)	Density of Bio-oil (g/cm^3)
1	330	50	20	38.67	28	0.898
2	330	50	20	38.44	25	0.898
3	330	50	40	38.91	23	0.988
4	330	50	40	38.77	20	0.898
5	330	50	60	38.70	14	0.899
6	330	50	60	37.90	19	0.900
7	330	50	80	38.25	8	0.898
8	330	50	80	38.35	11	0.898
9	330	50	120	36.45	8	0.897
10	330	50	120	38.241	10	0.896

It can be observed that high heating value of all the bio-oil samples lie between 36.45 – 38.84 MJ/kg. This HHV is high as compared to bio-crude produced by HTL from other biomasses like

27-30 MJ/kg from Chinese fir [67] , 30-36 MJ/kg from microalgae Desmodesmus sp. [71], 29-32 MJ/kg from macro-algae [72].

The results in Table 5 indicate that density of the bio-oil is not affected significantly by the increase of retention time. The density of all the bio-oil samples from 50% solid load lie between 0.896 – 0.900 g/cm^3 . Density values of bio-oil are not usually reported in HTL studies.

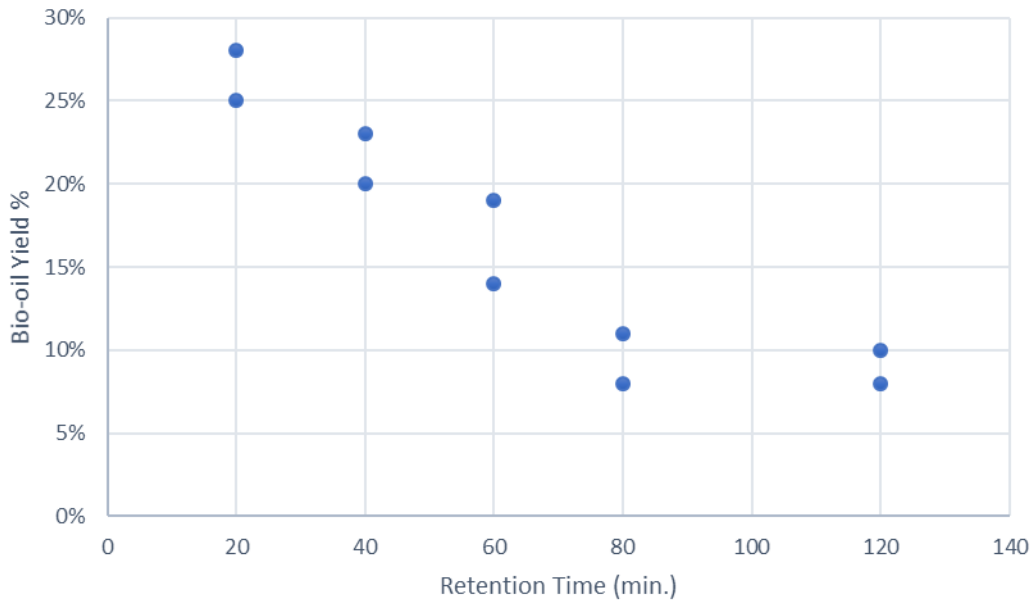


Figure 10: Bio-oil yield vs retention time for 330°C temperature and 50% solid load.

The Table 6 shows bio-oil yield, high heating value of bio-oil and density from changing the solid load of feedstock. In these experiments, temperature of 330°C was used with RT of 60 min and 3 different SL were tested – 25%, 50% & 75%. It is interesting to note that there was no bio-oil observed in 25% SL hydrolysate. This might have happened due to decomposition of oil phase to other fractions as the RT was 60 min. and the amount of feedstock to be converted was less. In accordance to the decreasing trend of bio-oil yield with retention time, it is possible that the yield of bio-oil became so low that it could not be extracted.

The bio-oil yield increased from 16±2.5% to 26±1% when the solid load was increased from 50% to 75%. The bio-oil from 75% SL experiment also showed a relatively higher density of 0.905 g/cm^3 in comparison to all the other bio-oil samples. High heating value saw a slight increase from 38.30±0.4 MJ/kg at 50% SL to 38.77±.09 MJ/kg at 75% SL. The 75% SL bio-oil's yield of 26±1%

was close to the highest yield observed in all experiments of 26.5±1.5% from 50%SL and 20 min. RT. Such high SL of 75% is not used in HTL studies due to issues of slurry pumping that might be faced later in a continuous process.[30] However, it is possible to pump animal fat in such cases due to its low melting point.

Table 6: HHV, yield% and density of bio-oils generated at varying solid loads with temperature at 330°C and 60 min. RT.

Experiment #	Temperature (°C)	Solid Load(SL) (%)	Retention time(RT) (min.)	High heating Value (MJ/kg)	Bio-oil yield (%)	Density (g/cm^3)
11,12	330	25	60	-	-[2]	-
5	330	50	60	38.70	14	0.899
6	330	50	60	37.90	19	0.900
13	330	75	60	38.77	27	0.905
14	330	75	60	38.60	25	0.904

[2]- there was no extractable bio-oil in the hydrolysate from 25% SL

4.1.3. **Composition of bio-oil**

The feedstock and bio-oils from various conditions were analyzed using DTG, FTIR and GC-MS for understanding the composition of the bio-oil and the changes that occurred due to the HTL process.

4.1.3.1. TGA/DTG Analysis

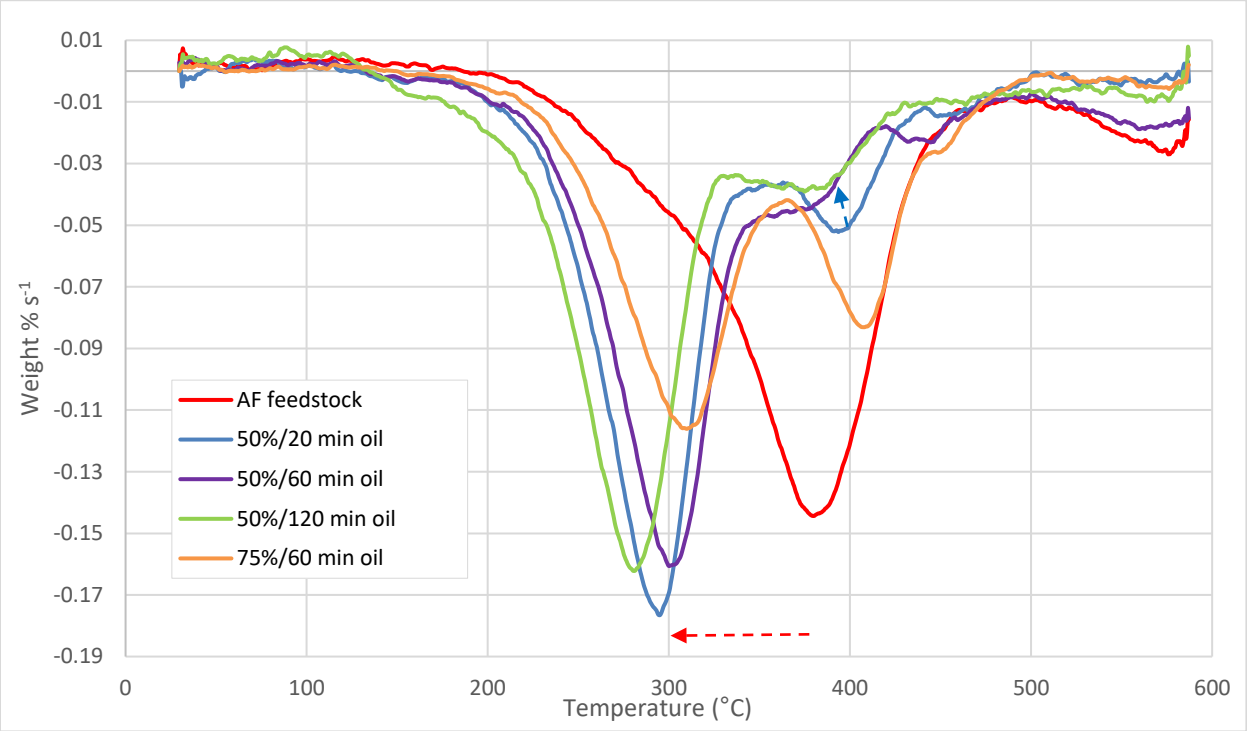
Figure 11(a) shows the DTG and TGA curves of AF feedstock and bio-oils from various conditions. The thermal decomposition of the feedstock seems to start around 150°C and gradually increases till 300°C. This is followed by a steeper curve as it approaches 400°C and this is the region of maximum decomposition. There also exists a small shoulder of degradation above 500°C. The DTG curve of the AF feedstock follows similar curve as rendering fat presented by other authors, although the small shoulder is lacking in their curve, main decomposition is seen around 400°C.[49], [73] Leon et al (2019) described the big peak near 400°C as decomposition of triglycerides and peak around 300°C corresponding to lighter fraction composed of free fatty

acids.[49] Similar conclusion was shared by Melzer et al(2013) as they conducted TGA on shear butter and jatropha oil which are also mainly triglycerides.[73]

