Influence of hydrothermal pre-treatment on biofuel production from micro algae

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The candidate confirms that the work submitted is his/her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

Algae are fast becoming a sought-after resource for the production of biofuels and platform chemicals. Macro algae contain high levels of carbohydrates and micro algae contain large amounts of lipids and have much faster growth rates, in comparison to terrestrial biomass. The main issues surrounding biofuels from algae is the processing methods, amount of upgrading required, and the costs associated with this.

Hydrothermal processing is an emerging biomass pre-treatment method, which at temperatures below 200°C, produces a carbonised material and also releases organic and inorganic material into the process waters. At temperatures between 200 and 375°C liquefaction occurs and produces an oil. This work sets out to investigate how to improve the quality of bio-oil produced from algae by studying the fate of heteroatoms, mineral content and biochemical components during hydrothermal processing.

The results show that hydrothermal pre-treatment results in solid algal residues of a higher energy density than the raw algae, with HHV ranging from 12 to 32 MJ/kg (d.a.f.). The process waters from hydrothermal pre-treatment are rich in organic and inorganic material and can be recycled into hydrothermal liquefaction as they are, or after being cleaned using Mg modified bio-chars.

Three conversion routes; pyrolysis, solvent extraction and hydrothermal liquefaction, of the raw and hydrothermally pre-treated algae, are investigated to establish which conversion routes produce the most appropriate bio-oils for use as biofuels. The bio-oils from hydrothermal liquefaction show lower nitrogen and phosphate content and higher HHV, in comparison to the bio-oils from pyrolysis and solvent extraction. Therefore, hydrothermal liquefaction is used as the conversion route for the remainder of the thesis.

Comparison of the bio-crudes from the raw and pre-treated micro algae; autotrophic and heterotrophic *Chlorella vulgaris, Spirulina platensis* and *Chlorogloeopsis fritschii*, show that different biochemical components react differently when liquefied. The lipids and proteins mostly contribute to the bio-

crude, with some of the carbohydrates contributing to the bio-crude but also being broken down into sugars and acids and released into the process waters.

The effect of formic acid during hydrothermal liquefaction is investigated on the autotrophic *Chlorella vulgaris*. The addition of formic acid has little effect on the bio-crude yield, but results in further decarboxylation of the bio-crude, which reduces the oxygen content of the bio-crude.

Overall, the results show that the quality of bio-crude from algae can be improved and the process waters from hydrothermal pre-treatment can be valorised during hydrothermal liquefaction.

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Abbreviations

HTL	Hydrothermal liquefaction
DW	Distilled water
PW	Process waters
CW	Cleaned water
GC-MS	Gas Chromatography - Mass Spectrometry
HPLC	High Performance Liquid Chromatography
a.r.	As received
d.b.	Dry basis
d.a.f.	Dry ash free
XRF	X-ray diffraction
Wt.	Weight

Chapter 1. Introduction

1.1. Background

The greatest challenge facing the world at present is climate change. A slight increase (2°C) in global temperature could have devastating effects on the living organisms on the planet with extinctions, collapse of ecosystems and the disappearance of food chains, resulting in food and water shortages, famine and conflict (Environmental Investigation Agency, 2016).

The world population in 2019, was 7.6 billion and increasing (Population Reference Bureau, 2019). The demand for energy is ever increasing due to the progress in technology and alongside this, the agricultural practices worldwide, have increased every year with the increasing population. The use of fossil fuels for transport is the 2nd major contributor of CO_2 to the atmosphere after energy production (Environmental Protection Agency, 2019).

At present approximately 80% of the energy used worldwide is derived from fossil fuels (Hallenbeck and Benemann, 2002). The main issue with using fossil fuels is the release of CO_2 as a result of combustion. This is problematic as the CO_2 is released to the atmosphere and causes global warming. Fossil fuels are a finite resource and global resources are diminishing. Due to this, alternative sources of energy are becoming ever more popular. Energy from renewable resources is paving the way for 'cleaner and greener' energy resources. In recent years, the interest in biomass, as an alternative feedstock to produce fuels, has increased.

Biomass can be used as an alternative feedstock to produce bio-fuels, which can be used to produce power, heat and also as transport fuels. It is a versatile feedstock as solid, liquid and gas products can be produced to be used as fuels. Biomass is considered to be CO_2 neutral, however this is not always the case, as the amount of energy required to transport and prepare the biomass for conversion, is not always taken into account (McKendry, 2002).

There are three main categories biofuels can be separated into; 1st generation, 2nd generation and 3rd generation biofuels. 1st generation biofuels refer to biofuels produced from biomass that is also a food source, for example, sugar cane to produce ethanol and biodiesel from rapeseed oil. 2nd generation biofuels are similar to 1st generation biofuels but are made from inedible biomass. These include lignocellulosic material such as wood and grasses. Although 2nd generation biofuels do not consist of food crops, there is still competition for the land used for growing the biomass. 3rd generation biofuels are quite different to 1st and 2nd generation biofuels as they are produced from alternative feedstocks such as algae, which do not require land to grow.

Algae are simple organisms able to undergo photosynthesis. There are two main types of algae; micro and macro. Algae contains lipids, proteins and carbohydrates which can be converted into biofuels. In comparison to terrestrial biomass, the yield of oil from micro algae is high, with a low amount of land required to grow it, whereas terrestrial biomass requires larger land areas and have lower oil yields (Shuvashish et al., 2015). There are however challenges to using algae as a feedstock, such as the high nitrogen content and requirement of phosphate. The nitrogen content is an issue as the bio-crude produced from the algae will also contain high levels of nitrogen. Phosphate is required during the cultivation of algae but becomes concentrated in the bio-crude. As phosphate is a finite resource, it is important to be able to recover this and re-use it.

With such a range of feedstocks, a variety of pathways from different sources and processes can be used to produce biofuels. The most common processes are classed mainly into biochemical and thermochemical processes. The most established biochemical processes include anaerobic digestion and fermentation. Fermentation is the process of converting sugars into ethanol and carbon dioxide using yeast, under anaerobic conditions. This technology is very well established in Brazil and is used to produce bioethanol, which is blended with conventional gasoline (Zanin et al., 2000). Anaerobic digestion (AD) is a process of converting feedstocks such as food waste and other organic wastes into biogas. The process consists of four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. During hydrolysis the proteins are broken down into amino acids, the lipids into fatty acids and carbohydrates into sugars. These are then converted into carbonic

acids, alcohols, ammonia, H_2 and CO_2 during acidogenesis. During acetogenesis, the organic acids are broken down further to acetic acid. In the final step, methanogenesis, the H_2 and CO_2 form methane (Adekunle and Okolie, 2015).

The most established thermo-chemical processes include gasification, pyrolysis and hydrothermal processing. Gasification involves the partial oxidation of a feedstock (>700°C) to produce a syngas, mainly consisting of CO, H_2 and CO_2. The process consists of five stages: drying, pyrolysis, combustion, cracking and reduction (Ahmad et al., 2016). Gasification is a well-established method, which was extensively used for coal before being applied to biomass. Liquid bio-fuel can be produced from the syngas produced from gasification by using the Fischer Tropsch process, which converts H_2 and CO to straight chain liquid hydrocarbons (Tijmensen et al., 2002).

Pyrolysis is the thermal decomposition in the presence of an inert gas. The pyrolysis products vary depending on the processing temperature, heating rate and residence time. Lower temperatures produce bio-char and higher temperatures favour the production of bio-oil. The oil resembles crude oil and requires upgrading before it can be used as a transport fuel. Although both gasification and pyrolysis are useful thermo-chemical routes for producing solid, liquid and gaseous fuels, they require the feedstock to be dry. This is not suitable for feedstock such as algae and food waste due to the high moisture content which would require the feedstock to be dried before processing.

Hydrothermal processing is a thermo-chemical process which can process both wet and dry feedstocks. The process involves converting biomass in the presence of water at elevated temperatures and pressures. This is similar to the natural processes that take place to produce fossil fuels such as coal, oil and natural gas. Hydrothermal processing can accelerate these processes and can produce either solid, liquid or gaseous hydrocarbon fuels within a much quicker time period depending on the temperature and pressure used (Savage et al., 2010b).

Whilst biomass may be an attractive feedstock for producing biofuels in terms of the carbon neutrality and sustainability of feedstocks, there are still challenges to using it as a feedstock due to the physiochemical properties of the biofuels being different to conventional fossil fuels. The bio-oils or bio-crudes produced from the different processing methods require extensive upgrading. This

work focuses on improving the quality of bio-crude produced from algae by pre-treating the algae before conversion into bio-crude.

1.2. Aims and objectives

The overall aim of this project is to develop approaches for improving the quality and yields of biofuels derived from algae by implementing hydrothermal pre-treatment to remove problematic components from the feedstocks (e.g. salts, nitrogen and other heteroatoms). This will allow an assessment to be made on the influence of pre-treatment on subsequent downstream processing of the biomass and to develop approaches to remove and recover valuable nutrients from the biomass in a form which allows its reuse. The approaches will be investigated with a range of algae with different biochemical content including phototrophic and heterotrophic micro algae and macro algae. Subsequent downstream processing of the biomass principally is focussed on hydrothermal liquefaction but for comparative purposes, solvent extraction and pyrolysis will also be investigated.

Objective 1: Conduct a literature review

The first objective is to conduct a literature review of previous work on pre-treatment and conversion of both autotrophic and heterotrophic micro algae and a phototrophic macro algae. Previous work into micro algae is used to determine which conversion routes to investigate. A review of the cultivation of algae is also included. Issues associated with bio-oils from algae and possible pre-treatment methods are reviewed. The potential uses of the process waters from hydrothermal processing are also explored.

Objective 2: Investigate the influence of temperature on hydrothermal pre-treatment

Investigate the influence of hydrothermal processing at different temperatures on the autotrophic micro and macro algae to find the optimum processing temperature. Particular focus is placed on the yields of products, the fate

of heteroatoms, the fate of biochemical components and the fate of mineral content in the process.

Objective 3: Investigate the potential of nutrient recovery and recycling from the process waters from hydrothermal pre-treatment

From the products produced in objective 2, the process waters are analysed further to investigate the potential of recovering and recycling nutrients from the process waters from hydrothermal pre-treatment. The removal of other problematic components such as metals and salts is also investigated. The removal and recovery of nutrients and problematic components will be conducted using carbon adsorbents. The carbon adsorbents are chemically modified using magnesium chloride. The ability to recover nutrients and metals from the modified bio-chars will also be investigated to see if the carbon adsorbents can be re-used.

Objective 4: Investigation of three different thermal conversion routes

Once suitable hydrothermal processing temperatures are determined in objective 2, suitable conversion methods for the production of oils, from the solid residues, from hydrothermal processing, are investigated. The conversion methods are chosen based on their suitability for wet feedstock, the methods chosen are pyrolysis, solvent extraction and hydrothermal liquefaction. From the analysis of the products from the selected conversion methods, the best suited method for the micro algae is chosen.

Objective 5: Hydrothermal liquefaction of algae

Hydrothermal liquefaction is the conversion method that is best suited for processing micro algae into oil. Hydrothermal liquefaction will be performed using high pressure reactors and will investigate the influence of different process variables such as temperature, feedstock type and the addition of additives on the quality of bio-crude produced. Two different liquefaction temperatures 300°C and 350°C will be investigated. A variety of different micro algae will be explored.

Objective 6: Comparison of hydrothermal liquefaction to assess the various process waters

This objective follows on from objective 5, continuing with hydrothermal liquefaction, however in this instance focusing on a comparison between a micro and macro algae. Three different water types will be used in the liquefaction process; distilled water, process waters from hydrothermal pre-treatment and cleaned process waters from hydrothermal pre-treatment. Recycling of process waters will assess the impact on yields and quality of products before and after separation of valuable nutrients (and problematic components).

1.3. Thesis outline

Chapter 2 is a literature review and addresses objective 1. It firstly gives a brief history of the use of micro algae to produce biofuels, followed by the characteristics of micro and macro algae along with cultivation methods. Then potential conversion methods of algae are explored, followed by the issues with the bio-oil produced. Pre-treatment technologies to reduce these issues are explored. Finally, the use of process waters from hydrothermal processing and the nutrient recovery of these process waters is discussed.

Chapter 3 provides the methods used throughout the thesis and is referred to in the results chapters. Firstly the materials used are described, then the methods are outlined chronologically in the order in which the experiments were carried out. The first section describes the pre-treatment process. The second section describes the conversion techniques used and the final section describes the analysis of the products.

Chapter 4 is the first results chapter and investigates hydrothermal pre-treatment of the feedstocks, covering objective 2. In this chapter, hydrothermal processing is used as a pre-treatment method for the algae. Various methods are used to analyse the products from this process.

Chapter 5 is the second results chapter and investigates three possible conversion routes for the solid residues of algae that have been deemed suitable after hydrothermal pre-treatment in Chapter 4. In this chapter, solvent extraction,

pyrolysis and hydrothermal liquefaction are assessed as potential conversion methods for producing oil. This chapter focusses on objective 4 of the thesis.

Chapter 6 is the third results chapter and investigates the effect of biochemical composition on the quality of bio-crude produced from hydrothermal liquefaction of micro algae. Comparisons are made between the different micro algae, both raw and pre-treated at 150°C. This chapter covers Objective 5 of the thesis.

Chapter 7 is the fourth results chapter and investigates the effect of the addition of formic acid on the quality of bio-crude produced from hydrothermal liquefaction of micro algae. Comparisons are made between the different micro algae, both raw and pre-treated at 150°C. This chapter also covers Objective 5 of the thesis.

Chapter 8 is the final results chapter and investigates the valorisation of the process waters from both hydrothermal pre-treatment and liquefaction. The liquefaction is carried out with process waters from the hydrothermal pre-treatment stage, with and without cleaning up with modified bio-chars. This chapter covers Objectives 3 and 5.

Chapter 9 concludes if the pre-treatment had an effect on the quality of the bio-crude and whether the quality of the bio-crude has improved due to the choice of conversion method and processing media.

Chapter 2. Literature review

2.1. Introduction

The term biomass covers a wide range of materials such as wood, grasses, fuel crops such as rapeseed and sugar cane, agricultural waste and animal by-products. Biomass (especially wood) has been used as a source of energy by humans for thousands of years. With the onset of the industrial revolution, fossil fuels such as coal and petroleum became predominant sources of energy; coal for both heating and electricity and petroleum for transport fuels. After the first petroleum crisis in 1973, it was recognised how unstable the future of crude oil was and as the price began to increase, more countries realised the need to research alternative fuels. In the early 2000's the need for alternative renewable fuels was considered a priority due to the damage that CO_2 from fossil fuels was having on the planet.

Biofuels could be an alternative to traditional fossil fuels, with their lower CO_2 emissions. First generation biofuels are made from food crops, such as sugarcane and rapeseed. However, when food prices began to increase due to the increase in use of first generation biofuels, other feedstocks were investigated. Second generation biofuels are produced from non-edible terrestrial crops and include grasses and lignocellulosic material. Although the competition for food had been removed by implementing second generation biofuels, it had been replaced with competition for land use and is still indirectly affecting the cost of food crops.

First and second generation biofuels have been extensively researched in the last twenty years, with improvements being made all the time. However, there is still the issue of competition for land for both food and energy crops. Therefore, research into third generation biofuels has increased in the last decade with algae becoming a more prominently researched feedstock, as it is not terrestrial and can be cultivated in wastewater.

2.2. Algae

Algae are aquatic plants which are an alternative to terrestrial biomass. There are two main types of algae; macro and micro. Macro algae are autotrophic organisms which mainly grow in salt water environments and contain high levels of carbohydrates. Micro algae can be autotrophic, mixotrophic and heterotrophic and can grow in fresh, brackish and salt water environments. Micro algae contain lower levels of carbohydrates than macro algae but higher levels of lipids. Carbohydrates and lipids are both desirable characteristics when producing biofuels.

2.2.1. Macro algae

Macro algae are photosynthetic organisms which vary in size, from a few centimetres to as much as 60 metres (Percival and McDowell, 1967). Macro algae anchor themselves to the seabed, but also need to be close enough to the surface of the water to be able to absorb a sufficient amount of sunlight, therefore they are most commonly found on the continental sea shelf, where they form dense underwater forests which do not allow much light to penetrate through (Lüning and Pang, 2003).

Macro algae are classified under three major groups based on their photosynthetic pigmentation: red (*Rhodophyta*), brown (*Phaeophyta*) and green (*Chlorophyta*) (John, 2011; Jung et al., 2013; Kraan, 2013). Overall, red macro algae is the most abundant, with over 6000 species in the group, followed by green which consists of over 4500 species and finally brown, which includes over 2000 species (Jung et al., 2013).

2.2.1.1. Structure of macro algae

Macro algae have a similar structure to terrestrial plants, with an example shown in Figure 2-1. The holdfast of the macro algae is similar to the roots of terrestrial plants and keeps the plant in place, however, unlike the roots of terrestrial plants, the holdfast does not absorb nutrients. The stipe of the macro algae acts as the stem does in terrestrial plants and the blade is not dissimilar to the leaf (Edwards

et al., 2012). Some macro algae also contain ‘air bubbles’ which help to keep them afloat and assist in capturing more sunlight.

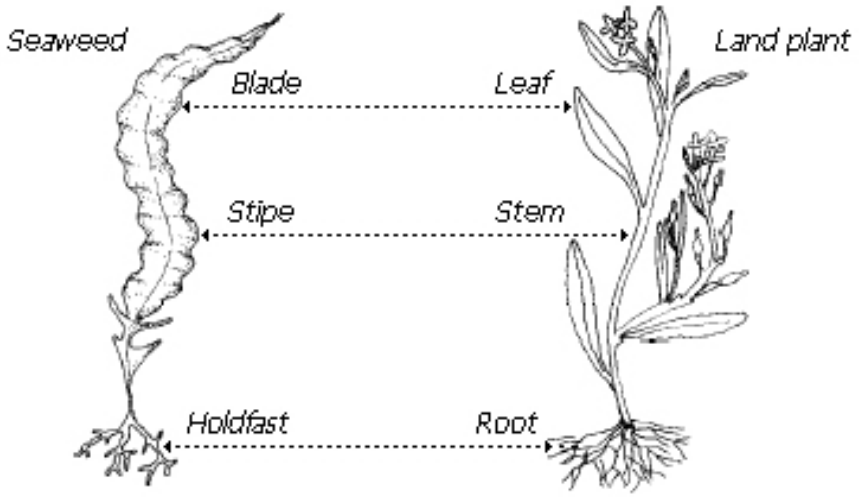


Figure 2-1: Diagram of macro algae and terrestrial plant structure (University of Washington, 2016)

2.2.1.2. Biochemical composition of macro algae

The biochemical composition of macro algae consists of carbohydrates, lipids and proteins. The biochemical composition and amount of ash present in the macro algae vary depending on the season. For example, the carbohydrate content is at the highest during the autumn months (Kraan, 2013). Macro algae generally consists of only 10-15% dry matter (Roseijadi et al., 2010), with 60% of this being made up of carbohydrates. Macro algae also contain mannan, ulvan, carrageenan, agar, laminarin, mannitol, alginate, fucoidin, fucose and uronic acid, which are not present in micro algae (Jung et al., 2013).

Red macro algae mainly consist of cellulose, glucan and galactan. The cell walls of red macro algae contain agar and carrageenan which have gel-forming abilities (Wei et al., 2013).

Green macro algae show similar evolutionary and biochemical traits to terrestrial plants (Roseijadi et al., 2010), with a similar chemical composition as there is cellulose and chlorophyll present in both (Jung et al., 2013). Green macro

algae have a higher lipid content than the red and brown macro algae, which both contain less than 5% (Ross et al., 2008).

Brown macro algae generally contain up to 55% dry weight of carbohydrates. They are rich in alginate and contain large quantities of laminarin and mannitol (Wei et al., 2013).

Overall, all three groups of macro algae contain high levels of alkali metals, which are even higher than those of terrestrial plants (Mautner, 1954).

2.2.1.3. Cultivation

Red algae mainly grow in water of over 10m in depth, away from tidal fluctuations (Percival and McDowell, 1967). Red algae grow especially well in inter-tropical zones (Bucholc et al., 2014).

Brown algae are predominantly found in water depths of 10 – 20m, below the tide level (Percival and McDowell, 1967). Brown algae grow mainly in tempered to very cold waters (Bucholc et al., 2014).

Due to the need for abundant amounts of sunlight for photosynthesis, green algae grow in shallow waters such as estuaries, bays and intertidal pools (Wei et al., 2013). Green algae can grow in either cold or warm water temperatures (Bucholc et al., 2014).

Traditionally seaweeds were collected from natural stocks or wild populations, however recently developments in cultivation techniques have allowed standardisation and increased harvesting. Large scale open water cultivation is a common cultivation technique that is employed by many Asian countries, whereas many other countries cultivate macro algae in tanks (Pereira and Yarish, 2008).

2.2.2. Micro algae

Micro algae are simple photosynthetic organisms that require only water, nutrients and a form of carbon to grow. Micro algae are fast growing and can be cultivated in a number of different conditions. They can be grown with the presence of light (autotrophic) or without light (heterotrophic). The carbon source for autotrophic growth is primarily carbon dioxide, whereas for heterotrophic growth,

the carbon source is organic carbon (Barreiro et al., 2013b). There are also strains of micro algae that can grow in a mixture of both autotrophic and heterotrophic conditions, these are known as mixotrophs.

2.2.2.1. Structure of micro algae

The part of the algae that absorbs light is the chlorophyll, which is located in the membrane of the algae. The four main types of chlorophyll are: *a*, *b*, *c* and *d*, with chlorophyll *a* being the most abundant in micro algae (Carlson and Simpson, 1996). The chlorophyll molecule is made up of a magnesium ion in the centre which is surrounded by a porphyrin ring containing nitrogen and a hydrocarbon chain (pytol chain) attached to the side of the ring. The variations in the hydrocarbon side chain are the cause of the differences in the types of chlorophyll (Encyclopedia Britannica, 2020). Figure 2-2 shows the structures of chlorophyll *a*, *b*, *c* and *d*.

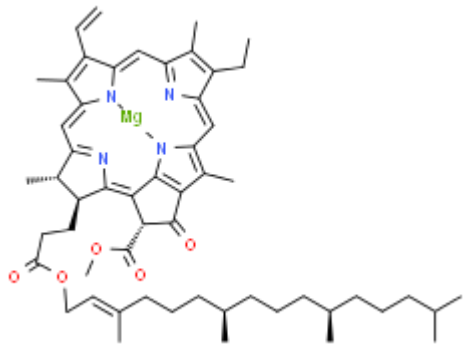
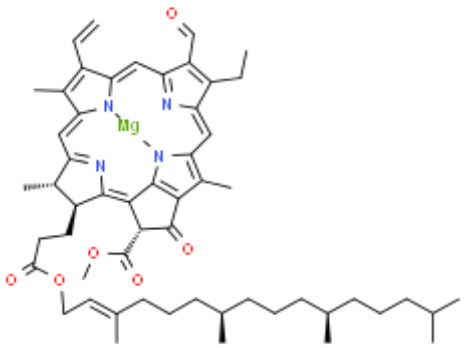
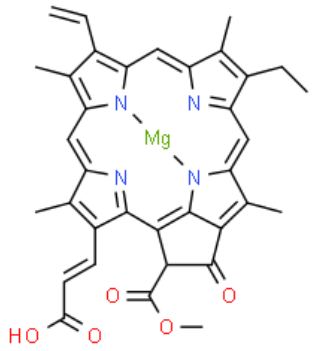
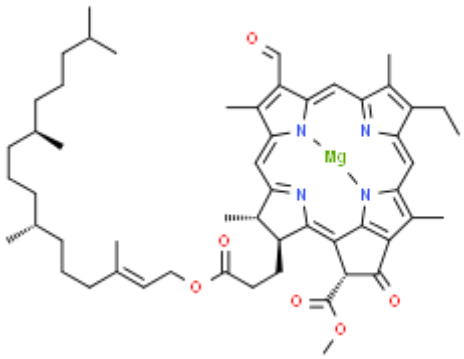
Chlorophyll *a* (Chemspider, 2020a)	**Chlorophyll *b*** (Chemspider, 2020b)
	
Chlorophyll *c* *(Chemspider, 2020c)*	**Chlorophyll *d*** *(Chemspider, 2020d)*
	

Figure 2-2: Structure of chlorophyll *a*, *b*, *c* and *d*

Unlike autotrophic micro algae, heterotrophically grown micro algae can be cultivated in the absence of light, although they still require water, nutrients and a source of carbon. For heterotrophic algae, the source of carbon is organic and is usually in the form of sugars. A review by Perez-Garcia et al. (2011) stated that for the glucose that is up taken by micro algae during heterotrophic growth, at least 85% of it is converted into polysaccharides.

The advantages of heterotrophically growing micro algae are that a high specific growth rate can produce high cell density algae (Azma et al., 2011). Gladue and Maxey (1994) and Running et al. (1994) both established a dry weight cell of 50g/L and 100g/L respectively of heterotrophic algae. An investigation by Miao and Wu (2004) also found that higher levels of lipid content are exhibited by heterotrophic growth in comparison to autotrophically grown micro algae.

2.2.2.2. Biochemical composition of micro algae

The biochemical composition of micro algae differs greatly from that of macro algae. Micro algae contain higher levels of lipids and have lower carbohydrate content than macro algae.

The nitrogen content of heterotrophic micro algae is lower than that of autotrophic micro algae. The main reason for this is due to the lack of chlorophyll in heterotrophic micro algae. The majority of the nitrogen found in heterotrophic micro algae is found in the proteins.

autotrophic and heterotrophic algae are cultivated under different conditions and therefore produce micro algae that have different properties. autotrophic algae contains chlorophyll which contains high amounts of nitrogen, whereas heterotrophic algae do not contain chlorophyll and therefore have considerably lower levels of nitrogen, but it is still present in the proteins in heterotrophic algae. The specific growth rates for heterotrophically grown algae are difficult to find as they are not usually stated in publications (Bumbak et al., 2011).

The biochemical composition of micro algae consists of lipids, proteins and carbohydrates. The lipids are made up of fatty acid triglycerides and act as part of the cell structure as they are located within the membrane, which consists of glycolipids and phospholipids. The lipids also act as energy reserves within the

micro algae and higher concentrations of fatty acids within the lipids result in the production of higher quality liquid biofuels (Williams and Laurens, 2010).

The lipid content of different strains of algae varies greatly, with many comparisons being made in the literature. An example of this is shown in Demirbas and Demirbas (2011) who found that 29.4% dry weight oil content of *Chlorella protothecoides*, whereas, Mata et al. (2010) found the oil content to be between 14.6 – 57.8% of dry weight.

Table 2-1 below has been adapted from Becker (1994) and shows a comparison of the lipid, protein and carbohydrate content between different strains of autotrophic micro algae.

Table 2-1: Biochemical composition of micro algae (dry matter basis %) (Becker, 1994)

Strain	Proteins	Carbohydrates	Lipids	Nucleic acid
Scenedesmus obliquus	50-56	10-17	12-14	3-6
Scenedesmus quadricauda	47	-	1.9	-
Scenedesmus dimorphus	8-18	21-52	16-40	-
Chlamydomonas rheinhardii	48	17	21	-
Chlorella vulgaris	51-58	12-17	14-22	4-5
Chlorella pyrenoidosa	57	26	2	-
Spirogyra sp.	6-20	33-64	11-21	-
Dunaliella bioculata	49	4	8	-
Dunaliella salina	57	32	6	-
Euglena gracilis	39-61	14-18	14-20	-
Prymnesium parvum	28-45	25-33	22-38	1-2
Tetraselmis maculate	52	15	3	-
Porphyridium cruentum	28-39	40-57	9-14	-
Spirulina platensis	46-63	8-14	4--9	2-5
Spirulina maxima	60-71	13-16	6-7	3-4.5
Synechoccus sp.	63	15	11	5
Anabaena cylindrical	43-56	25-30	4-7	-

The biochemical composition of micro algae also varies depending on the nutrients that they are supplied with. The main nutrients required for the growth of micro algae are potassium, nitrogen, phosphorous, sulphur and magnesium.

During the cultivation process, the amount of each nutrient supplied, affects the lipid, protein and carbohydrate content of the micro algae. Variations in just one nutrient such as nitrogen, can have a large effect on the biochemical composition of micro algae. Table 2-2 shows the effects of various amounts of nitrogen on the protein content of a variety of different strains of algae.

Table 2-2: Protein content on a % dry weight basis of different strains of algae with various concentrations of nitrogen (Piorreck et al., 1984)

Algal species	Nitrogen source	% nitrogen added					
		0.0003	0.001	0.003	0.01	0.03	0.1
Chlorella vulgaris	NH_4Cl	7.79	11.1	19.9	28.9	31.2	N/A
	KNO_3	12.6	6.75	14.5	30.7	31.1	32.2
Scenedesmus obliquus	NH_4Cl	9.36	9.43	22.0	33.2	34.4	N/A
	KNO_3	8.19	9.00	8.81	34.0	32.1	32.9
Anacystis nidulans	KNO_3	21.2	18.3	33.4	33.9	39.7	46.3
Microcystis aeruginosa	KNO_3	28.1	27.6	23.5	24.9	46.5	50.1
Oscillatoria rubescens	KNO_3	N/A	N/A	28.0	35.6	53.8	48.6
Spirulina platensis	KNO_3	N/A	25.8	26.6	33.4	52.1	47.4

For the *Chlorella vulgaris* it was found that nitrogen feeding reduces the lipid content as shown by Feng et al. (2011) and Mujtaba et al. (2012) who found that nitrogen feeding lowers the lipid content to 29.8% and 15.5% respectively. However, James et al. (2011) investigated the effect of nitrogen feeding on a different strain of micro algae, *Dunaliella salina,* and found that the lipid content was increased, this is similar to findings from Gao et al. (2013) who also found an increase in the lipid content with 54.15%. Therefore the biochemical composition of

micro algae varies between different strains and can be altered based on the growth conditions used.

2.2.2.3. Cultivation methods

Interest in micro algae is rapidly growing due to the fact that they are very quickly cultivated. There are many companies that have commercialised the use of micro algae for many different products but mainly for food supplements.

For autotrophic growth, the micro algae require a light source, water, nutrients and carbon in the form of CO_2. During the growth of autotrophic algae photosynthesis occurs, which converts the CO_2, minerals and water into carbohydrates using chlorophyll in the presence of a light source (Govindjee, 2014).

There are three main methods for cultivating micro algae, open raceway ponds, photo-bioreactors and fermenters. Both open raceway ponds and photo-bioreactors cultivate autotrophic algae in the presence of sunlight. Fermenters produce heterotrophic algae in the dark.

2.2.2.3.1. Open raceway ponds

Basic open raceway ponds simulate the normal growth conditions of algae. It is the simplest cultivation method for algae. Figure 2-3 shows a schematic of an open raceway pond. The ponds are usually located outdoors to expose the algae to direct sunlight so that autotrophic algae can be produced. With older raceway ponds, it was difficult for all of the algae to be mixed and receive enough CO_2 and nutrients, this has become easier at present due to the addition of paddle wheels to the newer open raceway ponds as is shown in Figure 2-3. The paddle wheels act as a mixer which stirs and circulates the slurry from the bottom upwards, towards the sunlight. There are also baffles that are in place to stop the flow of the slurry and cause vortexes, which also helps with mixing the algae (Moazami et al., 2012).

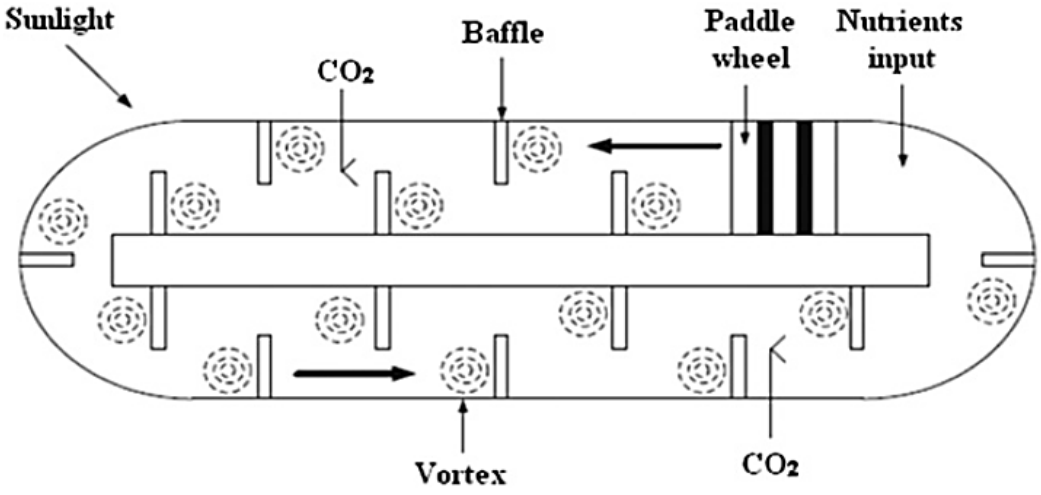


Figure 2-3: Open raceway pond schematic (Moazami et al., 2012)

There are however, limits to using open raceway ponds for cultivating algae. The main limitation is the amount of space required along with the amount of water required and the access to direct sunlight (Austin, 2014). Another concern is the contamination risk from bacteria in the air.

2.2.2.3.2. **Photo-bioreactors**

Photo-bioreactors otherwise known as closed loop systems, can also be used for cultivating algae. Figure 2-4 shows a schematic of the photo-bioreactor set-up. The air inlet valve allows CO_2 to be pumped into the water column which holds the medium used in the system, along with the nutrients, before it is pumped into the reactor tubes. The water is pumped into the reactor tubes which hold the algae and are exposed to the sunlight. The algae is harvested and any gas produced, is cleaned and removed through the exhaust vent.

The main advantage to using photo-bioreactors for the cultivation of algae is the amount of space required for a photo-bioreactor, as it is much less than the amount of space required for an open raceway pond. Another advantage is that photo-bioreactors can be attached to an exhaust vent to assimilate CO_2 from other process and prevent it from being released to the atmosphere. The algae produced is also uncontaminated.

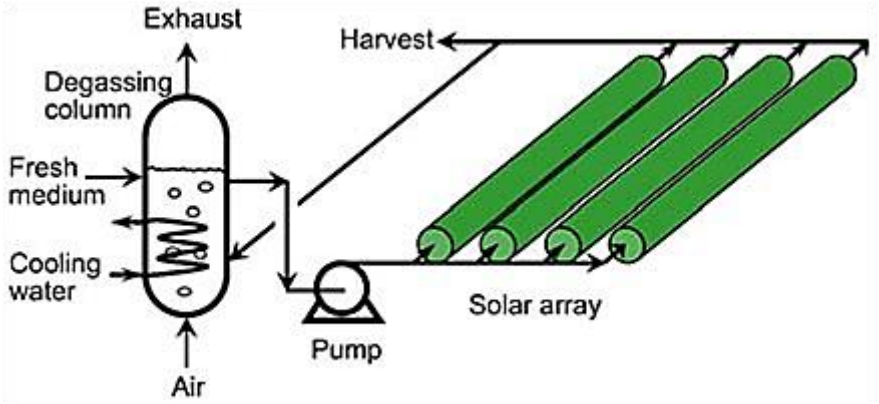


Figure 2-4: Photo-bioreactor schematic (Beranek-Collins and G, 2010)

Although there are advantages to using photo-bioreactors, there are also disadvantages. The main disadvantages include the high capital cost and the need to be able to receive direct sunlight to the reactor tubes, which means they have to be placed outside.

2.2.2.3.3. **Fermenters**

Fermenters are another method that can be used to cultivate algae. Figure 2-5 shows a schematic of a fermenter. As the algae that is cultivated is heterotrophic, CO_2 is not assimilated, instead a source of organic carbon is required. In most instances, sugars (such as glucose) are used, however some waste organic carbon streams are now being investigated.

There are advantages to using fermenters to cultivate algae, these include, producing algae with a high cell density, low capital cost and low cultivation costs (Xu et al., 2006). There is also a very good understanding of how fermenters work as they have been used previously for the production of yeast.

Although there are quite a few studies that compare the suitability of open raceway ponds and photo-bioreactors for the cultivation of algae, there are not so many that investigate fermenters as they are still considered a new technology and there is not much literature on the subject.

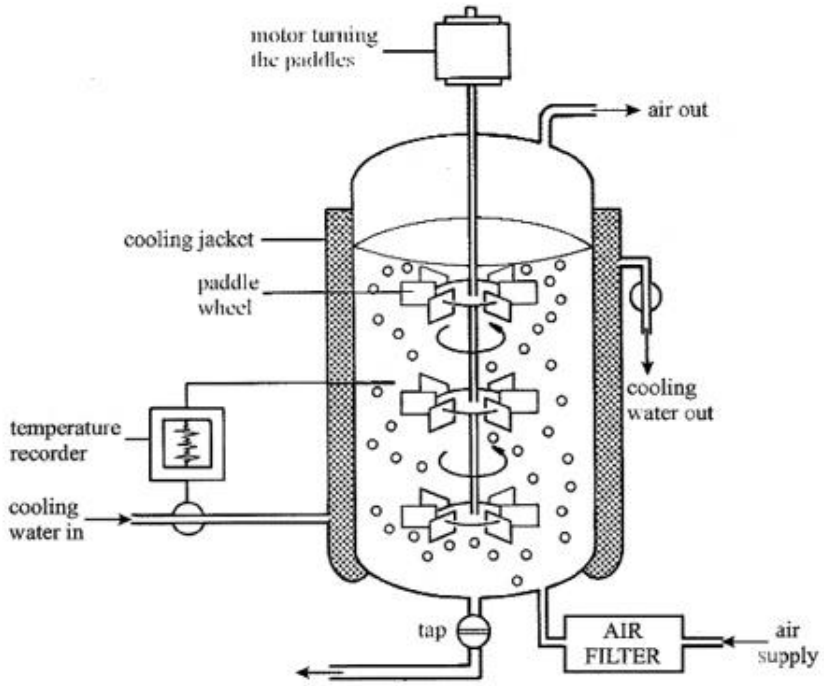


Figure 2-5: Fermenter schematic (Corfe, 2014)

Open raceway ponds and photo-bioreactors were compared on a life cycle analysis basis by Resurreccion et al. (2012) who found that open raceway ponds were more feasible as they had a longer lifespan. Jorquera et al. (2010) also carried out a lifecycle analysis and found that open raceway ponds were more economically feasible than photo-bioreactors.

2.3. Conversion methods to produce biofuels

There are many different types of technologies that can convert biomass (algae included) into biofuels, which can produce a char, liquid or syngas. All of the technologies fall into three main conversion categories: bio-chemical, chemical and thermochemical. Figure 2-6 shows the three main conversion routes, a variety of technologies that fall within each conversion route and the different types of biofuels that can be produced. The route of conversion is pivotal to the type of product that is produced and also to its characteristics as a fuel.

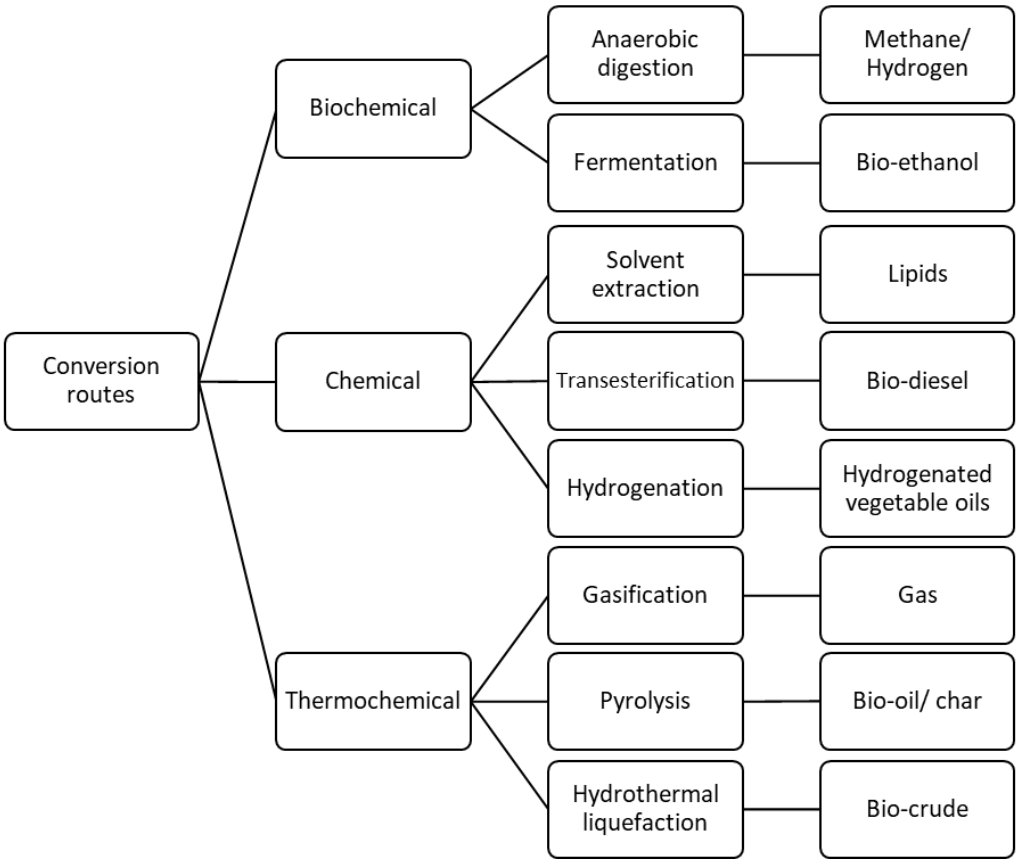


Figure 2-6: Conversion routes for micro algae (Gouveia, 2011)

2.3.1. Biochemical conversion

Two examples of biochemical conversion are anaerobic digestion and fermentation. Anaerobic digestion of micro algae produces biogas which is composed of mainly methane and hydrogen. Anaerobic digestion is one of the oldest technologies used for processing of micro algae, as shown in Golueke et al. (1956). Although anaerobic digestion has been a conversion technology for a long period of time, there are still limitations to using it as a conversion technology for micro algae, with the main issues being ammonia toxicity and carbon to nitrogen ratios (Ward et al., 2014).