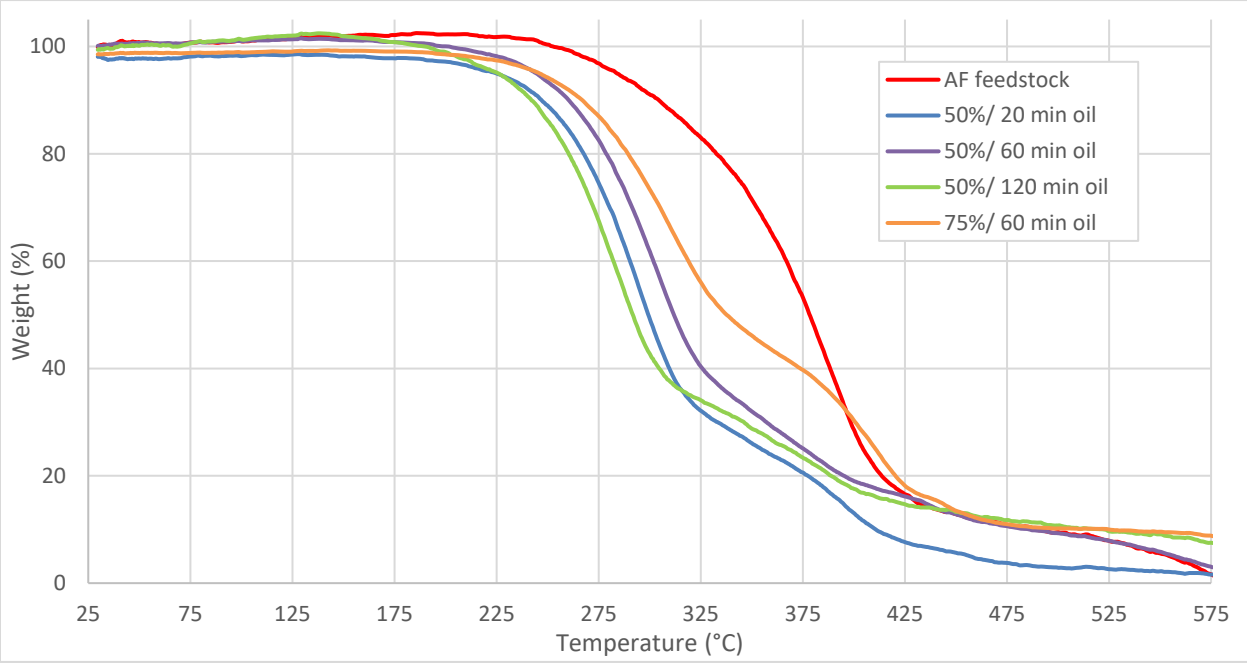
It can be observed from DTG curves of the bio-oils that their peaks have moved to lower temperature around 300°C in comparison to the curve of AF feedstock as shown by the red arrow in the Figure 11(a). As mentioned above, these lower temperature peaks represent lighter fractions. This clearly shows the transformation of triglycerides to lighter compounds due to the HTL process. Leon et al (2019) also shares this observation as their biocrude DTG curve at 290°C is similar to our 50% SL curves at 330°C. [49]

This observation is highlighted when we look at the curve for 75% SL at 60 min. In this case, it can be observed that percentage of conversion from triglycerides to free fatty acids is lower as the 2 major peaks are divided in approximately 60:40 ratio between near 300°C and 400°C. This can be due the fact that high mass of feedstock requires more RT which is more severe conditions for complete conversion. When we compare it to lower SL of 50% with the same RT of 60 min., the peak around 400°C decreases and a significantly bigger peak can be seen around 300°C, thereby showing a better conversion to lighter fraction for lower SL. Similar shift in peaks was observed by Leon et al (2019) when they increased the temperature from 250 to 290°C keeping the RT same. [49] They observed this trend by increasing the temperature and we observed it by increasing the RT, however both these parameters increase the severity of the conditions as indicated by the equation for severity factor given by Overend et al.[31]

The TGA curves in Figure 11 (b) also highlight the differences mentioned above in decomposition among AF feedstock and different bio-oils.



(a)



(b)

Figure 11: (a) DTG and (b) TGA curves of untreated AF feedstock and its bio-oils obtained at different conditions.

4.1.3.2. **FTIR Analysis**

FTIR spectra provides a comprehensive idea about functional groups of compounds present in the compound. Figure 12 shows the FTIR spectra of the animal fat feedstock in comparison to bio-oils from different parameter conditions. It is clear from the graph that all the bio-oils follow the same trendline and their curves are overlapped. However, in comparison to the feedstock, there are significant changes from feedstock spectra to the bio-oils. The bio-oil peaks have increased transmittance in contrast to feedstock at four instances – 3007 cm^{-1}, 2914 cm^{-1}, 2851 $cm^{-1}$and 719 cm^{-1}. The peaks at these wavenumbers represent: 3007 cm^{-1}: C–H aromatic rings; 2914 & 2851 cm^{-1} : Methylene C-H symmetric and asymmetric stretches indicating presence of C-H alkanes and 719 cm^{-1} : C–H alkynes and phenyl groups. Higher transmittance signifies lower absorption which means the bonds in this region have reduced in the bio-oils.

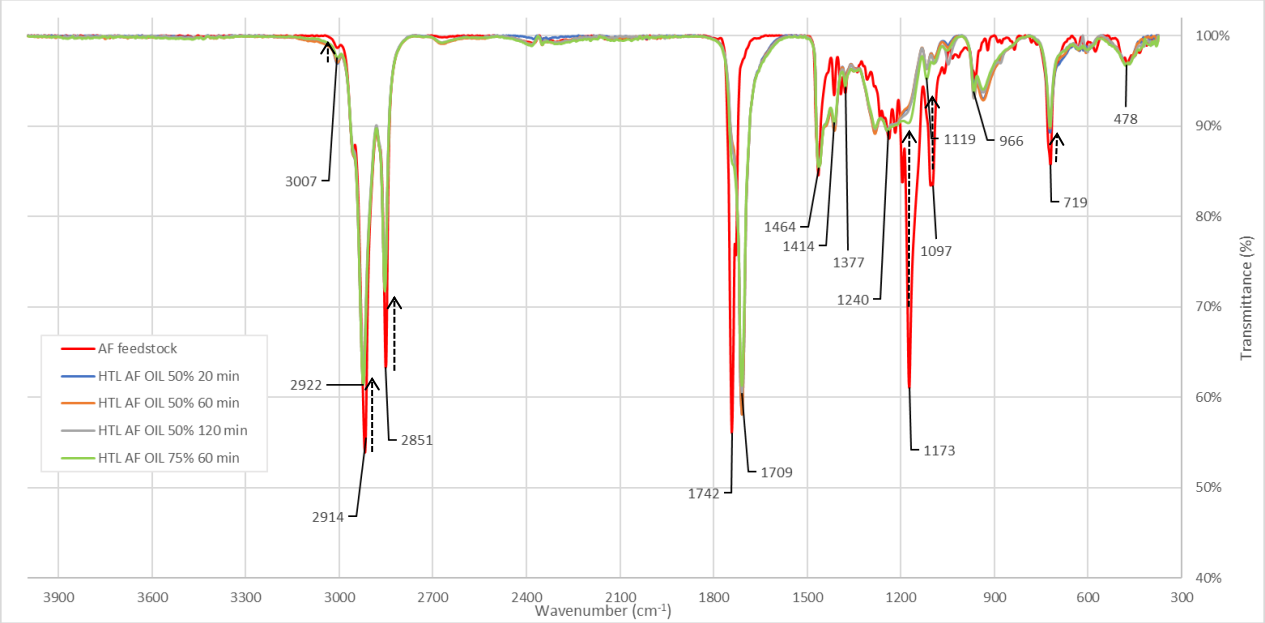


Figure 12: FTIR spectra of untreated feedstock and its HTL bio-oils obtained at different conditions.

The major difference is seen in the range of 1200-1050 cm^{-1} where two sharp peaks of feedstock do not exist for the bio-oils. These two peaks – 1173 and 1097 cm^{-1} represent alcohols or phenols. The other peaks that reveal data about the bio-oils are: 1709 cm^{-1} : C=O stretching vibrations indicate presence of esters and acids; 966, 1119 and 1240 cm^{-1} : C–O like acids and ethers. The FTIR spectra of the bio-oils presented here is similar to the spectra published by Yang et al (2019) for biocrude from HTL of pork, except for the peaks for N-H and C-N as our bio-oil lacks N. [50]

4.1.3.3. **Bio-oil analysis by GCMS**

The GCMS of the bio-oil was conducted by Intertek (Schweiz) AG and the results containing major compounds in the bio-oil are presented in Table 7 . The mixture of compounds includes fatty acids methyl and ethyl esters, long chain fatty acids and some hydrocarbons. The fatty acids can be explained by the breakdown of triglycerides of the animal fat. HTL of triglycerides produces fatty acids in the oil phase and glycerol in the water-soluble phase.[7], [14], [74], [75] This reaction occurs rapidly with temperatures above 280C [45]. Although fatty acids are relatively stable up to 300C, Denial et al pointed that some fatty acids can generate hydrocarbons like alkanes and alkenes through decarboxylation reaction.[7] This can explain the presence of the two hydrocarbons in the list. Posmanik et al also highlights this fact that high temperature HTL of lipid-rich biomass like meat waste will contain mostly fatty acids and long chain hydrocarbons. [75]

The list of compounds is similar to previous studies dealing with meat waste of different kinds or high lipid rich biomass. The major difference is lack of amides, N-heterocyclic and any nitrogen containing compounds in our bio-oil due to the lack of N in the biomass. [49], [50], [76] The presence of N compounds is a pressing concern for biocrudes generated from HTL of biomass containing protein. Biocrudes with high nitrogen levels require refining to comply with NOx emission regulations which increases their energy demand. [77] Thus, lack of nitrogen compounds in the AF bio-oil is a crucial environmental advantage.

The decomposition of glycerol in near-critical water hydrolysis has been reported to generate methanol, ethanol and allyl alcohol among other products. [78] When these alcohol groups interact with fatty acids of the triglycerides, esters of the fatty acids may be formed. This can explain the presence of fatty acid esters in the bio-oil. The abundance of fatty acids, its esters and amides in the HTL bio-crude of lipid-rich biomass has been reported before[79] but the mechanism of formation of methyl and ethyl esters is not mentioned.

Table 7: Major compounds identified using GCMS in the bio-oil from 330 °C, 60 min. and 50% solid load.

RT [min]	Formula	Name of component	Probability [%]
18.08	$C_{15}H_{30}O_2$	Tetradecanoic acid methyl ester	92.58
25.33	$C_{17}H_{34}O_2$	Hexadecanoic acid methyl ester	75.94

27.06	$C_{18}H_{36}O_2$	Hexadecanoic acid ethyl ester	87.43
27.82	$C_{10}H_{20}O_2$	Decanoic Acid	75.35
28.49	$C_{18}H_{34}O_2$	Hexadec-9(e)-enoic acid ethyl ester	72.6
34.63	$C_{19}H_{38}O_2$	Octadecanoic acid methyl ester	48.95
35.63	$C_{19}H_{36}O_2$	9-Octadecenoic acid methyl ester	14.52
36.01	$C_{20}H_{38}O_2$	9-Octadecenoic acid ethyl ester	74.12
36.97	$C_{20}H_{38}O_2$	Oleic acid ethyl ester	51.85
42.49	$C_{14}H_{28}O_2$	Tetradecanoic acid	45.34
44.55	$C_{14}H_{28}O_2$	Tetradecanoic acid	88.32
46.00	$C_{27}H_{44}$	Cholesta-7,14-diene	50.78
46.33	$C_{15}H_{30}O$	Z-9-Pentadecenol	20.66
46.49	$C_{15}H_{32}$	Pentadecane	84.89
47.28	$C_{16}H_{32}O_2$	12-Methyltetradecanoic acid methyl ester	70.87
48.67	$C_{15}H_{30}O_2$	Pentadecanoic acid	88.73
51.22	$C_{32}H_{62}O_3$	Palmitic anhydride	46.19
51.5	$C_{18}H_{24}O$	Estra-1,3,5(10)-trien-17β-ol	51.56

4.1.4. **Comparison with fuels**

The bio-oil was sent to Intertek (Schweiz) AG in Switzerland to check fuel properties like viscosity, total acid number (TAN), moisture content, caloric value and trace elements. The results from the report are presented here.

Table 8 shows the elemental composition of the AF bio-oil and comparison of the fuel properties of the AF bio-oil that was produced in this case study with two different quality of marine fuels – Distillate FAME grade B (DFB) and Residual Marine A30 (RMA 30). The comparison is made with marine fuels as the idea is to use this bio-oil directly or with least further processing. The specifications required for marine engines have a wider scope of restrictions than petroleum products for road vehicles. The high heating value of AF bio-oil is 90-95% of the grade of marine fuels mentioned here, thereby making it compatible to be blended the marine fuels mentioned. The viscosity and TAN are higher than permissible limit for DFB but well within the range for RMA 30. Tyrovola et al highlights the requirement of low sulphur content in the future of marine fuels

which is planned to be 0.5% from January 1, 2020. [80] As the sulphur content in the animal fat feedstock is very low, the %S in the bio-oil is merely 0.016%. This makes this bio-oil suitable for marine fuels now and in the future.

FAME in marine fuels were earlier restricted to 0.1% v/v as per EN ISO 8217:2012 but the standard in 2017 increased this limit to 7% in specific marine distillate grades(DF), thereby allowing blending of FAME containing biofuels to these specific distillates.[80] Our bio-oil does have a certain quantity of FAME as can be seen in the GCMS results; however it was not quantified.

Considering the comparison made above, properties of the AF bio-oil like viscosity, density, HHV, sulphur content and TAN are well within the range for blending with RMA 30 however it is not compatible with DFB due to higher viscosity and TAN. One of the issues is the high moisture content in the bio-oil which would require further processing of the bio-oil to make it suitable for marine uses.

Table 8: Elemental composition of AF bio-oil + Comparison of fuel properties of DFB, RMA 30 & AF bio-oil from this case study.

Sample	N	C	H	S	O[1]	HHV (MJ/kg)
AF bio-oil 330°C, 50% SL, 20 min RT	0	74.16	11.88	0	13.96	38.56 ± 0.12

[1] – determined by difference

Properties (Max. limit)	**DFB*** [81]	**RMA 30*** [81]	**AF Bio-oil**
Viscosity (mm^2/s)	11 (at 40°C)	30 (at 50°C)	21.69 (at 50°C)
Density at 15°C (kg/m^3)	900.0	960.0	896.0 – 899.0
Sulphur(% m/m)	1.50	-[1]	0.016
Water(% V/V)	0.30	0.50	6
Acid Number (TAN)(mgKOH/g)	0.5	2.5	1.46
Fatty acid methyl ester(FAME)	7.0	-[2]	-[3]
Heating Value (MJ/kg) [82]	42	40	37-38.84

** - The properties mentioned for DFB and RMA 30 are maximum permissible limits for the fuels. Values for the bio-oil are measured values.*

[1] – as requested by supplier; [2] – not mentioned; [3] – not quantified.

4.2. Case study 2: Using marine biomass – macroalgae (*Ulva* sp.)

4.2.1. Description of products of HTL

Figure 13 shows the actual pictures of the macroalgae used and the products generated after the HTL treatment. The macroalgae presented in the Figure 13(a) is the dried form as it was used in all the experiments conducted in this case study. The HTL process generated a liquid and solid phase which was converted to three products after extraction – bio-oil, biochar and organic rich aqueous phase. As it can be observed from the Figure 13(b), the energy dense bio-oil is a dark brown highly viscous oil. The biochar showed in Figure 13(c) is the final product after drying at 40C for 24 hours. It is a porous and crystallized char-like material. The aqueous phase mostly contains water with water-soluble organics as shown in Figure 13(d). The aqueous phase was subjected to drying at 40C for 24 hours to find the water-soluble solids in it which is shown in Figure 13(e).

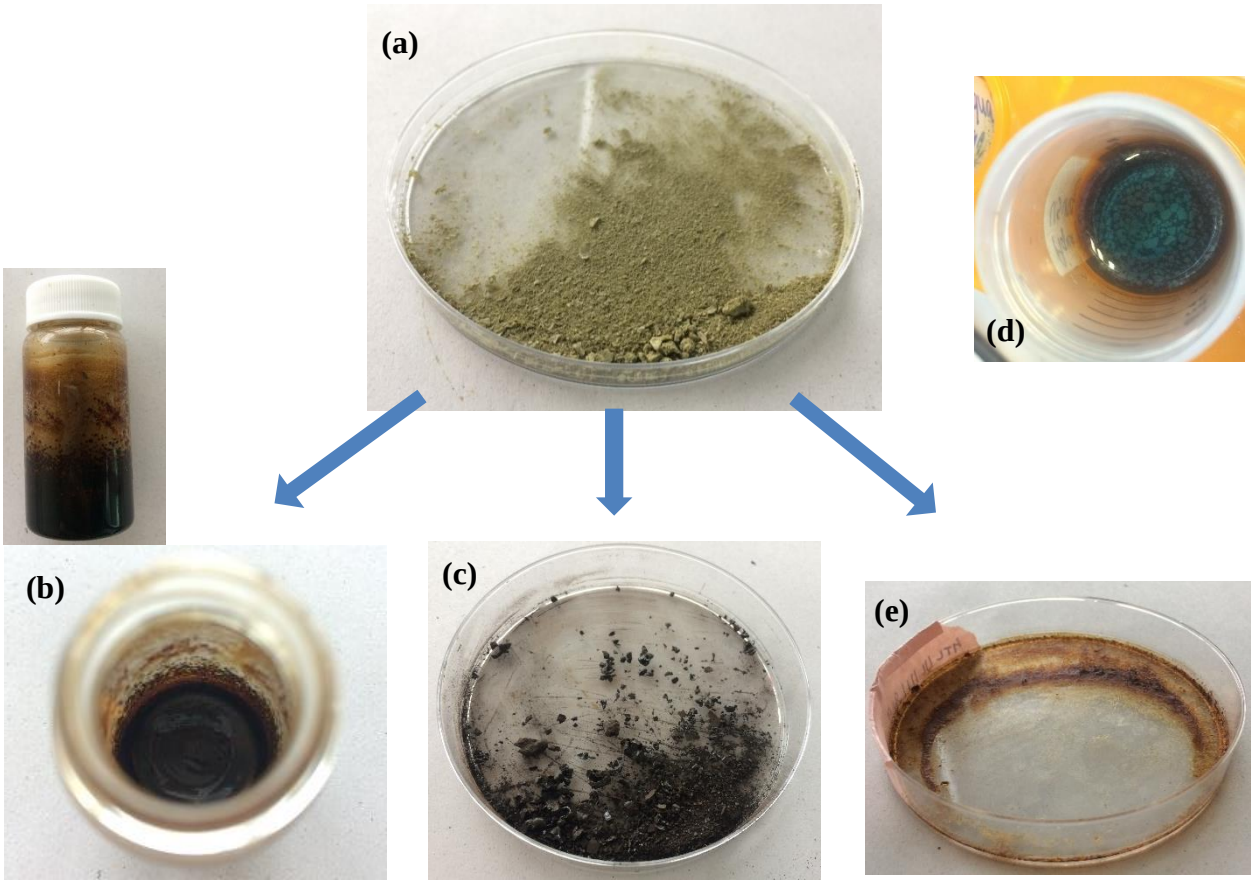


Figure 13: Pictures of Ulva sp. feedstock and its HTL products while experimentation: (a) dry Ulva Sp. feedstock (b) extracted bio-oil (c) dried biochar (d) aqueous phase (e) dried aqueous phase for mass balance.

The experiments were conducted using two harvest batches of *Ulva* sp. termed here as feedstock A and feedstock B. Table 9 shows the elemental analysis and ash content of these feedstocks. It can be noticed that the elemental composition for both the feedstocks is quite different even though being from the same genus. This can be due to different harvest time. Such variation in elemental composition can also be observed in Table 2 where same genus is used by different researchers (like *Enteromorpha prolifera* in [55], [59] and [60]) and they have significantly different compositions.

Table 9: Elemental Analysis and ash content of Ulva feedstocks.

Sample Name	**N**	**C**	**H**	**S**	**O[1]**	**Ash**	**Moisture content**
UL Feedstock A	7.96	33.4	5.73	4.1	31.51	17.28	14 – 15%
UL Feedstock B	1.45	24.90	4.89	6.73	25.28	36.74	14 – 15 %

All values presented in this table are percentages, [1] – O is calculated by O = 100 – (C + H + N + S + Ash).

4.2.2. **Effect of severity of HTL conditions on bio-oil yield**

Experimental conditions of 1 & 2 were conducted in the 1L reactor and the rest of the experiments were conducted in the 0.25L reactor. The parameter conditions of HTL and their yields of bio-oil and biochar are presented in Table 10. Among the conditions tested, the highest bio-oil yield of 7.93±0.85% is observed for experiment #3 with severity factor of 6.59 whose parameters were 290°C, 10 min RT and 14% SL. The other two conditions tested at 270°C and 350°C had a lower yield of 3.16% and 4.70% respectively.