Fermentation is also a conversion process that can be used for converting micro algae and also other biomass crops. During the fermentation process, there are two steps, hydrolysis and then fermentation. During the hydrolysis step, enzymes or acids are used to produce hydrolysate which can then be converted into bio-ethanol. Although fermentation is a well-established method for converting biomass into bio-

ethanol, but there are not many works in the literature for converting micro algae by fermentation. One of the few studies carried out on fermentation of micro algae, to produce bio-ethanol, was carried out by Ho et al. (2013). It was found that the micro algae that had a higher carbohydrate content resulted in a higher bio-ethanol yield.

2.3.2. Chemical conversion

Two examples of chemical conversion are transesterification and hydrogenation. Transesterification and hydrogenation are processes that involve converting lipids that are extracted from micro algae into oils.

The methods used to extract the lipids are solvent extraction and supercritical carbon dioxide. Solvent extraction is the most commonly used method when extracting lipids from micro algae. One of the first studies to investigate extracting lipids from micro algae by means of solvent extraction and then producing oils from the lipids was carried out by Dubinsky and Aaronson (1979) and more recently, similar studies were carried out by Bai, Xue et al. (2014).

Transesterification is a process that can be used for converting lipids into FAME (fatty acid methyl esters). The process involves producing two different phases; crude glycerine and FAME. The esters in the crude glycerine are converted into biodiesel (FAME) (Costa and Morais, 2011).

When the crude glycerine is converted into biodiesel, methyl ester fatty acids, are produced and the triglycerides are converted into glycerol, as is shown in Figure 2-7. There is a vast amount of literature that has been published on transesterification of micro algae and other biomass.

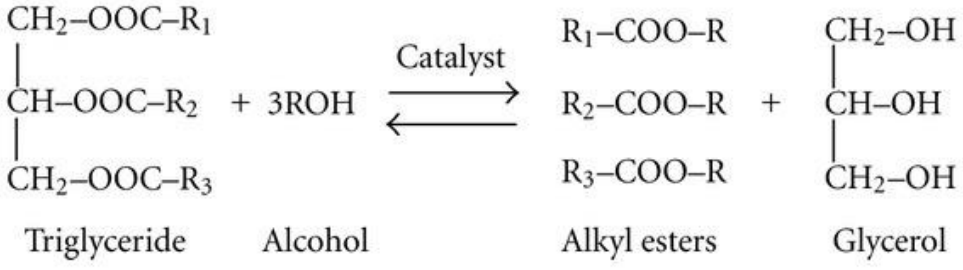


Figure 2-7: Process of transesterification (Koohikamali et al., 2012)

Hydrogenation is another chemical conversion process, which involves a catalyst being used to induce a chemical reaction by the addition of hydrogen, which is usually carried out under high temperatures and pressures (Amin, 2009). There are however, challenges to this approach which will be investigated in further detail in section 2.8.1.

2.3.3. Thermo-chemical conversion

Gasification, pyrolysis and hydrothermal processing are examples of thermo-chemical conversion.

2.3.3.1. Gasification

Gasification is a thermo-chemical conversion process which involves heating to produce mainly gas, and some chars. The gas is composed of mainly carbon dioxide, carbon monoxide, methane and hydrogen. Sanchez-Silva et al. (2013) and Guan et al. (2012), both carried out gasification of micro algae and produced hydrogen, carbon dioxide, carbon monoxide and methane, which indicated that the water shift gas reaction had taken place. Hirano et al. (1998) also carried out gasification at temperatures between 850°C and 1000°C on micro algae. The gasified micro algae was then used to produce methanol. A comparison was made between the yield of methanol for both woody biomass and micro algae. It was found that micro algae produced a yield of between 50-64% in comparison to the 45-50% yield from woody biomass.

2.3.3.2. Pyrolysis

Pyrolysis is another thermo-chemical conversion process which is similar to gasification as it applies heat to produce products, however, it is different as pyrolysis produces mainly oils and some chars. Pyrolysis was the method that was initially investigated for the conversion of micro algae into a biofuel, with works dating as far back as 1981 (Goldman et al., 1980).

There are two types of pyrolysis; slow and fast. The difference between both is the heating rate. Slow pyrolysis was carried out on micro algae by Grierson et al.

(2009) and Chaiwong et al. (2013) who both found that bio-oils and bio-chars were produced. Fast pyrolysis was carried out on micro algae, by Miao and Wu (2004), using a fluidised bed reactor. The temperature was increased from 400°C to 600°C, where the product yield increased, once 600°C was reached the yield of products began to decline.

Gasification and pyrolysis are both effective methods of thermo-chemical processing but they are not without limitations, the main one being the need to dewater feedstock before they can be used as the methods cannot deal with high water content. Hydrothermal processing is an alternative thermo-chemical processing technique that can process biomass and algae as received, even with a high water content.

2.3.3.3. Hydrothermal processing

Hydrothermal processing involves applying high temperature to organic matter, under high pressure. The process is similar to the natural process that occurs when fossil fuels are produced but within a much shorter time period. There is an extensive amount of literature available on hydrothermal processing of micro algae. Brown et al. (2010), Ross et al. (2010), Zhou et al. (2010) and Biller and Ross (2012), focus primarily on producing bio-crude oil from hydrothermal processing for the use as a transport fuel. Although bio-crude oil has been the focus in previous years, the literature available on hydrothermal processing has moved on to other products produced from hydrothermal processing. Depending on conditions, hydrothermal processing can produce chars, oils and gases through hydrothermal carbonisation, liquefaction and gasification. Changes to the pressure and/or temperature can result in different phases or products being produced as is shown in Figure 2-8.

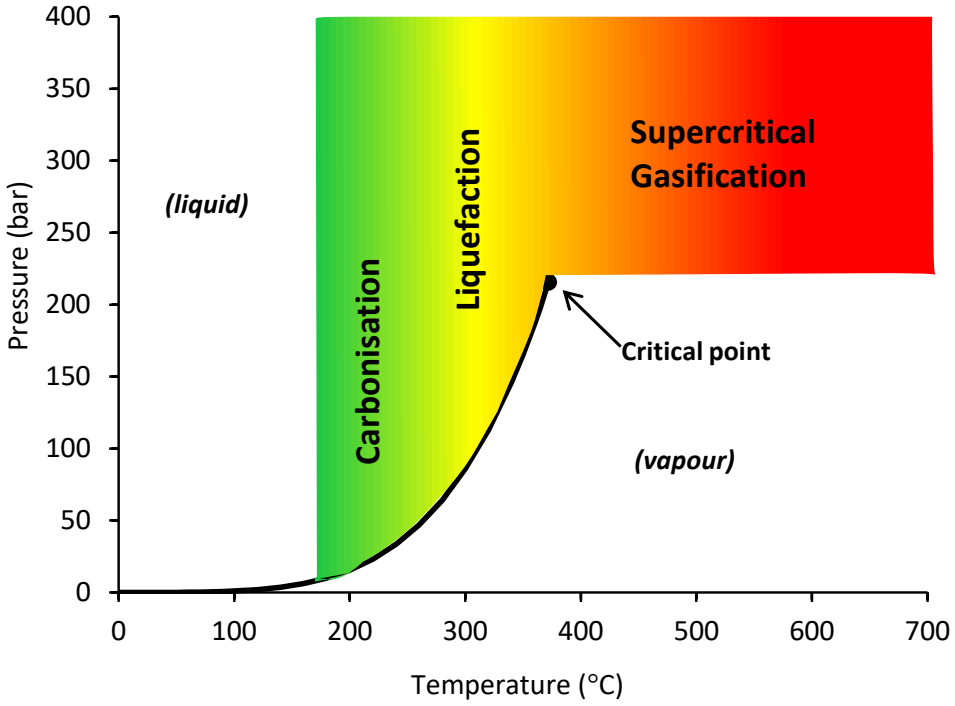


Figure 2-8: A pressure temperature phase diagram (Biller and Ross, 2016)

As hydrothermal processing is such a versatile conversion method, various feedstocks can be converted into useable products; switchgrass (Cheng et al., 2009), grassland perennials (Zhang et al., 2009), micro algae (Ross et al., 2010), macro-algae (Xu et al., 2015), duckweed (Duan, Peigao et al., 2013b) and lignocellulosic biomass feedstocks have been investigated (Savage et al., 2010a).

Hydrothermal carbonisation occurs at temperatures up to 200°C, resulting in the production of chars. The chars produced are often referred to as hydro-char and have the ability to be used for various applications. A study by Heilmann et al. (2010) found that the char produced from hydrothermal carbonisation had very similar qualities to those of bituminous coal. Bird et al. (2011) investigated the potential of bio-char as a means of providing nutrients to soil to improve the quality of soil and found that the use of bio-chars was beneficial to acidic soils as they enhanced the quality of the soil. It was also found that bio-chars are beneficial as a means of carbon sequestration.

At temperatures between 200°C and 375°C, an oil is produced and the process is referred to as hydrothermal liquefaction. The liquefaction process takes place in the following sequence as shown by Alba et al. (2012): hydrolysis of macromolecules which are then converted through dehydration and rearranged by means of condensation, cyclisation and polymerisation to eventually produce oil.

For temperature above 375°C, a syngas is produced and the process is referred to as hydrothermal gasification (Biller and Ross, 2012). The carbons present in the feedstock material are converted into longer hydrocarbon chains which can then be used as a natural gas fuel or can be used to produce other chemicals.

Of the three hydrothermal pathways, liquefaction is the focus of the majority of this study and is therefore explained in more detail in the next section.

2.3.3.3.1. **Reaction pathways for hydrothermal liquefaction**

There are three major steps that occur during degradation of biomass during hydrothermal liquefaction which are described by Toor et al. (2011):

i. Depolymerisation of the biomass
ii. Decomposition of monomers by cleavage, dehydration, decarboxylisation and deamination
iii. Recombination of reactive fragments

During the first stage, the macromolecules such as carbohydrates, proteins and lipids are hydrolysed. These molecules are broken down further at higher temperatures and then recombined into ‘heavy molecular weight’ materials. In the second stage, the macromolecules in the biomass lose a substantial part of the oxygen, which is removed by dehydration or decarboxylation. In the final stage the reactive fragments are recombined into longer chain, larger molecules.

For algal biomass, the biochemical components; proteins, lipids and carbohydrates, behave differently when hydrothermally liquefied.

The mechanism for oil formation from proteins in algae is a result of hydrolysis of the C–N peptide bond between the carboxyl and amine groups of the amino acids which are the building blocks of proteins. The amino acids formed by

this process subsequently degrade by decarboxylation and deamination (Peterson et al., 2008). Several amino acids have been studied during HTL to understand their reactivity and stability, which allows the behaviour of proteins from algae to be investigated. Abdelmoez et al. (2007) conducted a study on the kinetics of 17 amino acids in saturated subcritical water (230–290 °C) and found that for alanine and glycine, the pathways of deamination and decarboxylation were competing pathways. Glycine, alanine, valine, and proline formed as intermediate products from decarboxylation of complex amino acids. Another study by Sato et al. (2004) measured the decomposition of five amino acids (alanine, leucine, phenylalanine, serine, and aspartic acid) at 200–340 °C, 20 MPa, and 20–180 seconds and reported that the two main pathways, under these conditions, were deamination to organic acids and ammonia, and decarboxylation to amines and CO_2. Figure 2-9 shows the decomposition pathways of amino acids; glycine, alanine, serine and aspartic acid, during HTL.

For the production of bio-crude via HTL, high lipid content is preferred as experimental work by Biller and Ross (2011b) indicates that biocrude production follows the trend lipids > proteins > carbohydrates.

It is important to understand the hydrothermal reaction pathways of lipids and lipid model compounds. There are several studies which have investigated the hydrothermal reaction model compounds, such as acylglycerides, fatty acids, and fatty acid esters in both sub- and supercritical water, that have been summarised in studies such as Ruiz et al. (2013), Peterson et al. (2008) and Toor et al. (2011), however the main focus is on the whole biomass and there is little information on the reaction kinetics of lipid model compounds on a molecular level.

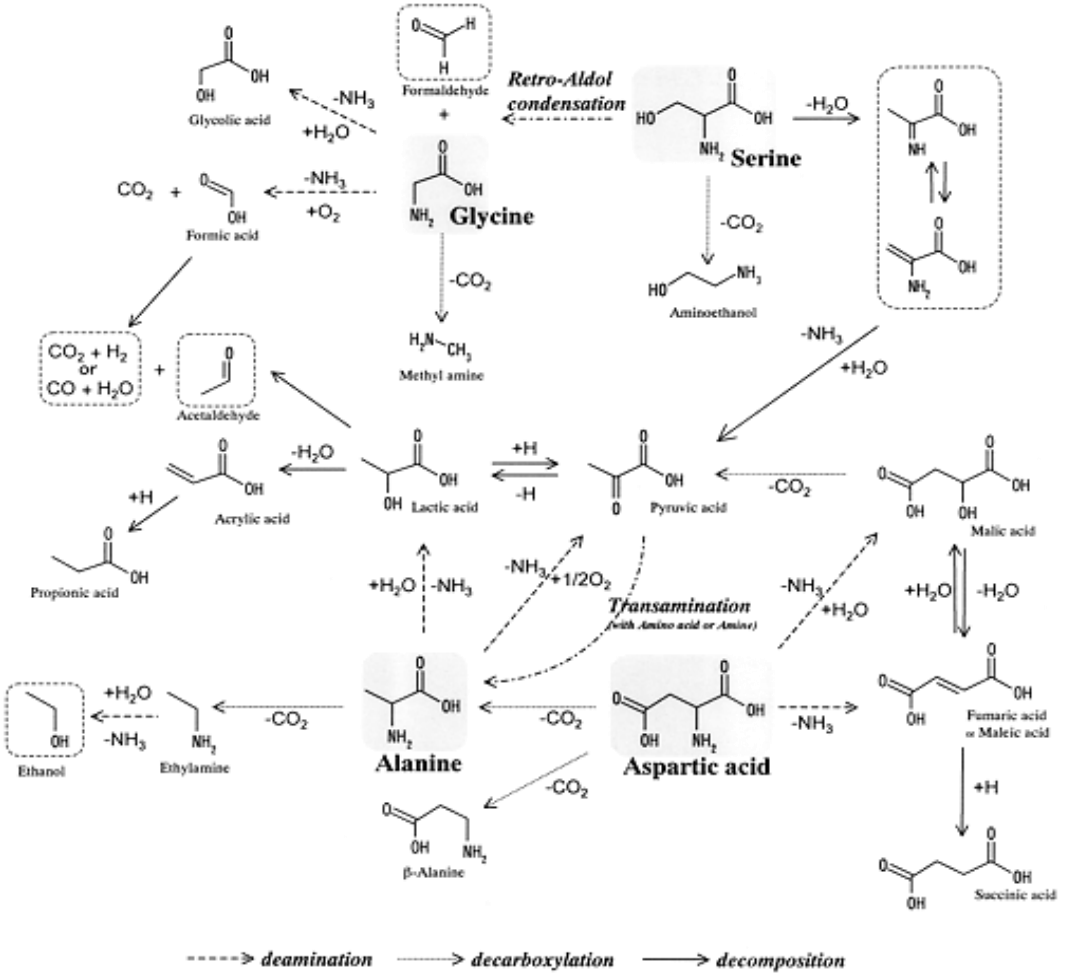


Figure 2-9: Decomposition pathways of amino acids during HTL (Sato et al., 2004)

Lipids can be further separated into polar lipids such as phosphoglycerides, glycosylglycerides, and sphingolipids, and non-polar lipids such as acylglycerols, sterols, free fatty acids, wax, and steryl esters (Chen et al., 2018). Non-polar lipids, such as acylglycerides hydrolyse to form fatty acids and glycerol. The saturated fatty acids tend to be stable in subcritical water and may begin to decompose in supercritical water, however, the conversion is low unless a catalyst is present (Changi et al., 2015). Further work is required on reaction pathways of polar lipids and unsaturated fatty acids as there is not much literature available at present.

There have been many studies investigating hydrothermal liquefaction of carbohydrates (e.g., cellulose, hemicellulose and starch) into mono-saccharides and oligosaccharides and their derivatives. Some of these are explored in more detail below.

Cellulose makes up the primary cell wall of micro algae and consists of glucose units linked by β-1,4-glycosidic bonds (Toor et al., 2011). The high crystallinity of cellulose makes it insoluble in water at room temperature, however, in water at near-critical conditions, the cellulose can be hydrolysed into low molecular weight oligomers which are soluble in water. These can be further hydrolysed into smaller water-soluble products such as glucose and fructose (Baig et al., 2013).

A study by Rogalinski et al. (2008) investigated the hydrolysis kinetics of a few different biopolymers, namely starch and cellulose (polysaccharides) and found that cellulose degrades in subcritical water at temperatures from 240–310°C and pressures from 20–25 MPa and can be described by a first-order rate law. Another study by (Sasaki et al., 2000) investigated the decomposition of cellulose in subcritical and supercritical water at temperatures between 320-400°C and pressure of 25MPa. It was found that at temperatures between 320 and 350°C decomposition products of glucose were the main products whereas at temperatures above 350°C the cellulose decomposed quickly and at 400°C, the product yield from hydrolysis was 77% and the cellulose conversion was almost 100% at residence times as short as 0.05s. Figure 2-10 shows the reaction pathways for hydrolysis of cellulose and glucose.

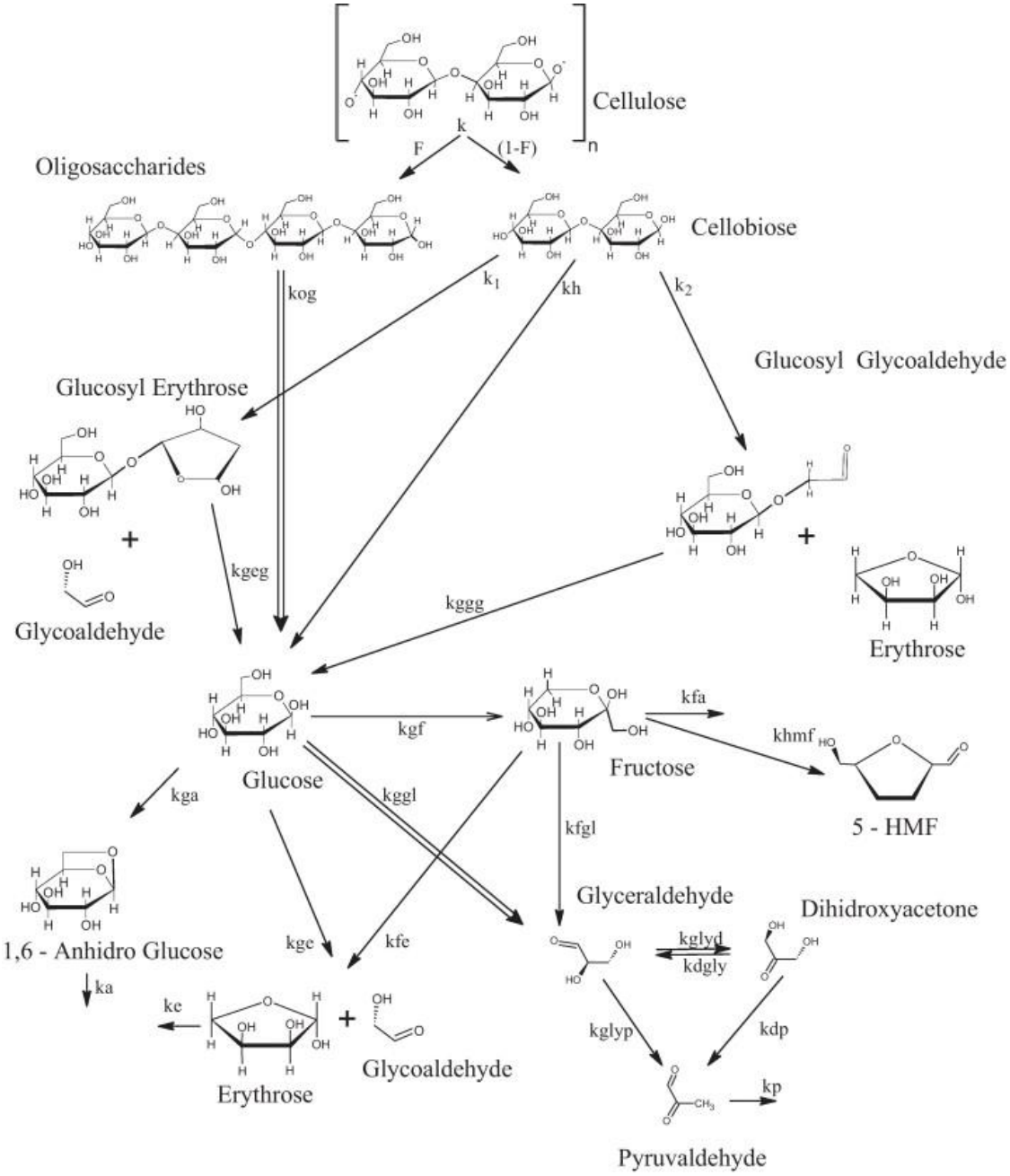


Figure 2-10: Reaction pathways for cellulose hydrolysis and glucose reactions during HTL (Cantero et al., 2013)

Hemicellulose is a polysaccharide which generally consists of d-xylose, l-arabinose, d-galactose, d-mannose, and d-glucose, with xylose being the most abundant (Zhou et al., 2013). Hemicellulose is found in the cell wall lining of micro algae and is less resistant than cellulose to hydrolysis. Hemicellulose is solubilized in water at temperatures above 180°C. At temperatures between 200 and 230°C, the hemicellulose is fragmented and the materials in the cell wall dissolve (Hashaikeh et al., 2007). The major hydrolysis product of hemicellulose at mild temperatures

(~200°C) is xylose, which can be dehydrated to furfural and then can be further converted into other products such as formic acid (Ruiz et al., 2013). Figure 2-11 shows the reaction pathways of xylose during hydrothermal processing.

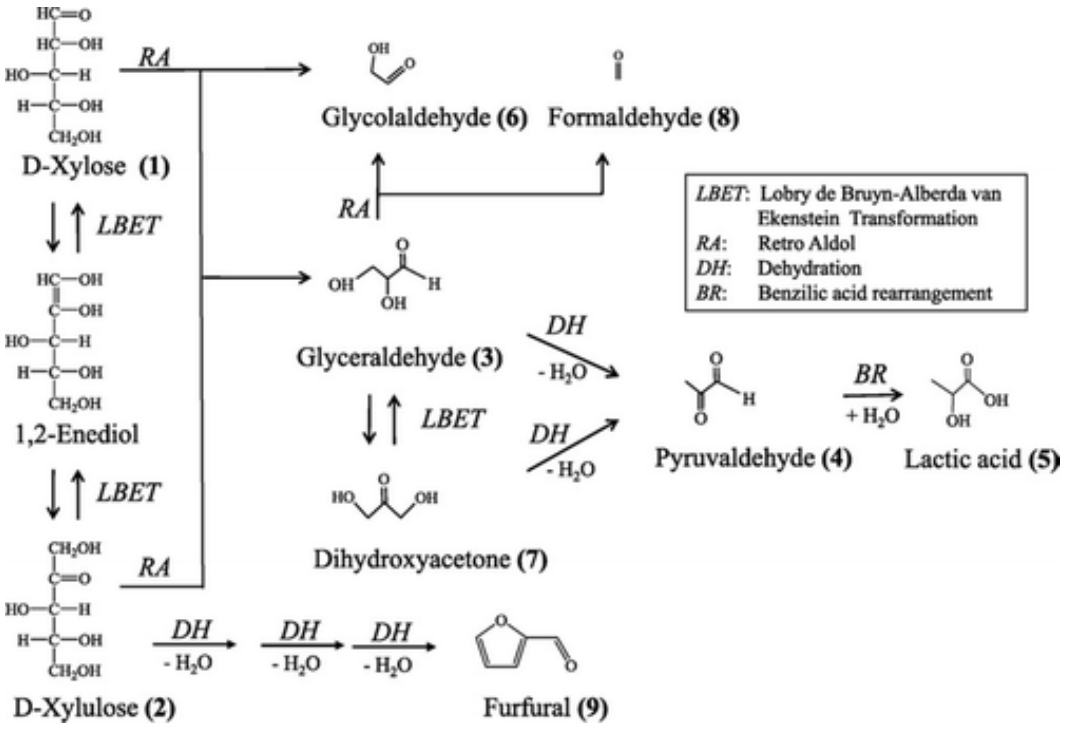


Figure 2-11: Reaction pathways of xylose during hydrothermal processing (Changi et al., 2015)

Starch is another polysaccharide which is mainly formed by β-1,6-glycosidic links. When depolymerised at 180-250°C, oligosaccharides and monosaccharides are produced, which include maltose, glucose, fructose, 5-hydroxymethylfurfural (5-HMF), and furfural (Orozco et al., 2012). The reaction pathways of glucose have previously been shown in Figure 2-10.

Overall, the severity of the temperature affects the different products produced from the proteins, lipids and carbohydrates. Low temperature hydrothermal processing shows more carbohydrates being broken down into polar water-soluble organics in comparison to high temperature hydrothermal processing which favours the carbohydrates being transformed into non-polar hydrocarbon structures along with the proteins and lipids.

2.3.3.1. Catalytic hydrothermal liquefaction

Whilst hydrothermal liquefaction of micro algae has been investigated quite extensively in recent years, there are some limitations to the bio-crude produced such as the yield, nitrogen and oxygen content and amount of upgrading required to make the bio-crude into a useable fuel. To overcome these limitations catalysts can be used, to increase the bio-crude yield, to improve the bio-crude quality, thus resulting in less upgrading being required.

Literature shows that both homogenous and heterogenous catalysts have been used for hydrothermal liquefaction of micro algae. The most common homogenous catalyst employed include Na_2CO_3 (Jena et al., 2012), CH_3COOH and KOH (Ross et al., 2010). The heterogenous catalysts used mainly focus on transition metal oxide catalysts such as Raney-Ni and HZSM-5 (Zhang et al., 2013) and palladium or platinum on carbon (Duan and Savage, 2010).

Duan and Savage (2010) carried out catalytic hydrothermal liquefaction of Nannochloropsis sp. using six different catalysts (Pd/C, Pt/C, Ru/C, Ni/SiO2−Al2O3, CoMo/γ-Al2O3 (sulfided), and zeolite) and found that all of the catalysts tested produced higher yields of bio-crude than just liquefaction of Nannochloropsis sp. However, the elemental compositions and heating values of the crude oil were largely unaltered by using the catalysts. Another study by Chen et al. (2015) investigated the use of two acid catalysts (ZrO2/SO422and HZSM-5) and two base catalysts (MgO/MCM-41 and KtB) and found that the base catalysts improved the conversion rate and bio-crude yield. In another study by Ross et al. (2010) investigated the addition of both alkali (potassium hydroxide and sodium carbonate) and organic acids (acetic acid and formic acid) and found that the yields of bio-crude were higher using an organic acid catalyst than an alkali one.

Although catalytic hydrothermal liquefaction seems to increase the yield and improve the quality of the bio-crude from micro algae, there is the major limitation of catalyst poisoning when using catalysts during hydrothermal liquefaction.

2.4. Issues associated with oils from algae

Although alternative biomass feedstocks such as algae, are able to produce bio-oils, there are still issues associated with them that prevent the oils from being used directly. Some of the issues are organic, such as nitrogen and others are inorganic, such as heavy metals. Overall there is a need to reduce these elements in the bio-crude.

2.4.1. Nitrogen

Nitrogen in bio-crude from algae is problematic as it will disassociate during combustion and undergo chemical transformation to form N_2, NO and NO_x. As N_2 is a major constituent of air, it is not considered a pollutant. NO_x however is more problematic as it can cause inflammation of the airways and damage the lungs of humans. There is legislation in place from both the UK and Europe, which is covered under the European directive (99/30/EC) and the Air Quality Strategy (UK), which states that the hourly limit of NO_x is 200µg m^3, with no more than 18 exceedances per year and 40µg m^3 annually, to be achieved by 2005 for the UK legislation and 2010 for the European directive (Air Quality Expert Group, 2004).

The nitrogen content of crude oils is typically less than 1% (Prado et al., 2017) and is therefore not considered problematic for liquid transport fuels, whereas for bio-oils from algae nitrogen can consist of up to 8% of the bio-oil, due to the high protein content in some algae (Obeid et al., 181).

2.4.2. Heavy metals

Heavy metals are another problematic component of the algae which are present in bio-crude from HTL, as they can cause issues in the refinery process such as corrosion and catalyst poisoning. Iron is one of the metals that can cause issues when deposited on catalysts, which results in reduced activity and efficiency of the catalysts. Iron is also difficult to remove from the bio-crude. Other metals, such as sodium, can form low-temperature melting compounds that lead to deposition and build-up on surfaces. However, sodium is easier to remove than iron and can be removed via various demineralisation methods (Jiang and Savage, 2018).

Jiang and Savage (2018) carried out a study on the effects of hydrothermal process variables (temperature, residence time, feedstock and solvent loading) on 13 different elements and found that P, Mg, Na, Ca were present in bio-crudes produced from lower temperatures, whereas the concentrations of Zn, Cu and Ni do not show much difference with variation of the HTL severity.

2.5. Pre-treatment technologies

There is a need for pre-treatment of algae, due to the rigidity of the cell wall. This can inhibit extraction of lipids during biofuel production. Therefore, by adding a pre-treatment step and disrupting the cell wall, the inner components of the algae such as the lipids, can be extracted more easily. As mentioned in the previous section, the main problematic components of the algae are the nitrogen and heavy metals. Pre-treatment of the algae can remove and reduce these components.

There are many different technologies that can be used for the pre-treatment of algae in the process of producing biofuels. These can be categorised into mechanical, physical, chemical and thermal techniques. The removal and reduction of the components from the algae are dependent on the type of technique used during pre-treatment (Onumaegbu et al., 2018).

2.5.1. Mechanical

High pressure homogenising (HPH) and bead milling are two examples of mechanical pre-treatment methods.

High pressure homogenisation is widely used for the pre-treatment of algae due to its ability to process wet biomass and rupture the cell walls to allow effective lipid extraction (Ekpeni et al., 2015). However, there are disadvantages to HPH as it is energy intensive and high cost (Onumaegbu et al., 2018).

Bead milling is more commonly used than HPH for cell disruption of algae. The process entails of the collision of small glass or ceramic beads spinning on high speed with the algae (usually micro algae). Although this is a simple, effective method, there are also disadvantages to its use. Several studies have noted that

proteins are released from the cells when the algae is pre-treated by bead milling (Donsì et al., 2013), (Balasundaram and Pandit, 2001), (Balasundaram et al., 2012).

2.5.2. Physical

Microwave and ultrasonic processing are examples of commonly used physical pre-treatment techniques.

Microwave pre-treatment works by the magnetic waves causing an increase in temperature which in turn increases the kinetic energy within a cell and causes cell disintegration (Onumaegbu et al., 2018).

Ultrasonic pre-treatment works by submerging the biomass into liquid media, which increases turbulence effects and shock waves in the liquid media which results in cell disintegration (Onumaegbu et al., 2018). Ultrasonic pre-treatment has been effectively used, for various micro algae, in a number of studies (Geciova et al., 2002; Gerde et al., 2012; Gupta et al., 2017).

2.5.3. Chemical

Chemical pre-treatment has been investigated using many different chemicals under various conditions and works by initiating a chemical reaction to disrupt the algae structure (Onumaegbu et al., 2018). Alkali and acidic reagents are used to solubilise the hemicellulose in micro algae to open them up to enzymatic break down.

2.5.4. Thermal

2.5.4.1. Hydrothermal

Hydrothermal pre-treatment is a relatively new method, which is not yet commonly used for pre-treating biomass. The hydrothermal pre-treatment can also be considered as hydrothermal carbonisation which produces a solid char like residue and a nutrient rich aqueous phase.

Recent work by Montero-Hidalgo et al. (2019), Costanzo et al. (2015) and Levine et al. (2013) focussed on hydrothermal pre-treatment of algae at mild

temperatures, to reduce the N and O content in the bio-crude produced by HTL of the pre-treated algae and to produce a nutrient rich aqueous phase for algal cultivation. From these studies it is evident that hydrothermal pre-treatment is having an affect on the resulting bio-crude from HTL.

2.6. Sequential hydrothermal processing

Sequential hydrothermal processing is mentioned in the literature as method which incorporates pre-treatment and conversion into one process. It works by exposing the algae to a mild hydrothermal pre-treatment step followed by hydrothermal liquefaction in the same reactor with no emptying and separating of products from the pre-treatment stage to the liquefaction stage.

A group of researchers at Washington State University developed a unique sequential hydrothermal liquefaction method for the production of bio-crude from liquefaction of micro algae. The method involves first applying a mild hydrothermal pre-treatment and then removing the polysaccharides from the process waters by precipitation with ethanol. The solid algal residue is then liquefied at 300°C to produce a bio-crude. The researchers, Miao et al. (2012), first carried out the method on heterotrophic *Chlorella sorokiniana* to determine the impact of reaction conditions on the bio-crude quantity. They found at 160 °C, 20 min and 1:9 biomass/water ratio a maximum yield of 32wt% of polysaccharides was obtained. They also found that sequential hydrothermal liquefaction always produced ~5% more bio-oil and ~50% less bio-char than direct hydrothermal liquefaction. In another study published by the research group, Chakraborty et al. (2012), sequential hydrothermal processing was again undertaken of *Chlorella sorokiniana*. It was found that the sequential hydrothermal liquefaction method extracted 26% of the polysaccharides present, which resulted in 63% less bio-char being produced in comparison to direct liquefaction. This suggests that the majority of the carbohydrates present in algal biomass were converted into bio-char. Although this technique appears to be a promising method for the production of bio-crude from micro algae, there are some issues that would need to be further investigated, such as the operational costs of the added step and disposal of used ethanol.

Another study by Jazrawi et al. (2015) undertook sequential hydrothermal processing with the aim to reduce the nitrogen content of the resulting bio-crude and found that sequential hydrothermal processing reduces the nitrogen content by 50%.

Although there have been some studies on sequential hydrothermal liquefaction and also on hydrothermal liquefaction following hydrothermal pre-treatment of the algae, there does not seem to be any literature that utilises the processing waters by extracting nutrients, organic and inorganics and recycling the process water back into the hydrothermal liquefaction stage, but instead focus primarily on using the process waters for cultivation.

2.7. Nutrient recycling in aqueous phase from hydrothermal processing

There have been many papers that have investigated recycling and reusing the aqueous phase from hydrothermal processing. Heilmann et al. (2010) carried out a study which investigated the potential of recycling the process water to grow more algae. Another study carried out by Onwudili et al. (2013) on the hydrothermal gasification of micro algae, investigated the aqueous phase that was produced and found that it could be used for the cultivation of micro algae. Both studies found that the aqueous phase has the potential to be recycled to be used for the cultivation of micro algae.

2.7.1. Phosphorous

Phosphorous is an essential element for living things as it makes up the back bone of DNA/RNA. It is also essential for the growth of algae. The biggest use of phosphorous is in fertilisers for agriculture, in the form of ammonium phosphate. This is produced from phosphate ore which is mined out of the ground. It is recycled through the food chain in the following order; absorbed by plants for growth, plants consumed by animals and humans, released as manure which can be used as fertiliser and then the phosphorous is returned to the soil or as sewage which goes into waste water treatment. With an increasing population the demand for phosphate ore will also increase. However, as phosphate ore is a finite resource, its use needs to

be controlled (Royal Society of Chemistry, 2017). The phosphorous consumed by humans is mostly converged at wastewater treatment plants. Although there have been many chemical treatments developed for the recovery of phosphorous from wastewater, biological treatments such as growth on algae and other microbial biomass remain as the primary treatments used (Withers et al., 2015).

The ability of the phosphorous to be recycled depends on the phosphorous speciation in the raw feedstock and products from treatment. This is as speciation largely controls phosphorous extractability and speciation in the liquid extracts from treatment of the biomass (Turner and Leytem, 2004) and (Hunger et al., 2005). Therefore, in this regard, the potential for phosphorous reclamation or recycling from the products from hydrothermal treatments is determined by the phosphorous speciation in the products.

In recent years there has been an increasing number of studies on the transformation of phosphorous during hydrothermal treatments of various biowastes, which have similar characteristics to biomass. These studies suggest that hydrothermal treatments can be tuned towards modulating phosphorous speciation and migration, which could help with specific phosphorous species reclamation or recycling. However, further work is required as understanding of the factors that control speciation are still not complete, which hinders the optimisation of techniques for phosphorous recovery (Huang et al., 2017).

2.7.2. Nitrogen

Nitrogen is an element which is required for the growth of crops. It is normally found in the form of ammonia. The ammonia can be used in various forms such as ammonium nitrate, ammonium sulphate and ammonium phosphate. Liquefied ammonia is usually used as a spray on fertiliser. Ammonia is commercially produced by the Haber-Bosch process (Zumdahl, 2017). As with phosphorous, excess nitrogen can be washed into water systems causing eutrophication. This can cause rapid algal blooms due to the excess nutrients available. However, once the algae die, there is a lack of oxygen in the water system and this is damaging to the fish population (Food and Agriculture Organization, 2017). Therefore there is a need to put in a place a bioenergy system which can

recover excess nutrients from processing algae and reuse the nutrients in either cultivation or for other purposes.

2.8. Upgrading of bio-oil

The bio-oils produced from biomass usually require upgrading to some extent to be able to use them as bio-fuels. There are a few different upgrading methods that can be employed such as hydrogenation and hydrothermal upgrading.

2.8.1. Hydrogenation

Hydrogenation is used in petroleum refining to increase the saturation of hydrocarbons and remove sulphur, nitrogen and oxygen and has also become a commonly used method for the upgrading of bio-oils (Ramirez et al., 2015). A by-product of the process is ammonia, which could also be used as a value added chemical. Rathsack et al. (2019) carried out a study to analyse the products of bio-crude from HTL of algae and found that the most common compounds present in the hydrogenated bio-crude were nitrogen and oxygen containing compounds and that there were more lower molecular weight hydrocarbons observed after hydrogenation.

Another study by Li, H.Y. et al. (2014) compared hydrogenated and non-hydrogenated bio-crude from HTL and found that the contents of acids, amides, phenols, and alcohols decreased, in the hydrogenated bio-crude, whereas hydrocarbons content increased. There were also more branched cyclic nitrogenous compounds detected in the hydrogenated bio-crudes but there was a decrease in the aromatic/hetero-aromatic functionality.

2.8.2. Hydrothermal upgrading

Hydrothermal upgrading is also a method that is becoming more frequently used for the upgrading of bio-oils. It involves carrying out hydrothermal processing with the addition of catalysts to remove heteroatoms, improve heating value and reduce viscosity of the bio-oil (Xu, D. et al., 2018).

There have been numerous studies on catalytic hydrothermal upgrading of bio-oils with many focused on algae. Catalysts such as platinum on gamma alumina (Duan, Peigao et al., 2013a), platinum on carbon, molybdenum carbide and HZSM-5 (Duan and Savage, 2011), amongst others, have been used.

A study by Xu, Y. et al. (2018) investigated the use of Ru/C (10wt%) for upgrading bio-crude from a range of micro and macro algae at 400 °C for 2 h and found that all the upgraded bio-oils had higher energy densities and significantly lower N, O, and S contents and viscosities than their corresponding raw bio-crudes. Another study by Bai, Xiujun et al. (2014) investigated the use of Ru/C and Raney-Ni combined and found that at 400°C in the presence of hydrogen, the quality of the bio-crude was improved and contained higher H and C contents.

Overall, the use of catalysts during hydrothermal upgrading of bio-crude has a positive effect on the bio-crude quality. Just as there are limitations to using catalysts for catalytic hydrothermal liquefaction, there are also limitations to using catalysts for upgrading, such as catalyst poisoning.

2.9. Summary

There are many methods which are currently used for the production of biofuels from algae, however, there are still gaps in the literature which need to be studied. Hydrothermal liquefaction, pyrolysis and solvent extraction are established methods which can be used for producing bio-fuels from algae, however, there is little literature available, which couples hydrothermal pre-treatment with these processing methods. Although there are established methods for producing biofuels from algae, there are still outstanding upgrading issues for the bio-oil. The majority of the bio-oils produced from the different conversion methods require extensive upgrading, thus, there is a need to look at different upgrading methods, which can also involve pre-treatment.

Nutrient recovery from process waters from hydrothermal liquefaction of algae mainly focus on re-cycling and re-use of the processing waters for cultivation of the algae, with very limited focus on re-using and re-cycling the processing waters from hydrothermal pre-treatment into hydrothermal liquefaction.

Overall, there are still gaps in the literature with regards to producing good quality biofuels from algae and this thesis investigates a potential route which has not yet been studied.

Chapter 3. Methodology

3.1. Introduction

The scope of the project covers the entire process from raw material preparation to production and use of bio-crude. This chapter is split into sections based on the order of research; materials, pre-treatment, conversion and analysis. The first part states what materials were used and how they were prepared. The next section explains the pre-treatment of the materials. The third section details the conversion methods undertaken. The final section covers the analysis of the products from the pre-treatment stage and the conversion methods.

Figure 3-1 shows the process in order of how experiments were carried out. First analysis of the raw algae was undertaken. The algae was then hydrothermally pre-treated. The products from hydrothermal pre-treatment were separated into solid residues and process waters. The solid residues were used to produce oil from the three conversion methods: pyrolysis, solvent extraction and hydrothermal liquefaction. The process waters from hydrothermal pre-treatment were used in hydrothermal liquefaction as received and also after passing through Mg bio-chars. These were compared to hydrothermal liquefaction in distilled water. The difference in the quality of oil was explored.

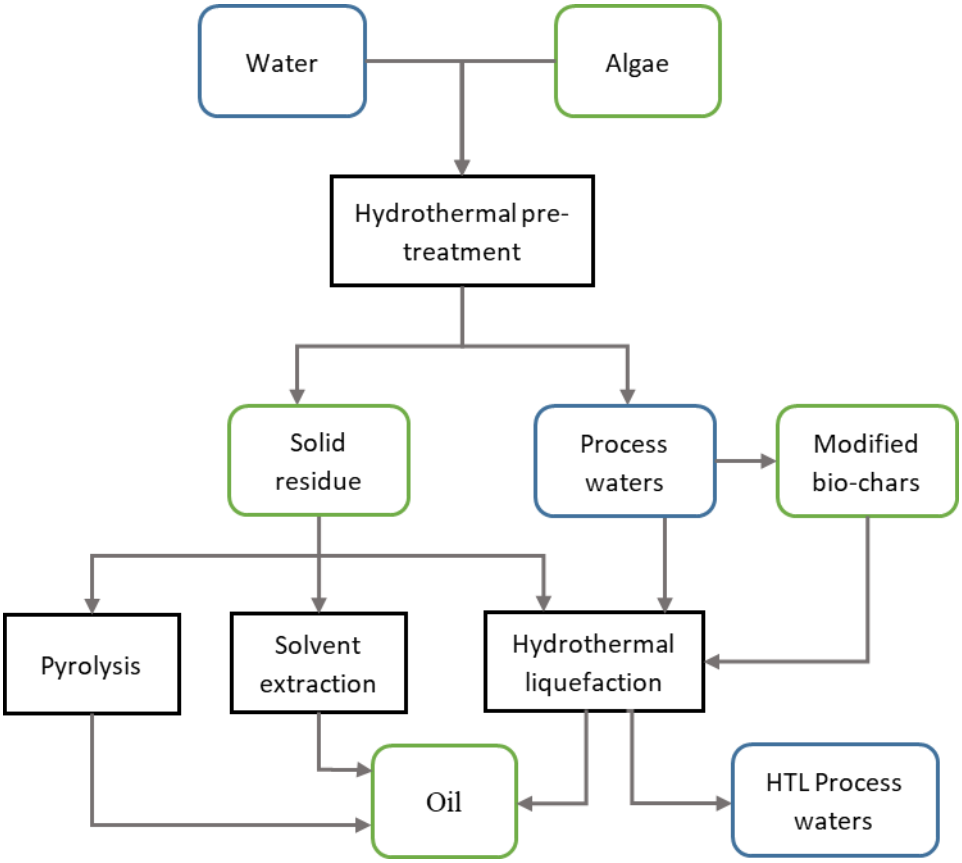


Figure 3-1: Flow chart of experimental process

3.2. Materials

Samples of autotrophic and heterotrophic micro algae, along with autotrophic macro algae are used as feedstocks in this investigation. The autotrophic micro algae, *Chlorella vulgaris*, was purchased from a commercial source in China. The *Ulva lactuca* was provided by Ocean Harvest Technology Ltd. The heterotrophic *Chlorella vulgaris*, was cultivated at and provided by the University of North Dakota. The *Spirulina platensis*, was cultivated at the University of Leeds. The *Chlorogloeopsis fritschii* was cultivated at the Plymouth Marine Laboratory. These strains of algae were chosen based on the variation in their biochemical content. All samples were received freeze dried and were used as received.

All chemicals used for experiments and analysis were of analytical grade and used as-received. Distilled water was produced in-house in the Energy building at the University of Leeds.

3.2.1. Sample preparation

All of the algae samples were cryogenically milled using a Retsch cryomill. The cryomill is an impact ball mill that is cryogenically cooled to -196°C using nitrogen on the outside of the mill. Freezing causes the algae to become brittle and allows it to break down easier. A cryomill is used as it keeps the sample cold and avoids volatile material being released as it is not being heated up. Approximately 10g of sample were milled per cycle. Once the samples were milled, they are passed through a 100μm sieve with any large pieces being re-milled. Figure 3-2 shows an example of the algae before and after milling. The main reason for using the cryomill was to make sure the feedstock was a uniform size and also to break down the cell walls and make pre-treatment easier.

Figure 3-2: Example of algae before (left) and after milling (right)

3.2.2. Biochemical analysis

Biochemical analysis was performed for the raw algae. The samples tested are *Ulva lactuca,* autotrophic *Chlorella vulgaris,* heterotrophic *Chlorella vulgaris, Spirulina platensis* and *Chlorogloeopsis fritschii.*

Lipid content was determined by solvent extraction using hexane, following the method outlined later in section 3.5.2.

Protein was determined using the DUMAS method. The method involves combustion of a sample (with a known mass) at temperatures of 800-900°C in the presence of oxygen, which produces a gas which consists mainly of carbon dioxide, water and nitrogen. The gas is bubbled through a solution of potassium hydroxide which absorbs the carbon dioxide and water and then the remaining nitrogen is

determined using a thermal conductivity detector (Oxford Dictionary, 2008). Then using the nitrogen content of the algae, a conversion factor is applied to determine the amount of proteins in the sample. The nitrogen to protein conversion factor of 6.25 is commonly used for most biomass samples, however, this has been criticised as being too high, resulting in over estimation of the protein content (Krul, 2019). A more reasonable factor of 4.78 has been reported and is often used for determining protein content in algae (Lourenço et al., 1998; Lourenço et al., 2004; Laurens et al., 2012; Lourenco et al., 2002). The lower conversion factor of 4.78 has been used in this work so not to overestimate the protein content of the algae.

The structural carbohydrates of the raw algae were determined by amylase-treated neutral detergent fibre content (aNDF) using the BS EN ISO 16472:2006 method. This analysis was carried out by the Institute of Biological, Environmental & Rural Sciences (IBERS) at Aberystwyth University. For the structural carbohydrate content of the pre-treated algae, the non-structural carbohydrates were calculated as described below and then the structural carbohydrates were determined by difference.

For the raw algae, the non-structural carbohydrates were determined by difference using Equation 3-1. For the pre-treated algae, the values for the non-structural carbohydrates were estimated for the three temperatures with 20%. 30% and 40% removal for the algae pre-treated at 100, 150 and 200°C respectively.

$$\text{Non structural carbs} = 100\% - \text{Ash} - \text{Lipids} - \text{Protein} - \text{Structural carbs}$$

Equation 3-1: Non-structural carbohydrates

3.3. Cultivation

Cultivation of the *Spirulina platensis* was carried out at the University of Leeds using a starter culture obtained from the Culture Collection of Algae and Protozoa (SAMS Research Services Ltd, Scottish Marine Institute, Oban, Scotland). The growth media used to cultivate the *Spirulina platensis* is outlined in Table 3-1.

Table 3-1: Nutrients in medium for cultivation of *Spirulina platensis*

Nutrients	g per litre
NaCl	1
$MgSO_4.7H_2O$	0.2
$CaCl_2.2H_2O$	0.04
$FeSO_4.7H_2O$	0.01
EDTA	0.08
K_2HPO_4	0.5
$NaNO_3$	2.5
K_2SO_4	1
$NaHCO_3$	15

Cultivation trials were also undertaken for the purpose of producing heterotrophic *Chlorella vulgaris* as it is not commercially available to purchase. However, the cultivation process was unsuccessful and therefore the heterotrophic algae was supplied by the University of South Dakota instead. The cultivation method for the attempt at the University of Leeds is described in detail in Appendix 1.

3.4. Hydrothermal pre-treatment

3.4.1. 600ml Parr reactor

Hydrothermal pre-treatment was performed in a 600 ml Parr bench top reactor (Parr, USA) (Figure 3-3) at 100°C, 150°C and 200°C. A Proportional Integral Derivative (PID) heat controller was used to regulate the temperature. The set-up of the reactor is shown in Figure 3-3. 20g of algae and 200ml of distilled water was loaded into a quartz glass liner, giving a 10% solid loading rate within the reactor. The reactor was then heated to 100°C, 150°C or 200°C at approximately 8 °C $minute^{-1}$ and the reaction temperature held for one hour. After one hour the reactor was removed from the heating jacket and allowed to air cool. Once cooled, the gas produced within the reactor is vented to the atmosphere. The solid and aqueous fractions are separated using vacuum Buchner filtration using 90mm

qualitative circles (Whatman, UK). The solid residue was allowed to air dry in a ventilated fume cupboard for a minimum of 48 hours.

The solid loading rate of 10% was determined by the literature available on hydrothermal carbonisation (HTC). Although an increase in solid loading is highly advantageous for HTC as low water to solid ratios is likely to lead to more favourable process economies, there is also a limitation to how much can be loaded due to the risk of saturation of the water and therefore reduced extraction of the inorganics from the biomass.

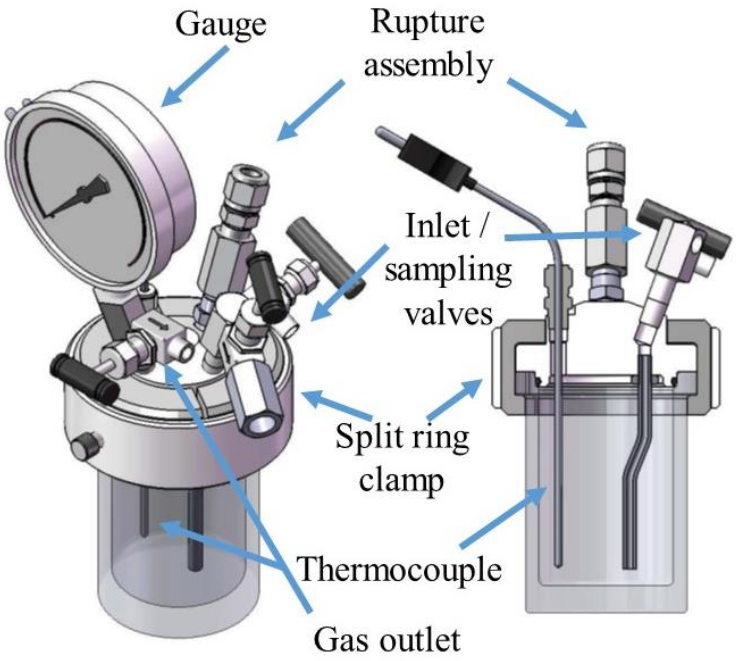


Figure 3-3: Schematic of 600ml parr reactor (Smith, 2018)

3.5. Conversion methods

3.5.1. Pyrolysis

Pyrolysis was carried out on the algae to produce oils, using the pyrolysis reactor shown in Figure 3-4. The reactor consists of a horizontal tube furnace with nitrogen flowing through and condenser to collect the oils and then any remaining gases. Under a flow of nitrogen at 3 litres per minute, 1g of algae was loaded into a ceramic boat, which was inserted into the tube furnace when the furnace had reached

a temperature of 600°C. The algae was held in the furnace for 1hr, after which the ceramic boat was removed from the tube furnace into the water cooled section of the tube, to stop any further reactions. The experiments were carried out in triplicate to produce enough oil for analysis.

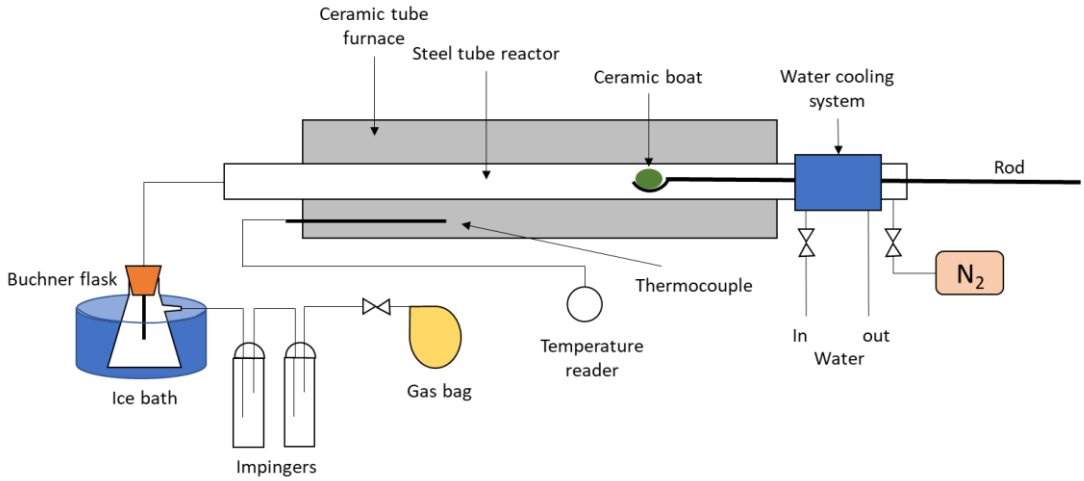


Figure 3-4: Schematic of pyrolysis reactor

3.5.2. Solvent extraction

Solvent extraction was carried out on the algae, to remove the lipids, using soxhlet extraction. The set-up of the soxhlet extraction is shown in Figure 3-5. The operation of the Soxhlet extractor relies on the refluxing and condensing of a solvent. In this instance the solvent used is hexane for the first step of the extraction and chloroform-methanol in a 2:1 ratio for the second step. 150ml of each solvent was used in a 250ml round bottomed flask. The solvent is heated to reflux, causing the solvent vapour to travel up the distillation arm and down into the main chamber where the thimble, containing approximately 5g of algae, is placed. The condenser is placed at the top of the extractor to make sure that the solvent is condensed back into the main chamber in order to fill it up to a point where the top of the siphon is. At this point, the main chamber, which contains the solvent and the dissolved lipids, empties itself automatically and returns the solvent back into the round bottom flask. The cycle is then repeated for 24 hours. The solvent is then transferred to a beaker and left to evaporate overnight in a fume cupboard. The lipids then remain.

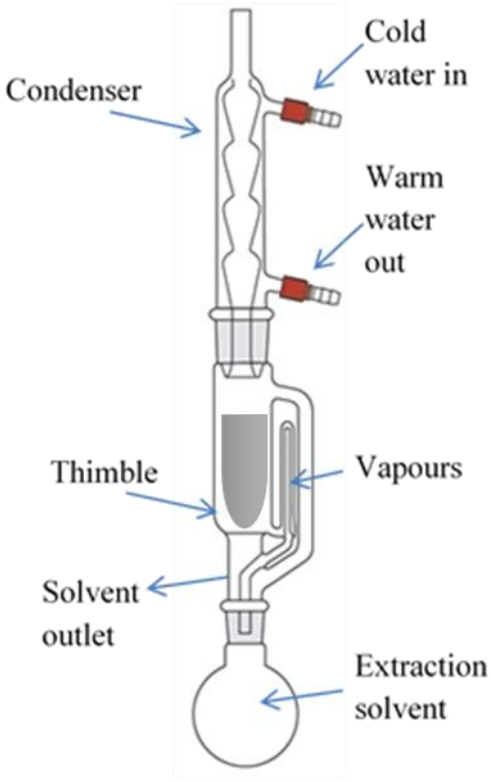


Figure 3-5: Schematic of a soxhlet extractor (Fischer Scientific, 2019)

3.5.3. Hydrothermal liquefaction - 25ml reactor

Hydrothermal liquefaction was initially performed on the autotrophic *Chlorella vulgaris* and *Ulva lactuca*. The liquefaction was performed in a custom built Swagelok reactor, constructed from 316 stainless steel 1.905cm x 10cm Swagelok pipe. One end of the reactor is capped whilst the other end is connected to a 1.905-0.635 cm reducer, fitted with a pressure release valve to allow the release of gas.

The methodology employed was taken from Biller et al. (2016). 1g of algae and 9ml of distilled water were added to the reactor. The sealed reactor was placed into a sand bath at 350°C for a residence time of 20mins. After 20mins, the reactor was removed from the sand bath and placed in cold water to quench and stop any further reactions taking place. The reactor was then dried with compressed air to remove any residual sand and to dry the reactor. The reactor was then weighed before and after venting the gas produced, to determine the mass of gas gravimetrically by difference. The solid residue and aqueous phase were decanted into a centrifuge tube. The bio-crude remained in the reactor and required rinsing

with dichloromethane (DCM) to remove from the reactor. Three aliquots of 3ml DCM were used to rinse the bio-crude out of the reactor and was decanted into a separate centrifuge tube.

The centrifuge tube containing the aqueous phase was centrifuged at 6500rpm for 7mins. Once centrifuged, the water was pipetted out into a vial and stored in a refrigerator at 5°C until required further. The solids remaining in the centrifuge tube were then washed with an aliquot of DCM. The contents of the centrifuge tube containing the solids and the centrifuge tube containing the bio-crude and DCM solution were subjected to vacuum filtration. DCM was passed through the filter paper until it appeared clear. The DCM was then decanted into a pre-weighed 30ml glass bottle and left to evaporate under a stream of nitrogen for approximately 8hrs until a constant weight was observed. The mass of solids was determined by drying the filter paper in an oven for 5hrs at 105°C.

This method was employed as it ensures there is no contact between the DCM and aqueous phase as it has been previously shown that DCM can extract additional compounds from the aqueous phase (Xu and Savage, 2014).

3.5.4. Hydrothermal liquefaction - 75ml reactor

Hydrothermal liquefaction was performed on the heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii*. Initially liquefaction was performed in a custom built 75ml Parr (USA) reactor following the methodology outlined previously in A.B.Ross et al. (2010).

3g of sample was mixed with either 27ml distilled water or 25ml distilled water with 2ml of formic acid. Liquefaction was performed at 350°C in distilled water and 350°C for the distilled water and formic acid mixture, for a period of 1 hour with a heating rate of 25°C min^{-1}. Once the reaction has taken place, the heating jacket is removed from the reactor and the reactor was allowed to cool in air. Once cooled, the reactor is weighed, the gas is released and the reactor is weighed again to account for the mass balance. The process water is emptied out of the reactor into a pre-weighed centrifuge tube. 50ml of DCM is added to the reactor to remove the oil. The DCM phase is separated using filtration with the remaining DCM being removed by evaporation to determine the mass of the remaining bio-

crude. The mass of the solids was determined by drying the filter paper in an oven for 5hrs at 105°C.

3.5.4.1. Pentane fractionation of bio-crude from HTL

Pentane fractionation of the bio-crude was performed using a method adapted from Bjelić et al. (2018). Instead of using centrifuge tubes and a sonic bath, a soxhlet extractor was used. The set-up is the same as used for the solvent extraction as shown in Figure 3-5. An empty soxhlet thimble was weighed and the mass noted. Approx. 100mg of the bio-crude from HTL was measured into the pre-weighed soxhlet thimble. The amount is approximate as the bio-crude is very difficult to work with. 200ml of pentane was added to the round bottom flask and placed in the heating mantle. As the boiling temperature of pentane is 36.1°C, the pentane was only heated to up to 40°C for the soxhlet reflux to work. The entire set-up was sealed and kept in place using parafilm to avoid losing any pentane through evaporation. The soxhlet was left to run for 24 hours. Once the soxhlet had cooled to room temperature. The thimble was removed and left to air dry in a fume cupboard. The thimble contained the pentane insoluble fraction of the bio-crude. The pentane in the round bottom flask was emptied into a pre-weighed beaker and left to evaporate at room temperature inside a fume cupboard. Once the pentane had evaporated, the pentane soluble fraction was weighed and the mass balance calculated.

3.6. Production of modified bio-chars

Oak woodchips were used to produce bio-chars following a methodology adapted from Zhang et al. (2012). 10g of oak woodchips (2x1x1cm) were mixed with 40g of magnesium chloride ($MgCl2{\cdot}6H2O$) in 60ml of distilled water. The mixture was stirred and left to stand for 2hrs at room temperature. It was then covered and heated for 24hrs at 100°C on a hotplate. The oak woodchips were then strained and pyrolysed in a single vertical tube furnace (Elite Thermal Systems Ltd., Model TSV12/100/750) under the flow of N2 ($10ml^{-1}$) at a heating rate of 10°C min^{-1} to 600°C. Bio-oils collected in the condenser catch pot and gases generated, were not analysed. Once cooled, the pyrolysed bio-char was ground using a pestle and

mortar to achieve a larger surface area and more consistency between the size of the individual pieces. The oak wood chips and bio-chars produced were characterised and the results are shown in section 8.2.1 of the results chapters.

3.6.1. Bio-char adsorption test

Bio-char adsorptions tests were carried out on the process waters from hydrothermal pre-treatment using the Mg modified bio-chars. 1ml of process waters was added to a 100ml volumetric flask and made to volume with distilled water. 20ml of this dilution was then added to 1g of Mg modified biochar. The bottle was shaken for 30 seconds to disperse the bio-char within the water and then left for 30mins to allow adequate time for adsorption to occur. After 30mins the sample was filtered using simple gravity filtration. The process waters were analysed for TOC, TN, ammonium and phosphate content. The bio-chars were dried at 60°C for 24hrs to remove any residual moisture and were then analysed by proximate and ultimate analysis.

3.7. Characterisation methods

3.7.1. Proximate analysis

Proximate analysis was carried out using Mettler Toledo TGA/DSC Star System . The programmed method was consistent on both instruments and involved heating the sample in nitrogen atmosphere from 30°C to 105°C at a rate of 25°C min-1, with a 10min hold at 105°C to remove any residual moisture. The temperature was then ramped to 900°C at 25°C min-1 and held for an additional 10min to ensure full devolatilisation. After the 10 minute hold the atmosphere was switched to pure bottled air at 900°C, which was held for an additional 15 minutes to enable complete combustion. The micro algae samples were analysed in 70µl alumina crucibles (Mettler Toledo, Switzerland). Due to the high salt content of the macro algae, 70µl platinum crucibles (Mettler Toledo, Switzerland), were used instead to avoid permeation of sodium through the alumina crucibles. The moisture, volatiles, ash and fixed carbon contents of the samples were calculated using Equation 3-2, Equation 3-3, Equation 3-4, and Equation 3-5.

$$\% \text{ Moisture} = \frac{Mass_{initial} - Mass_{105°C}}{Mass_{initial}} \times 100$$

Equation 3-2: Moisture fraction from proximate analysis

$$\% \text{ Volatile} = \frac{Mass_{105°C} - Mass_{900°C\ N_2}}{Mass_{initial}} \times 100$$

Equation 3-3: Volatile fraction from proximate analysis

$$\% \text{ Ash} = \frac{Mass_{900°C\ Air}}{Mass_{initial}} \times 100$$

Equation 3-4: Ash fraction from proximate analysis

$$\% \text{ Fixed Carbon} = 100 - (\% \text{ Ash} + \% \text{ Volatile} + \% \text{ Moisture})$$

Equation 3-5: Fixed Carbon fraction from proximate analysis

3.7.2. Ultimate analysis

Ultimate analysis was carried out using a CE Instruments Flash EA 1112 series elemental analyser, following the methodology laid out in the British Standard BS EN ISO 16948:2015. Calibration standards of atropine, methionine, cystine, sulphanilamide and BBOT (2,5 Bis–(5–Tert-Butyl- Benzoxazol-2-yl)-thiopene) (Elemental Microanalysis, UK) were used to calibrate the instrument, with a standard reference material of oatmeal. These were run as a control every 10 samples to check and maintain instrument performance. Approximately 2.5 – 3mg of sample and the standards were placed in individual tin foil capsules using laboratory balance with an accuracy to 0.005mg (Mettler Toledo, Switzerland) and crimped to remove any air. The elemental composition of the sample is automatically calculated based on the CO2, NOx and SO2 concentrations in the gas produced when the sample is combusted. These gases are then separated using gas chromatography – thermal conductivity detector. From the elemental composition, the Higher Heating Value can be calculated using the Dulong equation (Equation

3-6) which is commonly used in studies on bio-oils. The use of the Dulong equation however does not take into account the nitrogen content of the sample and therefore may not be the most accurate method to use for these particular feedstock as they contain high amounts of protein which also relays to a high nitrogen content.

$$\text{HHV } \frac{\text{MJ}}{\text{kg}} = 0.3383 \text{ x C} + 1.443 \text{ x H} - \frac{O}{8} + 0.0942 \text{ x S}$$

Equation 3-6: Dulong equation to calculate the Higher Heating Value

3.7.3. Pyrolysis gas chromatography mass spectrometry

Pyrolysis-GC-MS analysis was performed following the method set out in a study by Biller and Ross (2014). The apparatus consisted of a CDS 5000 series pyrolyser (CDS Analytical Inc., Oxford, PA, USA) connected to a Shimadzu QP2010E GC–MS (Shimadzu Corporation, Kyoto, Japan). A quartz tube (25 mm length and 0.5 mm inner diameter) was used to hold the sample in the pyrolyser. Quartz wool was placed at either side of the sample to hold it in place. A purge flow of helium was set at 25ml/min to remove any oxygen prior to pyrolysis of the sample. Pyrolysis was performed at 600°C a ramp rate of 20°C/ms with a hold time of 20 s. Volatiles were trapped on a TENAX adsorbent trap and then desorbed at 300°C onto a heated transfer line, also held at 300°C. The transfer line was connected to the split/splitless injector on the GC inlet port at 280°C with the split ratio set to 10:1. The products were separated onto a DB5ms column with an internal diameter of 0.25 and a film thickness of 0.25μm. The temperature programme begins with a starting temperature of 40°C, hold time 2 min, ramped up to 280°C (10°C/min) with a hold time of 10 min. The mass spectrometer ion source was set to 260°C and the interface to 280°C. Scanning took place in the range of 50-550m/z, once per second. 50 peaks were identified per sample, using the NIST mass spectral database versions 147 and 27. Pyrolysis-GC-MS was carried out on raw micro algae and the solid residue after hydrothermal pre-treatment.

3.7.4. High Performance Liquid Chromatography

The aqueous phases from the hydrothermal pre-treatment stage and also from the hydrothermal liquefaction step were analysed, for sugar and acid content by high performance liquid chromatography (HPLC). HPLC was undertaken using a Thermo Ultimate 3000 (USA) setup, fitted with a column oven (Shimadzu CTO-10AC, Japan), ultraviolet detector and a refractive index detector (Shodex RI-101, Japan). The samples were injected at a volume of 10µL onto a Supelcogel C-610H (30cm x 7.8 mm) column which was held at 30°C. The mobile phase used was 0.1% H_3PO_4 in distilled water with a flow rate of 0.5ml/min. The sugars were detected using the refractive index and the acids were detected using the UV at 210nm.

3.7.5. Gas Chromatography Mass Spectrometry

3.7.5.1. Bio-crude analysis

The bio-crude was analysed using gas chromatography mass spectrometry (GC-MS) following a method from Biller et al. (2016). The bio-crudes were derivatised using -methyl-N-trifluoroacetamide (MSTFA, 69479 Sigma Aldrich). Approximately 10mg of bio-crude was prepared for analysis by adding 50µl MSTFA, 850µl dichloromethane (DCM) and 100µl of internal standard. The internal standard is made up of 4-bromotoluene (B82200 Sigma Aldrich) at approximately 200ppm in DCM, resulting in a concentration of 20ppm in the derivatised sample. The sample was vortexed for 30s and then placed on a shaker board for a further 3hrs before analysis. The standards stated in the method were also run and are listed in the Appendix 5.

The analysis was performed on one of two GC-MS set-ups. The first is an Agilent 7890B gas chromatograph coupled to an Agilent 5977A quadrupole mass spectrometer, courtesy of Aarhus University, Denmark and the other a Shimadzu QP2010E GC–MS (Shimadzu Corporation, Kyoto, Japan) at the University of Leeds.

The Agilent was set up using the following conditions. The GC inlet temperature was set at 280°C, with 1µl of sample injected with a split ratio of 20:1. A VF-5MS column (60m x 0.25mm x 0.25µm, 5-m EZ-Guard) was used. The programme conditions started at 40°C with a hold of 5min, ramp at 10°C/min to

100°C, ramp at 4°C/min to 280°C, ramp at 10°C/min to 300°C and a final hold time of 10 min, resulting in a total run time of 68min. The mass spectrometer transfer line and ion source were held at 300°C. Electron impact ionization was employed at 70eV and the data was acquired in scan mode (35–500 m/z).

The Shimadzu QP2010E GC–MS (Shimadzu Corporation, Kyoto, Japan) was set-up using the same conditions, but using an RTX-5 column instead of the VF-5MS used in Denmark.

3.7.5.2. Aqueous phase analysis

The aqueous phases from both the hydrothermal pre-treatment step and the hydrothermal liquefaction step were analysed following a method from Madsen, René Bjerregaard et al. (2016). The aqueous phase was derivatised before analysis using methyl chloroformate (MCF). 200µl of the aqueous phase was mixed with 40µl of 5.0% w/w sodium hydroxide solution, 200µl of methanol, and 50µl of pyridine. 25µl of MCF was added and then the sample was vortexed. This step was repeated again so that there was a total of 50µl of MCF added to the sample. Immediately to this, 400µl of chloroform containing 4-bromotoluene (20.8µg mL^{-1}) was added and then vortexed for 10s. 400µl of 50mM sodium bicarbonate solution was added and the solution was vortexed for a further 10s. Two layers formed within the vial; an aqueous layer and the chloroform layer. The aqueous layer was removed and the chloroform layer was transferred into a clean vial. The standards stated in the method were also run and are listed in the Appendix 6.

Analysis was performed using either the Agilent 7890B gas chromatograph coupled to an Agilent 5977A quadrupole mass spectrometer, at Aarhus University, Denmark or the Shimadzu QP2010E GC–MS (Shimadzu Corporation, Kyoto, Japan) at the University of Leeds.

The programme conditions for the Agilent are as follows. The GC inlet temperature was set at 260°C, with 1µl of sample injected with a split ratio of 20:1. A VF-5MS column (60m x 0.25mm x 0.25µm, 5-m EZ-Guard) was used The column oven temperature was set at 60°C to begin with and was held for 3min. This was then ramped to 300°C at a ramp rate of 5°C min^{-1} and held for a further 3min, with a total run time of 56min. The mass spectrometer transfer line and ion source

were held at 300°C, with a solvent delay of 3min. Electron impact ionization was employed at 70eV and the data was acquired in scan mode (35–500 m/z).

The Shimadzu QP2010E GC–MS (Shimadzu Corporation, Kyoto, Japan) was set-up using the same conditions, but using an RTX-5 column instead of the VF-5MS used in Denmark.

3.7.5.3. Aldehydes analysis of aqueous phase

The aqueous phases from both the hydrothermal pre-treatment step and the hydrothermal liquefaction step were also analysed for aldehydes, using a Shimadzu 2010QE GC–MS (Shimadzu Corporation, Kyoto, Japan) at the University of Leeds, following a method adapted from Flannelly et al. (2015). The GC inlet temperature was set at 250°C, with 1µl of sample injected with a split ratio of 10:1. A Restek wax capillary column (30m, 0.25mm ID, and 0.25µm) was used with helium as the carrier gas. The programme conditions started at 40°C, ramped to 220°C at 20°C/min, with a final hold time of 5 min, resulting in a total run time of 14min. The mass spectrometer transfer line and ion source were held at 200°C, with a solvent delay of 2.6min. Electron impact ionization was employed at 70eV and the data was acquired in scan mode (35–500 m/z).

3.7.6. X-Ray fluorescence (XRF)

XRF was carried out on the process waters from hydrothermal pre-treatment and liquefaction. The following elements were tested for: Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Br, Mo, Cd, Sb, and Pb. The method allows concentrations from approximately 0.0001 % to be analysed for. The process waters were placed in 25ml crucibles with a 30mm surface diameter, which were covered with a polypropylene film. The crucibles were then placed in the XRF and analysed.

3.7.7. Total Organic Carbon

Total organic carbon was determined using a Hach Lange IL550 total carbon analyser (Germany). The process water samples were injected into a furnace at

1000°C, which converts the carbon within the sample to carbon dioxide. The carbon dioxide is passed through the gas chromatography column onto a thermal conductivity detector. The amount of carbon dioxide is calculated which is proportional to the amount of carbon in the sample.

3.7.8. Total nitrogen, ammonium, total phosphorous and orthophosphate

The total nitrogen, ammonium, total phosphorous and orthophosphate content of the process waters were determined using Hach Lange test kits LCK338, LCK303, LCK350 and LCK409 respectively and analysed with a Hach Lange Lasa 100 spectrophotometer.

The total nitrogen is determined by the inorganically and organically bonded nitrogen oxidising to nitrate by digestion with peroxo-disulphate. The nitrate ions then react with the 2.6-dimethylphenol in a solution of sulphuric and phosphoric acid to form a nitrophenol. The ammonium is determined when the ammonium ions react at a pH 12.6 with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside, as a catalyst, to form indophenol blue.

The total phosphorous was determined as the phosphate ions react with molybdate and antimony ions in an acidic solution to form an antimonyl phosphomolybdate complex, which is reduced by ascorbic acid to phosphomolybdenum blue.

The orthophosphate was determined when the phosphate ions react with vanadate-molybdate reagent to form a yellow dye.

3.8. Data analysis and statistics

All experiments were performed in duplicate to minimise the likelihood of unrepresentative results. The error margin is given in the results as standard errors where appropriate and calculated using Equation 3-7. For the colorimetric analysis,

the samples were analysed in duplicate with triplicates of each reading being taken and then taken as a mean figure.

$$\text{standard error} = \frac{\text{standard deviation}}{\sqrt{\text{numberof samples}}}$$

Equation 3-7: Standard error in analysis

Chapter 4. Pre-treatment of feedstocks

4.1. Introduction

Raw algae can be used as a feedstock to produce bio-oil or bio-crude from a number of different processes as mentioned in the literature review, however, there are certain properties of the algae that make the quality of these oils quite low, which result in the requirement for upgrading. The elements that have been identified as the most problematic within the algae, which are subsequently also present in the bio-crude, are nitrogen and phosphorous along with other inorganics, such as heavy metals. These are described in more detail in the review by Usman et al. (2019). Pre-treatment of the algae before processing can help with reducing the occurrence of these problematic elements in the bio-crude, by reduction or removal into the process waters during hydrothermal pre-treatment, before conversion of the algae into bio-crude.

Pre-treatment of the algae can help with reducing the amount of upgrading required for the resulting bio-crude (Biller et al., 2013). Pre-treatment of micro algae can be carried out using various methods such as microwave, hydrolysis and hydrothermal processing. With the hydrothermal pre-treatment step, there is the possibility of the nitrogen and phosphorous, along with other organics and inorganics, being released from the algae into the process waters, therefore hydrothermal processing was chosen as the pre-treatment utilised in this research. This results in a potentially 'cleaner' solid feedstock, which is advantageous for the production of bio-crude, as less upgrading can be required.

When undertaking this research, there was limited literature available and only Reza et al. (2013) had investigated the fate of inorganics in hydrothermal processing at temperatures of 200, 230 and 260°C with a short retention time of 5 minutes. Recently, Smith et al. (2016) have also reported on the subject by conducting an investigation on the influence of hydrothermal carbonisation on a various feedstocks such as food waste, secondary sewage sludge, micro and macro algae, to determine the behaviour of the hydro-char produced when used in

combustion. The hydrothermal processing was carried out at 200 and 250°C. Both these studies have temperatures at the higher end of the carbonisation range.

Inorganics are a particular issue during conversion routes such as pyrolysis and gasification for the production of biofuels from biomass. Alkali and alkaline earth metals, influence ash chemistry during conversion of the biomass to fuel and can result in slagging and fouling when the resulting fuel is combusted (Loo and Koppejan, 2008). The alkali and alkaline earth salts can be removed from the feedstock through dissolution of the salts into the process waters during hydrothermal pre-treatment, potentially removing a large portion of the mineral content of the feedstock and therefore reducing the problems arising from the ash.

Work by Saddawi et al. (2012) demonstrated that simply washing biomass in distilled water (at room temperature and atmospheric pressure), can dissolve and remove ionic salts, such as alkali chlorides. Hydrothermal pre-treatment causes the water to become subcritical, which has a lower density than water under atmospheric conditions (Wagner and Pruß, 2002). Therefore, removal of these simple ionic salts can be increased with the application of hydrothermal pre-treatment. Ionic bonded inorganics could also be removed from the feedstock during hydrothermal pre-treatment due to increased ionic dissociation constant (Bandura and Lvov, 2006) increased dielectric constant (Archer and Wang, 1990) and lower pH (Funke and Ziegler, 2010).

The main aim of this chapter is to address objective 2 of this thesis, to investigate how the composition of the algae and the process waters change during hydrothermal pre-treatment at different temperatures. The effect of the release of the organics and inorganics into the process waters on the solid algal residue will be taken into consideration. Figure 4-1 shows a flow diagram of the process of pre-treatment which is undertaken in this chapter. Firstly algae is added to distilled water and hydrothermally treated at either 100, 150 or 200°C. The products from hydrothermal pre-treatment are then separated into a solid residue and process waters, which are analysed separately. The solid residue contains lipids, proteins and structural carbohydrates. The process waters contain sugars from carbohydrates, phenols, cyclopentanones, nitrogen and phosphorous, along with inorganics from the algae. The solid residues are then further converted into bio-crude by hydrothermal liquefaction either in fresh distilled water or in the separated process

water, which will be investigated in Chapter 8. The removal of certain organic and inorganic material from the algae into the process water during hydrothermal pre-treatment can improve the resulting bio-crude produced after conversion.

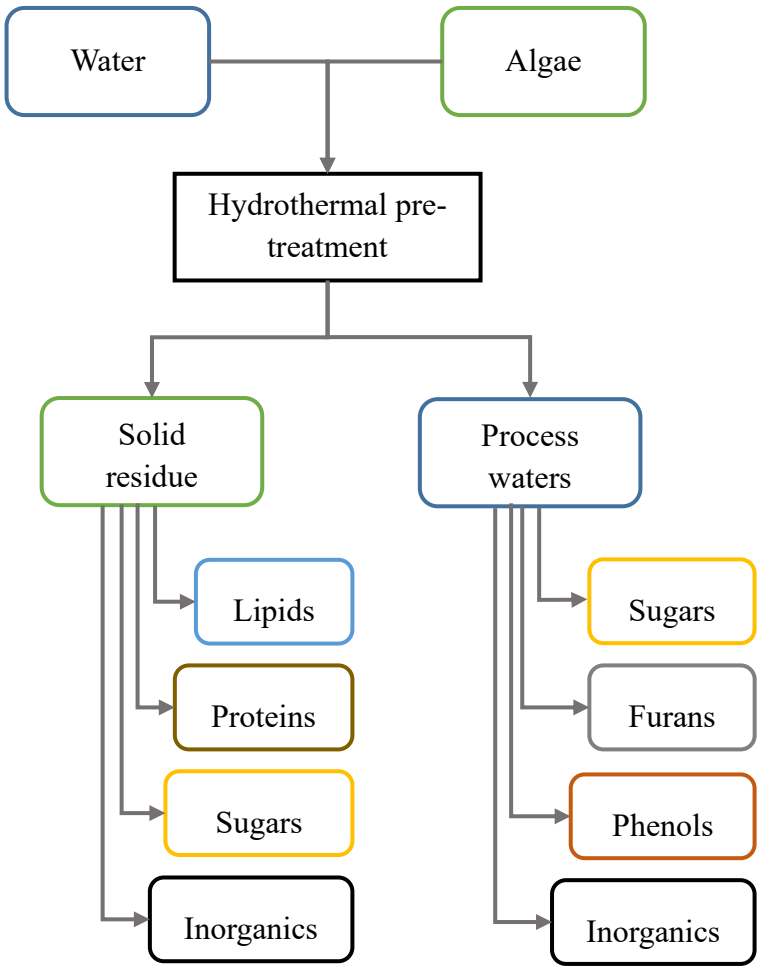


Figure 4-1:Flow diagram showing pre-treatment process and products produced

The first section of this chapter characterises the raw algae by determining the biochemical composition and undertaking ultimate and proximate analysis.

The second section of the chapter investigates hydrothermal pre-treatment by comparing a macro algae (*Ulva lactuca*) and a micro algae (autotrophic *Chlorella vulgaris*) at three different pre-treatment temperatures; 100°C, 150°C and 200°C, to determine if pre-treatment has an effect on the composition of the solid algal residue. The process waters are also analysed to determine what has been released

during pre-treatment. From this section an appropriate temperature pre-treatment is selected for the final section of the chapter.

The final section of the chapter investigates three different micro algae: heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii*, which are hydrothermally pre-treated at 150°C to investigate if differences in growth conditions and biochemical composition affect the quality of the feedstock. These feedstocks were chosen for hydrothermal pre-treatment due to their differences in biochemical composition and availability. This comparison was undertaken to investigate how micro algae of different compositions compare to the autotrophic *Chlorella vulgaris*, investigated in the first section of the chapter.

The products from the hydrothermal pre-treatment, i.e. solid residues and the process waters, are both analysed to determine the effects of pre-treatment. The solid algal residues are analysed by proximate and ultimate analysis. The process waters are analysed by GC-MS, HPLC, XRF and UV to determine the organics, sugars and metals present in the process waters along with the TOC, TN, ammonium, phosphate and orthophosphate content.