The conditions with the maximum yield was adopted from a recent study on HTL of *Ulva* prolifera, however they had a higher bio-oil yield of 12%. [60] This can be due to difference in the feedstock as their carbon content(46.2%) is higher and ash content(7%) significantly lower. The higher carbon content increases the bio-oil as carbon is the major component of all the compounds in the bio-oil. The carbon content in our feedstock is much lower 25 – 34%, which means proportionally there should be less bio-oil yield as expected because carbon converts to the bio-oi. Another reason can be difference in extraction protocol. In this study [60], the separation protocol is not mentioned clearly as the amount of solvent used and number of extractions performed is missing. Such information is important as then only a proper quantitative comparison can be made. Due to different extraction protocol followed, there can be additional oil left in the aqueous phase or

biochar after the extraction. Such an observation was made in aqueous phase of the experiments at severity of 6.59. Even after three continuous extractions using diethyl ether, there was an oil layer seen at the top of the aqueous phase when it was kept in the oven for drying, as shown in Figure 13(d) . As we are working with small volumes, such loses can amount to huge differences in bio-oil yield.

Table 10: Parameters of experiments conducted in Ulva sp. case study.

Experiment #	Water used	Temperature (°C)	Solid Load(SL) (%)	Retention time (RT) (min.)	Severity Factor (log R_0)	Bio-oil yield(daf) (%)
1	3.8% SW	180	8%	30	3.83	-
2	3.8% SW	270	8%	30	6.48	3.16
3a	3.8% SW	290	14%	10	6.59	8.78
3b	3.8% SW	290	14%	10	6.59	7.08
4a	DW	290	14%	10	6.59	7.88
4b	DW	290	14%	10	6.59	8.05
5a[1]	3.8% SW	350	15%	20	8.66	4.70
5b[1]	3.8% SW	350	15%	20	8.66	4.70

[1] – 5a & 5b have same bio-oil yield because they were extracted together. daf – dry ash free

Singh et al [83] conducted HTL on three different macroalga with conditions close to our experiment – 280°C, 15 min and 14% SL. They used two solvents to extract the bio-oil from the HTL products – DEE and acetone and reported separate yields of both solvents. The stated bio-oil yields for the DEE extraction are between 4-6% which is similar to our yields [83]. This can also explain lower yields in comparison to other studies as they use different solvents like chloroform [84], dichloromethane(DCM) [29], [56] or even two solvents [83].

4.2.3. **Change in morphology of biochar through SEM**

The SEM images provide information of the changes in morphology of the feedstock after HTL treatment. Figure 14 presents the SEM images of the *Ulva* feedstocks and their biochar. In the images of the feedstock, the intricate structure of the surface of the macroalgae can be seen. After applying the hydrothermal treatment, these structures are disintegrated to small and porous elements. If the biochar at different temperatures are compared, it can be observed that the highest temperature (350°C) biochar has the highest disintegration.

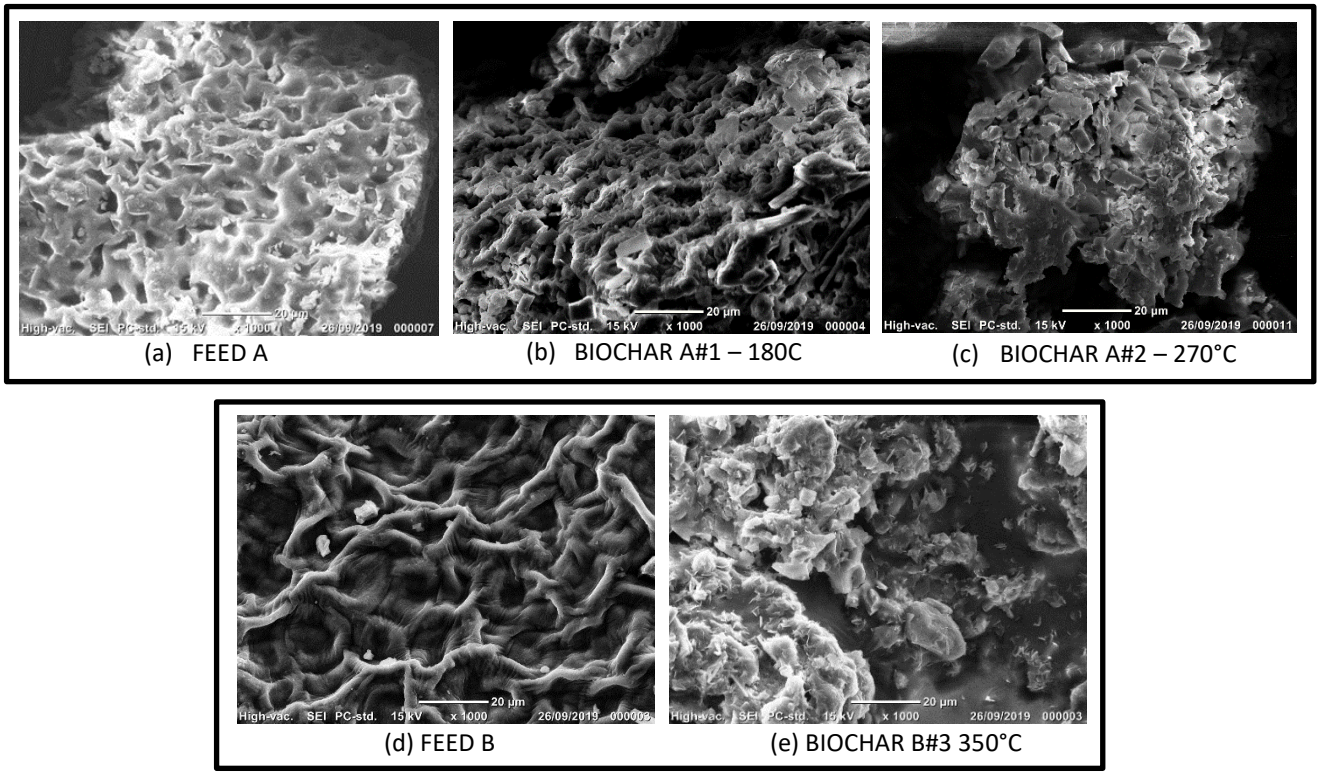


Figure 14: SEM images of two batches of feedstock - (a) & (d) and their biochars at temperatures - (b)180°C, (c)270°C & (e)350°C

4.2.4. **Analysis of biochar: FTIR**

FTIR spectra provides information about the functional groups present in the samples. The spectra of feedstocks and their biochar at different temperatures is presented in Figure 15. The difference in the composition of the two feedstocks can be noticed in Figure 15 as the red dashed line is feedstock A and red solid line is feedstock B. The peaks are shared by both feedstocks – A & B, however feedstock A has lower transmittance in most of the spectra – 3100-3500, 1300-1700, 900-1100 and 500-700 cm^{-1} thereby implying presence of more percentage of functional groups in these regions in A than B. Similar observation can be made about the spectra of biochar at various temperatures – 180, 270 and 350°C. The changes in composition due to difference in severity of the experiments is highlighted in the FTIR spectra.

The peaks between 600 – 900 cm^{-1} represent aromatic out of plane C-H bends which are shared by all biochar samples with varying intensity of the functional groups. The peak 872 cm^{-1} representing C=C of alkene is only present in the 350°C biochar. The peaks between 1020 – 1250 cm^{-1}, 1400 – 1600 cm^{-1} and 2840 – 3000 cm^{-1} are attributed to C-N stretch like amines, C-H bends of alkane and methylene C-H stretch of alkane group respectively. These peaks have higher intensity for 350°C condition in comparison to the other two thereby implying higher presence of these functional groups in 350°C biochar. This can be due to the higher severity of the reaction which led to a higher conversion to these functional groups.

The spectra of the feedstocks in the region 1550 – 1650 cm^{-1} have a weak peak which is attributed to C=C stretching of alkene group. If we focus on the spectra of biochar in the same region, this peak is split into two stronger peaks which is characteristic of amine group. The intensity of these two peaks is highest for 350°C biochar. The broad peaks between 3200 – 3550 cm^{-1} are attributed to OH groups which indicate potential presence of alcohols. [65] In case of biochar b#5 350C, this region seems to be a combination of two peaks – one similar to former representing alcohols and other one in 3400 – 3500 cm^{-1} for N-H stretch which signifies the presence of amines. This seems to be negligent in the other two biochar. This difference in peaks and intensity highlight the variation in conversion corresponding to severity of the conditions.

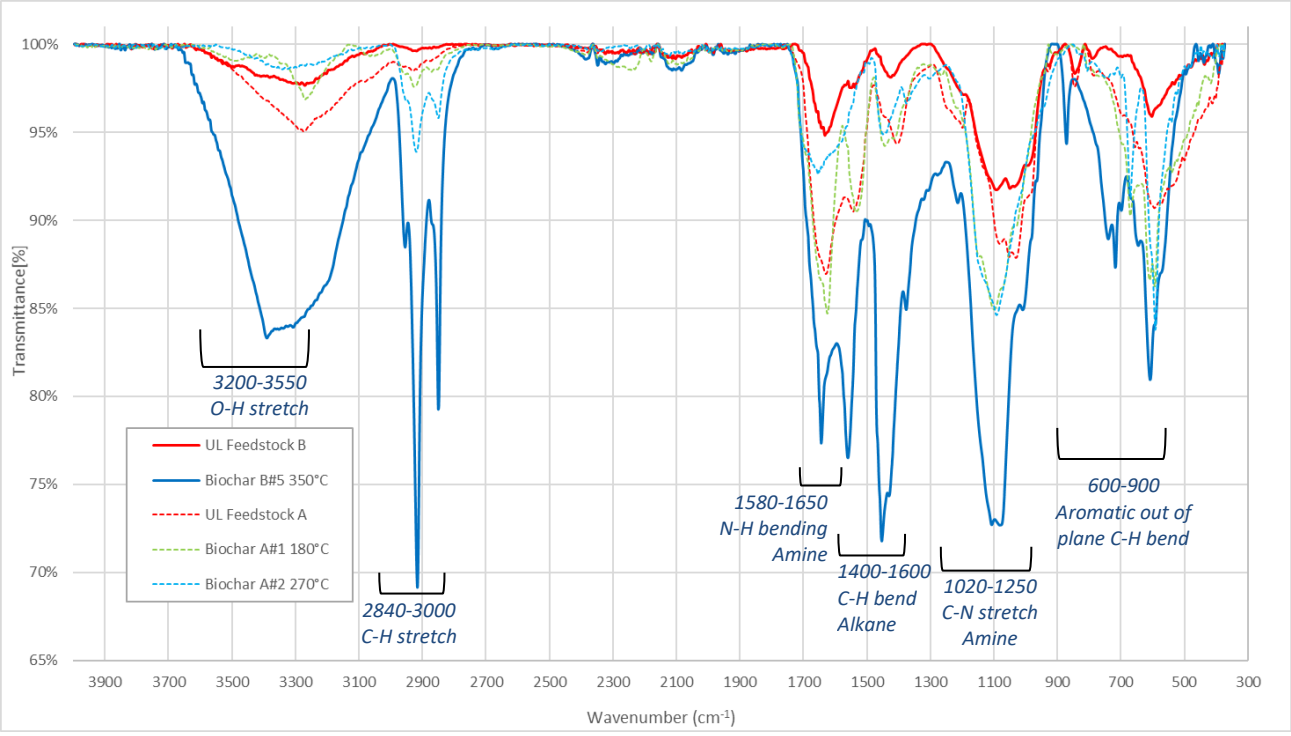


Figure 15: FTIR spectra of feedstocks A & B and their biochar - A#1 at 180°C, A#2 at 270°C and B#5 at 350°C, along with functional groups of different regions in the spectra are labelled.

4.2.5. **Analysis of bio-oil: GCMS**

The bio-oils extracted at three different temperatures – 180, 270 and 350 °C were subjected to GCMS to see the variation in the type of compounds that are attained. Figure 16 shows the chromatograms of these three bio-oils. There is significant change in the composition of compounds by varying the severity conditions. It can be observed that the density of the peaks shifts towards lower retention time of the GC column as the temperature is increased. In case of bio-oil of 180°C, majority of area of peaks lie after 10 min. However, for 270°C, the peaks do not go beyond 10 min and their majority peak area is between 3-7 min. Consequently, for the highest temperature tested – 350°C, there are negligible peaks after 6 min and the concentration of the peak area is within the first 4 minutes.

This can be attributed to the fact that depolymerization increases with more severe HTL conditions, thus shorter chain compounds are formed for the more severe conditions. The identified compounds in the bio-oil samples are grouped according to their carbon-chain size in Figure 17. These only include compounds where were matched with 70% or higher probability. It can be noticed that the high temperature of 350°C has highest shorter chain carbon C6-C10 compounds and least compounds above C16. Vice versa is true for bio-oil extracted from hydrothermal treatment at 180°C.

Table 11 shows the identified compounds and their molecular mass from the chromatographs in Figure 16 which had match probability of 70% or more with the NIST library. The bio-oils shown in the table contain ketones, aromatics, aldehydes, phenols, fatty acids, fatty acid amides, N-heterocyclic compounds and one fatty acid ester.

N-containing compounds which are formed from decomposition of proteins are present more in the higher temperatures of 270°C and 350°C in comparison to 180°C. This is due to the fact that proteins require more severe conditions to break down like for shorter RT of 5 min 200-225°C temperature is required and for less temperature of 170-200°C, longer RT of 60 min is needed start the breakdown. [85] The identified compound categories are similar to previously reported studies

on HTL of macroalgae [55], [60], [65], [86]. Also these compounds are from the categories present in the detailed pathway shown in Figure 4 from Barreiro et al. [29]

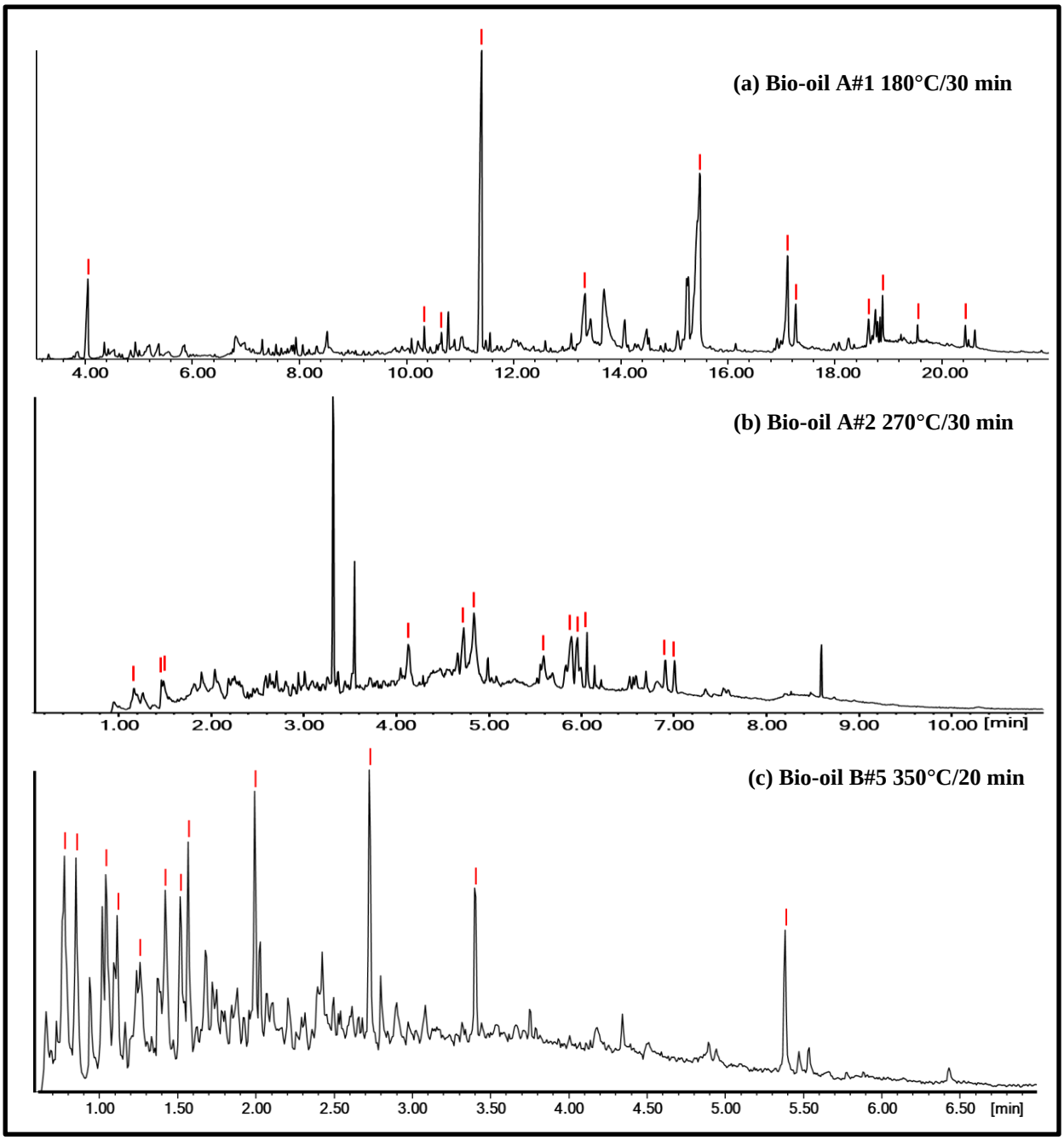


Figure 16: GCMS chromatograms of the bio-oils extracted after the HTL process with three different severity conditions – (a) 180°C for 30 min, (b) 270°C for 30 min and (3) 350°C for 20 min.

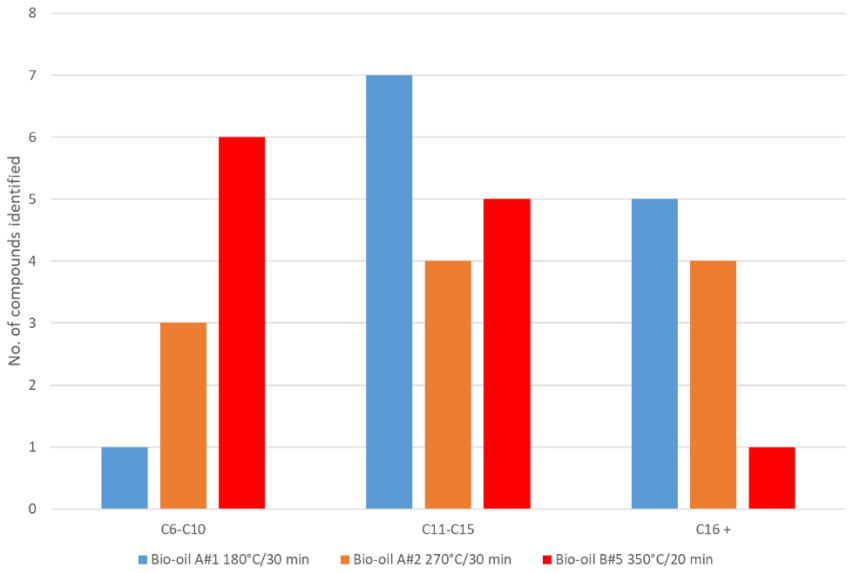


Figure 17: This chart shows number of compounds identified by GCMS for the three HTL conditions grouped according to carbon chain size of the compounds. The identified compounds were matched with 70% or more probability.

Table 11 : GCMS identified compounds the bio-oils extracted after the HTL process with three different severity conditions – (a) 180°C for 30 min, (b) 270°C for 30 min and (3) 350°C for 20 min.