4.2. Characterisation of raw algae

4.2.1. Biochemical composition of raw algae

Micro algae are used for a number of applications, from food supplements to potential feedstocks for biofuels. Depending on the desired use of the algae, different biochemical compositions are required. As a feedstock for food supplements, a high level of protein is favoured, whereas for biofuel production, a high lipid content is preferred as a higher yield of oil can be produced. A high carbohydrate content is favoured for extraction of sugars for fermentation into ethanol. The biochemical composition of both the *Ulva lactuca* and autotrophic *Chlorella vulgaris* were determined following the method described in section 3.2.2 of the methodology chapter.

Generally, macro algae are characterised by lower contents of proteins and lipids but higher carbohydrates content compared to micro algae (Monlau et al., 2014). The type of carbohydrates in macro algae differ to those in micro algae.

Macro algae contain cellulose, xylan, mannan and ulvan (Rioux and L.Turgeon, 2015), whereas micro algae mainly consist of algaenan, glucose, rhamnose, xylose, and mannose (Markou et al., 2012).

Table 4-1 shows the biochemical composition of the raw algae, with the remaining percentage consisting of the ash in the sample. The ash contains the majority of the inorganics such as metals, in the sample.

Table 4-1: Biochemical components of raw algae

Type of algae	Biochemical components (%) (a.r.)			
	Lipids	Proteins	Carbohydrates	
			Structural	Non-structural
Ulva lactuca	0.2	6.5	21.7	55.2
Auto *Chlorella V.*	15.6	40.5	14.0	22.0
Hetero *Chlorella V.*	6.2	29.9	7.9	49.5
Spirulina platensis	5.0	53.6	2.1	32.4
Chlorogloeopsis fritschii	7.0	44.3	37.8	7.8

The *Ulva* has a very low lipid and protein content but a very high carbohydrate content with the non-structural carbohydrates making up over 50% of the composition. The main carbohydrates in green algae, such as the *Ulva lactuca*, correspond to mannan, ulvan, starch and cellulose (Jung et al., 2011). Marine macro algae tend to store energy as carbohydrates whereas micro algae convert sugars into lipids, which is where the energy is stored (Chen and Johns, 1991). The autotrophic *Chlorella* has the highest lipid content of the five algae. The protein content makes up over 40% of the composition of the autotrophic *Chlorella*, with the remainder of the composition made up of carbohydrates. The carbohydrates in micro algae are the main constituent of the cell wall and are therefore mainly structural carbohydrates (Chen et al., 2013). The higher lipid content of *Chlorella* makes it the most desirable of the feedstocks studied for biofuel production, however the high protein content could prove problematic as this means the *Chlorella* also contains high levels of nitrogen. The heterotrophic *Chlorella* contains less lipids, proteins and structural carbohydrates than the autotrophic *Chlorella*, however the heterotrophic *Chlorella* contains a higher amount of non-structural carbohydrates than the autotrophic *Chlorella*. The *Spirulina* has a low lipid content in comparison to the autotrophic *Chlorella* but contains more protein, with over 50% of the *Spirulina* being made up

of protein. For the carbohydrate content, there is very little structural carbohydrates present with the majority being non-structural. The *Chlorogloeopsis* has a slightly higher lipid content than the *Spirulina* but is still lower than the autotrophic *Chlorella*. The protein content of the *Chlorogloeopsis* is lower than the *Spirulina* but higher than the autotrophic *Chlorella*. Carbohydrates make up 40% of the biochemical composition of the *Chlorogloeopsis*, however the quantity of the structural carbohydrates is very high and the amount of non-structural carbohydrates is very low compared to the other four algae. This makes the *Chlorogloeopsis* harder to break down and release material during hydrothermal pre-treatment. A study by Biller et al. (2015) investigated the same *Chlorogloeopsis* and found that it contains a resistant aliphatic biomacromolecule, similar to algaenan, which makes it difficult to break down. The five different strains of algae were chosen due to their differences in biochemical content to investigate what role the different biochemical components play during hydrothermal liquefaction.

4.2.2. Ultimate and proximate analysis of raw algae

TGA was used to determine the proximate analysis of the algae which consists of the moisture, volatiles, fixed carbon and ash fractions of the sample. The method for this is described in detail in section 3.7.1 of the methodology chapter. The moisture content is reported on an as received basis and is mainly used for mass balance calculations and also to determine the chemical composition of the algae on a dry ash free basis for characterisation. The proximate analysis is reported on a dry basis. An elemental analyser was used to determine the ultimate analysis of the algae which is reported on a dry ash free basis. The method for this is described in detail in section 3.7.2 of the methodology chapter.

Table 4-2 shows the proximate and ultimate analysis of the raw algae feedstocks. From the proximate analysis, the *Ulva* has the highest moisture and ash content of the five algae. For the micro algae, the autotrophic *Chlorella* has the highest ash content of the four micro algae and the *Chlorogloeopsis* has the lowest. The ash is the fraction of the algae which usually contains the inorganics, therefore a high ash content usually relates to a high content of inorganics. The purpose of applying a hydrothermal pre-treatment step would be to reduce the amount of inorganics present in the ash, along with a reduction in the ash fraction.

Table 4-2: Proximate and ultimate analysis of raw algae

Type of algae	As received % (a.r.)	Proximate (%) (d.b.)			Ultimate (%) (d.a.f.)					HHV (MJ/kg)
	Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*	
Ulva lactuca	21.9	21.0	76.6	2.4	28.1	6.1	1.7	0.0	64.1	10.2
Auto *Chlorella V.*	7.1	8.6	77.1	14.3	54.6	8.0	9.3	0.0	28.1	26.6
Hetero *Chlorella V.*	3.6	6.8	83.5	9.7	54.9	8.0	6.7	0.0	30.4	26.3
Spirulina platensis	7.9	7.5	75.4	17.1	52.7	7.8	12.1	0.0	27.4	25.6
Chlorogloeopsis fritschii	9.5	3.5	80.2	16.3	52.6	7.6	9.6	0.0	30.2	25.0

*Oxygen was quantified by difference

All five algae have relatively similar volatiles content. The autotrophic *Chlorella, Spirulina* and *Chlorogloeopsis* have similar fixed carbon contents The *Ulva* has the lowest fixed carbon content of the five algae and the heterotrophic *Chlorella* is also low. The reason for the low fixed carbon content may be due to the high non-structural carbohydrates present in both the *Ulva* and heterotrophic *Chlorella*.

The ultimate analysis shows that the carbon content of the *Ulva* is significantly lower than the four micro algae, which are all quite similar with the carbon content over 50%. The *Ulva*, has the lowest hydrogen content whereas the micro algae all have a similar hydrogen content. The *Spirulina* has the highest nitrogen content of the five algae. The nitrogen content of the autotrophic *Chlorella* and the *Chlorogloeopsis* are very similar. The heterotrophic *Chlorella* has a lower nitrogen content of the micro algae and the *Ulva* has the lowest overall. The low nitrogen content of the heterotrophic *Chlorella* along with the low ash content, makes it an appealing feedstock for biofuel production. However, the application of a hydrothermal pre-treatment stage will also try to reduce the nitrogen content of the algae and therefore potentially improving the biofuel quality. There is no sulphur detected in the algae using elemental analysis. This may be due to the algae having such a low content which was not detected by the analyser as it has a detection limit of <0.1%. The oxygen content of the *Ulva* is much higher than the four micro algae which are all quite similar. The *Ulva* has the lowest HHV in comparison to the four micro algae, which are again quite similar. Overall, the four micro algae could be considered as quite similar to one another but the *Ulva* is quite different.

4.3. Hydrothermal pre-treatment of micro and macro algae

Firstly, two algae species were selected to investigate whether there is a difference between micro and macro algae. The chosen micro algae is *Chlorella vulgaris* and the macro algae is *Ulva lactuca*. The details of how these algae were obtained and how they were cultivated are described in section 3.3 of the methodology chapter.

The algae were hydrothermally pre-treated at three different temperatures, 100, 150 and 200°C, in a 600ml Parr reactor. The method is described in detail in section 3.4 of the methodology chapter.

An investigation by Smith et al. (2016) on a range of temperatures (°C) and biomass, including a micro algae (*Chlorella spp*.) and macro algae (*Laminaria Hyperborea*), was considered when determining the temperatures to carry out hydrothermal pre-treatment. The temperatures chosen in this project (100, 150 and 200°C) are lower than those used by Smith et al. (2016) who used 200 and 250°C, as the results from their paper showed the lower of the two temperature they investigated (200°C), showed more change between the raw feedstock and the solid residue after hydrothermal treatment. Therefore it was decided that a lower range of temperatures would be investigated.

There are many methods that are used for pre-treatment but research into hydrothermal pre-treatment is still fairly limited. This section is an initial scoping exercise to determine if hydrothermal pre-treatment improves the quality of two different algae; autotrophic *Chlorella vulgaris* and *Ulva lactuca*.

4.3.1. Mass balance of the products from hydrothermal pre-treatment of micro and macro algae

Mass balance of the products were calculated by separating the process waters and solid residues after hydrothermal pre-treatment and then weighing each fraction. This was then calculated as a percentage of the amount of algae originally added to the reactor.

Table 4-3 shows the mass balance of the products from hydrothermal pre-treatment of both micro and macro algae at the three different temperatures. It is expected that the production of gaseous products will be low at these temperatures and therefore has been assumed as negligible. Both the *Ulva* and the autotrophic *Chlorella* follow the same trend, with the yield of solids reducing and the aqueous phase increasing, as the pre-treatment temperature increases. This suggests that a fraction of the algae is solubilised during hydrothermal pre-treatment. There is a lower solid yield at 200°C than at 100°C, for both the micro and macro algae which suggests that degradation of the different biochemical components are enhanced at

higher temperatures. In particular, the carbohydrates are released at lower temperatures and much more easily than the proteins and lipids in the algae. Therefore, the *Ulva* shows a higher liquid to solid ratio in comparison to the *Chlorella*, which has a lower carbohydrate content.

Table 4-3: Percentage of solids and aqueous phase products from hydrothermal pre-treatment of autotrophic *Chlorella vulgaris* and *Ulva lactuca*

Type of algae	Pre-treatment temperature (°C)	%	
		Solid residue	Aqueous phase
Ulva lactuca	100	53	47
	150	49	51
	200	33	67
autotrophic *Chlorella*	100	82	18
	150	68	32
	200	43	57

4.3.2. Composition of solid residues from hydrothermal pre-treatment of micro and macro algae

The composition of the solid residues of the *Ulva lactuca* and the autotrophic *Chlorella vulgaris*, from hydrothermal pre-treatment at 100, 150 and 200°C, were analysed to determine the biochemical composition along with the ultimate and proximate analysis. These were determined following the methods described in sections 3.2.2, 3.7.1 and 3.7.2 of the methodology respectively.

Table 4-4 shows the biochemical components of the solid residues from the *Ulva lactuca* and the autotrophic *Chlorella vulgaris* at the three different pre-treatment temperatures. There is a big difference between the biochemical composition of the *Ulva lactuca* and the autotrophic *Chlorella vulgaris*.

Table 4-4: Biochemical components of micro and macro algae pre-treated at 100, 150 and 200°C

Type of algae	Pre-treatment temperature (°C)	Biochemical components (a.r.) (%)			
		Lipids	Proteins	Carbohydrates	
				Structural	Non-structural
Ulva lactuca	100	0.2	8.5	19.1	44.2
	150	0.2	9.5	18.5	30.9
	200	2.4	5.9	32.5	18.6
Autotrophic *Chlorella V.*	100	16.8	35.0	21.9	17.6
	150	31.7	32.9	7.6	12.3
	200	50.3	30.7	0.5	7.4

The lipid content of the solid residues from *Ulva* pre-treated at 100 and 150°C, are the same as one another and are significantly lower than the raw *Ulva*. For the solid residue from pre-treatment at 200°C, the lipid content is higher than the solid residues from *Ulva* pre-treated at 100 and 150°C but lower than the raw. The protein content of the solid residues from *Ulva* pre-treated at 100 and 150°C, are similar, with the solid residue from pre-treatment at 200°C being slightly lower. However, all three pre-treated residues contain significantly more protein than the raw *Ulva*. The structural carbohydrate content of the solid residues from pre-treatment at 100 and 150°, decrease with increasing pre-treatment temperature. However, for the solid residue from pre-treatment of the *Ulva* at 200°C, the structural carbohydrate content increases and is higher than the raw *Ulva*. The non-structural carbohydrate content decreases with increasing pre-treatment temperature as they are easily broken down and therefore more is released into the process waters during hydrothermal pre-treatment due to the increasing severity of the hydrothermal processing. Overall, there obvious differences between the lipid, protein and non-structural carbohydrates of the pre-treated *Ulva* in comparison to the raw *Ulva*. From Table 4-4 and Figure 4-2, it seems as though the main material being extracted is non-structural carbohydrates due to the big difference between the raw and pre-treated values.

The lipid content of the solid residues from pre-treatment of the *Chlorella* show an increase with increasing pre-treatment temperature, although the lipid content of the *Chlorella* pre-treated at 100 and 150°C are lower than the raw *Chlorella*. The protein content of the solid residues from *Chlorella* decreases with increasing pre-treatment temperature. The structural carbohydrate content increases

from the raw *Chlorella* to the solid residue from *Chlorella* at 100°C, but then decreases significantly for the solid residues from pre-treatment at 150 and 200°C. The non-structural carbohydrates content of the solid residues from *Chlorella* decreases with increasing pre-treatment temperature. Overall, there is only a small difference between the raw *Chlorella* and the solid residue from pre-treatment at 100°C, but there is more of a difference for the solid residues at 150 and 200°C. The solid residue from pre-treatment of the *Chlorella* at 200°C shows the highest increase in lipid content and highest decrease in the protein, structural and non-structural carbohydrate content. The reason for this is due to the increased severity of the higher temperature pre-treatment releasing more carbohydrate derived soluble material such as sugars and oligomers into the process waters during hydrothermal pre-treatment. This is also the case for the protein as they are interacting with the carbohydrates and releasing nitrogen compounds into the process waters.

This in turn also affects the lipid content as the ratio of lipids to carbohydrates increases due to concentration of the lipids and removal of the carbohydrates from the solid residues after pre-treatment. This is a positive outcome in terms of pre-treatment as there is a high fraction of lipids and lower fraction of carbohydrates which can be problematic when converting the algae to bio-crude due to the reactions between the proteins and carbohydrates.

Of the two algae samples, the hydrothermal pre-treatment seems to have the highest effect on the autotrophic *Chlorella*, as there are significant differences between the raw *Chlorella* and the solid residues from pre-treatment at the three temperatures. The *Ulva* does not show much difference in the lipid content of the solid residues, but does show significant differences in the carbohydrate content.

Figure 4-2 shows the DTG curves from the raw algae and the solid residues from pre-treatment at 100, 150 and 200°C for both a) *Ulva lactuca* and b) autotrophic *Chlorella vulgaris*. The DTG curves from the *Ulva* show four definite peaks. The first peak appears at 100°C for all of the samples and is the moisture present. The second, third and fourth peaks (between 125 and 550°C) relate to the carbohydrates, lipids and proteins in the samples. There is a sharp peak that appears between 200 and 350°C which seems to relate to the carbohydrates. The solid residues from pre-treatment of the *Ulva* show a decrease in this peak with increasing pre-treatment temperature from the raw to the solid residue from pre-treatment at

150°C. The solid residue from pre-treatment at 200°C increases in comparison to the solid residue from pre-treatment at 150°C but is still smaller than for the raw *Ulva* and the *Ulva* pre-treated at 150°C. The peaks have also shifted to the right, suggesting that the solid residues from pre-treatment at the higher temperatures have a different composition of carbohydrates than the raw *Ulva*. This also correlates to the biochemical composition data in Table 4-4, which shows the difference in the structural and non-structural carbohydrate content of the solid residues after pre-treatment. The final peak relates to the carbonaceous material in the samples. There is again a decrease with increasing pre-treatment temperature in the solid residues, which suggests that there is more ash present in the pre-treated samples compared to the raw *Ulva* The most interesting of the four samples is the solid residue from pre-treatment at 200°C, as it forms 2 peaks, a smaller one and then a slightly larger second peak. This could be due to polymerisation producing humins, which would show as a separate peak (Gai et al., 2013).

The DTG curves from the autotrophic *Chlorella* show a different weight loss trend to the *Ulva*. There are three peaks that are clearly defined on the DTG curves for the autotrophic *Chlorella*. Again, the first peak appears at 100°C and represents the moisture in the sample. The second and third peaks refer to the carbohydrates, lipids and proteins in the samples. There is a drastic decrease between the raw *Chlorella* and the solid residues pre-treated at 150°C and 200°C. This indicates that the carbohydrates are released into the process waters during pre-treatment. For the third peak, there is little difference between the four samples as it seems this represents the lipids and proteins. The reason for this is that the lipids are not affected by hydrothermal treatment under 200°C, whereas the other biochemical components, such as the carbohydrates are. This also correlates with the biochemical composition data in Table 4-4. The final section of the DTG curve refers to the carbonaceous material content of the solid residue which shows an increase with increasing pre-treatment temperature. This also correlates to the biochemical composition data in Table 4-4, which shows an increase in the ration of lipids of the solid residues, with increasing pre-treatment temperature.

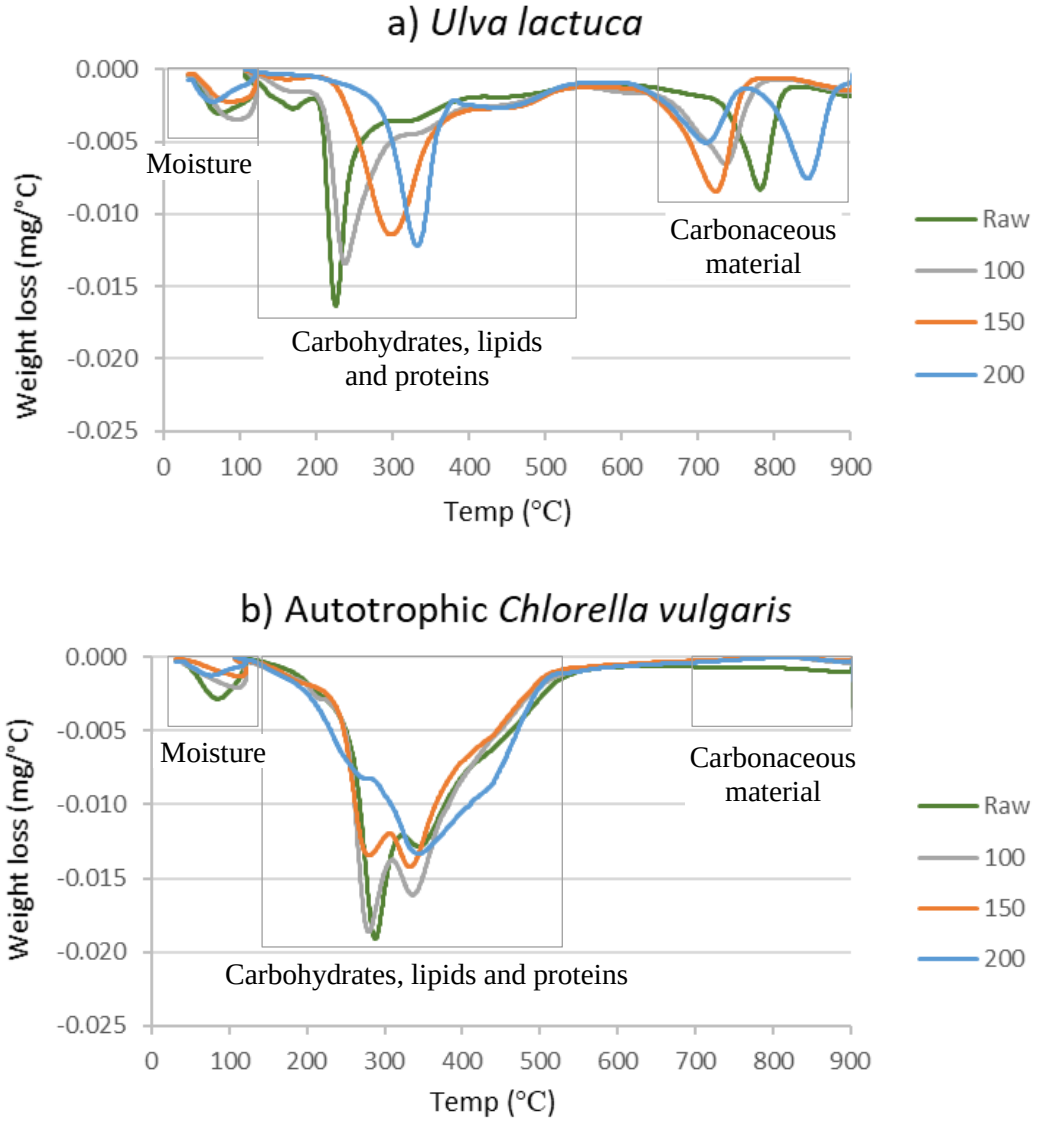


Figure 4-2: DTG curves from raw and pre-treated algae a) *Ulva lactuca* and b) autotrophic *Chlorella vulgaris*

Overall, these DTG curves show that there is an increase in the ratio of lipids and proteins in the pre-treated algae, due to the decrease in the carbohydrate content of the solid residues with increasing pre-treatment temperature, due to more material being released from the algae as the pre-treatment temperature increases. From the biochemical composition and the DTG curves, it is shown that after pre-treatment, the carbohydrate content decreases and the ratio of lipids increases in both the micro and macro algae. Therefore, the solid residue can be considered as a better feedstock than the raw algae, as this will result in the production of 'cleaner' oils after conversion, which will require less upgrading.

Table 4-5 shows the proximate and ultimate analysis of the raw algae and the solid residues from hydrothermal pre-treatment of the micro and macro algae at the three different temperatures. This analysis was carried out to determine if there is a difference between the raw algae and the solid residues from pre-treatment to investigate the effect of pre-treatment on the solid algae.

From the proximate analysis of the solid residues from the *Ulva lactuca,* the moisture content shows a decrease with increasing pre-treatment temperature. The ash content of the raw *Ulva* is significantly lower than for the pre-treated *Ulva*. The reason for this is due to the fact that the *Ulva* contains high levels of carbohydrates and therefore when these are removed by hydrothermal pre-treatment, the solid residue has a higher ash content as it contains more of the inorganics which are insoluble in the process waters during hydrothermal pre-treatment. These two points are again indicative that carbonisation is beginning to take place at the higher pre-treatment temperature (200°C) and the increase in the ash content could be a result of reabsorption of the organics on to the solid algae at the higher pre-treatment temperature. The volatile content of the pre-treated *Ulva* decreases with increasing pre-treatment temperature in comparison to the raw *Ulva*. The fixed carbon content of the pre-treated *Ulva* increases with increasing pre-treatment temperature except for the *Ulva* pre-treated at 150°C, which is lower than the value for the raw *Ulva*.

The ultimate analysis of the pre-treated *Ulva* shows that the carbon, hydrogen, nitrogen and oxygen content all increase for the solids residues from pre-treatment at 150°C but reduce when the pre-treatment temperature is increased to 200°C. Of the pre-treated *Ulva*, the sample pre-treated at 200°C is closest to the raw algae but there is still a difference between the two. The reason for this may be due to polycondensation reactions of the dissolved sugars and organics precipitating out of the process waters. This may be due to the algae at 200°C beginning to carbonise and therefore has char like properties causing it to re-absorb material released into the process waters. The raw *Ulva* has higher hydrogen, nitrogen and oxygen levels than the pre-treated *Ulva*, with the exception of the carbon content, which is higher for the pre-treated *Ulva*. The HHV of the *Ulva* increases with increasing pre-treatment temperature except for the sample pre-treated at 200°C, which is lower than the 100°C and 150°C but higher than the raw *Ulva*.

Table 4-5: Proximate and ultimate analysis of solid residues from hydrothermal pre-treatment of macro (*Ulva lactuca*) and micro (*Chlorella vulgaris)* algae

Type of algae	Pre-treatment temp (°C)	(a.r.)	Proximate (%) (d.b.)			Ultimate (%) (d.a.f.)					HHV (MJ/kg^{-1})
		Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*	
Ulva lactuca	Raw	21.9	21.0	76.6	2.4	28.1	6.1	1.7	0.0	64.2	10.3
	100	7.0	30.2	66.3	3.5	38.3	5.7	2.5	0.1	53.4	14.5
	150	4.2	42.6	55.9	1.5	49.2	6.8	3.5	0.0	40.6	21.4
	200	2.7	41.9	52.6	5.6	39.0	4.0	2.1	0.0	54.8	12.1
autotrophic *Chlorella*	Raw	7.1	8.6	77.1	14.3	54.6	8.1	9.3	0.0	28.1	26.6
	100	4.4	9.1	72.9	18.0	51.3	7.3	8.1	0.7	32.6	23.9
	150	3.6	16.1	67.7	16.2	57.4	7.9	8.2	0.4	26.1	27.6
	200	2.3	11.4	71.1	17.5	67.5	8.1	7.2	0.3	16.9	32.4

*oxygen determined by difference

The proximate analysis of the autotrophic *Chlorella*, show a different trend to the solid residues from the *Ulva lactuca*. The moisture content decreases with increasing pre-treatment temperature. This may be due to an increase in the concentration of hydrophobic lipids, which are present in a higher ratio than the carbohydrates after pre-treatment. The ash content increases with increasing pre-treatment temperature to 150°C but then decreases at 200°C. This is indicative that carbonisation is taking place at the higher pre-treatment temperature (200°C) as there is less moisture and more ash at the higher pre-treatment temperatures. The volatile content of the solid residues is fairly similar for the raw and pre-treated algae, with the exception of the *Chlorella* pre-treated at 150°C which is slightly lower. The fixed carbon content of the pre-treated *Chlorella* is higher than that of the raw *Chlorella*.

The ultimate analysis of the autotrophic *Chlorella* shows that with increasing pre-treatment temperature the solid residues have increasing carbon and hydrogen content but decreasing sulphur and oxygen content. The nitrogen content decreases with increasing pre-treatment temperature. The higher heating values of the solid residues also increase with increasing pre-treatment temperature, however the solid residue from pre-treatment at 100°C has a lower HHV than the raw *Chlorella.*

The decreased oxygen content in both the pre-treated macro and micro algae is mainly due to the decarboxylation and dehydration reactions that occur during hydrothermal treatment. The decarboxylation reaction degrades the carboxyl (-COOH) groups to form CO_2 and the carbonyl groups (C=O) to form CO and the dehydration reaction removes the hydroxyl groups (-OH) resulting in less hydrophilic functional groups. These reactions significantly reduce the oxygen content and increase the energy density of the algae (Smith et al., 2016). The solubilisation of the sugars from the algae into the process waters is also contributing to the reduction in oxygen content as there are less carbohydrates present in the solid residue.

The lower yields of solid residue obtained at the higher hydrothermal pre-treatment temperature (200°C) shown in Table 4-3, correspond with lower oxygen levels in the solid residue shown in Table 4-5, indicating that the decarboxylation and dehydration reactions are more favourable at higher hydrothermal pre-treatment

temperatures. This also corresponds with an increase in the HHV of the solid residues from hydrothermal pre-treatment.

Alba et al. (2011) measured the oxygen content of bio-crude from pre-treated *Desmodesmus sp* and found that between 175°C and 300°C, the oxygen content decreased compared to bio-crude from unprocessed *Desmodesmus sp*. This implies that major deoxygenation reactions occurred at temperatures below 300°C. This correlates to the results in this section.

The reduction in nitrogen content in the solid residues with increasing pre-treatment temperature correlates with the reduction in protein content of the solid residues as shown in Table 4-4. This is due to the interactions between the proteins and carbohydrates, which are being released into the process waters (Heilmann et al., 2011).

4.3.3. Composition of process waters from hydrothermal pre-treatment of micro and macro algae

The process waters from hydrothermal pre-treatment of the macro and micro algae are also analysed to determine what components are released from the algae into the process waters. Table 4-6 shows the total compounds, analysed for using an Agilent 7890B GC-MS, in process waters from hydrothermal pre-treatment at 100°C, 150°C and 200°C for the *Ulva lactuca* (macro) and autotrophic *Chlorella vulgaris* (micro). The method used is described in section 3.7.5 of the methodology chapter. Formic acid, lactic acid and the glucose, fructose, ribose and mannose where analysed for, using the HPLC method described in section 3.7.4 of the methodology chapter. The content of the process waters varies for the three samples from both the micro and macro algae. The additional data of the individual compounds for both the micro and macro algae can be found in Appendix 2.

The notable acids in the process waters are acetic, levulinic, succinic and glutaric acid and are shown in Figure 4-3 for the a) *Ulva lactuca* and b) autotrophic *Chlorella*. These acids are present in much higher quantities than the other acids that were analysed for. The process waters from the *Ulva*, show an increase in all four of the noted acids with increasing pre-treatment temperature, with the succinic acid showing the largest increase. Of the three process waters for the autotrophic

Chlorella, the 200°C process water, contains the highest amount of acetic and succinic acid, but the lowest amount of levulinic acid.

Table 4-6: Total compounds in process waters after hydrothermal pre-treatment of macro algae (*Ulva lactuca*) and autotrophic micro algae (*Chlorella vulgaris*) at 100, 150 and 200°C

	mg/l					
Compounds	**Macro**			**Micro**		
	100°C	**150°C**	**200°C**	**100°C**	**150°C**	**200°C**
Acids	11021.0	13648.9	14334.1	197.0	683.7	7057.8
Nitrogen	1194.2	1540.9	5965.0	1227.0	868.2	1122.6
Cyclopentanones	176.4	204.6	434.7	182.6	216.4	459.2
Phenols	0.0	0.0	0.6	0.0	0.0	4.5
Sugars	0.9	2.7	2.5	0.8	0.6	0.2

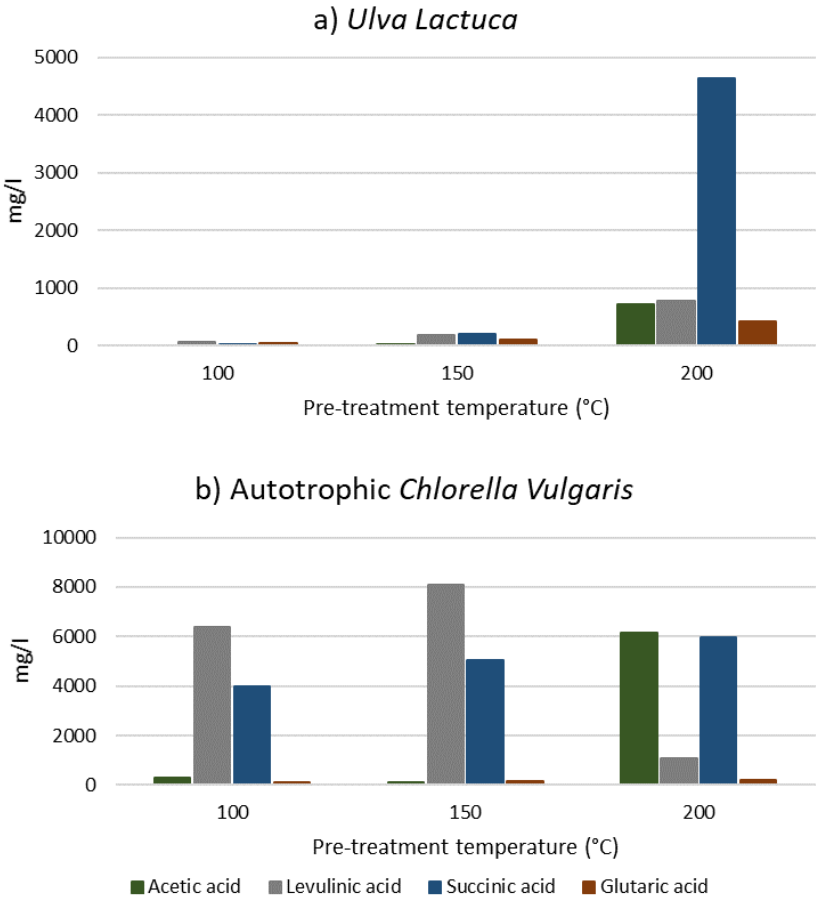


Figure 4-3: Notable acids in the process waters from hydrothermal pre-treatment of a) *Ulva lactuca* and b) autotrophic *Chlorella*

From Table 4-6 it is evident that there are significant amounts of nitrogen in the process waters. The nitrogen compounds appear to be formed from the Maillard reactions during hydrothermal pre-treatment, which seem to play an important role in the behaviour of the proteins in the algae. The nitrogen that is extracted appears to remain as organic compounds such as nitrogen heterocycles, pyrazines and pyridines, with ionic nitrogen (nitrate, nitrite, ammonia) making up only a relatively small portion of the extracted nitrogen, indicating they are formed from the degradation of proteins (P.Biller and A.B.Ross, 2011). Danso-Boateng et al. (2015) found that Maillard reactions become significant during hydrothermal processing at 180°C as there was an increase in the nitrogen compounds present in the process waters identified by GC-MS. Therefore, it appears that the higher hydrothermal pre-treatment temperature of 200°C has increased the amount of nitrogen compounds in the process waters due to increased severity of thermal degradation and increased interactions between the proteins and carbohydrates (Heilmann et al., 2011). Figure 4-4 shows the nitrogen compounds present in the process waters from hydrothermal pre-treatment of a) *Ulva lactuca* and b) autotrophic *Chlorella*. The macro algae process waters contain lower levels of nitrogen in comparison to the process waters from the micro algae.

The reaction pathways of HMF during hydrothermal pre-treatment are shown in Figure 4-5. As carbohydrates are pre-cursors of furanic derivatives such as furfural and 5-HMF (Monlau et al., 2014), it is expected that the process waters from the *Ulva* will contain high amounts of these furans. However as there is no HMF or furfural detected with the GC-MS method that was employed these have been omitted from the results in Table 4-6. It is expected that furanic compounds will be present in the process waters as it has recently been reported in literature that furanic compounds are released after thermal and thermo-chemical pre-treatments of algal biomass by Jung et al. (2011), Park et al. (2011) and Park et al. (2013). However, the concentration of the furanic compounds depends on several factors such as type of pre-treatment and operating conditions (i.e. pressure, temperature, pH, solid loading, operating time) (Mussatto and Roberto, 2004). Therefore, the conditions used in this chapter may not have been the right ones to produce the furanic compounds as the temperatures are not high enough which also suggests that carbonisation is not taking place during the pre-treatment due to this.

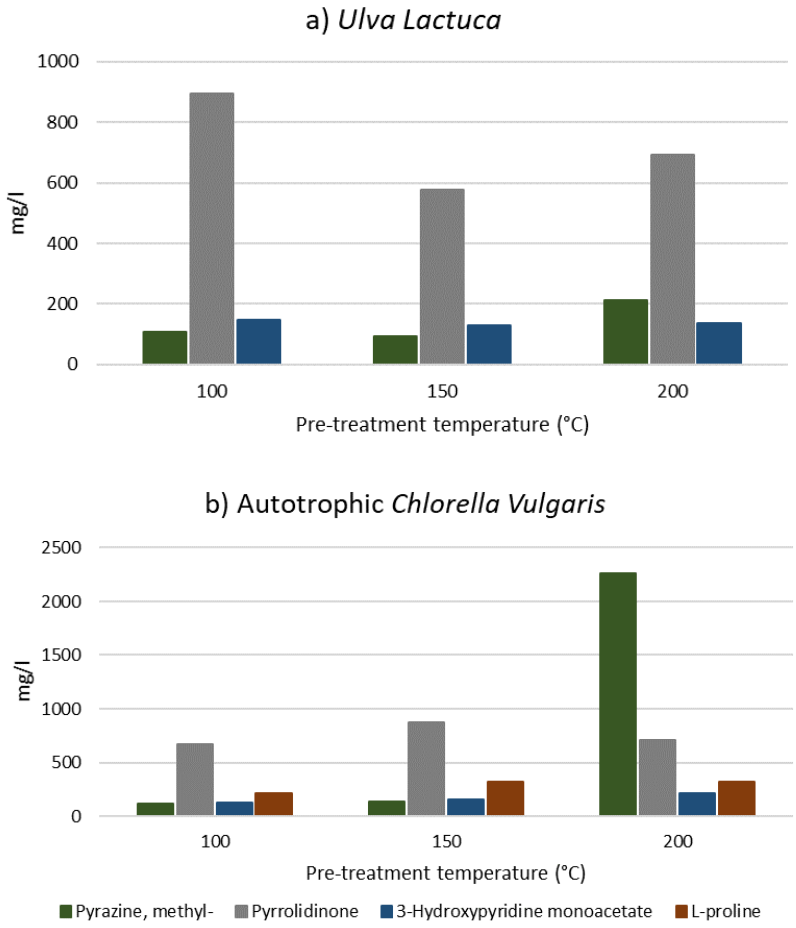


Figure 4-4: Notable nitrogen compounds in the process waters from hydrothermal pre-treatment of a) *Ulva lactuca* and b) autotrophic *Chlorella*

The cyclopentanones are present in relatively similar quantities for the three process waters from micro algae and are present in even lower quantities in the macro algae. There is no phenol or p-Cresol present in the process waters at 100°C and 150°C and only a very small amount in the process waters at 200°C for both the micro and macro algae.

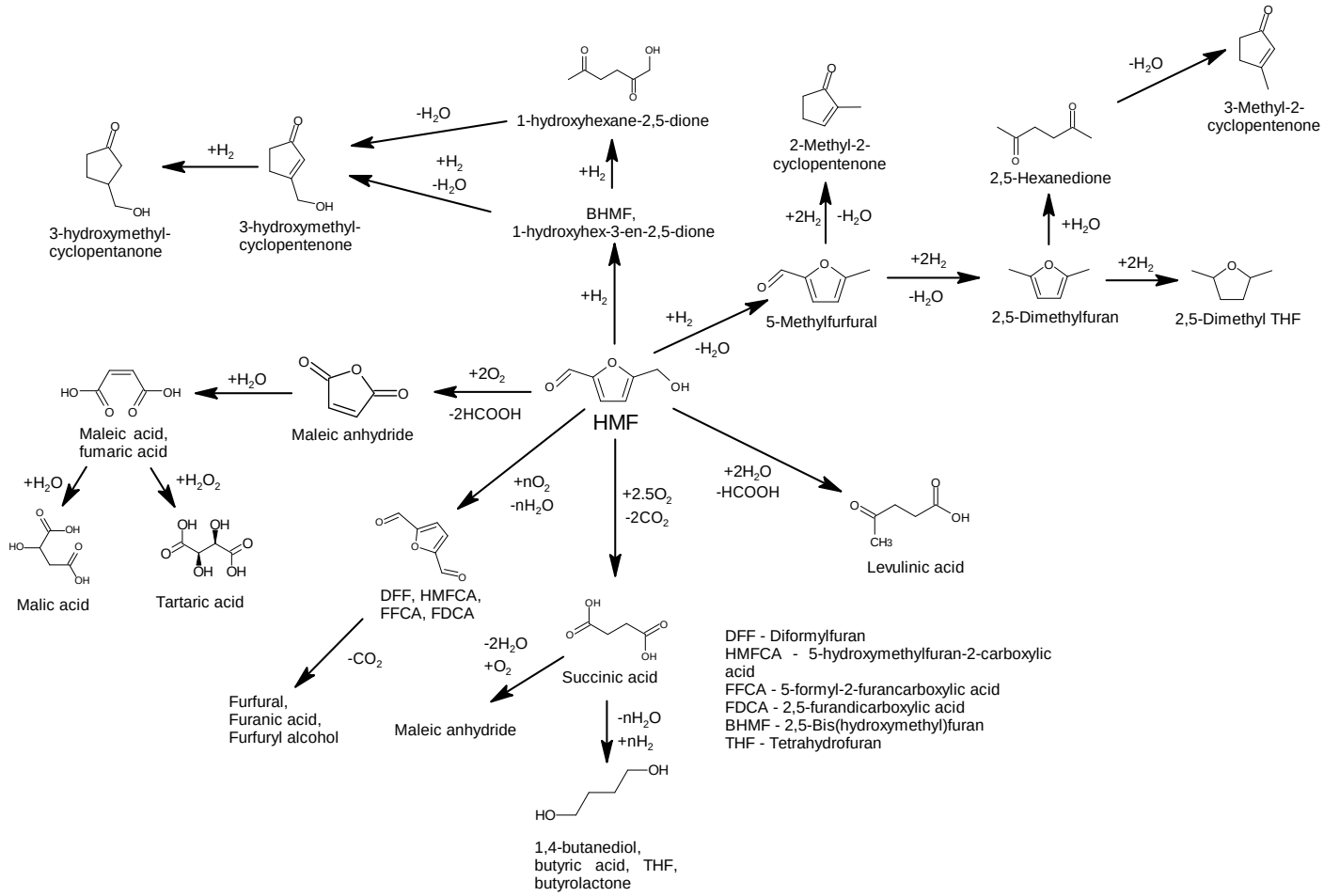


Figure 4-5: Reaction pathways of HMF during hydrothermal pre-treatment (Hammerton, 2020b)

The sugars content of the micro algae is higher than the macro algae. This may be due to the macro algae containing higher levels of structural carbohydrates than the micro algae, which are harder to break down than non-structural carbohydrates. Srokol, Z. et al. (2004) carried out a study on hydrothermal liquefaction at 340°C, of some monosaccharide model compounds, after acid hydrolysis and found that glucose broke down and resulted in the formation of formic, acetic, lactic and acrylic acid. This may be why the amount of glucose detected in the process waters is so low or has not been detected, as it has been broken down and formed the acids mentioned, via the routes shown in Figure 4-6. Although the four acids mentioned previously have not been detected in all of the process water samples, there is still some present. The process waters from the micro algae show high levels of acetic acid, with the process water from pre-treatment at 200°C showing a very high amount.