(a) Bio-oil A#1 180°C/30 min			
RT(min)	Name	Formula	Mass
4.05	2-hydroxy-3-methyl-2-Cyclopenten-1-one	$C_6H_8O_2$	112
10.09	2,6,10-trimethyl-Tetradecane	$C_{17}H_{36}$	240
10.3	Butylated Hydroxytoluene	$C_{15}H_{24}O$	220.2
10.78	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180
11.4	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
13.3	Tetradecanoic acid	$C_{14}H_{28}O_2$	228
15.47	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	210
17.1	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282
17.26	Octadecanoic acid	$C_{18}H_{36}O_2$	284
18.63	Cyclo-(l-leucyl-l-phenylalanyl)	$C_{15}H_{20}N_2O_2$	260
18.88	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	$C_{14}H_{16}N_2O_2$	244
19.53	Docosanoic acid	$C_{22}H_{44}O_2$	340
20.61	3,6-bis(phenylmethyl)-2,5-Piperazinedione,	$C_{18}H_{18}N_2O_2$	294
(b) Bio-oil A#2 270°C/30 min			
RT(min)	Name	Formula	Mass`
1.164	2-ethyl-6-methyl-Pyrazine	$C_7H_{10}N_2$	122.1
1.464	2,6-diethyl-Pyrazine	$C_8H_{12}N_2$	136.1

1.486	2,5-dimethyl-3-ethyl-Pyrazine	$C_8H_{12}N_2$	136.1
4.137	2-(Diacetylmethyl)cyclooctanone oxime	$C_{13}H_{21}NO_3$	239.1
4.71	l-Valine, n-propargyloxycarbonyl-, decyl ester	$C_{19}H_{33}NO_4$	339.1
4.84	3-Aminocarbazole	$C_{12}H_{10}N_2$	182.1
5.59	3-benzyl-6-isopropyl-2,5-Piperazinedione	$C_{14}H_{18}N_2O_2$	246.1
5.88	cis,anti-4,5,6,6a,6b,7,8,12b-Octahydrobenzo(j)fluoranthene	$C_{20}H_{20}$	260.1
5.96	Cyclo-(l-leucyl-l-phenylalanyl)	$C_{15}H_{20}N_2O_2$	260.1
6.06	(Z)-9-Octadecenamide	$C_{18}H_{35}NO$	281.3
6.91	2-Benzyloxy-6-ethoxy-8-aminoquinoline	$C_{18}H_{18}N_2O_2$	294.1

(c) Bio-oil B#5 350°C/20 min

RT(min)	Name	Formula	Mass
0.778	2-ethyl-6-methyl-Pyrazine	$C_7H_{10}N_2$	122.1
0.852	2,3-dimethyl-2-Cyclopenten-1-one	$C_7H_{10}O$	110.1
1.046	3-ethyl-2,5-dimethyl-Pyrazine	$C_8H_{12}N_2$	136.1
1.098	1-Phenylcarbamoylmethyl-piperidine-4-carboxylic acid amide	$C_{14}H_{19}N_3O_2$	261.1
1.262	Pilocarpine	$C_{11}H_{16}N_2O_2$	208.1
1.425	2-Naphthuric acid	$C_{13}H_{11}NO_3$	229.1
1.52	(Z)-2,5-dimethyl-3-(1-propenyl)-Pyrazine	$C_9H_{12}N_2$	148.1
1.57	1-butyl-2-Pyrrolidinone	$C_8H_{15}NO$	141.1
1.996	Decahydroquinolin-10-ol	$C_9H_{17}NO$	155.1
2.729	Butylated Hydroxytoluene	$C_{15}H_{24}O$	220.2
3.404	[(2,4,6-triethylbenzoyl)thio]-Acetic acid	$C_{15}H_{20}O_3S$	280.1
5.383	(Z)-9-Octadecenamide	$C_{18}H_{35}NO$	281.3

4.2.6. **Mass Balance**

The mass recovery of four experiments are shown in Table 12. Bio-oil and biochar yields are calculated by measured dry weights. Solids in aqueous phase are extrapolated from measuring dried sample of 5 ml of the aqueous phase. The mass closure is within the range of previously reported by other studies 70-90%.[56] This mass balance does not include the gas phase. Various studies have used different approaches to measure or estimate gas phase. It can be estimated through ideal gas law using residual pressure however that requires the exact composition of gas phase. [56] Some studies calculate it by subtracting all other products from 100% but this can lead to erroneous values as it fails to account for the losses during collection and separation.[55], [65] Another good method is to measure the weight of the whole reactor before the heating and after

the cooling, but unfortunately this was not paid attention to during the experiments. This method can be used in future experiments to estimate the gas phase.

The gas yield can be estimated to be around 7-8% as reported by Singh et al as their HTL conditions and DEE bio-oil yield are similar to our experiments #3 & #4. [83] Our products of interest are bio-oil and biochar thus we have focused to measure them accurately. The remaining mass can be accounted in losses during collection from the reactor as bio-oil can be trapped in pipes of the reactor; biochar is at times difficult to remove from the stirrer and sampling tube, and light volatiles are lost during evaporation of solvent DEE. [56] These losses can even amount to 5-10% of the mass.

Table 12: Mass recovery of products of HTL of Ulva sp.

Experiment	Conditions	Bio-oil yield(daf) (%)	Biochar yield (%)	Aq. Ph solids (%)	Total HTL products (%)
#4a	290C | 10 min | 14% | DW	7.88	19.16	46.87	73.91
#4b		8.05	19.33	48.15	75.53
#3a	290C | 10 min | 14% | SW	8.78	25.78	53.80	88.35
#3b		7.08	21.10	50.44	78.61

Most of the organic byproducts are recovered in the aqueous phase which is consistent with previous HTL studies. [56], [57], [64], [87] This is considered one of the issues with commercialization of HTL as it limits the energy yield of the process. Different catalysts are being tested to shift this yield towards bio-oil and they show some potential. [87] Although our primary goal is bio-oil, we have a much higher yield of biochar for all the cases. This can be explained by high ash content in the feedstock as it is reported to catalyze the reaction towards char and gas formation. [65], [88]

4.2.7. **Comparison between DW and SW**

As mentioned before, for all the experiments seawater was used as a medium except one. The exception was conducted using deionized water to compare the results with seawater. From Table 12, it is interesting to note that the bio-oil yields for both the cases #3 & #4 are very close to each other. If we consider the biochar yield, it is higher in SW experiment (#3) which can be due to the catalysis towards biochar due to presence of salts as previously mentioned. [65], [88] This was a preliminary experiment to check difference of yield between DW and SW. It suggests that similar

yields can be attained using either DW or SW. A further enquiry needs to be made on the effect of SW on the reactor to get a more detailed picture on the long-term use of SW as a reaction medium.

4.2.8. **Change in elemental composition due to hydrothermal treatment**

Table 13 shows the elemental content of one of the feedstocks and its products after hydrothermal treatment at different temperatures. As expected, the carbon content has increased in the products in comparison to the feedstock. In we compare different conditions, C% is higher in biochar of 270°C than of 180°C. Hydrothermal treatment has increased the carbon content and decreased the oxygen content thereby increasing the HHV from 14 MJ/kg to 22.9 MJ/kg in case of 270°C. The maximum concentration of energy can be seen in the bio-oil as the HHV increased by more than 2 times in comparison to the feedstock. HHV of 30.5 MJ/kg is within the range of 28 – 33 MJ/kg as reported by previous studies of HTL of different macroalgae. [29], [55], [64], [84]

Table 13: Elemental composition of the Ulva sp. feedstock & its products after HTL treatment.

Sample	N	C	H	S	O[1]	HHV (MJ/kg)
UL Feedstock A	7.96	33.4	5.73	4.1	31.51	14
Biochar A#1 180°C	6.69	35.73	5.38	2.66	29	17±0.21
Biochar A#2 270°C	4.33	49.12	5.84	4.16	5.75	22.9±0.31
Bio-oil A#2 270°C	9.18	63.27	7.38	0	20.16	30.58±0.49

[1] – by difference.

The nitrogen content is quite high which is also consistent with previous studies based on macroalgae. [29], [64], [65] High nitrogen content is due to the breakdown of proteins present in the *Ulva* feedstock.[7] Such bio-oil would require further refining and treatment like hydrotreating and hydrocracking as suggested by previous research. [89], [90] Considering the quality of bio-oil product from macroalgae, Barreiro et al 2015 suggested that HTL might not be best technology for converting macroalgae to bio-oil. Instead, it might be a better idea to convert residues to bio-oil after higher-value products like proteins are extracted from it in a co-product biorefinery. [29]

5. Summary and conclusions

In this work, HTL was tested on two feedstocks which come from two diverse origins – one is a form of waste which is accompanied by the humungous meat slaughter industry – animal fat and second is known as a third-generation aquatic feedstock – marine macroalgae *Ulva* sp. Various parameters of the HTL process like temperature, retention time, solid load and kind of water were tested to see its effects on the biofuel generated. The bio-oil was compared for its compatibility with fuels in the current infrastructure.

In case of animal fat as a feedstock, the maximum bio-oil yield of 26.5±1.5% was achieved at 330°C, 50% SL and 20 min of RT. The HHV of 38.5±0.16 MJ/kg was quite high in comparison to other HTL feedstocks and it is 90-95% of the marine grade fuels. The nitrogen and sulphur content were negligible making it ideal for use as a fuel considering strict regulations against NOx and SOx emissions.[80] The fuel properties like TAN, water content and viscosity were tested and compared to current marine fuel regulations. Apart from the higher water content which would require further processing, other properties indicate that bio-oil from HTL of animal fat has a substantial potential as a biofuel. One of the major benefits of converting this waste to biofuel would be its aversion from landfills which will reduce contribution to GHG emissions [34], or open disposal which can lead to diseases.

The results for macroalgae *Ulva* sp. are less promising in comparison to the animal fat. The highest yield of 7.93±0.85% for conditions 290°C, 10 min RT and 14% SL. is lower than previously reported for HTL of macroalgae for which the major reason can be the composition of the macroalgae used. Other reasons for lower yields have been mentioned in detail in section 4.2.2 like choice of extraction solvent and protocol followed. The HHV of 30.58±0.49 MJ/kg is on the lower side considering previously reported range of 28-36 MJ/kg. Negligible sulphur content is noteworthy, however the high nitrogen content of the bio-oil generated is a significant problem as it will lead to high NOx emissions when used as a fuel. Therefore, such a bio-oil would require further refining to pass the fuel regulations, making it less energy efficient. [77]

An interesting note from the macroalgae case study was the comparison between yields from same conditions tested for deionized water and sea water as reaction medium. The yields were similar with higher yield of biochar for the SW which might be due to the extra salt content because of

SW. These results suggest that SW can be a fascinating option to explore as a reaction medium as it would reduce the energy expenses of washing the macroalgae and sourcing distilled water for the process. This was a preliminary experiment; thus, it requires further investigation about impact of SW on HTL equipment in the long run as it can be corrosive to the metals at such severe conditions of operation.

When we compare the results from the two case studies, the effect of feedstock composition should be seen on the bio-oil produced. The presence of proteins in Ulva sp. lead to high nitrogen content in its bio-oil whereas nitrogen content was negligible in AF bio-oil as the starting animal fat contained only fat with no protein. Bio-oil with negligible N would require less further refining thereby making it a more energy-efficient. This shows the importance of considering the composition of feedstock for HTL.