Alba et al. (2011) identified the following organics in the aqueous phase: acetone, polyols, amines, amino acids, nitrogen containing aromatics and pyrrolidones (in high concentration). This corresponds with the compounds present in the process waters from hydrothermal pre-treatment for the micro and macro algae as shown in Table 4-6.

The total organic carbon, total nitrogen, ammonium and organic nitrogen, along with the total phosphate, orthophosphate and organic phosphate content was analysed for the process waters from hydrothermal pre-treatment of the autotrophic *Chlorella* and *Ulva lactuca* at 100, 150 and 200°C. The additional data can be found in Appendix 3.

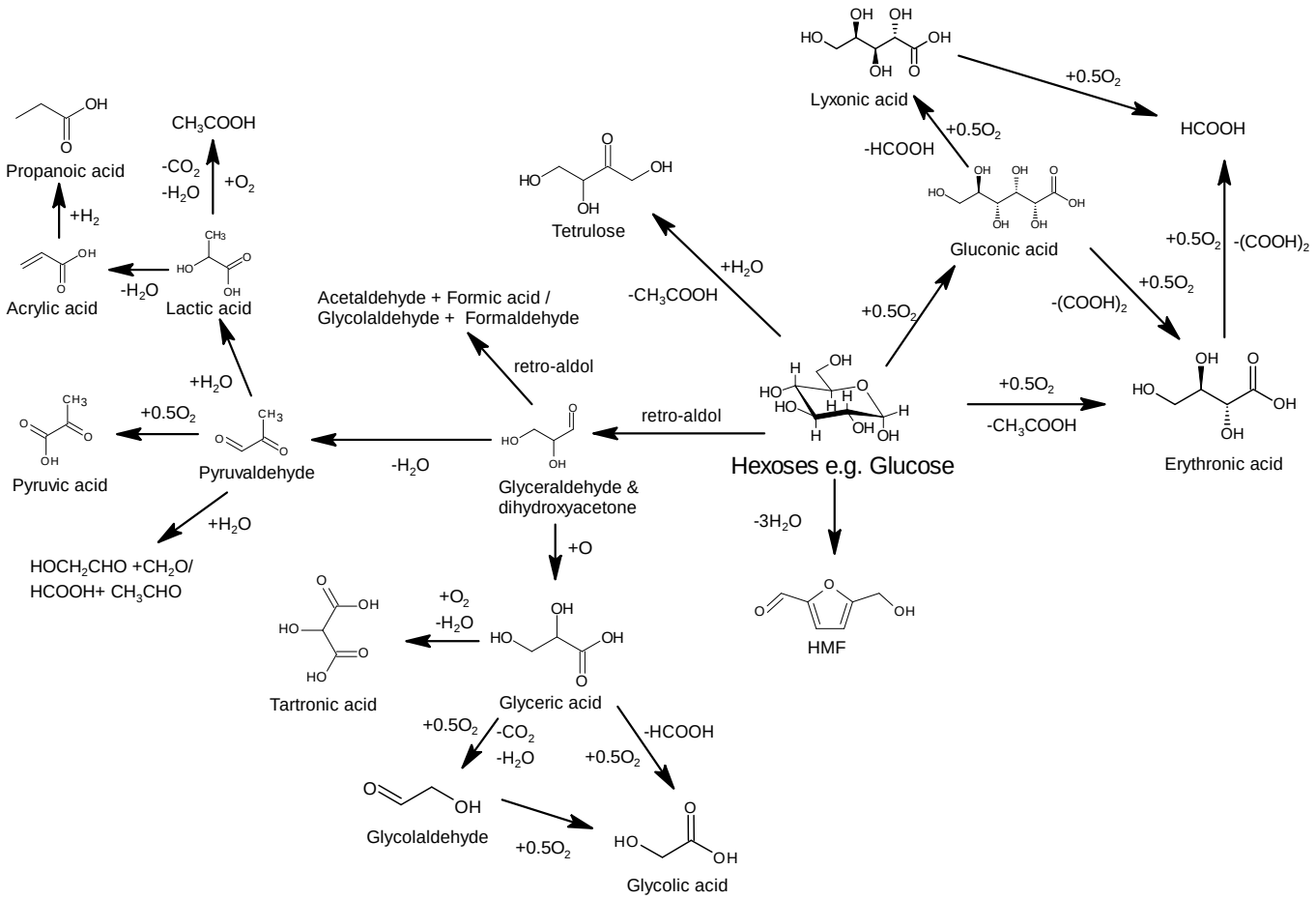


Figure 4-6: Reaction pathways of glucose during hydrothermal pre-treatment (Hammerton, 2020a)

Figure 4-7 shows the total organic carbon content of the process waters. For the *Ulva* there is an increase in the total organic carbon with increasing pre-treatment temperature, with a large increase between the process waters at 100°C and 150°C but only a small difference between the process waters at 150°C and 200°C. The autotrophic *Chlorella* shows a similar trend for the total organic carbon with an increase in the total organic carbon with increasing pre-treatment temperature. The total organic carbon content correlates with the solid residue yield from hydrothermal pre-treatment. The higher the hydrothermal pre-treatment temperature, the higher the total organic carbon content and the lower the solid residue yield.

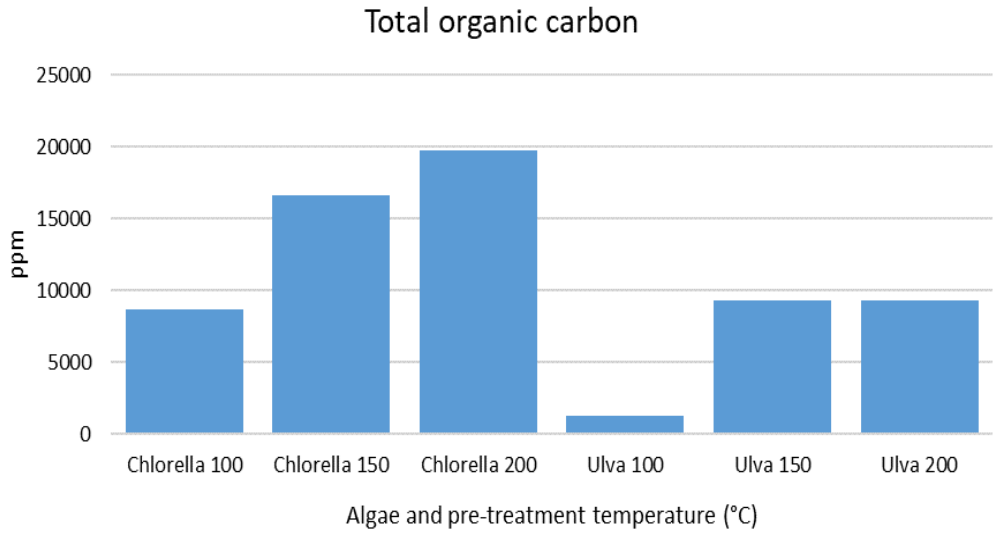


Figure 4-7: Total organic carbon distribution in the process waters from hydrothermal pre-treatment of *Ulva lactuca*

Figure 4-8 shows the nitrogen content of the process waters from *Ulva*. The total nitrogen content of the process waters from the *Ulva* are quite similar with the 100°C water giving the highest value. For the ammonium content, the samples pre-treated at 100°C and 200°C are very similar, whereas the sample at 150°C is much higher. However, the organic nitrogen shows the complete opposite of this with the process waters from 100 and 200°C being higher than the process water at 150°C. The phosphate content of the *Ulva* shows a decrease in all three components with increasing pre-treatment temperature. Again, the reason for the bigger decrease at

the higher pre-treatment temperature may be due to the solid residue acting as a char and reabsorbing the phosphate, instead of releasing it to the process waters.

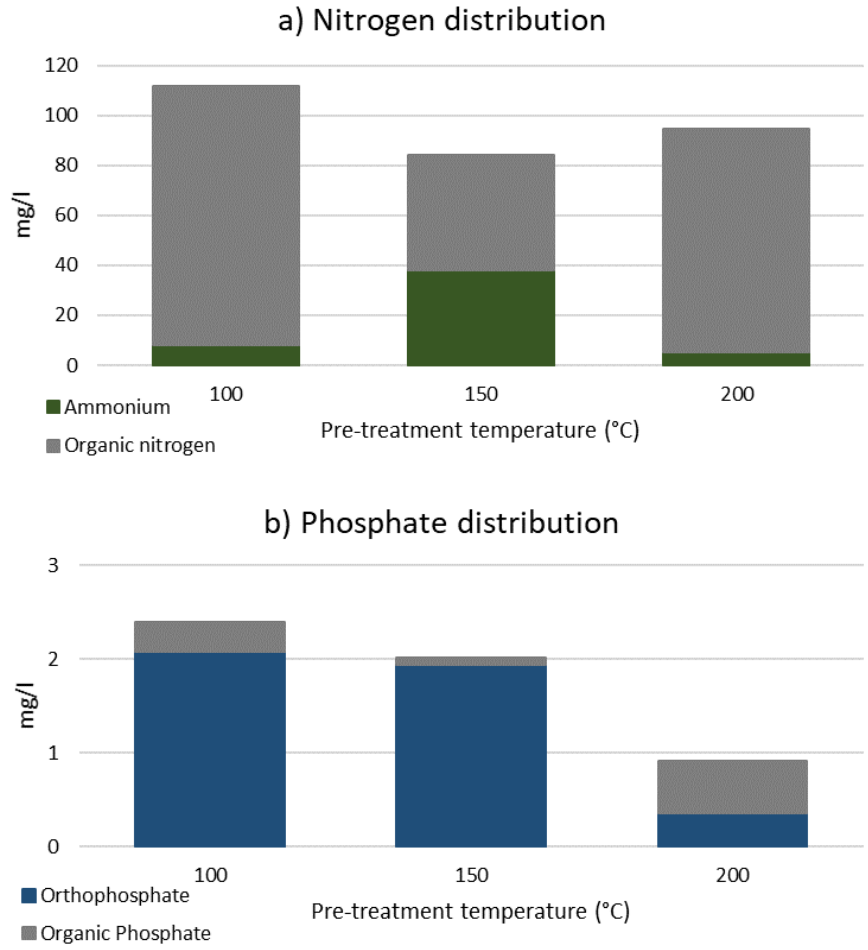


Figure 4-8: a) nitrogen distribution and b) phosphate distribution in the process waters from hydrothermal pre-treatment of *Ulva lactuca*

The percentage nitrogen and phosphate distribution in the process waters from the *Ulva* is shown in Figure 4-9. The total nitrogen content of the process waters at 100°C and 200°C from autotrophic *Chlorella* are very similar, however there is a decrease in the process water at 150°C. For the total ammonium content of the autotrophic process waters, there is very little difference between the 100°C and 150°C samples, but there is a big increase in the 200°C sample. This could be due to more nitrogen being released and broken down into ammonium at 200°C, which is when carbonisation begins to occur. This also correlates to the organic nitrogen content which decreases from 100 to 150°C but increases for the process waters at

200°C, which suggests more organic nitrogen is being released from pre-treatment at the higher temperature. For phosphate content of the process waters, the total phosphate, orthophosphate and organic phosphate all increase from 100°C to 150°C, but then there is a decrease between 150°C and 200°C. The reason for this decrease may be due to the solid residue acting as a char and reabsorbing the phosphate, instead of releasing it to the process waters.

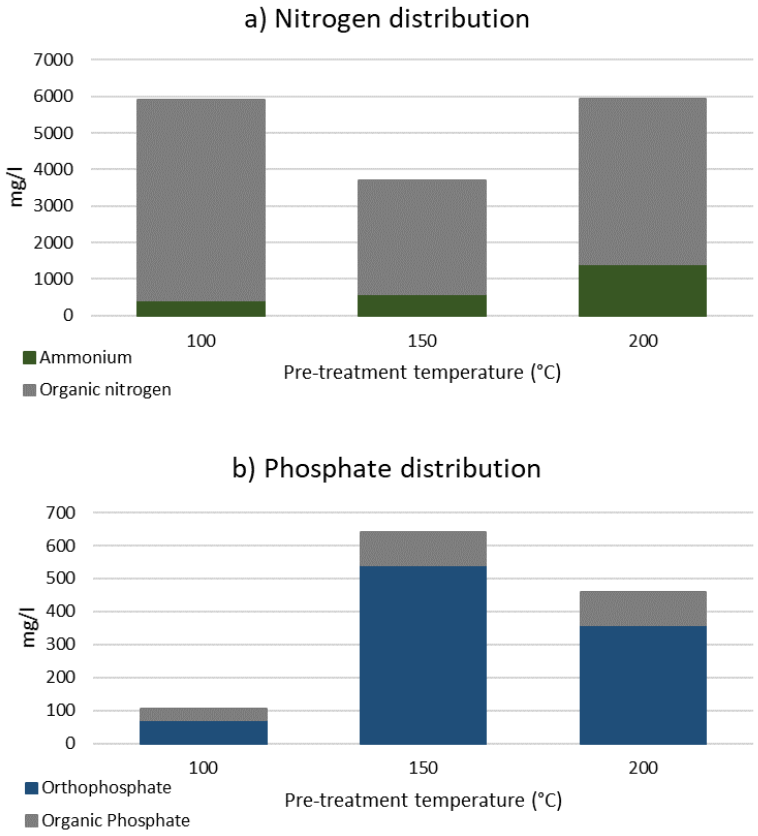


Figure 4-9: a) nitrogen distribution and b) phosphate distribution in the process waters from hydrothermal pre-treatment of autotrophic *Chlorella vulgaris*

The total nitrogen and ammonium content of the process waters corresponds with the deamination of amino acids, which results in the release of nitrogen as organic compounds, with ionic nitrogen (ammonia) making up a small fraction of the total extracted nitrogen.

Phosphorous occurs in algae both in its ionic form and organically in the protein, phospholipids and nucleic acids. Due to this association with the organic

matter in the algae, the phosphorous is less easily extracted than from its ionic form (Smith et al., 2016).

Table 4-7 shows the XRF of the process waters from hydrothermal pre-treatment of the micro and macro algae. Although XRF covers a larger range of metals, only the ones present in the process waters are shown in Table 4-7.

Table 4-7: XRF of process waters from macro and micro algae after hydrothermal pre-treatment at 100, 150 and 200°C

	Ulva lactuca (ppm)			*Chlorella vulgaris* (ppm)		
Metal	100°C	150°C	200°C	100°C	150°C	200°C
Na	13790	ND	ND	ND	ND	ND
Mg	2315	1720	2000	627	ND	370
Al	163	ND	93	120	133	97
Si	158	278	135	125	100	113
P	1093	1147	1084	1905	1970	2172
S	3103	3284	2316	122	197	334
Cl	5341	5174	5623	256	240	197
K	1749	1801	2065	432	405	416
Ca	764	2259	588	362	235	298
Fe	ND	ND	24	ND	ND	16
Br	33	33	45	ND	ND	ND
Sr	9	ND	10	ND	ND	ND

For the *Ulva*, sodium is only detected in the process waters from pre-treatment at 100°C using XRF. The magnesium and chlorine contents decrease from 100°C to 150°C and then increases again for the 200°C process water. The aluminium content decreases between the 100°C and 200°C process waters but is not present in the 150°C process water. The potassium, bromine and strontium content of the process waters increases with increasing pre-treatment temperature. The silicon, phosphorous, sulphur and calcium contents of the process waters increases from 100°C to 150°C but then decreases for the 200°C process water.

There is no sodium detected in any of the process waters for the autotrophic *Chlorella*. This may be due to the amount being too low to be analysed using XRF. The process waters from the *Chlorella* pre-treated at 100 and 200°C contain magnesium, however, the process water at 150°C does not. There is also a reduction in the amount of magnesium in the process water with increasing pre-treatment temperature, with the 200°C process water showing a decrease of almost half,

compared to the 100°C. The aluminium content of the process waters increases from 100°C to 150°C but then decreases for the 200°C process water. The silicon, sulphur, potassium and calcium content decreases from 100°C to 150°C and then increases again for the 200°C process water. The phosphorous and chlorine contents of the process waters increases with increasing pre-treatment temperature. This indicates that hydrothermal pre-treatment is releasing phosphorous into the process waters. Iron is only detected in the process water at 200°C. There is no bromine or strontium present in the micro algae as it is a freshwater strain.

For both the *Chlorella* and *Ulva*, there is a decrease in the extraction of metals at 200°C. It has been shown that the increased number of carboxylic groups on the surface of the higher temperature residues, can increase the cation exchange capacity and increase the surface functionality of the residues, thus allowing metals to reabsorb from the process waters into the algal residue (Libra et al., 2011). This could be the reason why there is a decrease in the metals present in the process waters at 200°C instead of 100°C and 150°C. Another reason that there is a decrease in the metals present in the process waters at 200°C instead of 100°C and 150°C could be due to the build-up of hydrolysed products on the surface of the algal residue, which reduces the extraction efficiency of smaller molecules (M.Mosteiro-Romero et al., 2014). There is also a slight decrease in the ash content of the solid residues from the algae pre-treated at 200°C in comparison to the algae pre-treated at 150°C, although the ash content is still higher than for the raw algae and for the algae pre-treated at 100°C for both the macro and micro algae as shown in Table 4-5.

4.3.4. Discussion of micro vs macro algae

Hydrothermal pre-treatment is affecting the biochemical composition of the algae. The protein is breaking down as the pre-treatment temperature increases to 150°C and then reduces again at 200°C. This may be due to the algae acting as a char and reabsorbing the nitrogen. This is shown through the amount of nitrogen compounds in the process waters. The lipids seem to be unaffected by the pre-treatment at any of the three temperatures investigated. The amount of sugars which are released into the process waters increases with increasing pre-treatment temperature, however the structural carbohydrates are not being broken down as the

pre-treatment temperatures are not high enough. Although they are not being broken down, they are becoming concentrated as the other components (protein and non-structural carbohydrates) are being released. The ash increasing also suggests that some of the organic fraction of the algae is being released into the process waters. This improves the quality of the solid algal residue, effectively producing a new improved feedstock.

The micro and macro algae have very different bio-chemical compositions. The macro algae contains high levels of carbohydrates whereas the micro algae has higher lipid content (which are beneficial for producing biofuels). As this section has proved that mainly the protein and non-structural carbohydrates are released at the temperatures investigated, and the carbohydrates are of less interest when producing bio-fuels, the macro algae will not be further investigated in this chapter.

Of the pre-treatment temperatures investigated, 150°C shows the most difference for the solid algal residue in comparison to the raw algae. This temperature was chosen to investigate the difference in the composition of three other micro algae against the autotrophic *Chlorella*.

4.4. Effect of micro algae composition on pre-treatment

From the comparison between the micro (autotrophic *Chlorella vulgaris*) and macro algae (*Ulva lactuca*), in section 4.3, it was decided that micro algae would be the main focus for the remainder of this project, as it yielded the most interesting results due to its high lipid and protein content in comparison to the macro algae. A further three species of micro algae were investigated to compare to the autotrophic *Chlorella*. The three other species of micro algae which have been studied include heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii*.

These feedstocks were hydrothermally pre-treated at 150°C only, as the initial hydrothermal pre-treatment carried out on the autotrophic *Chlorella vulgaris* and the *Ulva lactuca*, showed that this temperature released higher amounts of material such as nitrogen, phosphate and metals, into the process waters than either 100°C or 200°C.

Hydrothermal pre-treatment was carried out using the same reactor and methodology as used previously for the comparison between the micro and macro algae (section 3.4). The analysis of products was also carried out using the same methods for the solid residues and process water samples with the exception of the GC-MS analysis, which was undertaken on a different GC-MS using the same methodology as shown in section 3.7.5.2.

This section compares four micro algae of different biochemical content, to investigate if pre-treatment has an effect on the biochemical composition of the algae and whether the solid algal residue is improved as a feedstock for biofuel production, in comparison to the raw algae. Based on the finding from the comparison between the micro and macro algae at three different temperatures, in the previous section, it is hypothesised that the micro algae with higher protein and carbohydrate content will release more nitrogen into the process waters and concentrate the lipids to produce an improved feedstock.

4.4.1. Mass balance of products from hydrothermal pre-treatment of various micro algae

Table 4-8 shows the percentage of solids and aqueous phase of the products from pre-treatment of autotrophic *Chlorella vulgaris,* heterotrophic *Chlorella vulgaris, Spirulina platensis* and *Chlorogloeopsis fritschii* at 150°C.

From the four pre-treated samples, the *Chlorogloeopsis fritschii* gave the highest yield of solids and lowest yield of aqueous phase, followed by the autotrophic *Chlorella* and the *Spirulina platensis.* The heterotrophic *Chlorella* has the lowest solid yield of less than half of the original sample added. Overall, the four micro algae all behave slightly differently when hydrothermally pre-treated. The reason for this may be due to the difference in biochemical composition.

Table 4-8: Percentage of solids and aqueous phase products of hydrothermal pre-treatment at 150°C of autotrophic *Chlorella vulgaris*, heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii*

Type of algae	%	
	Solids	Aqueous phase
Autotrophic *Chlorella vulgaris*	68	32
Heterotrophic *Chlorella vulgaris*	41	59
Spirulina platensis	61	39
Chlorogloeopsis fritschii	82	18

4.4.2. Composition of solid residues from various micro algae

Table 4-9 shows the biochemical composition of the four micro algae pre-treated at 150°C. There are differences shown between the raw micro algae and the micro algae pre-treated at 150°C. The autotrophic *Chlorella* contains the highest lipid content and the lowest carbohydrate content. Whereas, in comparison, the heterotrophic *Chlorella* contains a much lower lipid content but a much higher carbohydrate content. The *Spirulina* and *Chlorogloeopsis* contain low levels of lipids but have significantly higher proteins contents.

Table 4-9: Biochemical components of various micro algae pre-treated at 150°C

Type of algae	Biochemical components (%)			
	Lipids	Proteins	Carbohydrates	
			Structural	Non-structural
Auto *Chlorella V.*	31.7	32.9	7.6	12.3
Hetero *Chlorella V.*	12.0	27.3	30.7	27.7
Spirulina platensis	10.0	49.6	14.2	18.1
Chlorogloeopsis fritschii	14.6	42.5	30.2	4.4

A comparison of the raw micro algae vs the pre-treated micro algae is shown in Figure 4-10. The autotrophic *Chlorella*, pre-treated at 150°C shows that the lipid content has doubled in comparison to the raw autotrophic *Chlorella*. The protein content decreases by almost 10% and the structural and non-structural carbohydrates have also decreased by approximately half of the original content in the raw autotrophic *Chlorella*. This shows that the composition of the solid residue after pre-treatment has more favourable characteristics for biofuel production than the raw autotrophic *Chlorella*. In comparison to the raw heterotrophic *Chlorella*, the solid residue from pre-treatment at 150°C shows an increase in the lipid content but a

slight decrease in the protein content, along with an increase of <20% in the structural carbohydrates and a significant reduction of <20% in the non-structural carbohydrates content. The *Spirulina* pre-treated at 150°C shows an increase in the lipid content and a small reduction in the protein content in comparison to the raw *Spirulina*. The structural carbohydrates in the solid residue from *Spirulina* increase by <10%, whereas the non-structural carbohydrates decrease by half. In comparison to the raw *Chlorogloeopsis*, the solid residue from pre-treatment at 150°C shows an increase in the lipid content and a slight decrease in the protein content, with both the structural and non-structural carbohydrates showing a slight decrease.

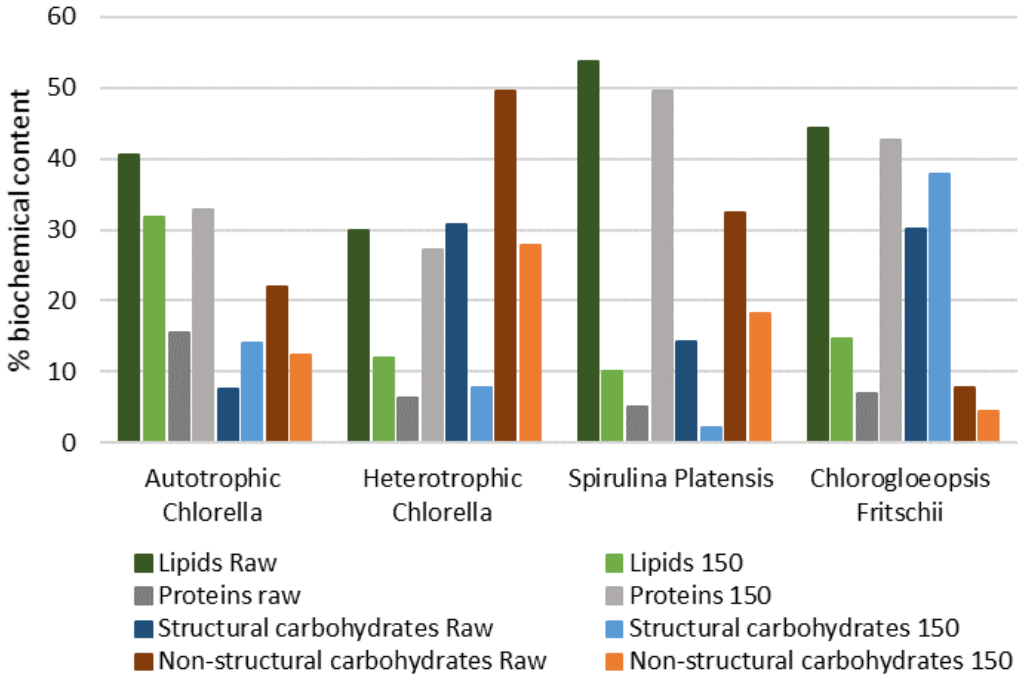


Figure 4-10: Biochemical composition of the various micro algae for both the raw and pre-treated at 150°C solid residues

Of the four micro algae, The solid residue after pre-treatment at 150°C, from the autotrophic *Chlorella*, contains the highest lipid content and the heterotrophic *Chlorella* contains the lowest. The reason for this is as of the four micro algae, the raw autotrophic *Chlorella* contained the most lipids, whereas the heterotrophic *Chlorella* contained the least. Although there is a difference between the raw and pre-treated solid residues, the lipid content does not change but merely the ratio increases, due to a reduction in the ratio of the other biochemical components such as the carbohydrates. Therefore hydrothermal pre-treatment at 150°C is not affecting

the lipids directly. The non-structural carbohydrates decrease in the solid residues from pre-treatment at 150°C for the heterotrophic *Chlorella*, *Spirulina* and *Chlorogloeopsis*, but increase for the autotrophic *Chlorella*. The structural carbohydrates reduce in the solid residues from pre-treatment at 150°C for all four micro algae but in particular for the heterotrophic *Chlorella* and *Spirulina*. There is also a smaller difference between the protein content of the raw and pre-treated heterotrophic *Chlorella* and *Spirulina* as they contain the highest non-structural carbohydrate content, which is released first during hydrothermal pre-treatment.

The high non-structural carbohydrate content of the raw micro algae also affects the yields from hydrothermal pre-treatment. The trends shown in Table 4-1 and Table 4-9, show that the raw micro algae which contains higher quantities of non-structural carbohydrates, results in lower solid residue yields and higher aqueous phase yields from hydrothermal pre-treatment as more of the sugars are released into the process waters, in particular for the heterotrophic *Chlorella* and *Spirulina.*

Figure 4-11 shows the DTG curves from the raw algae and the solid residues from pre-treatment at 150°C for: a) autotrophic *Chlorella*, b) heterotrophic *Chlorella vulgaris*, c) *Spirulina platensis* and d) *Chlorogloeopsis fritschii*. There are three main peaks that are clearly defined on the DTG curves. The first peak which appears at 100°C relates to the moisture in the samples. The second peak relates to the carbohydrates, the third peak relates to the lipids and finally the last peak relates to proteins, except for the heterotrophic *Chlorella*. The DTG curve from the autotrophic *Chlorella* was previously discussed in section 4.4.2.

For the heterotrophic *Chlorella,* the DTG curve is different to the DTG curves for the other three samples. Again the first peak is moisture in the samples. From the values in Table 4-1 and Table 4-9, it seems as though the merged peak from 150 to 200°C relates to the lipids, the peak between 200 and 300°C relates to the proteins and the peak between 300 and 450°C relates to the carbohydrates present and shows a change in the content of the structural and non-structural carbohydrates in the solid residue from pre-treatment at 150°C.

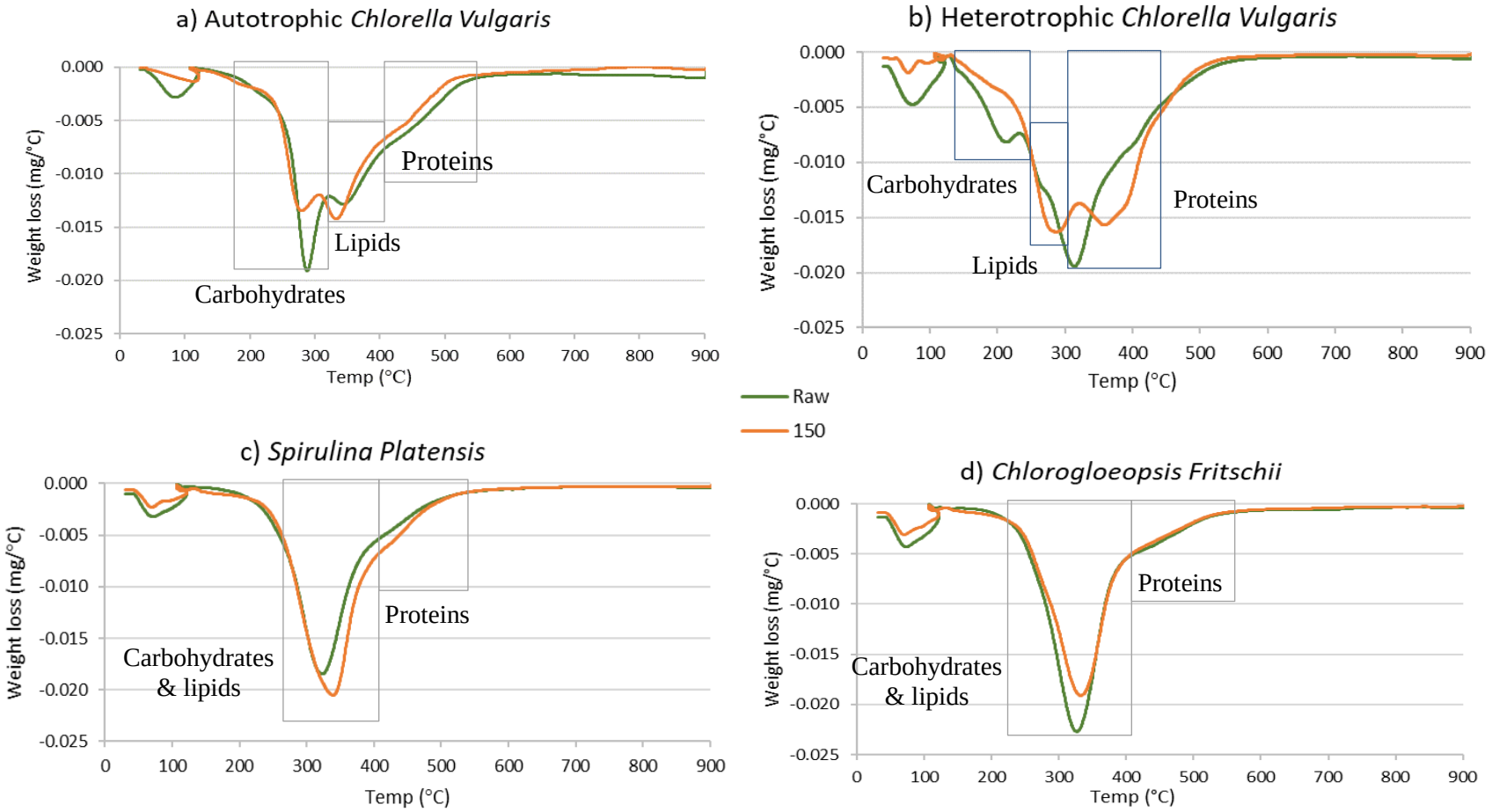


Figure 4-11: DTG curves from raw and pre-treated micro algae: a) autotrophic *Chlorella*, b) heterotrophic *Chlorella vulgaris*, c)*Spirulina platensis* and d) *Chlorogloeopsis fritschii*

The DTG curve from the *Spirulina* shows two main peaks. The first peak again relates to the moisture present in the sample. The second peak differs for the raw algae and the solid residue from pre-treatment at 150°C. The peak for the pre-treated solid residue becomes larger and wider than the peak of the raw algae. The second peak also increases for the *Spirulina* pre-treated at 150°C in comparison to the raw *Spirulina*. This is a combination of the carbohydrates and lipids. The change in the width of the curve may be due to the change in the ratio of structural and non-structural carbohydrates in the *Spirulina* pre-treated at 150°C. The small bump in the curve at 450°C relates to the proteins, although there is not much difference in the amount of proteins present shown in the biochemical components of both samples in Table 4-9, the composition of the proteins may have been altered during pre-treatment and therefore there is a difference in the curve for both samples.

For the *Chlorogloeopsis* the DTG curve shows a similar trend to the *Spirulina*. There again two main peaks present, with the first being moisture in the samples. The second peak relates to the carbohydrates which shows a decrease in the *Chlorogloeopsis* pre-treated at 150°C in comparison to the raw *Chlorogloeopsis*. This correlates with the biochemical components data in Table 4-9. The lipids are part of this peak as they are usually seen between 350 and 400°C. The small peak between 400 and 500°C relates to the protein content.

Overall, all four micro algae are very different. It was expected that the heterotrophic *Chlorella* and autotrophic *Chlorella* wouldn't be too dissimilar but from the biochemical analysis and DTG curves, it shows how different they are to one another. The *Spirulina* and *Chlorogloeopsis* show similar biochemical composition and DTG curves to one another, but are very different to the two *Chlorella* samples, which the data suggests are much more complex than the *Spirulina* and *Chlorogloeopsis*.

Table 4-10 shows the ultimate and proximate analysis of the solid residues from hydrothermal pre-treatment at 150°C for the four micro algae; autotrophic *Chlorella vulgaris*, heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii*. Comparison of the raw and pre-treated micro algae shows that all four samples are different from one another and that pre-treatment also affects the algae.

The moisture content of the raw algae is higher than for the pre-treated algae apart from the *Spirulina*. The ash content of the two raw *Chlorella* samples is higher than the pre-treated *Chlorella*, whereas the solid residues from the pre-treated algae are higher than the raw algae for the *Spirulina* and *Chlorogloeopsis*. The volatile content decreases for the pre-treated autotrophic *Chlorella* and the *Chlorogloeopsis*, but increases for the heterotrophic *Chlorella* and stays the same for the *Spirulina*. The fixed carbon content decreases from the raw to the pre-treated for the heterotrophic *Chlorella* and *Chlorogloeopsis* but shows an increase in the fixed carbon content of the pre-treated autotrophic *Chlorella* and *Spirulina*. The carbon content of the pre-treated algae is lower than the raw algae for all the samples except for the autotrophic *Chlorella* which shows an increase. The hydrogen content of the pre-treated algae is slightly higher than the raw algae for all the samples. The nitrogen content increases for the pre-treated autotrophic *Chlorella* and *Spirulina* but decreases for the pre-treated heterotrophic *Chlorella* and *Chlorogloeopsis*. There is no traceable sulphur present in the raw algae, and is also not present in the pre-treated residues, except for the autotrophic *Chlorella*. The reason for this may be due to the sulphur becoming more concentrated within the sample as other components are released into the process waters during pre-treatment. The oxygen content of the pre-treated algae decreases for the autotrophic *Chlorella* but increases for the heterotrophic *Chlorella*, *Spirulina* and *Chlorogloeopsis* after pre-treatment. The HHV increases for the autotrophic *Chlorella* but decrease for the other three algae, in comparison to the raw algae.

Overall, the solid residues are of a higher energy density with lower moisture and volatile content than the raw algae. The difference in biochemical composition affects the distribution of carbon, nitrogen and ash in the solid residues after pre-treatment.

Table 4-10: Proximate and Ultimate analysis of solid residues from hydrothermal pre-treatment of autotrophic *Chlorella vulgaris,* heterotrophic *Chlorella vulgaris, Spirulina platensis* and *Chlorogloeopsis fritschii* at 150°C

Type of algae	Proximate (%) (d.b.)				Ultimate (%) (d.a.f.)					HHV (MJ/kg^{-1})
	Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*	
autotrophic *Chlorella*	3.6	16.1	67.7	16.2	57.4	7.9	8.2	0.4	26.1	27.6
heterotrophic *Chlorella*	3.6	2.4	87.2	10.4	50.7	8.4	6.8	0.0	34.1	25.0
Spirulina platensis	6.1	8.6	75.1	16.3	35.8	8.1	12.4	0.0	43.7	18.3
Chlorogloeopsis fritschii	7.8	9.7	73.3	17.0	36.1	8.2	10.7	0.0	45.1	18.3

*Oxygen was quantified by difference

4.4.3. Composition of process waters from various micro algae

The process waters from hydrothermal pre-treatment of the four different micro algae were analysed using a Shimadzu QP2010E GC-MS. The description of this methodology is in section 3.7.5.2 The formic acid, glucose, galactose, xylose and mannose, where analysed for using the HPLC method described in section 3.7.4.

Table 4-11 shows the compounds, analysed for using GC-MS, in process waters from hydrothermal pre-treatment of autotrophic *Chlorella vulgaris,* heterotrophic *Chlorella vulgaris, Spirulina platensis* and *Chlorogloeopsis fritschii* at 150°C. The notable acids in these process waters are lactic, acetic, butyric, levulinic and succinic acid, which are present in the process waters in much higher quantities than the other acids that were analysed for. The process water from *Spirulina* contains the most lactic and acetic acid and the autotrophic *Chlorella* contains the highest quantity of levulinic and succinic acid.

The process water from the autotrophic *Chlorella* is the only sample in which nitrogen compounds were detected. The reason for this is due to the release of proteins from the algae during hydrothermal pre-treatment which is shown in Table 4-9, therefore resulting in more nitrogen compounds in the process waters. The cyclopentanones are present in relatively low quantities for the four process waters from micro algae. There are no phenols present in any of the process waters.

For the sugar content of the process waters, only the process waters from the *Chlorella* contain glucose. with *Spirulina* and *Chlorogloeopsis* showing similar amounts. The autotrophic *Chlorella* contains very low amounts of glucose and fructose but no ribose and mannose. The heterotrophic *Chlorella* contains higher levels of glucose, fructose and ribose than the other process waters. Only fructose, was detected in the *Spirulina* and ribose in the *Chlorogloeopsis*.

These results show that a lot of the compounds were not detected for the heterotrophic *Chlorella, Spirulina* or *Chlorogloeopsis*. The reason for this is may be due to the process waters containing only a very small amount which was not detected by the GC-MS or that these experiments were carried out and analysed at a later date, at which point there could have been an issue with the GC-MS or the process waters.

Table 4-11: Compounds in process waters after hydrothermal pre-treatment of autotrophic *Chlorella vulgaris,* heterotrophic *Chlorella vulgaris, Spirulina platensis* and *Chlorogloeopsis fritschii* at 150

		mg/l			
	Compound	Auto	Hetero	*Spirulina*	*Chlorogloeopsis*
Acids	Formic acid	0.2	7728.6	ND	7.0
	Lactic acid	1.5	ND	15326.3	4854.1
	Acetic acid	126.0	225.9	634.2	532.4
	Butyric acid	0.5	51.7	254.8	252.0
	Crotonic acid	0.8	0.0	ND	ND
	Isovaleric acid	0.8	47.1	ND	ND
	Valeric acid	6.3	34.1	ND	ND
	3-methyl-Pentanoic acid	ND	ND	ND	ND
	4-methyl-Pentanoic acid	1.0	ND	ND	ND
	Hex-5-enoic acid	12.4	ND	ND	ND
	Malonic acid	21.1	ND	ND	ND
	Methyl Malonic acid	4.6	ND	ND	ND
	Levulinic acid	8104.7	22.2	ND	ND
	Succinic acid	5028.2	384.6	124.5	221.1
	Benzoic acid	4.8	6.5	ND	ND
	Glutaric acid	143.4	ND	ND	ND
	Hydrocinnamic acid	10.5	ND	ND	ND
Nitrogen compounds	Pyrazine	27.9	ND	ND	ND
	Pyrazine, methyl-	133.3	ND	ND	ND
	2,5-dimethyl-Pyrazine	7.5	ND	ND	ND
	Ethyl-Pyrazine	10.2	ND	ND	ND
	Trimethyl- Pyrazine	11.1	ND	ND	ND
	Pyrrolidinone	877.8	ND	ND	ND
	3-Hydroxypyridine monoacetate	152.9	ND	ND	ND
Cyclopentanones	Cyclopentanone	29.8	ND	ND	ND
	2-methyl-2-Cyclopenten-1-one	29.1	ND	ND	ND
	3-methyl-2-Cyclopenten-1-one	100.4	ND	ND	ND
	2,3-dimethyl-2-Cyclopenten-1-one	45.2	ND	ND	ND
Phenols	Phenol	ND	ND	ND	ND
	p-Cresol	ND	ND	ND	ND
Sugars	Glucose	2.0	443.6	ND	ND
	Fructose	0.7	1198.2	ND	322.8
	Ribose	ND	1118.2	100.9	ND
	Mannose	ND	ND	ND	ND

Table 4-12 shows the total organic carbon, nitrogen and ammonium, along with the orthophosphate and phosphate content of the process waters from hydrothermal pre-treatment of the autotrophic *Chlorella vulgaris*, heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii* at 150°C.

The heterotrophic *Chlorella* and *Spirulina* pre-treated at 150°C are very similar and have a significantly higher total organic carbon content than the autotrophic *Chlorella* and *Chlorogloeopsis* which are also both very similar. The heterotrophic *Chlorella* and *Spirulina* also have a very similar total nitrogen content. The *Chlorogloeopsis* is slightly higher than these and the auto *Chlorella* has the lowest total nitrogen content. The ammonium content of the autotrophic and heterotrophic *Chlorella* is very similar to the *Chlorogloeopsis* but the *Spirulina* is lower. The reason for the low ammonium content may be due to the *Spirulina* being a cyanobacteria, which has a different biochemical composition to the *Chlorella*. Both the raw *Chlorella* have high non-structural carbohydrate content, whereas, the *Spirulina* has a lower non-structural carbohydrate content but higher protein content. The non-structural carbohydrates are released into the process waters during hydrothermal pre-treatment where they enable reactions with the nitrogen compounds in the protein. This does not occur as much with the *Spirulina* as it contains less non-structural carbohydrates. This could affect how the nitrogen is decomposed into different compounds within the process waters, forming compounds such as amines instead of ammonium. Ammonium is mainly produced by deamidation of the amino acids or peptide bonds in the algae. The increase in hydrothermal pre-treatment temperature causes an increase in protonation which favours the deamination of the amino acids or other nitrogen compounds, thus resulting in increased ammonium content in the process waters (YutakaDote et al., 1996).

The total phosphate content of the heterotrophic *Chlorella* and *Spirulina* are very similar and are over double the content of the autotrophic *Chlorella* and *Chlorogloeopsis* which are also similar to each other. The majority of the total phosphate in all four algae is orthophosphate and organic phosphate only makes up a small fraction of the total phosphate.

Table 4-12: Total organic carbon, nitrogen, ammonium, orthophosphate and phosphate content of process waters from pre-treatment of autotrophic *Chlorella vulgaris,* heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii* at 150°C

	mg/l						
Type of algae	Total Organic Carbon	Total Nitrogen	Ammonium	Organic nitrogen	Total Phosphate	Ortho-phosphate	Organic Phosphate
autotrophic *Chlorella*	16637.1	3700.0	574.0	3126.0	640.0	540.0	100.0
heterotrophic *Chlorella*	28590.6	5120.0	602.0	4518.0	1350.0	1050.0	300.0
Spirulina platensis	28133.3	5960.0	360.0	5600.0	1270.0	940.0	330.0
Chlorogloeopsis fritschii	16662.1	6760.0	554.0	6206.0	680.0	630.0	50.0

Table 4-13 shows the XRF of the process waters from hydrothermal pre-treatment of the four micro autotrophic *Chlorella vulgaris*, heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii*, at 150°C.Although XRF covers a larger range of metals, only the ones present in the process waters are shown.

Table 4-13: XRF of process waters from autotrophic *Chlorella vulgaris*, heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii* after hydrothermal pre-treatment at 150°C

	ppm			
Metal	Auto *Chlorella*	Hetero *Chlorella*	*Spirulina*	*Chlorogloeopsis*
Na	ND	ND	ND	ND
Mg	ND	ND	ND	421
Al	133	77	109	86
Si	100	127	156	139
P	1970	1722	1795	1353
S	197	196	300	172
Cl	240	ND	236	ND
K	405	1179	1495	378
Ca	235	289	247	316
Fe	ND	24	ND	15
Br	ND	ND	ND	ND
Sr	ND	ND	ND	ND

There is no sodium, bromine or strontium present in any of the micro algae, which is the expected result as they are not marine water algae. With the exception of the *Chlorogloeopsis*, there is no magnesium present in the other algae. The aluminium content of the heterotrophic *Chlorella* and *Chlorogloeopsis* are similar at 77 and 86ppm, as are the autotrophic *Chlorella* and *Spirulina* at 133 and 109ppm, however these are higher than the other two samples. The autotrophic *Chlorella* has the lowest silicon content at 100ppm with the process waters from the heterotrophic *Chlorella*, *Chlorogloeopsis* and *Spirulina* having higher silicon contents at 127, 156 and 139ppm respectively. The autotrophic *Chlorella* has the highest phosphate content at 1970ppm and the *Chlorogloeopsis* has the lowest at 1353ppm. The phosphate content of the heterotrophic *Chlorella* and *Spirulina* are similar at 1722 and 1795ppm respectively and fall within the middle of the other two samples. The high phosphate content in the process waters suggests that phosphate has been removed from the algae during hydrothermal pre-treatment and has been released

into the process waters. The sulphur content of the autotrophic and heterotrophic *Chlorella* are almost identical (197 and 196ppm), with the *Chlorogloeopsis* being slightly lower at 172ppm. The *Spirulina* has the highest sulphur content of 300ppm. Only the autotrophic *Chlorella* and *Spirulina* have chlorine present. Both the values are almost identical at 240 and 236ppm respectively. The autotrophic *Chlorella* and *Chlorogloeopsis* have similar potassium content (405 and 378ppm), as do the heterotrophic *Chlorella* and the *Spirulina* (1179 and 1495ppm), however their content is higher than the other two algae. The calcium content of the autotrophic *Chlorella* and the *Spirulina* are similar (235 and 247ppm), as are the calcium contents of the heterotrophic *Chlorella* and the *Chlorogloeopsis* (289 and 316ppm), which are higher than the other two samples. The process waters from the autotrophic *Chlorella* and *Spirulina* do not contain and iron, but the heterotrophic *Chlorella* and *Chlorogloeopsis* contain similar amounts 24 and 15ppm respectively). None of the micro algae process water samples contain bromine or strontium.

4.4.4. Discussion of micro algae pre-treated at 150°C

The autotrophic *Chlorella* differs greatly to the heterotrophic *Chlorella*. The main reason causing these differences is the variation in the biochemical composition of both *Chlorella* samples. As stated previously, the biochemical composition of both the *Chlorella* samples varies greatly due to a slight differences in the amount of lipids and proteins present and a major difference in the non-structural carbohydrates. The high protein content of the four algae is problematic as it means that there are high levels of nitrogen in the feedstock. Pre-treatment breaks down these proteins and releases some into the process waters along with the non-structural carbohydrates. Thus, improving the solid algal residue that remains after pre-treatment and effectively creating a new feedstock. This will improve the quality of products produced from further processing and has fulfilled the hypothesis set out in the begin of the section.

4.5. Conclusion

This study has shown that hydrothermal processing is a promising pre-treatment method for both the micro and macro algae as it produces a higher energy density solid residue and releases problematic inorganics into the process waters.

During the hydrothermal pre-treatment, the quantity of the biochemical components are altered which in turn results in the biochemical composition also transforming due to the altered reaction pathways. The release of carbohydrates into the process waters results in a reduction in the solid residue. This also increases the ratio of lipids and proteins to carbohydrates, thus resulting in an improved feedstock for the production of biofuels.

The results from hydrothermal pre-treatment of the five algae samples, suggests that different algae can be used to produce improved solid residues for producing high protein, high lipid and high carbohydrate content feedstocks. From the five algae samples, the best algae to use to produce a high protein feedstock after hydrothermal pre-treatment at 150°C is the *Spirulina*. For the production of a high lipid content feedstock is the algae to start with is the autotrophic *Chlorella* and to hydrothermally pre-treat at 200°C. For a feedstock with high carbohydrate content the *Ulva* should be used raw with no pre-treatment. The heterotrophic *Chlorella* pre-treated at 150°C can be used for solid residues with the lowest ash content.

In the first section of the chapter, the raw algae was characterised and it was found that the autotrophic *Chlorella* consisted of a higher lipid content than the *Ulva*, which suggests that bio-oil of a higher quantity could be produced from the *Chlorella*.

In the second section of the chapter, the comparison of a micro and macro algae; *Chlorella vulgaris* and *Ulva lactuca* showed that micro algae provided the biggest difference between the raw and pre-treated samples. The comparison of the three hydrothermal pre-treatment temperatures (100°C, 150°C and 200°C) showed that various components of the algae break down at different rates. 150°C was the most suitable temperature for both feedstocks, with it being the most effective for the *Chlorella vulgaris*. Macro algae (*Ulva lactuca*) is less suitable than the micro algae due to the high inorganics content. Hydrothermal pre-treatment lowers the

inorganics and ash content of the algae, resulting in a higher energy density solid residue.

In the final section of the chapter, the comparison between the four micro algae; autotrophic *Chlorella vulgaris*, heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii*, showed that hydrothermal pre-treatment at 150°C improved the quality of the solid residue by releasing inorganics and other unfavourable components into the process waters, resulting in an improved solid residue. There are differences between the four micro algae, that are due to the difference in the biochemical composition of the algae. The biochemical composition plays a key role in what is broken down and how, due to the ratio of the proteins, carbohydrates and lipids present. The process waters from hydrothermal pre-treatment of the four micro algae at 150°C differ greatly, but show that problematic components such as inorganics are being released into the process waters. The results suggest that the cations have an influence on the process chemistry and can overcome the unfavourable aspects of the algae as a feedstock for biofuels.

Chapter 5. Potential conversion routes

5.1. Introduction

Converting raw biomass feedstock into an oil is an energy intensive and costly process, with the majority of the cost and energy required contributing to the processing and upgrading of the final fuel. The energy and cost required can vary greatly for different conversion methods, as can the post conversion upgrading required to make the oil a useable fuel.

Chapter 4 investigated the hydrothermal pre-treatment of various macro and micro algae at temperatures of 100°C, 150°C and 200°C. The results showed that hydrothermal pre-treatment changes the composition of the solid residue with some evidence that this improves its quality for further processing by releasing inorganics into the process waters. In this chapter the macro algae, *Ulva lactuca*, and the autotrophic micro algae, *Chlorella vulgaris*, are further explored to investigate whether pre-treating algae improves the quality of oil from the three different conversion methods.

This chapter focuses on the differences and similarities between the *Ulva lactuca* and autotrophic *Chlorella vulgaris*, when processed using three different conversion routes. The three conversion routes investigated are pyrolysis, solvent extraction and hydrothermal liquefaction. Once the hydrothermal pre-treatment stage was undertaken at the three temperatures: 100°C, 150°C and 200°C, (covered in the first results chapter, section 4.3) a comparison of the three conversion routes was performed to determine which route produced the highest yield and quality of oil, for the *Ulva lactuca* or the autotrophic *Chlorella vulgaris*, for either the raw or pre-treated algae. The ideal characteristics of a good quality oil are high yield and HHV and low moisture, ash and fixed carbon content. Figure 5-1 shows a flow diagram of the conversion processes and the products produced by each conversion route.

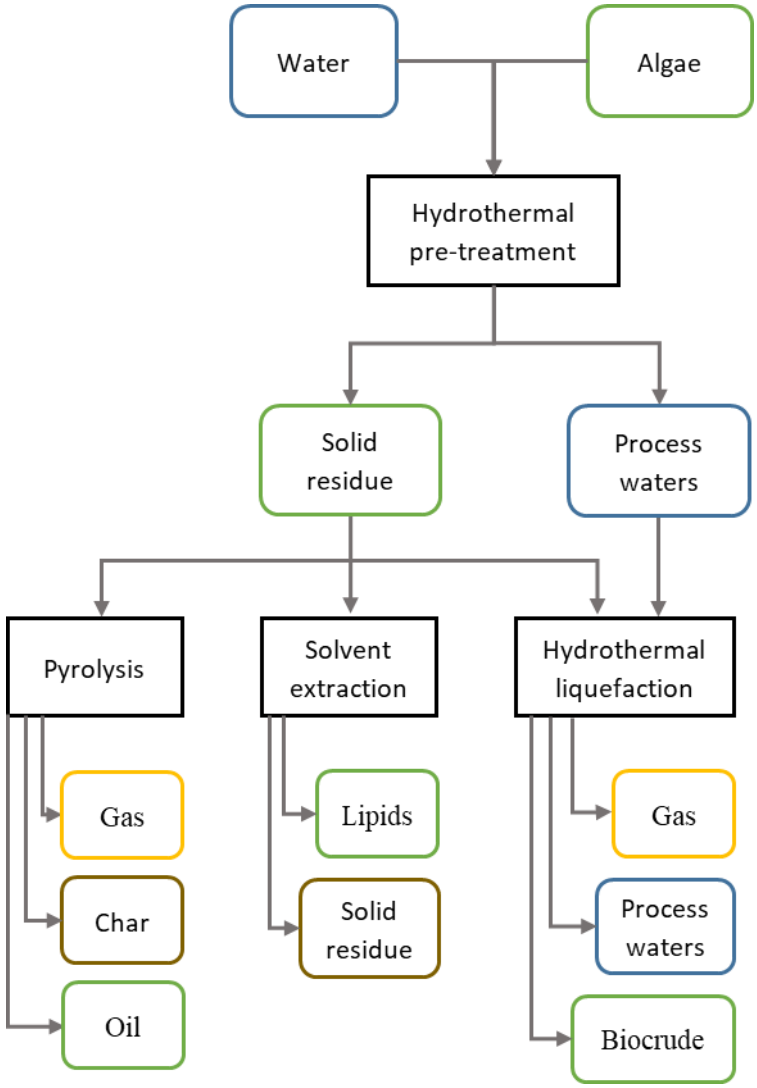


Figure 5-1: Flow diagram of conversion processes and products

The final section of this chapter investigates the amount of hydrogen required during upgrading of the bio-oils from the three different conversion methods.

5.2. Pyrolysis

Pyrolysis was chosen as one of the conversion method, as it is a technique that has gained popularity over the past 50 years, for processing biomass to produce oils (Yang, C. et al., 2019). Pyrolysis is a thermochemical process which decomposes biomass into char, liquid and gas, in the absence of air. The different products are produced in varying quantities based on the heating rate, hold temperature and residence time (Basu, 2010).

In this section, pyrolysis of the *Ulva lactuca* and autotrophic *Chlorella vulgaris* was carried out at 600°C and held for 1 hour, following the method described in section 3.5.1 of the methodology chapter.

The hypothesis that is proposed for this conversion route is that the quality of the pyrolysis oil will improve with the use of the solid residues from hydrothermal pre-treatment of the *Ulva lactuca* and the autotrophic *Chlorella*, at the three different temperatures (100, 150 and 200°C). This is expected as some of the problematic material present in the raw algae will be removed during pre-treatment. This hypothesis is investigated by analysis of the quality of the oils generated in comparison to those from raw algae.

5.2.1. Yields from pyrolysis

Table 5-1 shows the yields of oil, char and gas from pyrolysis of the *Ulva lactuca* and the autotrophic *Chlorella vulgaris*. In this instance, the oil yields represent the condensable oils at room temperature and the gaseous yields include, non-condensable gases and liquids.

Table 5-1: Yields of oil, char and gas from pyrolysis of micro and macro algae

Type of algae	Pre-treatment temperature	%		
		Oil	Char	Non-condensable gases
Ulva lactuca	Raw	4.9	58.3	36.8
	100	3.6	53.8	42.6
	150	4.0	41.6	54.4
	200	6.2	24.3	69.4
autotrophic *Chlorella*	Raw	42.9	28.8	28.3
	100	24.4	27.2	48.4
	150	28.4	27.6	44.0
	200	12.9	31.4	55.7

Of the four *Ulva* samples, the *Ulva* pre-treated at 200°C gave the highest yield of oil at 6%, with the lowest yield of char and highest yield of gas. In contrast, the *Chlorella* shows a decrease in oil yield with increasing pre-treatment temperature with the highest yield of oil (42.9%) at 100°C. In comparison to the autotrophic *Chlorella*, the *Ulva* has a much lower yield of oil and higher char and

gas yields. The oil yields from the *Ulva* are 4.9-6.2% whereas for *Chlorella* the yields are 12.9-42.9%. The reason for this is likely due to the *Ulva* having a lower initial lipid content. A study by Ross et al. (2009) found that carbohydrates in macro algae favour char formation during pyrolysis. Therefore, the higher char yield may also be due to higher carbohydrate content of the *Ulva*. In comparison, the *Chlorella* has a higher initial lipid content and lower carbohydrate content and therefore has higher oil yields and lower char yields. Although the char content of both algae increases with increasing pre-treatment temperature for both algae, with the *Chlorella* having a lower yield of 27-31% in comparison to the *Ulva* of 24-58%. The non-condensable gases also increase with increasing pre-treatment temperature for both the *Ulva* and *Chlorella*.

5.2.2. Ultimate and proximate analysis of pyrolysis oils

Table 5-2 shows the ultimate and proximate analysis of the pyrolysis oils from the autotrophic *Chlorella vulgaris* and *Ulva lactuca*.

The proximate analysis of the pyrolysis oils from the *Ulva* vary greatly between the four different samples. The oil from the raw *Ulva* has a much higher moisture content than the oils from the pre-treated *Ulva*, however, this may be an incorrect measure of moisture content. The reason for this is as there may be very light molecular weight hydrocarbons present in the oil, which are driven off at the beginning stage of the TGA analysis. The ash content of the oils show an increase with increasing pre-treatment temperature from 4.6% to 8.8%. The reason for this increase with the increasing pre-treatment temperature may be due to the concentration of the inorganics in the solid residue after pre-treatment due to the mainly carbohydrates being removed. The volatiles content of the oils from the pre-treated *Ulva* are similar (76.5-81.8%), whereas the raw *Ulva* is higher at 88.7%. The pyrolysis oil from the raw *Ulva* has the lowest fixed carbon content of the four oil samples (6.7%) and the *Ulva* pre-treated at 100°C has the highest (16.8%). The oils from the *Ulva* pre-treated at 150°C and 200°C are both similar.

The pyrolysis oils from the *Ulva*, pre-treated at the three different temperatures, have very similar carbon, hydrogen, nitrogen and sulphur content to each other and the raw *Ulva*. Although all the oils have a similar carbon content, the raw *Ulva* has the highest at 81.8%. On the flipside, the pyrolysis oil from the raw

Ulva has the lowest hydrogen content of the four samples, with the pyrolysis oil from the *Ulva* pre-treated at 100°C being very similar. The hydrogen content of the oils from the *Ulva* pre-treated at 150 and 200°C are slightly higher but not significantly. The nitrogen content of the pyrolysis oils from the pre-treated *Ulva* are slightly lower (7.3-8.5%) than the oil from the raw *Ulva* (9.0%). The sulphur content of the pyrolysis oils from the raw and pre-treated *Ulva* are all similar and below 1%. The oxygen content of the oils increases from 3.5% to 16.2% from the raw to the *Ulva* pre-treated at 100°C then decreases for the *Ulva* pre-treated at 150°C (13.2%) and then further for the *Ulva* pre-treated at 200°C (8.6%). The HHV is similar for the four samples with the lowest value shown for the *Ulva* pre-treated at 100°C. Overall the four pyrolysis oils from the *Ulva* are quite similar.

The proximate analysis of the pyrolysis oils from the *Chlorella* varies greatly between the four different samples. The oils from pre-treatment have lower moisture contents than the raw *Chlorella*, with the *Chlorella* at 200°C showing the lowest content at 6.24%. As pyrolysis oils normally contain moisture and are emulsions, the reason for the reduction at 200°C may be due to the change in the feedstock composition resulting in the formation of less water during pyrolysis.

The levels of inorganics associated with oils are often not reported and assumed to be low, however, this work has shown that the levels of ash in the oils can be quite significant. This has implications for upgrading or utilisation of these oils as inorganics often result in fouling or catalyst poisoning. The level of ash in the raw *Chlorella* pyrolysis oils is quite high (18.7% d.b.). The oils from the *Chlorella* pre-treated at 150°C and 200°C, have a lower ash content at 11.3% and 13.8% respectively whereas the highest ash content of 22.4% is observed after pre-treating at 100°C. These levels of ash in the oils are very high and while it is likely that the levels of inorganics will be affected by reactor design, this highlights an under reported phenomena which is extremely significant for these high ash containing feedstocks. The higher ash content of the feedstocks therefore results in some of the inorganics being partitioned within the pyrolysis oil, probably due to solubilisation of inorganics in the water fraction of the oil. This can also be detrimental when upgrading the oil.

The volatiles content of the oils from the pre-treated *Chlorella* are higher than the pyrolysis oil from the raw *Chlorella*. The oil from the *Chlorella* pre-treated

at 200°C has the highest volatiles content of 70% d.b. The fixed carbon content of the oil from the raw *Chlorella* is the highest at 15.1%. The oils from the pre-treated *Chlorella* are all above 10%. Fixed carbon content is an indicator of quality of the oil produced and the results show that there is higher molecular weight material in the oils resulting in more polymerisation to fixed carbon during proximate analysis. This therefore suggests that a lower quality oil has been produced. The reason for this is high fixed carbon content is an indicator of high aromatics in the oil and also results in the oil being more prone to coking which can be problematic during upgrading (Mierzwa-Hersztek et al., 2019).

The elemental analysis of the pyrolysis oils from the *Chlorella* shows more difference in the carbon, hydrogen, nitrogen and sulphur contents of the four oils than the *Ulva*. The pyrolysis oil from the raw and pre-treated at 100°C *Chlorella*, have very similar carbon content of 77.5% and 76.7% respectively. The carbon content of the oil from the *Chlorella* pre-treated at 150 and 200°C are also similar (67% and 65.4% respectively) but are lower than that of the oil from the raw *Chlorella*. The hydrogen content of the pyrolysis oils decreases from the raw *Chlorella* (12.1%) to the *Chlorella* pre-treated at 100°C (11.3%) to the *Chlorella* pre-treated at 150°C (8.9%) which is the lowest, then increases again for the *Chlorella* pre-treated at 200°C (10.3%). Although the nitrogen content of the raw *Chlorella* and *Chlorella* pre-treated at 100°C are similar 4.6% and 6.2%), the nitrogen content of the oil from the *Chlorella* pre-treated at 150°C is higher at 12.4%. the nitrogen then decreases again for the *Chlorella* pre-treated at 200°C to 5.3%. No sulphur was detected in the pyrolysis oils from *Chlorella*. This was expected as there was no sulphur detected in the raw and pre-treated *Chlorella* solid residues. The oxygen content of the pyrolysis oils increases with increasing pre-treatment temperature from 5.7% to 19.0%.. The HHV shows the opposite trend and decreases with increasing pre-treatment temperature from 43MJ/kg to 34.6MJ/kg.

In comparison, the oils from both the *Chlorella* and the *Ulva* are quite different to each other. The oils from the *Chlorella* have higher ash and moisture content than the oils from the *Ulva*, with the oil from the raw *Chlorella* having the highest ash content. The *Ulva* however, has higher volatiles and fixed carbon content than the *Chlorella*. The carbon content of the oils from *Chlorella* are lower than the oils from *Ulva*, whereas the hydrogen content is higher. The nitrogen

content of the oils from *Chlorella* are also lower than the *Ulva*. The oils from *Chlorella* contain no sulphur and the oils from *Ulva* contain less than 1%. The oxygen content of the oils from the *Ulva* are higher than the oils from the *Chlorella*. The HHV values of the *Chlorella* are higher than those of the *Ulva*. The lower HHV of the *Ulva* and the decreasing HHV values for the *Chlorella*, indicate that the oil is less energy dense which therefore suggests the oils will require further upgrading. Overall, with regards to the quality of the oils, the *Chlorella* have better characteristics than the *Ulva* for biofuel production.

Du et al. (2012) carried out a similar study on *Nannochloropsis oculate* using higher temperatures and shorter reaction times during the hydrothermal pre-treatment stage followed by pyrolysis at 500°C, using a Py-GC-MS. It was found that the pyrolysis oils from the hydrothermally pre-treated samples had higher carbon contents and lower nitrogen content compared to the pyrolysis oils from the raw algae. The results in Table 5-2 show that the carbon content of the pyrolysis oils from both the pre-treated *Ulva* and *Chlorella* decrease with increasing pre-treatment temperature, which is opposite to the trend observed in the study. The nitrogen content of the pyrolysis oils from both the *Chlorella* and *Ulva* also do not follow the trend observed in the study by Du et al. (2012), as the nitrogen content in the oils from the pre-treated *Ulva* and *Chlorella* is higher than that of the oils from the raw algae. These differences may be due to reactor design and experimental parameters. They may also be due to the difference in biochemical composition which could have an effect on the nitrogen content of the pyrolysis oil produced.

Table 5-2: Ultimate and proximate analysis of pyrolysis oils from *Ulva lactuca* and autotrophic *Chlorella*

Type of algae	Pre-treatment temperature (°C)	Proximate (%) (d.b.)				Ultimate (%) (d.a.f.)					HHV (MJ/kg^{-1}) (d.a.f.)
		Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*	
Ulva lactuca	Raw	10.4	4.6	88.7	6.7	81.8	4.9	9.0	0.9	3.4	34.4
	100	1.7	6.7	76.5	16.8	70.5	5.4	7.3	0.6	16.2	29.6
	150	1.8	7.4	81.8	10.7	70.5	8.1	7.3	0.9	13.2	34.0
	200	3.1	8.8	78.5	12.7	73.7	8.0	8.5	1.1	8.6	35.5
Auto *Chlorella*	Raw	10.2	18.7	66.0	15.1	77.5	12.1	4.6	0.0	5.8	43.0
	100	8.7	22.4	66.6	11.0	76.7	11.3	6.2	0.0	5.8	41.6
	150	9.6	11.3	76.9	11.8	67.0	8.9	12.4	0.0	11.7	34.0
	200	6.24	13.8	75.5	10.7	65.4	10.3	5.3	0.0	19.0	34.6

*Oxygen calculated by difference

5.2.3. Discussion for conversion by pyrolysis

The quality of the oil from pyrolysis of the *Chlorella* and *Ulva* vary greatly. The oil produced from *Chlorella* has high ash and moisture content, making it problematic for use as a biofuel. The high oxygen and hydrogen content is also an issue as it requires more upgrading to make the oil into a useable fuel. The oils from the *Ulva* have lower values for the ash and moisture, making them more appealing for use as biofuels as they require less upgrading. The HHV of both oils is lower than what is expected.

The pyrolysis oils from *Chlorella* also contain high levels of nitrogen, with the oils from the pre-treated *Chlorella* residues containing higher levels than the raw *Chlorella*. This correlates with the results in the previous pre-treatment chapter which shows that the amount of nitrogen in the algae has become concentrated by pre-treatment as other material has been removed. The removal of this material could also be a reason as to why there are higher levels of nitrogen, as there is less material which usually reacts with the nitrogen during pyrolysis, thus altering the reaction pathways of the nitrogen which results in increasing nitrogen in the pyrolysis oils. This is not good for biofuel production from the pyrolysis oil but may be better for producing platform chemical feedstocks instead.

Pyrolysis is a well-established method which was initially used for making charcoal, however it has since proven popular for the production of oils from various biomass, including algae. As there is an abundance of literature on pyrolysis of coal and biomass, it is easier to determine the best conditions for pyrolysis of algae. Although pyrolysis is a well-established method for producing oils from biomass, it does have some limitations for producing oils from algae. One of the drawbacks of this work is that slow pyrolysis is the method employed. The slow reaction rate of the pyrolysis results in higher yields of char and gases, which affects the bio-oil yield in a negative manner (Fermoso et al., 2017).

Another drawback of using pyrolysis as a conversion method for algae is that pyrolysis requires a dry feedstock. In this instance, pyrolysis was applicable to the work as the algae were freeze dried before use, however, in most other work on algae, it is not usually dried but instead used as a wet feedstock. These drawbacks indicate that pyrolysis may not be the best method for converting algae into bio-oils due to the constrictions of the reactor and feedstock.

5.3. Solvent extraction

The difficulty in producing bio-oil from biomass such as algae is in releasing the lipids in the most economical and energy efficient method, which involves avoiding the use of large amounts of solvent (Scott et al., 2010). Soxhlet extraction is a method which can be utilised to extract lipids using only a small amount of solvent, which is recycled. It is similar to stripping off solvent in industry. This method was chosen as literature shows it has commonly been used to extract lipids such as triglycerides from various high lipid feedstocks. In this instance, the lipids are the fraction of the algae that will be used for producing biofuels. The type of lipids extracted are based on the solvent used, for example, polar lipids are extracted using a polar solvent such as methanol and non-polar lipids are extracted using a non-polar solvent such as hexane.

Solvent extraction of the autotrophic *Chlorella vulgaris* and *Ulva lactuca* was carried out using the method described in section 3.5.2 of the methodology chapter. The method was adapted from Bligh and Dyer (1959) and combined with a soxhlet extractor. Bligh and Dyer's method uses chloroform:methanol in a 2:1 ratio. From studying the literature (Ramola et al., 2019; Avinesh et al., 2015), it was found that hexane enhanced the extraction of lipids from algae, therefore, the method utilised in this work incorporated a secondary extraction step using hexane as the solvent. The reason for this is as the chloroform is mid-polarity and the methanol is polar, thus both neutral and polar lipids are extracted. With regards to the hexane, due to it being a non-polar solvent, it extracts non-polar lipids from the algae (Prommuak et al., 2012).

The hypothesis that is proposed for this conversion route is that the quality of the extracted oils will improve with the use of the solid residues from hydrothermal pre-treatment of the autotrophic *Chlorella* and *Ulva lactuca*, at the three different temperatures (100, 150 and 200°C), in comparison to the raw algae. It is expected that the yield of the extractable oils will also increase as there are less problematic materials present in the pre-treated algae in comparison to the raw algae.

5.3.1. Yields from solvent extraction

Table 5-3 shows the yields of total extractable oils and solids from solvent extraction of *Ulva lactuca* and autotrophic *Chlorella vulgaris.*

The *Ulva* pre-treated at 100°C and 150°C gives a very similar yield of oil and solids to the raw *Ulva*. The *Ulva* pre-treated at 200°C only shows a slight increase in the oil content but this is not very noticeable. The low oil extraction is due to the *Ulva* having a very low initial lipid content. The *Chlorella* pre-treated at 100°C gives a very similar yield of oils and solids to that of the raw *Chlorella*. The *Chlorella* pre-treated at 150°C gives a yield of oil that is double of the yield of the raw *Chlorella* and the *Chlorella* pre-treated at 200°C is higher than that at 150°C.

Table 5-3: Yields of oil and solids from solvent extraction of micro and macro algae

Type of algae	Pre-treatment temperature (°C)	%	
		Extractable oils	Solids
Ulva lactuca	Raw	0.2	92.4
	100	0.2	99.8
	150	0.2	95.7
	200	2.4	97.6
autotrophic *Chlorella*	Raw	15.6	84.4
	100	16.8	83.2
	150	31.7	68.3
	200	36.3	63.8

The oils from the raw *Chlorella* which have been solvent extracted do not just consist of lipids, but also other material extractable in hexane, chloroform and methanol. This does not seem to be the case for either the raw or pre-treated *Ulva* as the percentage of extracted oils is so low and unlikely to contain any other solvent soluble material. For the pre-treated solid algal residues from the *Chlorella*, the lipids are more concentrated as there is less of the other solvent extractable material present as this has been released into the process waters during pre-treatment. Pre-treatment may have also liberated some soluble hydrocarbons from the *Chlorella* using the solvents, as well as hydrolysing some of the carbohydrates from the *Chlorella*, therefore, concentrating the lipids in the solid algal residue from pre-treatment.

5.3.2. Ultimate and proximate analysis of lipids from solvent extraction

Table 5-4 shows the ultimate and proximate analysis of oils extracted from solvent extraction of *Ulva lactuca* and autotrophic *Chlorella vulgaris*. From the proximate analysis of the *Ulva* it is shown that moisture content of the lipids from the four samples is very low (<2%), which is a favourable characteristic for oils. The raw *Ulva* and *Ulva* pre-treated at 100 and 200°C have similar ash contents (3.4, 3.0 and 3.7% respectively). The *Ulva* pre-treated at 150°C has the lowest ash content 1.4%. The volatiles content of both the raw and pre-treated *Ulva* samples is very similar and falls within 95.7% and 96.1%. The fixed carbon content of the raw *Ulva* and *Ulva* pre-treated at 100 and 200°C is similar (0.5, 0.7 and 0.6% respectively), whereas the *Ulva* pre-treated at 150°C has a higher fixed carbon content of 1.1%.

For the ultimate analysis, the carbon content of the extracted oils, increases from the raw to the *Ulva* pre-treated at 100°C from 73.7% to 82.1%. The carbon content then decreases from the *Ulva* pre-treated at 100°C to 150°C from 82.1% to 77.9%, then increases again for the *Ulva* pre-treated at 200°C to 79.1%. The hydrogen content of the raw *Ulva* is the lowest at 8.1%, which then increases for the oil from the *Ulva* pre-treated at 100°C to 10.3%, then decreases again to 8.7% for the *Ulva* pre-treated at 150°C (similar to the raw) and then increases again to 9.5% for the *Ulva* pre-treated at 200°C. The nitrogen content of the four extracted oils are fairly similar, with the *Ulva* pre-treated at 100°C showing the highest value at just 1.2%. The sulphur content of the oils from the raw and pre-treated *Ulva* is ≤1%. The oxygen content decreases from 16.5% for the raw *Ulva* to 5.8% for the *Ulva* pre-treated at 100°C. It then increases to 11.9% for the *Ulva* pre-treated at 150°C and finally decreases for the *Ulva* pre-treated at 200°C (9.5%). The HHV increases from 34.6MJ/kg for the oil from the raw *Ulva* to 42MJ/kg for the *Ulva* pre-treated at 100°C. It then decreases to 37.4MJ/kg for the *Ulva* pre-treated at 150°C before increasing again to 39.4MJ/kg for the *Ulva* pre-treated at 200°C. The HHV of the oils from the pre-treated *Ulva* are all higher than the oil from the raw *Ulva*. This suggests that the oils are improving slightly. Overall, the oils extracted from the *Ulva* for the four different solids show little difference between them.

From the proximate analysis of the oils from the *Chlorella*, it is shown that the moisture content of the oils from the pre-treated *Chlorella* have higher moisture

content than the oil extracted from the raw *Chlorella*. This may be an incorrect value of moisture as low molecular weight molecules may have also been driven off at this stage in the TGA analysis. The oil extracted from the *Chlorella* pre-treated at 100°C has the highest moisture content, however this may be due to the low amounts of material being released during hydrothermal pre-treatment, which resulted in more material being available for extraction in the solvents. The ash content of the oils extracted from the pre-treated autotrophic *Chlorella* are higher (6.8-7.5%) than the oil extracted from the raw *Chlorella* (1.3%) with the oil from the *Chlorella* pre-treated at 150°C containing the most ash (7.5%). The raw *Chlorella* has a higher volatiles content of 85.6% than the pre-treated *Chlorella* which ranges within 82.1-82.8%, but there is not much difference between the four oils. The fixed carbon content of the oils decreases from 12.8% for the raw *Chlorella* to around 10% for the three pre-treated *Chlorella*.

From the ultimate analysis, the carbon content of the oils from the pre-treated *Chlorella* decrease with increasing pre-treatment temperature and are lower than the carbon content of the raw *Chlorella* (77.3%). The hydrogen content of the lipids extracted from the autotrophic pre-treated *Chlorella* are similar (7.3% to 7.8%) which are lower than the raw *Chlorella* at 9.6%. The nitrogen content of the lipids increase with increasing temperature, from 2.9% for the raw *Chlorella* to 10.5% for the *Chlorella* pre-treated at 200°C. This suggests that material other than lipids is also being extracted into the solvent as the content has increased instead of decreasing. This is not ideal as the purpose of pre-treatment was to reduce problematic material such as the nitrogen from the oils. No sulphur was detected in the lipids from the autotrophic *Chlorella*. The oxygen content of the pre-treated *Chlorella* are slightly higher than the oil from the raw *Chlorella*. The higher heating value of the oils from *Chlorella* decrease with increasing pre-treatment temperature from 38.7MJ/kg for the raw to 33.1MJ/kg for the *Chlorella* pre-treated at 200°C.

In comparison to the *Ulva lactuca* the autotrophic *Chlorella* is quite different. The oils extracted from the *Chlorella*, have higher moisture content than the oils extracted from the *Ulva*, whereas the *Ulva*, have higher volatiles content than the *Chlorella.* The oils from the *Chlorella* have lower carbon and oxygen content than the *Ulva*, but higher hydrogen and nitrogen contents. Overall, the solvent extracted oils from the two different algae vary greatly from one another.

Table 5-4: Ultimate and proximate analysis of solvent extracted oils from *Ulva lactuca* and autotrophic *Chlorella*

Type of algae	Pre-treatment temperature (°C)	Proximate (%) (d.b.)				Ultimate (%) (d.a.f.)					HHV (MJ/kg^{-1})
		Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*	
Ulva lactuca	Raw	1.0	3.4	96.1	0.5	73.7	8.1	0.9	0.8	16.5	34.6
	100	1.5	3.0	96.2	0.7	82.1	10.3	1.2	0.6	5.8	42.0
	150	1.6	1.4	97.5	1.1	77.9	8.7	0.6	0.8	11.9	37.4
	200	0.8	3.7	95.7	0.6	79.1	9.5	1.0	1.0	9.5	39.4
Auto *Chlorella*	Raw	2.3	1.3	85.6	12.8	77.3	9.6	2.9	0.0	10.2	38.7
	100	4.4	6.8	82.8	10.0	75.8	7.8	3.7	0.0	12.7	35.3
	150	2.8	7.5	82.1	10.1	74.2	7.3	6.4	0.0	12.1	34.2
	200	2.5	6.7	82.1	10.9	69.3	7.8	10.5	0.0	12.4	33.1

*Oxygen calculated by difference

5.3.3. Discussion for conversion by solvent extraction

The quality of the oils extracted from the *Ulva* are better than from the *Chlorella* as they contain less ash and moisture (although there is still some present), and have higher HHV's however, the high volatiles content makes the oils difficult to store and use. The low oil yields also make *Ulva* an unlikely feedstock to be considered for biofuel production from lipid extraction.

The quality of the oil from the *Chlorella* is considered low, due to the high levels of ash and moisture which are problematic for use of the oils as biofuels. The ash and moisture that are present in the solvent extracted lipids may be due to salts dissolving in the solvents from the solid feedstock during Soxhlet extraction. This may be due to the size and design of the reactor however, this may be less of a problem with a larger scale process. The high nitrogen content of the oils from the *Chlorella* is also an issue as this would require more upgrading to make it into a useable fuel.

Overall, the quality of the oils from both the *Ulva* and the *Chlorella* are low and require a significant amount of upgrading before they can be used as biofuels.

While this section focuses on lipid extraction using solvents, the lipids are not the only material being extracted in the solvents. Labile hydrocarbon material which are soluble in the extraction solvents, are also being extracted. This affects the quality of the oil and in turn increases the amount of upgrading required to make the oil into a useable biofuel.

Although the quality and quantity of the oils extracted is not very high, there are advantages to using solvent extraction as a method to extract oils from algae. The main advantage is that a lower amount of energy is required in the extraction plant. Another advantage is that the solvents are cycled in the Soxhlet which reduces the amount of solvent required for the extraction. There are also limitations to using Soxhlet extraction to produce oils. High purity solvents are expensive and difficult to dispose of. With this method, the solvents are left to evaporate off leaving the lipids remaining. This is problematic as the solvents can be toxic and dangerous, therefore they need to be handled in a fume cupboard at all times. This separating method results in a loss of the solvents, when they could be collected and re-used if a better separating technique was employed at this stage. This process only applies

to lab scale Soxhlet extraction. The solvent extraction process would be different on an industrial scale and may be challenging due to the variables that affect lipid extraction from algae not being very well understood or studied. It is also difficult to assess the suitability of the extracted lipids for biodiesel production as most studies fail to include this and instead focus on nutraceutical or maricultural applications (Halim et al., 2011). Therefore it is difficult to assess the feasibility of biodiesel production of lipids from solvent extraction.

5.4. Hydrothermal liquefaction

Hydrothermal liquefaction was chosen as a conversion method as it is able to convert whole feedstocks into oils without the need to dry them and is becoming more of a commonly used method for this purpose (Tian et al., 2014). Hydrothermal liquefaction is the process of applying high temperature and pressure to a slurry of feedstocks to produce oils (Barreiro et al., 2013a).

Hydrothermal liquefaction of the autotrophic *Chlorella vulgaris* and *Ulva lactuca* was carried out at 350°C using small 25ml bomblet reactors, as described in section 3.5.3 of the methodology chapter. The 25ml reactors were used as they have a fast heating rate and are able to be quenched after reaching the desired temperature and residence time, to prevent any further reactions taking place.

The hypothesis that is proposed for this conversion route is that the quality of the bio-crude produced from hydrothermal liquefaction will improve with the use of the solid residues from hydrothermal pre-treatment, at the three different temperatures (100, 150 and 200°C). This is as some of the problematic material present in the raw algae will be removed during pre-treatment and therefore will not be converted into the bio-crude as it is not present, resulting in bio-crude of improved quality in comparison to the raw algae. It is expected that the higher the pre-treatment temperature the more improved the bio-crude will become.

5.4.1. Yields from hydrothermal liquefaction

Table 5-5 shows the bio-crude, char, gas and water yield from hydrothermal liquefaction of *Ulva lactuca* and autotrophic *Chlorella vulgaris*.