Another interesting point to mention from the comparison is the difference in extraction procedure for the bio-oil in both the case studies. In case of AF, there is no need for an organic solvent, the phases are separated by centrifugation only. However, in case of macro algae, there is a need of solvent of at least 3 times the volume of aqueous phase for extracting the bio-oil. At commercial scale, this would mean humongous amounts of solvent would be needed for separation from the aqueous phase.

Considering the results shown, HTL seems to have a good potential as a technique to convert biomass to liquid fuel. The quality of bio-oil generated, however has a strong dependence on the kind of feedstock used as can be seen from the two feedstocks used in this study. The optimal conditions for maximum yield for a particular biomass may be found experimentally as the elemental composition of the same genus of biomass can vary greatly as shown in the literature review for macroalgae case study.

6. Future Directions

All the experimentation mentioned in this work was conducted in batch reactors. The large-scale production of biofuels is not feasible in batch reactors, thus further experimentation of animal fat to biofuel should be conducted in continuous system to see its potential on a commercial scale.

This will also aid in generating large quantities of AF bio-oil which are needed to test it in actual marine engines, which is the next step towards its use as biofuel.

As mentioned in the previous section, the use of seawater for the reaction medium seems promising. The long-term effects of seawater on HTL equipment can be examined.

The AF bio-oil was extracted without the need of a solvent. An idea given by my supervisor is to do a co-liquefaction of animal fat and macroalgae so that oil from both the feedstocks can be extracted without a solvent.

7. References

[1] "Energy and the environment explained Greenhouse gases and the climate," *US Energy Information Administration*. [Online]. Available: https://www.eia.gov/energyexplained/energy-and-the-environment/greenhouse-gases-and-the-climate.php. [Accessed: 25-Nov-2019].

[2] R. Gardner, "The Outlook for Energy: A View to 2040," in *2015 EDI*, 2015.

[3] M. H. Langholtz, B. J. Stokes, and L. M. Eaton, "2016 Billion-ton report: Advancing domestic resources for a thriving bioeconomy, Volume 1: Economic availability of feedstock," *Oak Ridge Natl. Lab. Oak Ridge, Tennessee, Manag. by UT-Battelle, LLC US Dep. Energy*, vol. 2016, pp. 1–411, 2016.

[4] I. Billege, "700 Refineries supply oil products to the world," *Nafta*, vol. 60, no. 7–8, pp. 401–403, 2009.

[5] D. Spencer, "BP Statistical Review of World Energy Statistical Review of World," *Ed. BP Stat. Rev. World Energy*, vol. 68th Editi, pp. 1–69, 2019.

[6] K. B. Tokarska, N. P. Gillett, A. J. Weaver, V. K. Arora, and M. Eby, "The climate response to five trillion tonnes of carbon," *Nat. Clim. Chang.*, vol. 6, no. 9, pp. 851–855, 2016.

[7] M. Déniel, G. Haarlemmer, A. Roubaud, E. Weiss-Hortala, and J. Fages, "Energy valorisation of food processing residues and model compounds by hydrothermal liquefaction," *Renewable and Sustainable Energy Reviews*. 2016.

[8] Y. Zhu, K. O. Albrecht, D. C. Elliott, R. T. Hallen, and S. B. Jones, "Development of hydrothermal liquefaction and upgrading technologies for lipid-extracted algae conversion to liquid fuels," *Algal Res.*, 2013.

[9] Z. Li and P. E. Savage, "Feedstocks for fuels and chemicals from algae: Treatment of crude bio-oil over HZSM-5," *Algal Res.*, 2013.

[10] A. A. Peterson, F. Vogel, R. P. Lachance, M. Fröling, M. J. Antal, and J. W. Tester, "Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies," *Energy Environ. Sci.*, vol. 1, no. 1, pp. 32–65, 2008.

[11] P. Biller and A. B. Ross, "Production of biofuels via hydrothermal conversion," in *Handbook of Biofuels Production: Processes and Technologies: Second Edition*, 2016.

[12] Y. Zhang, "Hydrothermal liquefaction to convert biomass into crude oil," *Biofuels from Agric. wastes Byprod.*, vol. 201, 2010.

[13] H. Weingärtner and E. U. Franck, "Supercritical water as a solvent," *Angew. Chemie - Int. Ed.*, vol. 44, no. 18, pp. 2672–2692, 2005.

[14] S. S. Toor, L. Rosendahl, and A. Rudolf, "Hydrothermal liquefaction of biomass: A review of subcritical water technologies," *Energy*. 2011.

[15] J. Woods and D. O. Hall, “Bioenergy for development-Technical and environmental dimensions-FAO Environment and energy paper 13,” *FAO-Food Agric. Organ. United Nations, Rome, Italy*, 1994.

[16] S. Sharma, R. Meena, A. Sharma, and P. kumar Goyal, “Biomass Conversion Technologies for Renewable Energy and Fuels: A Review Note.,” *IOSR J. Mech. Civ. Eng.*, vol. 11, no. 2, pp. 28–35, 2014.

[17] D. Castello, T. H. Pedersen, and L. A. Rosendahl, “Continuous hydrothermal liquefaction of biomass: A critical review,” *Energies*, vol. 11, no. 11, 2018.

[18] A. R. K. Gollakota, N. Kishore, and S. Gu, “A review on hydrothermal liquefaction of biomass,” *Renew. Sustain. Energy Rev.*, vol. 81, no. August 2016, pp. 1378–1392, 2018.

[19] V. Feddern *et al.*, “Animal fat wastes for biodiesel production,” in *Biodiesel-Feedstocks and Processing Technologies*, IntechOpen, 2011.

[20] L. Yang, L. Nazari, Z. Yuan, K. Corscadden, C. C. Xu, and Q. S. He, “Hydrothermal liquefaction of spent coffee grounds in water medium for bio-oil production,” *Biomass and Bioenergy*, 2016.

[21] L. Qian, S. Wang, and P. E. Savage, “Hydrothermal liquefaction of sewage sludge under isothermal and fast conditions,” *Bioresour. Technol.*, 2017.

[22] K. Malins, V. Kampars, J. Brinks, I. Neibolte, R. Murnieks, and R. Kampare, “Bio-oil from thermo-chemical hydro-liquefaction of wet sewage sludge,” *Bioresour. Technol.*, 2015.

[23] D. J. Zastrow and P. A. Jennings, “Hydrothermal Liquefaction of Food Waste and Model Food,” *2013 AIChE Annu. Meet. Online Proc.*, no. 336978, pp. 1–9, 2013.

[24] R. Singh, A. Prakash, B. Balagurumurthy, R. Singh, S. Saran, and T. Bhaskar, “Hydrothermal liquefaction of agricultural and forest biomass residue: comparative study,” *J. Mater. Cycles Waste Manag.*, vol. 17, no. 3, pp. 442–452, 2015.

[25] W. S. L. Mok and M. J. Antal, “Uncatalyzed Solvolysis of Whole Biomass Hemicellulose by Hot Compressed Liquid Water,” *Ind. Eng. Chem. Res.*, vol. 31, no. 4, pp. 1157–1161, 1992.

[26] M. Nagamori and T. Funazukuri, “Glucose production by hydrolysis of starch under hydrothermal conditions,” vol. 233, no. June 2003, pp. 229–233, 2004.

[27] C. Xu and N. Lad, “Production of heavy oils with high caloric values by direct liquefaction of woody biomass in sub/near-critical water,” *Energy and Fuels*, vol. 22, no. 1, pp. 635–642, 2008.

[28] Z. Shuping, W. Yulong, Y. Mingde, I. Kaleem, L. Chun, and J. Tong, “Production and characterization of bio-oil from hydrothermal liquefaction of microalgae Dunaliella tertiolecta cake,” *Energy*, 2010.

[29] D. López Barreiro, M. Beck, U. Hornung, F. Ronsse, A. Kruse, and W. Prins, “Suitability of hydrothermal liquefaction as a conversion route to produce biofuels from macroalgae,” *Algal Res.*, 2015.

[30] D. C. Elliott, P. Biller, A. B. Ross, A. J. Schmidt, and S. B. Jones, “Bioresource Technology Hydrothermal liquefaction of biomass : Developments from batch to continuous process,” *Bioresour. Technol.*, vol. 178, pp. 147–156, 2015.

[31] R. P. Overend and E. Chornet, “Fractionation of lignocellulosics by steam-aqueous pretreatments,” *Philos T R Soc A*, vol. 321, no. 25959, pp. 523–536, 1987.

[32] J. L. Faeth, P. J. Valdez, and P. E. Savage, “Fast hydrothermal liquefaction of nannochloropsis sp. to produce biocrude,” *Energy and Fuels*, vol. 27, no. 3, pp. 1391–1398, 2013.

[33] E. J. and Berglin, “Review and Assessment of Commercial Vendors / Options for Feeding and Pumping Biomass Slurries for Hydrothermal Liquefaction,” no. November, p. 94, 2012.

[34] R. Davis *et al.*, “Process design and economics for the conversion of lignocellulosic biomass to hydrocarbons: dilute-acid and enzymatic deconstruction of biomass to sugars and biological conversion of sugars to hydrocarbons,” National Renewable Energy Lab.(NREL), Golden, CO (United States), 2013.

[35] K. Bitnere and S. Searle, “Effective policy design for promoting investment in advanced alternative fuels,” *White Pap. Int. Counc. Clean Transp.*, 2017.

[36] N. Emissieautoriteit, “Feedstocks and double-counting-Claiming deliveries-Energy for Transport-Dutch Emissions Authority,” 2018.

[37] J. Bruinsma, *World agriculture: towards 2015/2030: an FAO study*. Routledge, 2017.

[38] H. Ritchie and M. Roser, “Meat and Dairy Production,” *Our World Data*, 2019.

[39] B. B. Adhikari, M. Chae, and D. C. Bressler, “Utilization of slaughterhouse waste in value-added applications: Recent advances in the development of wood adhesives,” *Polymers (Basel).*, vol. 10, no. 2, 2018.

[40] C. R. Hamilton, “Real and perceived issues involving animal proteins,” *Protein sources Anim. Feed Ind.*, pp. 255–276, 2004.

[41] C. Walsh, “The use of Animal By-products The improving opportunities to add value to the beef and sheep slaughtering sectors,” 2014.

[42] M. Alm, “Animal Fats.” 2015.

[43] D. Kusdiana and S. Saka, “Effects of water on biodiesel fuel production by supercritical methanol treatment,” *Bioresour. Technol.*, 2004.