Table 5-5: Yields of bio-crude, char, gas and water from hydrothermal liquefaction of *Ulva lactuca* and autotrophic *Chlorella*

Type of algae	Pre-treatment temperature (°C)	%			
		Bio-crude	Char	Gas*	Aqueous phase
Ulva lactuca	Raw	10.2	11.0	6.9	72.0
	100	16.4	23.1	14.8	45.8
	150	12.8	39.4	4.0	43.8
	200	12.0	52.1	3.8	32.1
autotrophic *Chlorella*	Raw	28.7	5.4	12.0	53.9
	100	37.0	5.7	14.3	43.0
	150	41.3	6.8	11.5	40.3
	200	48.8	23.4	7.2	20.6

*calculated by difference

The bio-crude yield of the *Ulva* shows an increase between the raw *Ulva* and the *Ulva* pre-treated at 100°C, however, the bio-crude yields for the *Ulva* pre-treated at 150°C and 200°C are lower than the bio-crude yield from the *Ulva* pre-treated at 100°C and are actually very similar to the raw *Ulva* yield. The reason that there is little difference between the bio-crude yield from the raw and pre-treated *Ulva* is due to the low initial lipid and protein content and high carbohydrate content of the raw *Ulva*. The yield of char for the *Ulva* significantly increases with increasing pre-treatment temperature. The gas yield of the *Ulva* doubles from 6.9% for the raw to 14.8% for the *Ulva* pre-treated at 100°C but then decreases for the *Ulva* pre-treated at 150°C and 200°C to lower than the raw *Ulva* gas yield. The aqueous phase yield decreases with increasing pre-treatment temperature. Overall, the liquefaction from the *Ulva* at 100°C shows the highest bio-crude yield whereas the bio-crude yield from the *Ulva* at 150 and 200°C are very similar to the raw *Ulva* yield.

The bio-crude yield of the autotrophic *Chlorella vulgaris*, increases with increasing pre-treatment temperature. The *Chlorella* pre-treated at 200°C has the highest bio-crude yield and is the most suited towards liquefaction. This is could be due to the high initial lipid content of the solid residue after pre-treatment which is a

result of the removal of carbohydrates and other components, which favour char formation, during hydrothermal pre-treatment. The char yield from liquefaction is very similar for the raw *Chlorella* and the *Chlorella* pre-treated at both 100°C and 150°C, however the *Chlorella* pre-treated at 200°C has a much higher char yield following liquefaction. The gas yield increases from 12% for the raw to 14.3% for the *Chlorella* pre-treated at 100°C but then decreases again for the *Chlorella* pre-treated at 150 and 200°C. From the literature, it has been determined that the gas yield is mainly composed of CO_2, which suggests that the oxygen is being removed from the feedstock via dehydration and decarboxylation during hydrothermal liquefaction (Balat, 2008). This also correlates with the ultimate analysis data of the oils from liquefaction in Table 5-6, which shows the same trend as the gas yield. The percentage of aqueous phase decreases with increasing pre-treatment temperature and shows a significant decrease for the *Chlorella* pre-treated at 200°C. Overall the bio-crude from liquefaction of the *Chlorella* pre-treated at 200°C shows the most difference to the bio-crude from liquefaction of the raw *Chlorella*, as it has the highest bio-crude and char yield of the four samples and also the lowest gas and aqueous phase yields. This correlates with the high initial lipid content of the solid algal residue from pre-treatment at 200°C.

Miao et al. (2012) carried out a study on sequential hydrothermal liquefaction of *Chlorella Sorokiniana* and found that sequential HTL produced ~5% more bio-oil and ~50% less bio-char than just direct hydrothermal liquefaction. This correlates with the data in Table 5-5.

5.4.2. Ultimate and proximate analysis of bio-crude from Hydrothermal liquefaction

Table 5-6 shows the ultimate and proximate analysis of the oils produced from hydrothermal liquefaction of *Ulva lactuca* and autotrophic *Chlorella vulgaris.*

The proximate analysis of the HTL oils from the *Ulva lactuca,* shows that the moisture content of the oils from the pre-treated *Ulva* are lower than the oil from the raw *Ulva*. The ash content of the oils shows a decrease from the raw at 7.7% to 5.1% for the *Ulva* pre-treated at 100°C. The ash content then increases again to 6.9% for the oil from the *Ulva* pre-treated at 150°C and then decreases again to

5.4% for the *Ulva* pre-treated at 200°C. The volatiles content of the HTL oils from the *Ulva* pre-treated at 100°C (92.5%) and 150°C (91.8%) are very similar to the volatiles content of the bio-crude from the raw *Ulva* (91.4%). The *Ulva* pre-treated at 200°C has a lower volatiles content of 81.7%. The fixed carbon content of the *Ulva* pre-treated at 200°C is the same as the fixed carbon content of the bio-crude from the raw *Ulva* at 0.9%, whereas the fixed carbon content of the bio-crude produced from the *Ulva* pre-treated at 100°C and 150°C are higher.

From the ultimate analysis of the *Ulva*, the carbon content of the bio-crudes shows an increase for the bio-crude from the pre-treated *Ulva* (80.5-81.6%) in comparison to the bio-crude from the raw *Ulva* at 75.0%. The hydrogen content of the bio-crude from the raw *Ulva* and the *Ulva* pre-treated at 150°C and 200°C are similar and within the range of 10.3-11.0%, however the bio-crude from the *Ulva* pre-treated at 100°C shows a decrease to 5.9%. The bio-crude from the pre-treated *Ulva* contain none or very little quantities of sulphur, whereas the raw *Ulva* contains 3.8%. The oxygen content of the bio-crude from the *Ulva* pre-treated at 100°C shows an increase to 10.1% in comparison to the raw *Ulva* of 7.5%, however the *Ulva* pre-treated at 150°C and 200°C is lower. The HHV decrease from 39.9MJ/kg for the bio-crude from the raw *Ulva* to 34.5MJ/kg for the *Ulva* pre-treated at 100°C but then increases for the bio-crude from the *Ulva* pre-treated at 150°C and 200°C.

The proximate analysis of the bio-crude from the autotrophic *Chlorella* contain similar amounts of ash (3.0-3.9%), moisture (1.4-1.5%) and volatiles (93.4-95.5%) except for the bio-crude from the *Chlorella* pre-treated at 150°C, which has a higher moisture content (2.6%), lower ash content (1.9%) and higher volatiles content (98.0%).

The fixed carbon content of the HTL oils from the raw and pre-treated *Chlorella* are all quite low (<1.3%) with the exception of the *Chlorella* pre-treated at 100°C which is slightly higher at 3.6%. The low fixed carbon content is an attractive characteristic for the use of the bio-crude as there is a lower chance of coking.

Table 5-6: Ultimate and proximate analysis of hydrothermal liquefaction oils from *Ulva lactuca* and autotrophic *Chlorella*

Type of algae	Pre-treatment temperature (°C)	Proximate (%) (d.b.)				Ultimate (%) (d.a.f.)					HHV (MJ/kg^{-1})
		Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*	
Ulva lactuca	Raw	6.2	7.7	91.4	0.9	75.0	10.5	3.3	3.8	7.5	39.9
	100	1.2	5.1	92.5	2.4	80.6	5.9	3.5	0.0	10.1	34.5
	150	2.4	6.9	91.8	1.4	81.6	11.0	4.6	0.0	2.8	43.2
	200	2.0	5.4	81.7	0.9	80.5	10.3	4.6	0.1	4.5	41.6
Auto *Chlorella*	Raw	1.5	3.9	95.2	0.9	78.9	11.3	6.0	0.0	3.8	42.5
	100	1.4	3.0	93.4	3.6	78.5	11.1	5.6	0.0	4.7	42.1
	150	2.6	1.9	98.0	0.1	84.2	8.9	5.3	0.0	1.6	41.2
	200	1.5	3.2	95.5	1.3	81.3	9.8	5.1	0.0	3.8	41.1

*Oxygen calculated by difference

From the ultimate analysis of the autotrophic *Chlorella* of the bio-crude from the raw *Chlorella* and the *Chlorella* pre-treated at 100°C, the carbon contents are very similar at 78.9% and 78.5% respectively. There is then an increase to 84.2% and 81.3% for the *Chlorella* pre-treated at 150°C and 200°C respectively. The hydrogen content of the bio-crude from the raw *Chlorella* and the *Chlorella* pre-treated at 100°C are very similar at 11.3% and 11.1% respectively. The hydrogen content of the bio-crude from the *Chlorella* pre-treated at 150°C and 200°C are also similar to each other (8.9% and 9.8% respectively) but are lower than the other two bio-crudes. The nitrogen content of the bio-crudes from the *Chlorella* decrease with increasing pre-treatment temperature from 6.0% to 5.1%. Although the reduction in nitrogen is quite small there is still a reduction (even though the limit of detection is 0.1%). This shows that the hydrothermal pre-treatment stage is effective at removing nitrogen compounds when the solid residue is hydrothermally liquefied into a bio-crude. There is no sulphur detected in any of the bio-crudes from the *Chlorella* samples. The oxygen content of the bio-crude from the *Chlorella* pre-treated at 200°C is the same as the bio-crude from the raw *Chlorella* at 3.8%. The bio-crude from the *Chlorella* pre-treated at 100°C shows an increase to 4.7% and then the bio-crude from the *Chlorella* pre-treated at 150°C decreases to 1.6%.

The oils from HTL of the *Ulva lactuca* and the autotrophic *Chlorella* have some similarities and some differences. The moisture contents of oils from both of the algae are very similar. The ash content of the oils from the *Ulva* are higher than the bio-crude from the *Chlorella*. The oils from the *Ulva* have slightly lower volatiles content than the oils from the *Chlorella*. The fixed carbon content of the oils from both algae are also quite similar. The carbon and nitrogen content of the bio-crude from the *Chlorella* are higher than the bio-crude from the *Ulva*. The bio-crude from the *Ulva* have higher hydrogen and oxygen content than the bio-crude from the *Chlorella*. The *Chlorella* have HHV values than the bio-crude from the *Ulva*.

5.4.3. Discussion for conversion by hydrothermal liquefaction

Hydrothermal liquefaction is a good method for producing bio-crude from algae. There are a few reasons for this which include; the solvent used in the process, is water, which is very low cost and easily disposable; the feedstock can be

either wet or dry and the whole algae is converted into oil instead of just extracting the lipids.

The quality of the bio-crude from HTL is similar to raw petroleum crude oil as they have similar characteristics, which means the bio-crude from HTL can be used as a biofuel but requires some upgrading before it can be utilised. The *Chlorella* has low ash and moisture content which is favourable for oils used for biofuels, however, the *Ulva* has higher ash content which could prove to be problematic when used as a fuel. The ash content of the bio-crudes does not change too much between the samples but does show a decrease in the bio-crude produced from the pre-treated *Chlorella*, in comparison to the bio-crude from the raw *Chlorella*. This shows there is a decrease in the inorganics in the bio-crudes but it is a small decrease. The reason that there is such little difference may be due to the solid algal residue acting as a char and reabsorbing material during the liquefaction stage, which also correlates with the nitrogen content of the bio-crudes from *Chlorella*. The lower oxygen content of the bio-crude from liquefaction makes the fuel more chemically stable and results in less upgrading to hydrocarbons for fuel (Balat, 2008). Although the bio-crudes from HTL can be considered as similar to crude oil, there are some major differences. Crude oil contain lower levels of nitrogen than the bio-crude from HTL.

There also some drawbacks to using HTL to produce bio-crude from micro algae. One of the drawbacks to using HTL as conversion method is the amount of energy required to heat the water to super-critical. Although it is not as much energy as required for pyrolysis, HTL requires more energy than Soxhlet extraction.

Another major drawback of producing bio-crude from micro algae, using HTL, is the high nitrogen content of the bio-crude which is a result of the nitrogen content of the algae. As discussed previously, the nitrogen content of the bio-crudes decreases with increasing pre-treatment temperature of the solid algal residues. This change in nitrogen content may also affect the molecular weight of the bio-crudes and change the distribution of light and heavy molecular weight material. This reduction in nitrogen content is a measure of improvement for the bio-crude produced. The high nitrogen content of the bio-crude also means that it would require a significant amount of upgrading to become a useable fuel.

The usual upgrading method is hydrogenation which requires large amounts of hydrogen to remove the nitrogen. This also produces ammonia as a by-product which could be used as a value added chemical. One solution to the issue of the oils containing high levels of nitrogen would be to use low nitrogen containing feedstocks with high lipid and low protein content. Feedstocks of varying biochemical compositions are investigated in Chapter 6, to establish if this can be achieved.

5.5. Comparison of the quality of oil from the three conversion methods

A comparison of the three conversion methods was made to determine which method was the most effective for producing the highest yield of oil. Figure 5-2 shows the percentage oil yield from the three different conversion routes for the raw and pre-treated algae for a) *Ulva lactuca* and b) *Chlorella vulgaris*. Of the three conversion methods, hydrothermal liquefaction gave the highest oil yields. The reason for this is due to the bio-crude from HTL compromises of not only lipids but also the proteins and carbohydrates in the algae, unlike the pyrolysis oils and solvent extracted oils which mainly comprise of the lipids (Xu, D. et al., 2018). The yields from the three different conversion routes show notable differences for the *Chlorella* samples but do not show much difference between the *Ulva* samples. This is due to the low initial lipid content of the raw *Ulva*, which has a low ratio of lipids whether it is raw or pre-treated, in comparison to the *Chlorella,* which shows an increase in the ratio of lipids and therefore also an increase in the quantity of oil produced.

Comparison of the three conversion methods in this chapter has not previously been undertaken for algae. However comparison of hydrothermal liquefaction and pyrolysis of some micro algae has previously been carried out in literature. (Jena and Das, 2011) carried out liquefaction and pyrolysis on *Spirulina platensis* and reported that in comparison to pyrolysis, liquefaction resulted in higher bio-oil yields, lower char yields and used less energy during conversion. (Duan et al., 2015) also undertook a comparison between liquefaction and pyrolysis of micro algae (*Chlorella Pyrenoidosa*) and reported similar findings, with liquefaction producing

more bio-oil than pyrolysis. These studies correlate with the findings in this chapter for the bio-oil from pyrolysis and liquefaction.

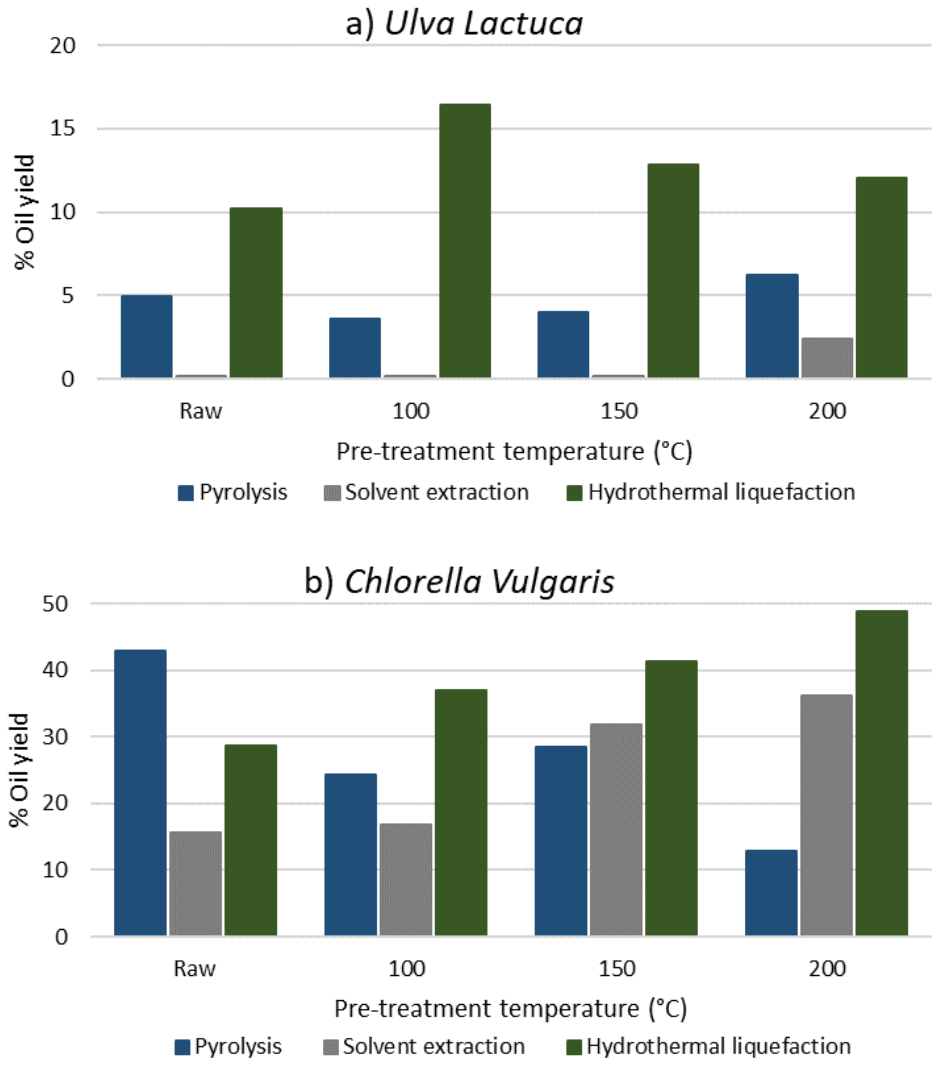


Figure 5-2: % Oil yield from pyrolysis, solvent extraction and hydrothermal liquefaction of raw and pre-treated a) *Ulva lactuca* and b) *Chlorella vulgaris*

For the proximate and ultimate analysis of the two different algae, there is little difference between the oils from the four *Ulva* samples for the different conversion methods, however, there is a notable difference in the oil samples from the four samples of *Chlorella* for the three different conversion methods.

Figure 5-3 shows the moisture content of the oils from pyrolysis, solvent extraction and hydrothermal liquefaction of the raw and pre-treated a) *Ulva lactuca* and b) *Chlorella vulgaris*.

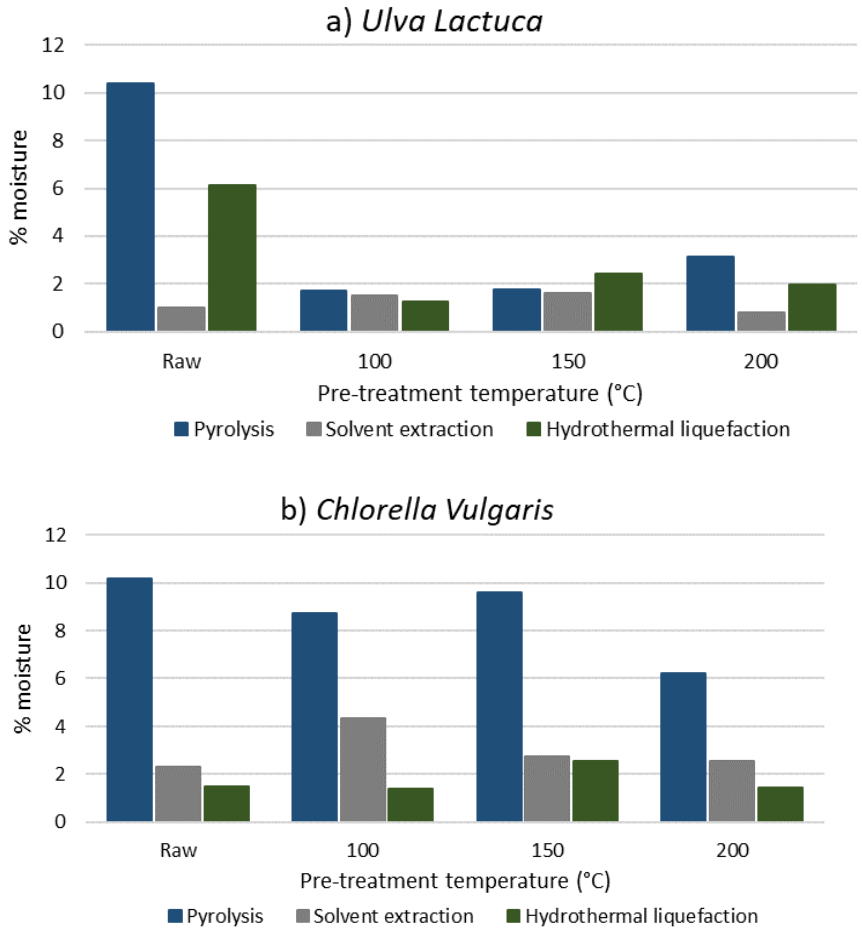


Figure 5-3: % moisture content (a.r.) of the oils from pyrolysis, solvent extraction and hydrothermal liquefaction from the raw and pre-treated a) *Ulva lactuca* and b) *Chlorella vulgaris*

The oils from pyrolysis and hydrothermal liquefaction of the raw *Ulva* contain high levels of moisture in comparison to the oils from the pre-treated *Ulva* which show little difference between the three conversion routes. The moisture content of the oils from *Chlorella* differs for the three conversion methods. The oils from pyrolysis of the *Chlorella*, contain similar amounts of moisture with the raw *Chlorella* containing the most. The lipids from solvent extraction contain less moisture than the oils from pyrolysis, with the *Chlorella* pre-treated at 100°C containing the highest yield. The moisture content of the oils from liquefaction of the *Chlorella* are similar for all four samples and are significantly lower than the

moisture content of the oils from pyrolysis and solvent extraction. Although the moisture contents differ greatly, they may not be accurate as there may be low molecular weight material also being drawn out at that stage of the TGA, which then gives an inaccurate measurement of the moisture content. The are other methods that could be used to determine the moisture content more accurately than TGA such as NMR spectroscopy (David et al., 2012).

Figure 5-4 shows the ash content of the oils from the three conversion routes. The *Ulva* shows little difference between the oils from the three conversion routes for the raw and pre-treated algae. The pyrolysis oils contain the most ash for both the *Ulva* and the *Chlorella*. The pyrolysis oils from the *Chlorella* contain the most ash, with the *Chlorella* pre-treated at 100°C having the highest ash content (22.4% d.b.).

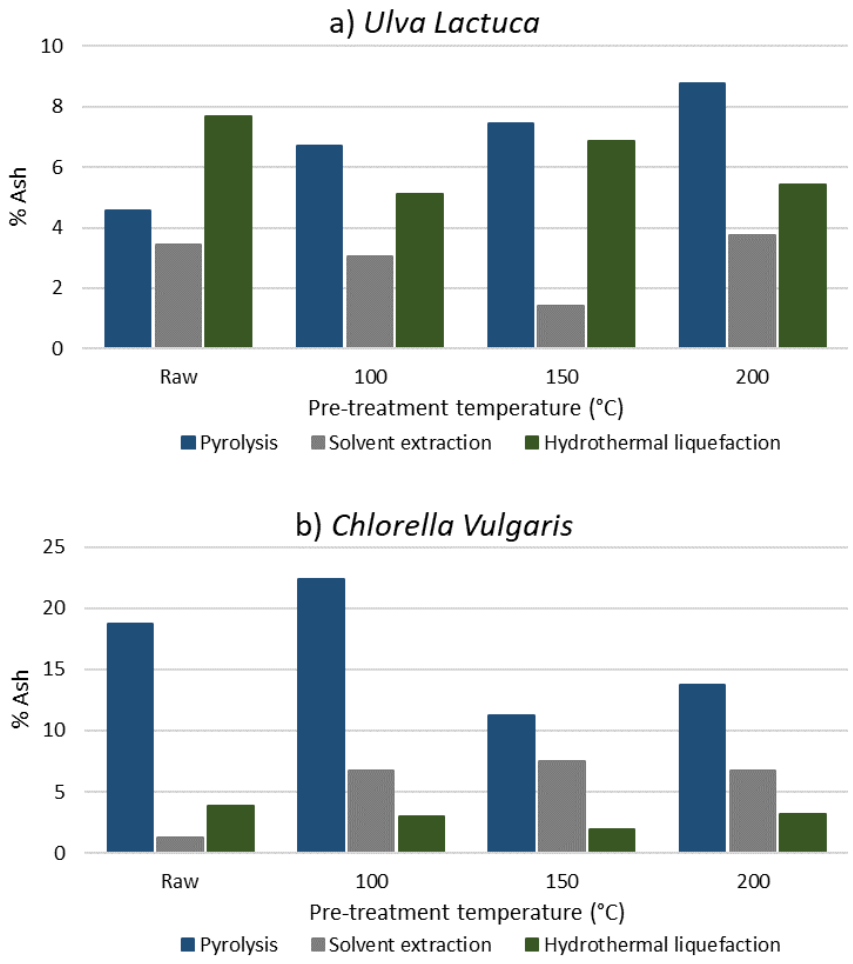


Figure 5-4: % ash content of the oils from pyrolysis, solvent extraction and hydrothermal liquefaction from the raw and pre-treated a) *Ulva lactuca* and b) *Chlorella vulgaris*

The ash content of the oils from solvent extraction are lower than the pyrolysis oils, with the *Chlorella* pre-treated at 150°C having the highest ash content (7.5% d.b.). The bio-crude from HTL have the lowest ash content of the oils from the three conversion routes with the *Chlorella* pre-treated at 150°C having the lowest ash content (1.9% d.b.). High ash content can be an issue as it can cause problems with upgrading such as poisoning of catalysts by the inorganics and coking, therefore pyrolysis may not be the best conversion route to utilise as the pyrolysis oils contain a significant amount of ash.

Figure 5-5 shows the fixed carbon content of the oils from pyrolysis, solvent extraction and hydrothermal liquefaction of the raw and pre-treated algae.

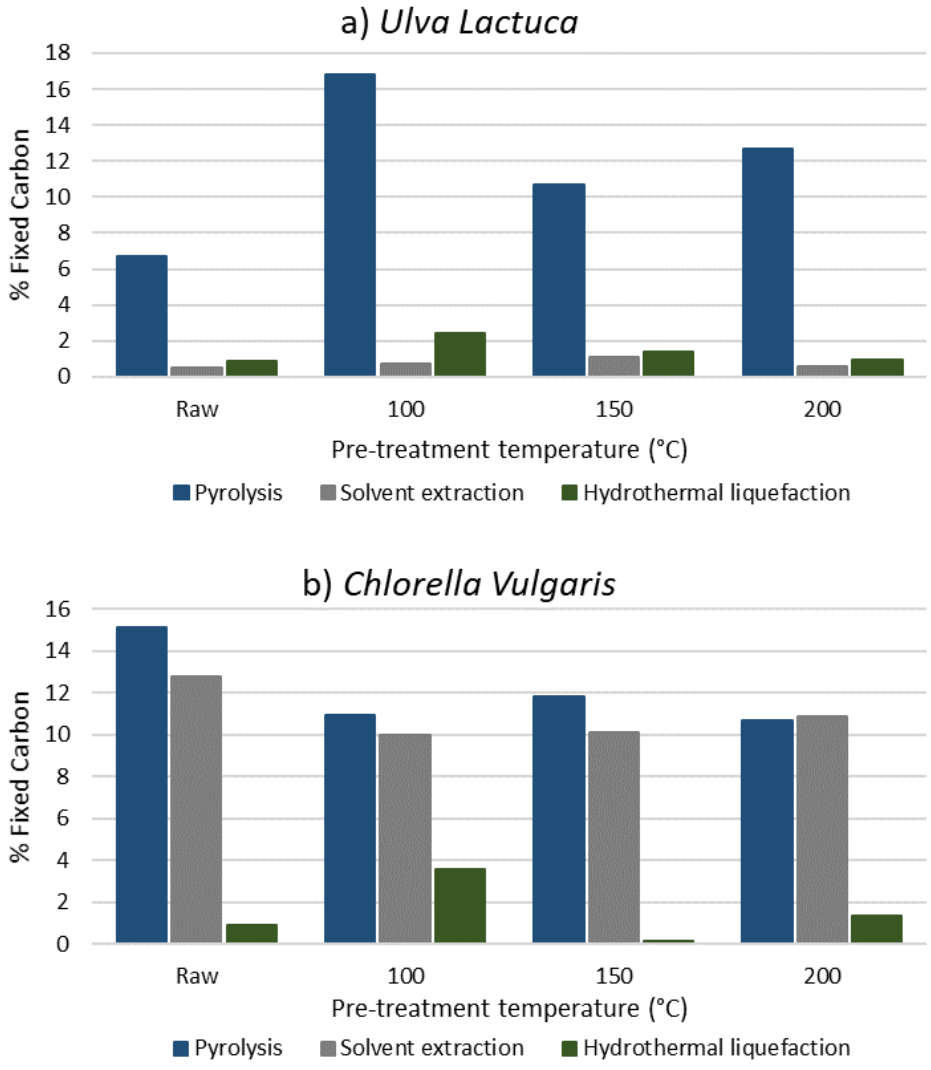


Figure 5-5: % fixed carbon content (d.b.) of the oils from pyrolysis, solvent extraction and hydrothermal liquefaction from the raw and pre-treated a) *Ulva lactuca* and b) *Chlorella vulgaris*

The fixed carbon content of the oils from the three conversion methods follows the same trend as the ash content, with the pyrolysis oils containing the most, then the solvent extracted oils and the bio-crude from HTL containing the least fixed carbon. The pyrolysis oils from the *Chlorella* contain the most fixed carbon (10.7-15.1% d.b.), with the raw *Chlorella* having the highest ash content. The fixed carbon content of the oils from solvent extraction are lower than the pyrolysis oils (10.0-12.8% d.b.), with the raw *Chlorella* again having the highest fixed carbon content. The bio-crude from HTL have the lowest fixed carbon content of the oils from the three conversion routes (0.1-3.6% d.b.), with the *Chlorella* pre-treated at 150°C having the lowest fixed carbon content at 0.1%. High fixed carbon content can also be an issue as it can also cause problems with upgrading such as poisoning of catalysts by the inorganics and coking, similar to the issues from high ash containing oils, therefore pyrolysis may not be the best conversion route to utilise as the pyrolysis oils contain a significant amount of fixed carbon.

Figure 5-6 shows the nitrogen content of the oils from pyrolysis, solvent extraction and hydrothermal liquefaction of the raw and pre-treated algae. The nitrogen content of the oils from the raw and pre-treated *Ulva* are similar to each other within the same conversion route. The pyrolysis oils have the highest nitrogen content of the three conversion routes and the solvent extracted oils have the least. For the oils from the *Chlorella*, the nitrogen content differs for each conversion method. The pyrolysis oils show an increase in the nitrogen content from the raw (4.6%) to the *Chlorella* pre-treated at 150°C (12.4%), then it decreases slightly to 5.3% for the oil from the *Chlorella* pre-treated at 200°C. The solvent extracted oils show an increase in the nitrogen with increasing pre-treatment temperature from 2.9% to 10.5%. The nitrogen content of the bio-crude from liquefaction of the *Chlorella* reduces with increasing pre-treatment temperature from 6.0% to 5.1%. The nitrogen content of the bio-crude is also lower than the nitrogen content of the oils from pyrolysis and solvent extraction. This reduction in nitrogen content is promising as it shows that hydrothermal pre-treatment works for the autotrophic *Chlorella* when converted into oils by hydrothermal liquefaction.

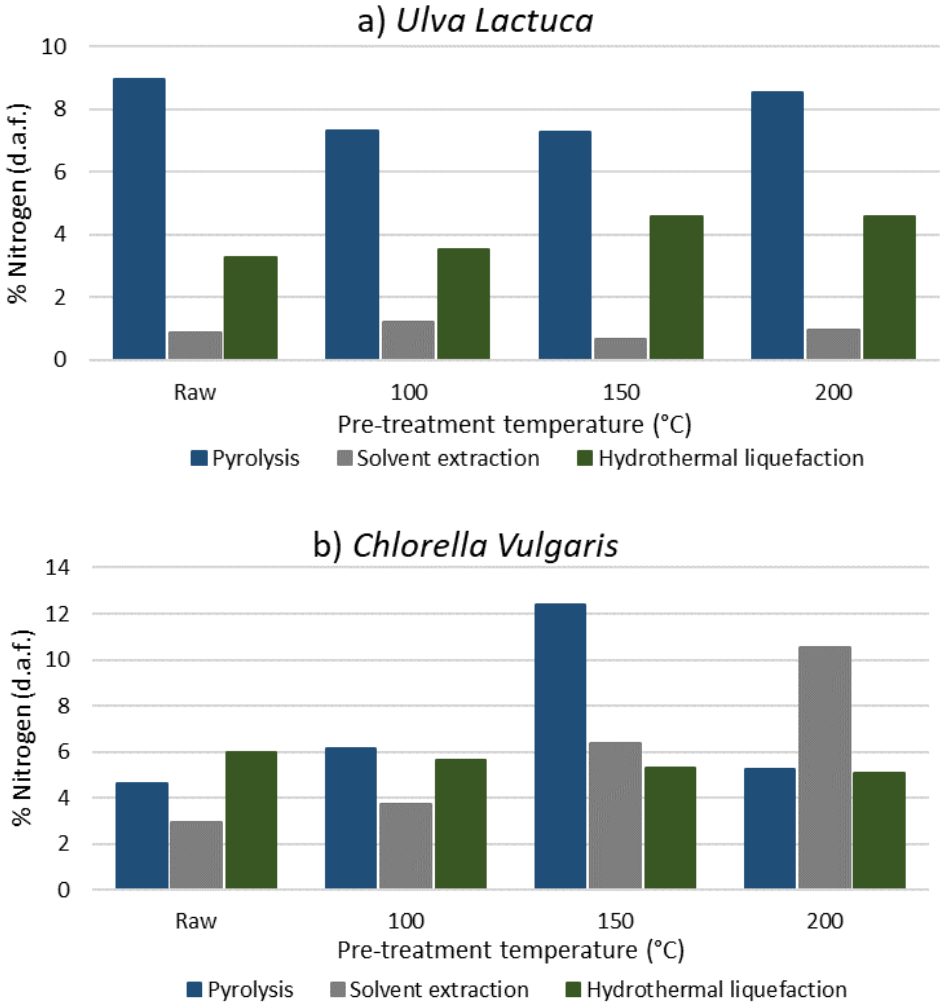


Figure 5-6: % nitrogen content of the oils from the raw and pre-treated *Ulva lactuca* and *Chlorella vulgaris* from pyrolysis, solvent extraction and hydrothermal liquefaction

Figure 5-7 shows the HHV of the oils from the three conversion methods. The oils from solvent extraction of the *Chlorella* have the lowest HHV of 33.1-38.7MJ/kg. The pyrolysis oils have slightly higher HHV of 34.0-43.0MJ/kg and the bio-crude from liquefaction have the highest HHV of 41.1-42.5MJ/kg. The HHV is indicative of the calorific value of the oil.

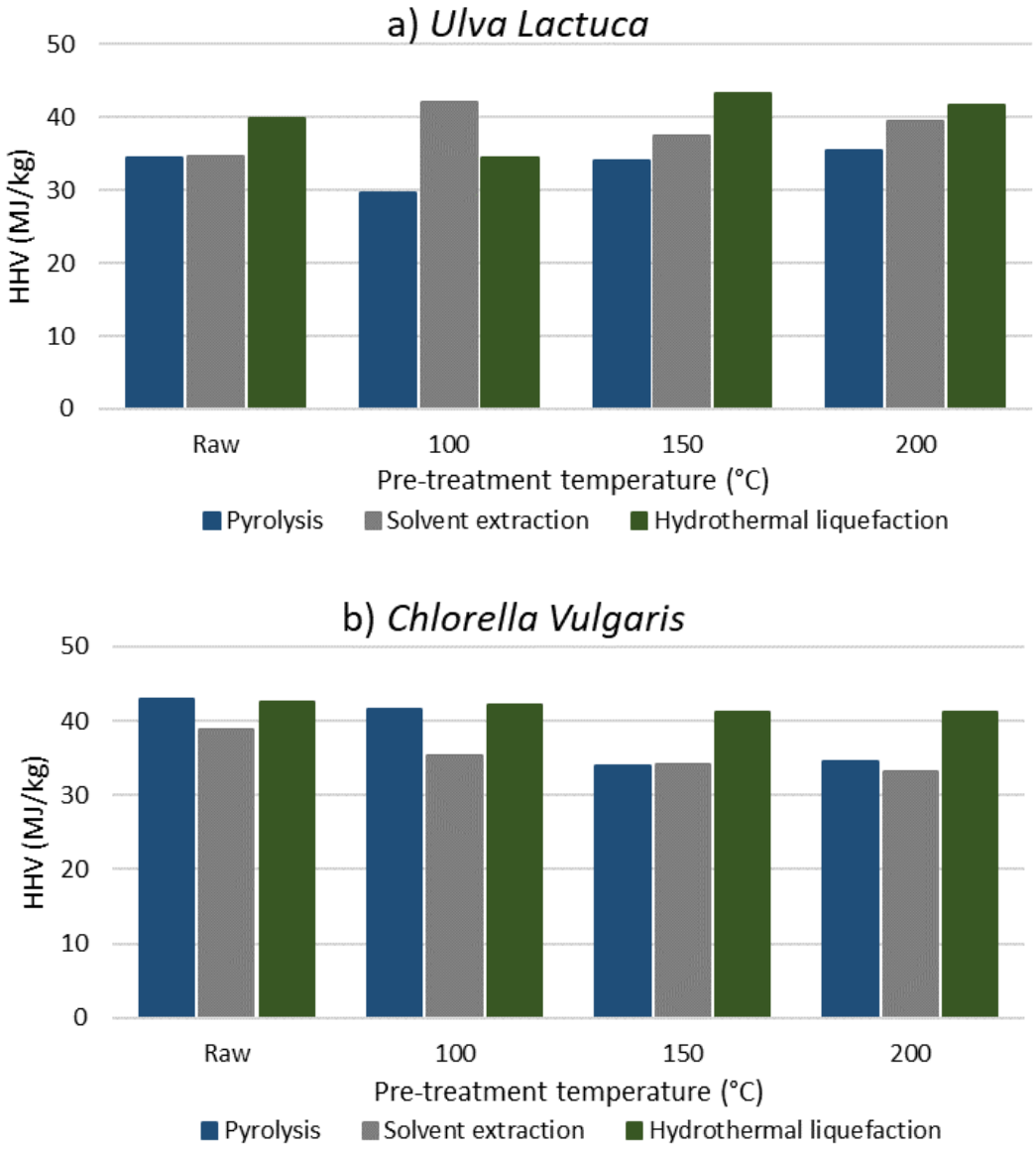


Figure 5-7: HHV of the oils from pyrolysis, solvent extraction and hydrothermal liquefaction from the raw and pre-treated a) *Ulva lactuca* and b) *Chlorella vulgaris*

5.5.1. Upgrading of oils

As mentioned in the previous section, the bio-oils from the three different conversion methods contain a mixture of aromatics and have different compositions. Although bio-oil has been produced, it is not able to be used directly as a fuel due to its undesirable properties such as high viscosity, high corrosiveness and low heating value. Therefore, the bio-oils require upgrading in order to be used as valuable hydrocarbons, which can be used as liquid biofuels or for the production of platform chemicals. Upgrading of the bio-oils is usually carried out in a process called hydrogenation. This has been discussed in detail in the literature review.

Upgrading of the oils using hydrogen, was calculated for the oils produced from the three conversion routes for both the *Ulva* and *Chlorella* samples. The method used was taken from Frank et al. (2012) who carried out a stoichiometric calculation to calculate the hydrogen demand for hydrotreating. Hydrogen demand of 0.060 g H2/g bio-crude was calculated for HTL bio-crude containing 71% C, 9.2% H, 11% O and 5.7% N using Equation 5-1.

$$\text{g H per g Oil} = fc(\text{H/C})_{\text{RD}} + (1-f)ac(\text{H/C})_{\text{light}} + 3n + 2[o - 2(1-f)(1-a)c] - h$$

Equation 5-1: hydrogen demand for upgrading oil

where:

f = 0.95 (carbon efficiency of the carbon molar fraction split)

a = 0.95 (light hydrocarbons of the carbon molar fraction split)

c, h, n, o = moles of carbon, hydrogen, nitrogen and oxygen per gram of feed oil

$(\text{H/C})_{\text{RD}}$ = 2.0 (hydrogen/carbon ratio in renewable diesel)

$(\text{H/C})_{\text{light}}$ = 2.67 (hydrogen/carbon ratio in the light hydrocarbon fraction of the oil)

The values for f, c, $(\text{H/C})_{\text{RD}}$ and $(\text{H/C})_{\text{light}}$ are taken from values used for upgrading of vegetable oils as calculated in Holmgren et al. (2008).

The results of the calculations for upgrading the oils from the three conversion routes in this chapter are shown in Table 5-7. The amount of hydrogen required for upgrading varies between the three different conversion routes for the two different algae. Based on the g of hydrogen required per g of oil, the data for the *Ulva* shows that the pyrolysis oils require the most hydrogen for upgrading, then the lipids from solvent extraction and finally the bio-crude from HTL. This is different to the trend for the oils from *Chlorella*, which show that the pyrolysis oils and bio-crude from HTL require similar amounts of hydrogen for upgrading to renewable diesel but the lipids from solvent extraction require more hydrogen. In both instances, the bio-crude from HTL requires the least amount of hydrogen for upgrading into a useable biofuel.

Based on the g of hydrogen per kg of algae required for the *Ulva*, the pyrolysis oil requires the least amount of hydrogen, whereas the solvent extracted lipids and bio-crudes from HTL require more. For the *Chlorella*, the solvent extracted lipids require the least amount of hydrogen and is similar for the pyrolysis oils, whereas the bio-crudes from HTL require significantly more hydrogen per kg of algae feedstock.

Table 5-7: Hydrogen requirement for upgrading of oils from the three conversion routes from *Ulva lactuca* and autotrophic *Chlorella*

	Type of algae	Pre-treatment temperature (°C)	Conversion route		
			Pyrolysis	Solvent extraction	Hydrothermal liquefaction
g hydrogen per g oil	*Ulva lactuca*	Raw	0.112	0.066	0.038
		100	0.101	0.045	0.097
		150	0.070	0.061	0.041
		200	0.073	0.052	0.048
	Auto *Chlorella*	Raw	0.027	0.054	0.038
		100	0.037	0.074	0.039
		150	0.065	0.081	0.066
		200	0.043	0.077	0.055
g hydrogen per kg algae	*Ulva lactuca*	Raw	11.588	1.122	11.076
		100	8.960	11.506	14.649
		150	18.545	29.827	28.364
		200	5.544	36.750	28.266
	Auto *Chlorella*	Raw	1.770	0.132	3.949
		100	1.195	0.087	16.155
		150	0.926	0.119	5.462
		200	0.877	1.241	5.978

5.6. Conclusion

The purpose of the work in this chapter was to investigate if the quality of oil, from *Ulva lactuca* and autotrophic *Chlorella vulgaris*, when processed using pyrolysis, solvent extraction and hydrothermal liquefaction, could be improved by using algae pre-treated at 100, 150 and 200°C, in comparison to the raw algae. The criteria for the oil to be considered of good quality are high yield, high HHV, along with low moisture, ash and fixed carbon content.