[44] H. L. Barnebey and A. C. Brown, “Continuous fat splitting plants using the colgate-emery process,” *J. Am. Oil Chem. Soc.*, vol. 25, no. 3, pp. 95–99, 1948.

[45] V. M. H and K. McClain, “Fat Hydrolysis,” *Ind. Eng. Chem.*, vol. 41, no. 9, pp. 1982–1985, 1949.

[46] L. Lascaray, “Mechanism of Fat Splitting,” *Ind. Eng. Chem.*, vol. 41, no. 4, pp. 786–790, 1949.

[47] A. Sturzenegger and H. Sturm, “Hydrolysis of Fats at High Temperatures,” *Ind. Eng. Chem.*, vol. 43, no. 2, pp. 510–515, 1951.

[48] O. J. Ackelsberg, “Fat splitting,” *J. Am. Oil Chem. Soc.*, vol. 35, no. 11, pp. 635–640, 1958.

[49] M. León, A. F. Marcilla, and Á. N. García, “Hydrothermal liquefaction (HTL) of animal by-products : Influence of operating conditions,” vol. 99, pp. 49–59, 2019.

[50] C. Yang, S. Wang, M. Ren, Y. Li, and W. Song, “Hydrothermal Liquefaction of Animal Carcass for Biocrude Oil,” *Energy & Fuels*, p. acs.energyfuels.9b03100, Oct. 2019.

[51] Z. Baicha *et al.*, “A critical review on microalgae as an alternative source for bioenergy production: A promising low cost substrate for microbial fuel cells,” *Fuel Processing Technology*. 2016.

[52] K. A. Jung, S.-R. Lim, Y. Kim, and J. M. Park, “Potentials of macroalgae as feedstocks for biorefinery,” *Bioresour. Technol.*, vol. 135, pp. 182–190, May 2013.

[53] J. W. Van Hal and H. Den Uil, “Biorefinery of sustainable biomass from land and sea,” no. November 2014, 2014.

[54] I. K. Chung, J. Beardall, S. Mehta, D. Sahoo, and S. Stojkovic, “Using marine macroalgae for carbon sequestration: A critical appraisal,” *J. Appl. Phycol.*, vol. 23, no. 5, pp. 877–886, 2011.

[55] D. Zhou, L. Zhang, S. Zhang, H. Fu, and J. Chen, “Hydrothermal liquefaction of macroalgae enteromorpha prolifera to bio-oil,” *Energy and Fuels*, vol. 24, no. 7, pp. 4054–4061, 2010.

[56] K. Anastasakis and A. B. Ross, “Hydrothermal liquefaction of the brown macro-alga Laminaria Saccharina: Effect of reaction conditions on product distribution and composition,” *Bioresour. Technol.*, vol. 102, no. 7, pp. 4876–4883, 2011.

[57] K. Anastasakis and A. B. Ross, “Hydrothermal liquefaction of four brown macro-algae commonly found on the UK coasts: An energetic analysis of the process and comparison with bio-chemical conversion methods,” *Fuel*, 2015.

[58] R. Singh, B. Balagurumurthy, and T. Bhaskar, “Hydrothermal liquefaction of macro algae: Effect of feedstock composition,” *Fuel*, vol. 146, pp. 69–74, 2015.

[59] W. Yang, X. Li, S. Liu, and L. Feng, “Direct hydrothermal liquefaction of undried macroalgae Enteromorpha prolifera using acid catalysts,” *Energy Convers. Manag.*, 2014.

[60] L. Yan *et al.*, “Hydrothermal liquefaction of Ulva prolifera macroalgae and the influence

of base catalysts on products," *Bioresour. Technol.*, vol. 292, no. March, p. 121286, 2019.

[61] R. Singh, T. Bhaskar, and B. Balagurumurthy, "Effect of solvent on the hydrothermal liquefaction of macro algae Ulva fasciata," *Process Saf. Environ. Prot.*, vol. 93, pp. 154–160, 2015.

[62] Y. Hu *et al.*, "Co-pyrolysis and co-hydrothermal liquefaction of seaweeds and rice husk: Comparative study towards enhanced biofuel production," *J. Anal. Appl. Pyrolysis*, 2018.

[63] C. Yuan *et al.*, "Optimization of hydrothermal co-liquefaction of seaweeds with lignocellulosic biomass: Merging 2 nd and 3 rd generation feedstocks for enhanced bio-oil production," *Energy*, 2019.

[64] N. Neveux *et al.*, "Biocrude yield and productivity from the hydrothermal liquefaction of marine and freshwater green macroalgae," *Bioresour. Technol.*, 2014.

[65] M. Parsa, H. Jalilzadeh, M. Pazoki, R. Ghasemzadeh, and M. Abduli, "Hydrothermal liquefaction of Gracilaria gracilis and Cladophora glomerata macro-algae for biocrude production," *Bioresour. Technol.*, vol. 250, pp. 26–34, Feb. 2018.

[66] "ISS AGRI facility - The New Rendering Process - BR017002 EN v 1.0 C," 2017. [Online]. Available: https://celitron.com/download/agri-facility-en.pdf.

[67] Y. Qu, X. Wei, and C. Zhong, "Experimental study on the direct liquefaction of Cunninghamia lanceolata in water," *Energy*, 2003.

[68] S. Karagöz, T. Bhaskar, A. Muto, Y. Sakata, and M. A. Uddin, "Low-temperature hydrothermal treatment of biomass: Effect of reaction parameters on products and boiling point distributions," *Energy and Fuels*, vol. 18, no. 1, pp. 234–241, 2004.

[69] Y. Guo, W. Song, J. Lu, Q. Ma, D. Xu, and S. Wang, "Hydrothermal liquefaction of Cyanophyta: Evaluation of potential bio-crude oil production and component analysis," *Algal Res.*, 2015.

[70] J. Akhtar, N. Aishah, and S. Amin, "A review on process conditions for optimum bio-oil yield in hydrothermal liquefaction of biomass," *Renew. Sustain. Energy Rev.*, vol. 15, no. 3, pp. 1615–1624, 2011.

[71] L. Garcia Alba *et al.*, "Hydrothermal treatment (HTT) of microalgae: Evaluation of the process as conversion method in an algae biorefinery concept," *Energy and Fuels*, vol. 26, no. 1, pp. 642–657, 2012.

[72] S. Raikova, C. D. Le, T. A. Beacham, R. W. Jenkins, M. J. Allen, and C. J. Chuck, "Towards a marine biorefinery through the hydrothermal liquefaction of macroalgae native to the United Kingdom," *Biomass and Bioenergy*, 2017.

[73] M. Melzer, J. Blin, A. Bensakhria, J. Valette, and F. Broust, "Pyrolysis of extractive rich agroindustrial residues," *J. Anal. Appl. Pyrolysis*, 2013.

[74] P. Biller and A. B. Ross, "Potential yields and properties of oil from the hydrothermal

liquefaction of microalgae with different biochemical content," *Bioresour. Technol.*, 2011.

[75] R. Posmanik, D. A. Cantero, A. Malkani, D. L. Sills, and J. W. Tester, "Biomass conversion to bio-oil using sub-critical water: Study of model compounds for food processing waste," *J. Supercrit. Fluids*, 2017.

[76] M. Leon, A. N. Garcia, A. Marcilla, I. Martinez-Castellanos, R. Navarro, and L. Catala, "Thermochemical conversion of animal by-products and rendering products," *Waste Manag.*, vol. 73, pp. 447–463, 2018.

[77] E. D. Larson, "A review of life-cycle analysis studies on liquid biofuel systems for the transport sector," *Energy Sustain. Dev.*, vol. 10, no. 2, pp. 109–126, 2006.

[78] W. Bühler, E. Dinjus, H. J. Ederer, A. Kruse, and C. Mas, "Ionic reactions and pyrolysis of glycerol as competing reaction pathways in near- and supercritical water," *J. Supercrit. Fluids*, 2002.

[79] I. Leonardis *et al.*, "Characterization of bio-oil from hydrothermal liquefaction of organic waste by NMR spectroscopy and FTICR mass spectrometry," *ChemSusChem*, vol. 6, no. 1, pp. 160–167, 2013.

[80] Theodora Tyrovola, George Dodos, Stamatis Kalligeros, and Fanourios Zannikos, "The Introduction of Biofuels in Marine Sector," *J. Environ. Sci. Eng. A*, vol. 6, no. 8, 2017.

[81] "Petroleum Products –Fuels (Class F) –Specifications of Marine Fuels, FDIS8217:2017," 2017.

[82] C. Y. Lin, "Effects of biodiesel blend on marine fuel characteristics for marine vessels," *Energies*, vol. 6, no. 9, pp. 4945–4955, 2013.

[83] R. Singh, B. Balagurumurthy, and T. Bhaskar, "Hydrothermal liquefaction of macro algae: Effect of feedstock composition," *Fuel*, 2015.

[84] D. Li *et al.*, "Preparation and characteristics of bio-oil from the marine brown alga Sargassum patens C. Agardh," *Bioresour. Technol.*, 2012.

[85] P. Biller and A. B. Ross, "Hydrothermal processing of algal biomass for the production of biofuels and chemicals," *Biofuels*, vol. 3, no. 5, pp. 603–623, 2012.

[86] Y. P. Xu, P. G. Duan, and F. Wang, "Hydrothermal processing of macroalgae for producing crude bio-oil," *Fuel Process. Technol.*, 2015.

[87] A. R. Maag, A. D. Paulsen, T. J. Amundsen, P. E. Yelvington, G. A. Tompsett, and M. T. Timko, "Catalytic hydrothermal liquefaction of food waste using cezrox," *Energies*, vol. 11, no. 3, pp. 1–14, 2018.

[88] J. Li *et al.*, "Deoxy-liquefaction of three different species of macroalgae to high-quality liquid oil," *Bioresour. Technol.*, 2014.

[89] P. Biller, B. K. Sharma, B. Kunwar, and A. B. Ross, "Hydroprocessing of bio-crude from continuous hydrothermal liquefaction of microalgae," *Fuel*, 2015.

[90] A. Cole *et al.*, “From macroalgae to liquid fuel: Via waste-water remediation, hydrothermal upgrading, carbon dioxide hydrogenation and hydrotreating,” *Energy Environ. Sci.*, vol. 9, no. 5, pp. 1828–1840, 2016.