The comparison of the three conversion routes determined that the hydrothermal liquefaction route produced the highest yield of oil from the three conversion methods. The *Chlorella vulgaris* pre-treated at 200°C gave the highest yield for both the solvent extraction and hydrothermal liquefaction routes, whereas the raw *Chlorella* showed the highest yield from pyrolysis. Of the three types of oils produced, the bio-crude from hydrothermal liquefaction of the *Chlorella vulgaris* required the least amount of hydrogen for upgrading.

From the three conversion methods utilised, the solvent extraction required the least energy as a soxhlet extractor was used. The pyrolysis and hydrothermal liquefaction both required significantly more energy to heat up to 600°C for the pyrolysis and to heat water in the reactor up to super-critical for hydrothermal liquefaction. The pyrolysis and solvent extraction favour dry feedstocks whereas hydrothermal liquefaction can utilise both wet and dry feedstocks.

Overall, there is at least one limitation for each conversion method, however from the comparison undertaken within this chapter, it is decided that hydrothermal liquefaction is the chosen conversion route which will continue to be utilised throughout the remainder of this work due to the low moisture, ash and nitrogen content of the bio-crude produced. It was also decided that further hydrothermal liquefaction analysis would be carried out on the autotrophic *Chlorella vulgaris* along with the other micro algae (heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii*), discussed previously in the first results chapter. This is investigated in the next results chapter.

Chapter 6. Influence of pre-treatment on bio-crude quality from hydrothermal liquefaction

6.1. Introduction

Bio-crudes of different quality, require varying amounts of upgrading. By pre-treating the feedstocks or selecting feedstocks based on their biochemical composition, the quality of the bio-crude produced, can be improved, which results in a reduction in the amount of upgrading required. Although pre-treatment is a useful step to have in the conversion process for algae, another method which could help to improve the quality of the bio-crude and also reduce the amount of upgrading required is the selection of the algae based on the biochemical composition. There have been many studies focusing on the lipid content of the micro algae, such as the study by Li, H. et al. (2014) who carried out a comparison between a low lipid, high protein micro algae and high lipid protein micro algae and found that the higher lipid containing algae produced a higher yield of oil from liquefaction. Another study by Biller and Ross (2011a) investigated the yields and properties of the oil from hydrothermal liquefaction of micro algae with different biochemical contents and found that the lipids and proteins converted to oil the most efficiently without a catalyst whilst the carbohydrates were best processed using Na_2CO_3. Therefore, micro algae with higher lipid and protein content would result in higher bio-crude yield when processed in water alone.

The main aim of this chapter is to address objective 5 of this thesis, to investigate the influence of different process variables such as temperature and feedstock type on the quality of bio-crude produced. The criteria for good quality biofuels are high bio-crude yield, high HHV and low nitrogen content. This chapter focuses on assessing the quality of the bio-crude from hydrothermal liquefaction of micro algae with different biochemical compositions. The feedstocks used include

algal strains cultivated in both marine and freshwater including a cyanobacteria and green algae.

This chapter compares intermediates produced from four different micro algae: autotrophi*c Chlorella vulgaris*, heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii*, during liquefaction in distilled water. A comparison of the raw micro algae and the four micro algae pre-treated at 150°C are compared to determine which can produce bio-crude of a higher yield and of better quality.

6.2. Hydrothermal liquefaction of raw and pre-treated micro algae

This section focuses on the hydrothermal liquefaction of the raw and pre-treated micro algae to investigate if there is a difference between the bio-crudes from the micro algae with different biochemical compositions and also between the raw and pre-tread algae. Due to the small quantities of sample, hydrothermal liquefaction was carried out in the 75ml parr reactor, at 350°C with a residence time of 1 hour. The method is described in detail in section 3.5.4 of the methodology chapter. These conditions were chosen based on literature from Biller and Ross (2011a).

The biochemical composition of the raw and pre-treated micro algae have previously been discussed and are shown in Table 6-1 and Table 1-10 respectively.

Table 6-1 shows the yields of products from hydrothermal liquefaction of the four micro algae: autotrophic *Chlorella vulgaris*, heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii* for both the raw and the algae pre-treated at 150°C. For the raw algae, the heterotrophic *Chlorella* contains the highest percentage of bio-crude at 31.0% and is not too dissimilar to the autotrophi*c Chlorella* (28.0%). The bio-crude yield of the *Chlorogloeopsis* is slightly lower than the two *Chlorella* at 26.3%, whereas the *Spirulina* has the lowest bio-crude at 15.0%. The char yields are below 5% for the autotrophic and heterotrophic *Chlorella* and *Spirulina*, however the yield is much higher for the *Chlorogloeopsis* at 14.0%. The autotrophic and heterotrophic *Chlorella* have very similar aqueous

phase yield 67.0 and 66.7% respectively, with the *Chlorogloeopsis* giving a slightly lower yield of 59.7%. The *Spirulina* has the highest aqueous phase yield at 81.7%, suggesting that more material has been released into the aqueous phase.

For the pre-treated algae, the heterotrophic *Chlorella,* produced the highest percentage of bio-crude at 25.6%, with the autotrophic *Chlorella* and *Chlorogloeopsis* showing similar bio-crude yields, of 28.7 and 26.7% respectively, although slightly lower than the heterotrophic *Chlorella.* There is a significant difference in the bio-crude yield from the *Spirulina*, which is much lower than the other three bio-crude yields at 19.7%. The autotrophic *Chlorella* produced the highest yield of char at 8%. The *Spirulina* and *Chlorogloeopsis* have the same char yield of 5.3%, which is slightly lower than the autotrophic *Chlorella*. Of the four algae, the heterotrophic *Chlorella* has the lowest char yields at 2.3%. The higher char yield may be due to the higher protein content in the raw algae. The autotrophic *Chlorella* has the lowest aqueous phase yield of 66.4%, with the heterotrophic *Chlorella* and *Chlorogloeopsis* having slightly higher yields of 68.0 and 69.0% respectively. The *Spirulina* has the highest aqueous phase yield at 75.0%, which suggests more material is released into the process waters during HTL of the *Spirulina* than the other micro algae.

In comparison to the liquefaction yields from the raw micro algae, the micro algae pre-treated at 150°C, shows lower yields for the bio-crude produced from the autotrophic and heterotrophic *Chlorella*. The pre-treated *Spirulina* shows a higher yield of bio-crude than the raw *Spirulina*, whereas both the raw and pre-treated *Chlorogloeopsis* have the same bio-crude yield. The overall bio-crude yield is lower due to large amounts of the carbohydrates breaking down to polar water-soluble organics during the hydrothermal pre-treatment stage instead of to non-polar hydrocarbon type structures which are present in bio-crude. The char yields of the pre-treated autotrophic *Chlorella* and *Spirulina* are higher than for the raw algae. This is not considered a good quality for bio-crude from liquefaction as less of the feedstock is being converted into an oil and more upgrading will be required to make the bio-crude into a useable fuel. The heterotrophic *Chlorella* does not show any change in the char yield, whereas the char yield for the pre-treated *Chlorogloeopsis* is significantly lower than for the raw *Chlorogloeopsis.* The lower char yield suggests that more of the *Chlorogloeopsis* is being converted into bio-

crude after pre-treatment in comparison to the raw algae. This shows that pre-treatment is having a positive affect on the *Chlorogloeopsis*. The aqueous phase yield is higher for the raw autotropic *Chlorella* and *Spirulina* than the pre-treated counterparts. On the other hand, the heterotrophic *Chlorella* and *Chlorogloeopsis* show a higher yield for the pre-treated samples in comparison to the raw algae.

Table 6-1: Yields of bio-crude, char and water from hydrothermal liquefaction of raw and pre-treated micro algae

	Type of algae	%		
		Bio-crude	Char	Aqueous phase*
Raw	Auto *Chlorella*	28.0	5.0	67.0
	Hetero *Chlorella*	31.0	2.3	66.7
	Spirulina platensis	15.0	3.3	81.7
	Chlorogloeopsis fritschii	26.3	14.0	59.7
Pre-treated at 150°C	autotrophic *Chlorella*	25.6	8.0	66.4
	heterotrophic *Chlorella*	28.7	2.3	69.0
	Spirulina platensis	19.7	5.3	75.0
	Chlorogloeopsis fritschii	26.7	5.3	68.0

*by difference

Previous researchers such as Biller and Ross (2011a) and Sawayama et al. (1999) have stated that algae which contains high lipid content results in higher bio-crude yields. The results shown in Table 6-1 confirm this. This also corresponds with the fact that the bio-crude yields from HTL are not just based on the lipid content but also the protein and carbohydrate content as these are also converted into bio-crude (Biller and Ross, 2011a; Minowa et al., 1995; Yang et al., 2015).

Figure 6-1 shows the reaction pathways for hydrothermal liquefaction of micro algae. Between 0-100°C the protein is hydrolysed into amino acids, the lipids are hydrolysed into glycerol and long chain fatty acids and the carbohydrates are hydrolysed into sugars (Gai, Chao et al., 2015).

From 100-200°C, the amino acids, fatty acids and sugars undergo further decomposition (Barreiro et al., 2013a). Decarboxylation of the amino acids occurs with the carboxyl group from this process producing CO_2 by removing the oxygen from micro algae. Some of the amino acids will also undergo deamination and produce carboxylic acids. Ammonia is also produced from the amine group from the

deamination process, which results in removal of the nitrogen from the micro algae. There are also some alkanes and alkenes produced from decarboxylation of the long chain fatty acids and amino acids from the hydrolysis of the lipids and proteins. Cyclic oxygenates are also produced in this temperature range from the reducing sugars from hydrolysis of the carbohydrates (Gai, Chao et al., 2015).

Above 200°C, the ammonia from the deamination of the amino acids replace the hydroxyl groups in the log chain fatty acids to produce aliphatic amine compounds. A certain amount of the long chain fatty acids react with the alcohols from the reduction of the amino acids (after deamination) producing esters. Nitrogen and oxygen heterocyclic compounds are produced from the Maillard reaction between the amino acids and reducing sugars from hydrolysis of the proteins and carbohydrates. Some of the amino acids may also repolymerise into aromatic ring-type compounds (Gai, Chao et al., 2015).

The residence time (1hr) and the severity of the conditions in this study will allow the smaller organic materials, produced by the decarboxylation and deamination steps, to re-polymerise the proteins into longer chain hydrocarbons and aromatic ring-type structures such as nitrogen heterocycles, phenols, indoles and pyrroles.

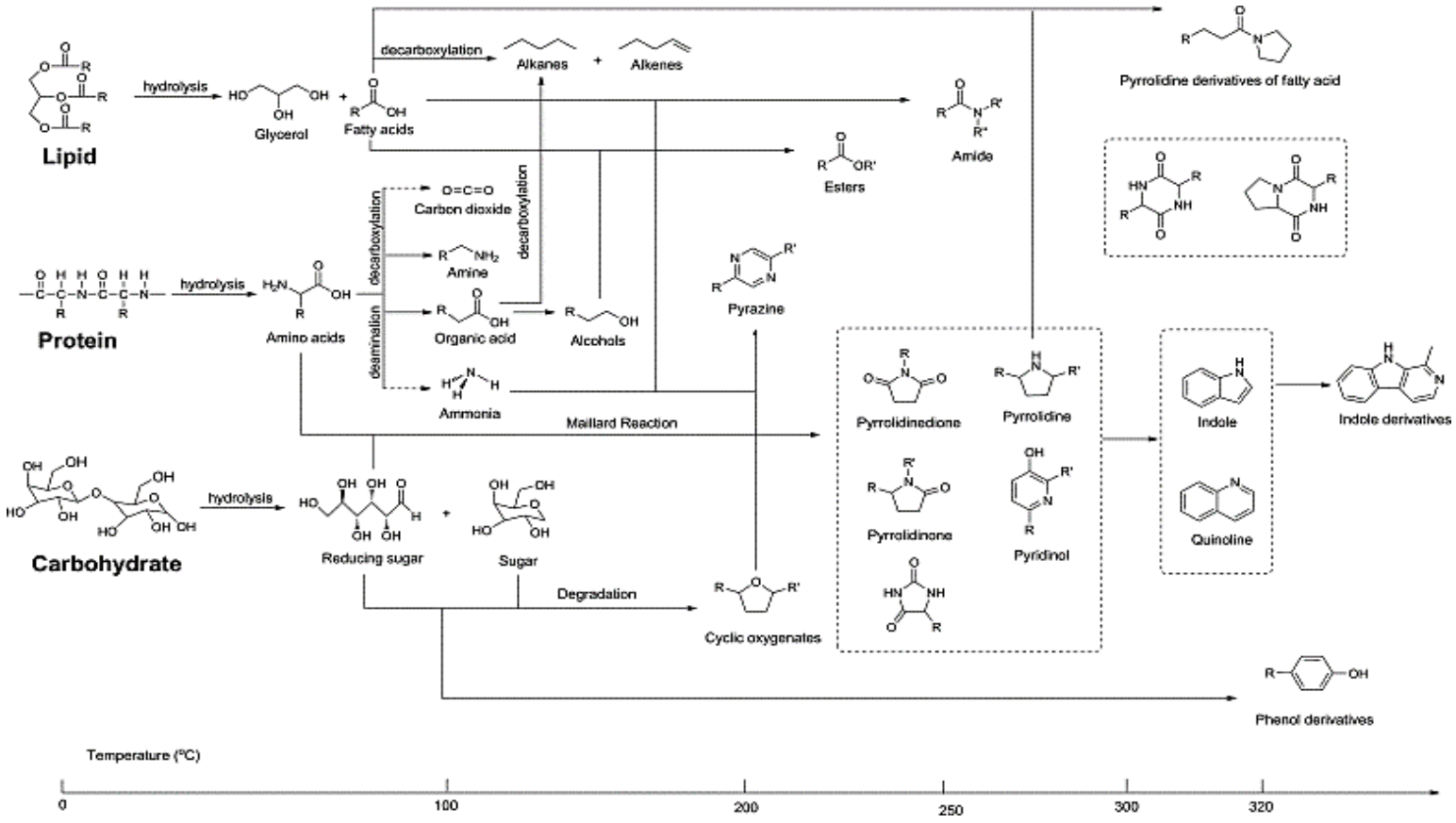


Figure 6-1: reaction pathways of lipids, proteins and carbohydrates during hydrothermal processing from (Gai, Chao et al., 2015)

Yang, W. et al. (2019) carried out a study on HTL of a selection of polysaccharides, proteins and lipids as model components of algae and found that when polysaccharides and lipids were co-liquefied at 220°C, the polysaccharides may promote the formation of bio-crude, whereas when the temperature is increased to 260-300°C, the polysaccharides cause the partial decomposition of the bio-crude, which is due to the interaction between polysaccharides and lipids forming a solid residue instead of bio-crude. For the co-liquefaction of proteins and lipids, at 220°C, the yield of bio-crude was higher than calculated from the theoretical yield, which indicated that the potential interaction between the proteins and lipids at this temperature increase the bio-crude yield, but do not make a difference to the yield at 260-300°C. The results from this study, suggest that higher temperature HTL such as the temperature used in this work (350°C), result in interactions between the carbohydrates and lipids but not the protein and lipids.

6.2.1. Ultimate and proximate analysis of the bio-crude from HTL of the raw algae

The ultimate and proximate analysis of the raw and pre-treated micro algae have previously been discussed and are shown in Table 4-2 and Table 4-5 respectively. Figure 6-2 and Figure 6-3 show the proximate and ultimate analysis of the bio-crude produced from hydrothermal liquefaction of the raw and pre-treated micro algae. The raw data is shown in Appendix 4.

A comparison of the moisture, ash and fixed carbon of the bio-crudes from the raw and pre-treated micro algae are shown in Figure 6-2. The moisture content of the bio-crudes from the pre-treated algae are higher than for the raw algae, with the exception of the heterotrophic *Chlorella*, which is higher for the bio-crude from the raw algae. The ash content of the bio-crudes from the pre-treated algae is lower than the bio-crudes from the raw algae, with the exception of the *Chlorogloeopsis*, which shows a higher ash content for the bio-crude from the pre-treated algae than for the raw. The lower ash content of the bio-crudes from the pre-treated algae suggests that pre-treatment is removing some of the inorganics from the algae into the process waters. This is good as there are less inorganics in the bio-crude which

results in less upgrading required. The bio-crude from the raw micro algae, contain higher levels of fixed carbon than the bio-crude from the pre-treated algae. This suggests that the fixed carbon is linked to the carbohydrate content of the algae and as the raw heterotrophic *Chlorella* contains high levels of non-structural carbohydrates (which are released into the process waters during pre-treatment), there are less left, which results in a lower fixed carbon content.

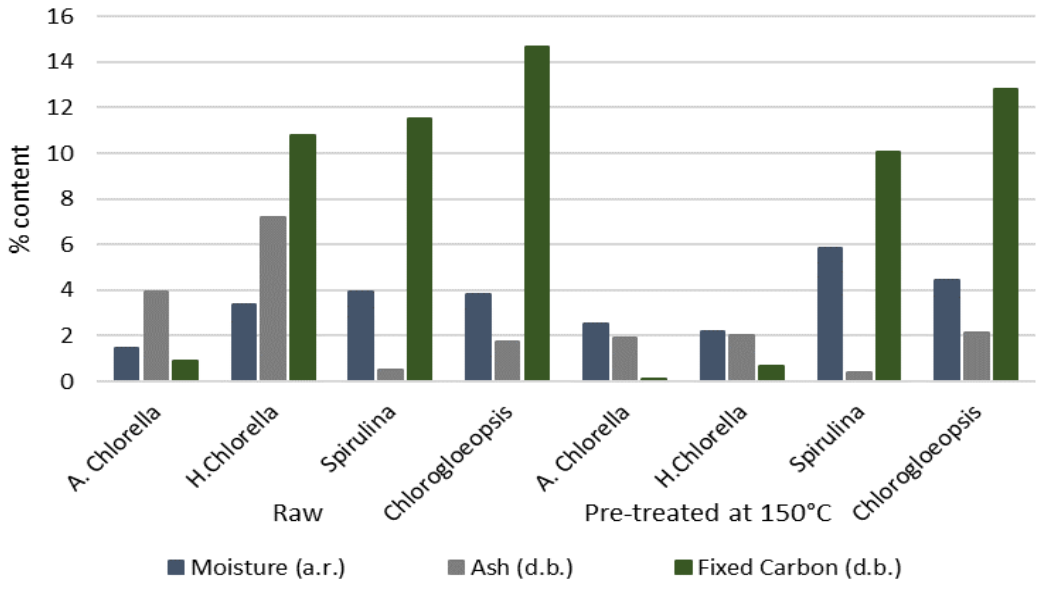


Figure 6-2: % moisture, ash and fixed carbon content of the bio-crudes from the raw and pre-treated autotrophic *Chlorella*, heterotrophic *Chlorella*, *Spirulina* and *Chlorogloeopsis*

Figure 6-3 shows the % hydrogen, nitrogen and oxygen (d.a.f.) content of the HTL oils from the raw micro algae and the micro algae pre-treated at 150°C. The hydrogen content of the HTL oils from the pre-treated algae is lower than the HTL oils from the raw algae, for all the micro algae with the exception of the *Chlorogloeopsis*. The nitrogen content of the HTL oils from the pre-treated algae is lower for the two *Chlorella* samples but is very similar to the HTL oils from the raw algae, for the *Spirulina* and *Chlorogloeopsis*. The oxygen content of the bio-crude from the pre-treated algae is significantly higher than the HTL oils from the raw algae, for the two *Chlorella* samples but is higher than the HTL oils from the raw algae, for the *Spirulina* and *Chlorogloeopsis*. These results show that the quality of the bio-crude from the pre-treated algae was of better quality than for the raw algae

for the autotrophic and heterotrophic *Chlorella*, but made little difference to the *Spirulina* and *Chlorogloeopsis*.

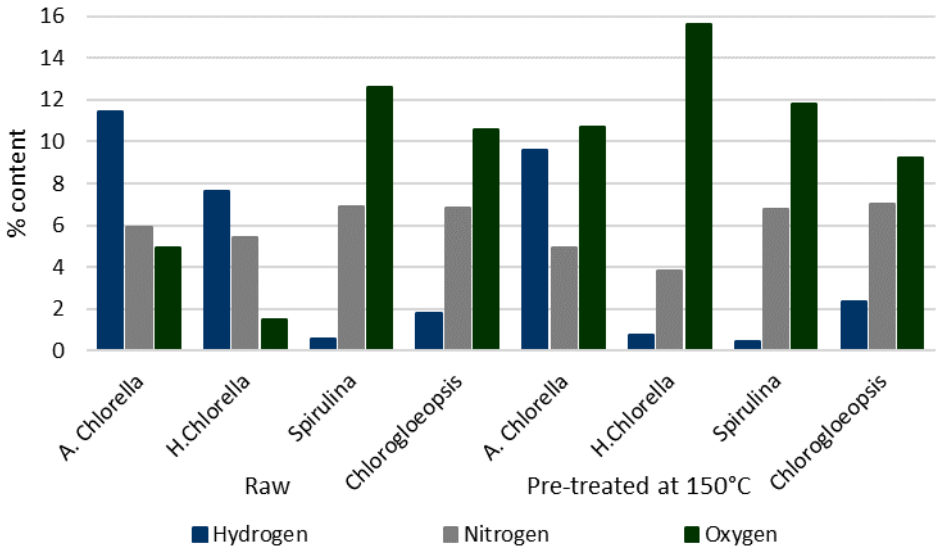


Figure 6-3: % hydrogen, nitrogen and oxygen (d.a.f.) content of the bio-crudes from the raw and pre-treated autotrophic *Chlorella*, heterotrophic *Chlorella*, *Spirulina* and *Chlorogloeopsis*

The HHV of the raw and pre-treated algae is shown in Figure 6-4. The pre-treated autotrophic and heterotrophic *Chlorella* are lower than their raw counterparts. This reduction in the HHV shows that the pre-treatment is not necessarily improving the quality of the bio-crude as it has reduced in comparison to the bio-crude from the raw algae. The HHV of the HTL oils from the raw and pre-treated *Spirulina* are very similar. The *Chlorogloeopsis* shows a slightly higher HHV for the bio-crude from the pre-treated algae than for the bio-crude from the raw *Chlorogloeopsis*.

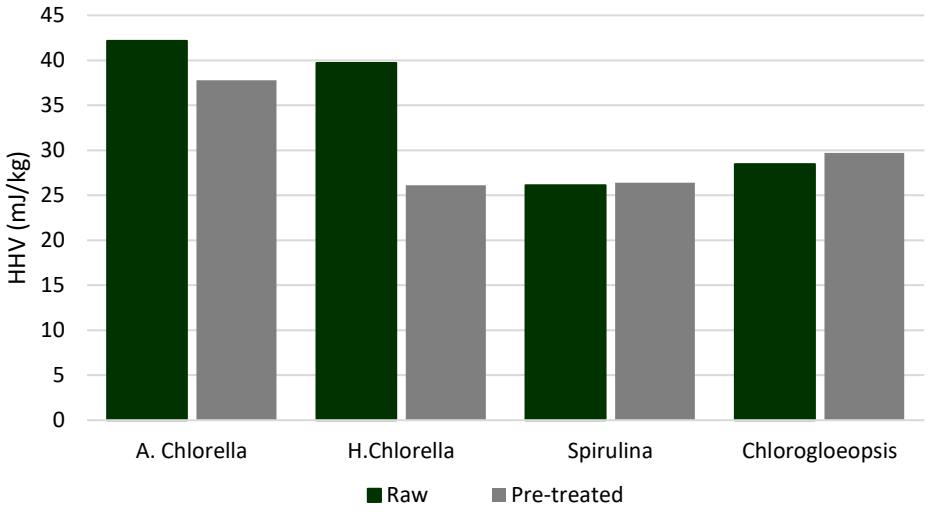


Figure 6-4: Higher heating value of the bio-crudes from the raw and pre-treated autotrophic *Chlorella,* heterotrophic *Chlorella, Spirulina* and *Chlorogloeopsis*

Overall, from the proximate and ultimate analysis, the bio-crude from the heterotrophic *Chlorella* shows the most improvement from the raw algae to the pre-treated algae, with lower moisture, ash, fixed carbon, hydrogen and nitrogen contents along with higher oxygen content. Both the *Spirulina* and *Chlorogloeopsis* show the least difference between the raw and pre-treated bio-crudes.

Table 6-2 shows a comparison of the nitrogen content and HHV of bio-crude from HTL of the four micro algae (studied in this chapter) by others. The nitrogen content of the four micro algae reported in this study for the raw algae, are very similar to the values from the literature for the raw algae, with <0.6% difference between the different autotrophic *Chlorella* investigations, <1.2% difference between the *Spirulina* and no difference between the *Chlorogloeopsis*. The HHV of the four micro algae reported in this study vary to the values from the literature. The HHV of the autotrophic *Chlorella* in this chapter is higher (42.2MJ/kg) than those shown in Table 6-2 (35.1-37.5MJ/kg). The HHV of the *Spirulina* studied in this chapter (26.1MJ/kg) is lower than those in the literature, which range from 35.3 to 36.8MJ/kg. The same trend is observed for the *Chlorogloeopsis*, with the results in this chapter showing a lower value of 28.5MJ/kg than that in the literature of 32.0MJ/kg. There is no literature at the time of this study which investigates bio-crude production from HTL of heterotrophic algae.

The desirable characteristics of bio-crude from hydrothermal liquefaction have high carbon content and low nitrogen and oxygen content. The main reason for desiring low nitrogen content is that less upgrading will be required of the bio-crude. Another reason for desiring low nitrogen content is due to the fact that nitrogen present in fuel directly forms NOx compounds during processing, which are problematic for the environment and are also regulated in legislation for that reason. The elemental analysis of the samples shows that the liquefaction of the raw algae produces bio-crude with the desirable carbon, nitrogen and oxygen contents.

Table 6-2: Comparison of nitrogen and HHV values for the four micro algae from literature

Type of algae	Nitrogen % (d.a.f.)	HHV (MJ/kg)	HTL conditions	Reference
Autotrophic *Chlorella vulgaris*	5.9	42.2	350°C, 1hr	This study
	5.9	35.1	350°C, 1hr	(Biller and Ross, 2011a)
	5.3	37.5	300°C, 1hr	(Biller et al., 2012)
	5.9	35.1	350°C, 1hr	
Heterotrophic *Chlorella vulgaris*	5.4	39.7	350°C, 1hr	This study
Spirulina platensis	6.9	26.1	350°C, 1hr	This study
	7.0	36.8	350°C, 1hr	(Biller and Ross, 2011a)
	6.3	35.3	350°C, 1hr	(Jena, Umakanta et al., 2011)
	8.1	35.8	350°C, 30min	(Vardon et al., 2012)
	6.3	36.1	300°C, 1hr	(Biller et al., 2012)
Chlorogloeopsis fritschii	6.8	28.5	350°C, 1hr	This study
	6.8	32.0	300°C, 1hr	(Biller et al., 2012)

6.2.2. GC-MS of HTL oils from raw micro algae

Table 6-3 shows the compounds found in the oils from hydrothermal liquefaction of the raw and pre-treated micro algae, using GC-MS.

The nitrogen compounds that were calibrated for have not been detected in the bio-crudes from either the raw or the pre-treated algae. This is also the case for the cyclopentanones. This is not what was expected but despite not detecting nitrogen compounds from the GC-MS analysis, the ultimate analysis of the oils shows that there is nitrogen present. This suggested that the nitrogen in the oils is of high molecular weight and is therefore not detected for the compounds by GC-MS.

During hydrolysis of the algae, amino acids and sugars are formed simultaneously. These then react via the Maillard reaction and lead to the formation of nitrogen containing cyclic organic compounds such as pyridines and pyrroles. These compounds also act as free radical 'scavengers', which inhibit the free radical chain reactions which are important for the formation of gas at subcritical conditions (Kruse et al., 2007). Therefore the lack of nitrogen compounds detected from GC-MS analysis may be due to the nitrogen compounds being used to inhibit the free radicals in the bio-crude and forming higher molecular weight material.

The bio-crudes from all four of the raw micro algae contain pentadecane, with the autotrophic *Chlorella* and *Spirulina* having similar contents to one another (6.4 and 6.5mg/l respectively), as do the heterotrophic *Chlorella* and *Chlorogloeopsis* (9.3 and 9.2mg/l respectively). Whereas, for the bio-crudes from the pre-treated algae, pentadecane is only available for the heterotrophic *Chlorella* at 17.8mg/l and the *Spirulina* at 6.6mg/l. For the hexadecane, only the bio-crudes from the raw and pre-treated heterotrophic *Chlorella* and raw *Spirulina* contain hexadecane at 3.9, 6.2 and 5.0mg/l respectively.

For the phenolic compounds, phenol, p-Cresol and 4-ethylphenol are present in the bio-crudes from both the raw and pre-treated micro algae. There is a small decrease in the phenol content of the bio-crudes from the pre-treated autotrophic and heterotrophic *Chlorella* and *Chlorogloeopsis* in comparison to the bio-crudes from the raw algae. In contrast, the *Spirulina* shows a very small increase in the pre-treated *Spirulina* in comparison to the raw algae. No 2,3-Dimethylphenol, or 2,6-Dimethoxyphenol were detected in any of the bio-crudes apart from in the bio-crude

from the raw autotrophic *Chlorella* at <100mg/l. The lack of the 2,3-Dimethylphenol and 2,6-Dimethoxyphenol was expected as algae do not contain lignin that produce these compounds when broken down.

Of the fatty acids and glycerol compounds that were calibrated for using standards, glycerol is the most prevalent with similar quantities present in the bio-crudes from both the raw and pre-treated algae. There is also a similar amount of hexadecenoic acid present in each of the bio-crudes for both the raw and pre-treated algae. Both the raw and pre-treated bio-crudes from the autotrophic and heterotrophic *Chlorella* and *Chlorogloeopsis* contain similar amounts of oleic acid with approximately 1000mg/l detected in each. There is no oleic acid detected in either of the bio-crudes from the *Spirulina*.

Table 6-3: GC-MS of bio-crudes from HTL of raw and pre-treated micro algae

Compounds (mg/l)		Raw algae				Algae pre-treated at 150°C			
		Auto *Chlorella*	**Hetero *Chlorella***	***Spirulina***	***Chlorogloeopsis***	**Auto *Chlorella***	**Hetero *Chlorella***	***Spirulina***	***Chlorogloeopsis***
Nitrogen		ND	ND	ND	ND	ND	ND	ND	ND
Cyclopentanones		ND	ND	ND	ND	ND	ND	ND	ND
Alkanes	Pentadecane	6.4	9.3	6.5	9.2	ND	17.8	6.6	ND
	Hexadecane	ND	3.9	5.0	ND	ND	6.2	ND	ND
Phenols	Phenol	279.9	247.0	283.9	324.9	243.0	242.3	292.2	289.4
	p-Cresol	186.7	131.5	175.5	208.6	143.9	138.4	176.4	174.2
	4-Ethylphenol	77.0	51.5	104.2	103.8	52.5	49.1	94.5	83.3
	2,3-Dimethylphenol	98.3	ND	ND	ND	ND	ND	ND	ND
	2,6-Dimethoxyphenol	ND	ND	ND	ND	ND	ND	ND	ND
Fatty acids & glycerol	Hexadecanoic acid	2435.6	2143.9	2206.0	2209.0	2179.0	2432.1	2173.2	2081.9
	Oleic acid	989.9	1012.9	ND	1000.0	972.9	1130.0	ND	969.0
	Glycerol	2398.7	2385.1	2407.0	2388.5	2448.1	2400.7	2388.9	2381.7

6.2.3. Pentane fractionation of HTL oils from raw micro algae

From the analysis of the whole bio-crude from hydrothermal liquefaction, it was proposed that solvent fractionation maybe a rapid method for fractionating the high molecular weight material from the bio-crude. Solvent fractionation was carried out using pentane, following the method outlined in section 3.5.4.1. This is a standard method used for fractionating petroleum resulting in the isolation of asphaltenes, which are high molecular weight components that are detrimental for some upgrading and conversion routes. While it is not expected that the high molecular weight material from algal liquefaction will be the same as in petroleum, this fractionation approach was thought to be useful in determining the potential levels of high molecular weight material in liquefaction oils.

Figure 6-5 shows the yields from pentane fractionation of the bio-crudes produced from hydrothermal liquefaction of the raw and pre-tread micro algae. The insoluble fraction contains the asphaltenes. From this data it is shown that all four of the micro algae have higher pentane soluble fractions than pentane insoluble fractions for the raw algae, however for the pre-treated algae, the trend is the opposite with the insoluble fraction being higher than the soluble fraction. This may be due to more heavy molecular weight material being formed due to Maillard reactions between the sugars and amino acids, as there are more carbohydrates present in the raw algae in comparison to the pre-treated algae. Overall, it seems as though pre-treating the algae results in more material in the bio-crude, becoming pentane soluble.

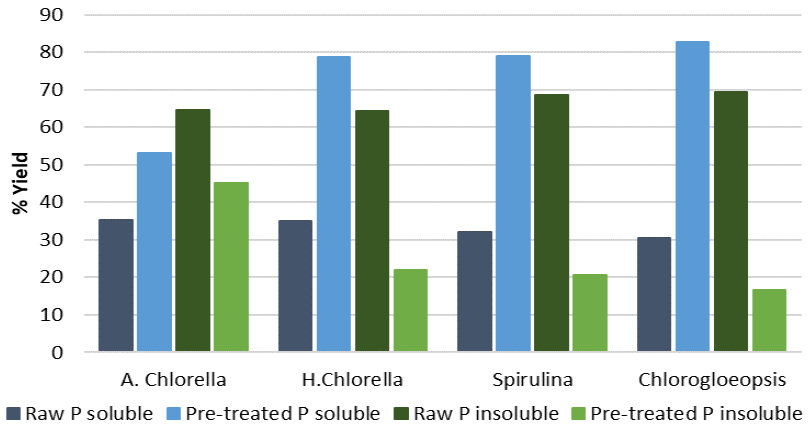


Figure 6-5: % pentane soluble and insoluble fractions of the bio-crudes from the raw and pre-treated autotrophic *Chlorella*, heterotrophic *Chlorella*, *Spirulina* and *Chlorogloeopsis*

6.2.4. Ultimate and proximate analysis of pentane fractions from HTL oils of raw micro algae

Once the pentane fractionation was carried out on the bio-crudes from hydrothermal liquefaction of the raw and pre-treated algae, both the soluble and insoluble fractions were analysed for ultimate and proximate analysis, which is shown in Table 6-4.

The moisture contents of the pentane soluble and insoluble fractions from the bio-crudes from both the raw and pre-treated micro algae show little difference and are all ≤2%, which is not a significant amount.

The ash content of the pentane fractions from both sets of bio-crudes shows that the insoluble fractions from the autotrophic *Chlorella* and the *Chlorogloeopsis* are higher for the pre-treated algae than for the raw. The heterotrophic *Chlorella* show a higher ash content for the pentane insoluble fraction of the raw bio-crude in comparison to the pre-treated bio-crude. This suggests that the bio-crude from the raw heterotrophic *Chlorella* contains more inorganic material than the bio-crudes from the other three algae. For the pentane soluble fractions, the pre-treated autotrophic *Chlorella*, *Spirulina* and *Chlorogloeopsis* have a higher ash content than the soluble fractions of their raw counterparts. The heterotrophic *Chlorella* shows little difference between the raw and pre-treated soluble fractions.

For the % fixed carbon content of the pentane fractions, there is little difference between the raw and pre-treated insoluble fractions for all four micro algae. There is also little difference in the fixed carbon content of the raw and pre-treated soluble fractions for all four micro algae. There is however a big difference between the insoluble and soluble fractions for all four micro algae, with the insoluble fractions containing significantly higher (>15%) fixed carbon than the soluble fractions. This suggests that there is more higher molecular weight material in the insoluble fraction.

The hydrogen content shows little difference between the insoluble fractions from both the raw and pre-treated algae, however, there is a notable difference between the soluble fractions. The soluble fractions from the bio-crude produced from the pre-treated algae contain a significantly higher amount of hydrogen than the soluble fraction from the bio-crude from the raw algae.

For the % nitrogen content there is little difference between the raw and pre-treated insoluble fractions for all four micro algae. There is also little difference in the nitrogen content of the raw and pre-treated soluble fractions for all four micro algae. There is however a difference between the insoluble and soluble fractions for all four micro algae, with the insoluble fractions containing higher nitrogen content than the soluble fractions.

There is little difference in the oxygen content between the raw and pre-treated insoluble fractions for the autotrophic *Chlorella*, *Spirulina* and *Chlorogloeopsis* whereas the heterotrophic *Chlorella* shows an increase in the soluble fraction from the pre-treated heterotrophic *Chlorella*. The oxygen content of the soluble fractions is higher than the insoluble fractions with the exception of the insoluble fraction of the pre-treated heterotrophic *Chlorella*. The soluble fractions from the bio-crude of the raw algae contain higher oxygen content than the pre-treated algae for the four algae with the autotrophic *Chlorella* showing the biggest difference between the raw and pre-treated bio-crudes.

Overall, there is little difference between the pentane insoluble from the raw and pre-treated micro algae. This is also the case for the soluble fractions from both the raw and pre-treated algae. However, there is a difference between the insoluble and soluble fractions for both the raw and pre-treated algae.

Table 6-4: Ultimate and proximate analysis of the pentane fractions of the bio-crudes from the raw and pre-treated micro algae

	Pentane fraction	Type of algae	Proximate (d.b.)				Ultimate (d.a.f.)					HHV (MJ/kg^{-1})
			Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*	
Raw	Insoluble	Auto *Chlorella*	0.2	3.0	75.5	21.4	78.9	8.5	6.1	0.8	5.7	38.3
		Hetero *Chlorella*	0.2	4.9	67.7	27.5	76.3	7.6	6.0	0.7	9.5	35.6
		Spirulina platensis	0.8	3.6	69.0	27.3	74.5	7.3	7.4	0.9	9.9	34.6
		Chlorogloeopsis fritschii	0.4	2.3	68.0	29.6	75.3	7.4	6.8	0.6	9.9	35.0
	Soluble	Auto *Chlorella*	1.9	1.0	96.9	2.1	73.7	4.4	4.4	0.0	17.5	29.1
		Hetero *Chlorella*	1.2	0.3	98.5	1.2	77.4	3.2	3.2	0.0	16.2	28.8
		Spirulina platensis	0.1	2.0	95.1	2.9	76.0	4.4	4.4	0.0	15.2	30.1
		Chlorogloeopsis fritschii	0.2	2.1	95.8	2.1	78.3	4.3	4.3	0.0	13.1	31.0
Pre-treated at 150°C	Insoluble	Auto *Chlorella*	0.0	3.5	74.9	21.5	77.8	8.2	6.6	0.8	6.6	37.4
		Hetero *Chlorella*	1.4	0.7	70.6	28.7	72.5	6.6	6.2	0.5	14.3	32.3
		Spirulina platensis	1.0	3.6	68.4	28.0	75.0	7.5	7.2	1.0	9.3	35.1
		Chlorogloeopsis fritschii	0.3	3.5	69.1	27.4	76.1	7.5	7.0	0.8	8.6	35.6
	Soluble	Auto *Chlorella*	0.2	2.3	95.9	1.9	78.9	11.8	3.9	0.9	4.6	43.2
		Hetero *Chlorella*	1.2	0.3	98.5	1.2	78.9	7.3	2.9	0.0	10.9	35.9
		Spirulina platensis	0.0	2.7	94.1	3.3	76.9	6.9	5.4	0.0	10.8	34.6
		Chlorogloeopsis fritschii	0.7	2.7	94.5	2.7	78.6	9.4	4.5	0.0	7.5	39.3

*Oxygen by difference

6.3. Conclusion

Hydrothermal liquefaction of the raw and pre-treated micro algae with the different biochemical compositions showed that the raw autotrophic and heterotrophic *Chlorella* gave a higher yield of oil than the pre-treated *Chlorella*. Whereas the *Spirulina* gave a higher yield of oil for the pre-treated algae than for the raw. The *Chlorogloeopsis* show no difference between the raw and pre-treated algae.

Analysis of the whole bio-crudes showed that the bio-crudes from the raw micro algae contained higher fixed carbon than the bio-crudes from the pre-treated algae. This is beneficial as a lower fixed carbon content means there will be less upgrading required. The hydrogen and nitrogen content of the bio-crude from the pre-treated algae is lower than for the raw algae, which suggests that there is some interactions with the nitrogen compounds (Maillard reactions) which are reducing the nitrogen content of the bio-crudes. The bio-crude from the pre-treated heterotrophic *Chlorella* contains the least hydrogen and most oxygen but also has the lowest HHV.

The results from this chapter suggest that the biochemical composition of the algae greatly influences the bio-crude yield, nitrogen and oxygen content and the HHV of the bio-crude produced. Hydrothermal pre-treatment plays a role in the ratio of the biochemical components present. The variation in the biochemical content has a different affect each time based on the ratio of lipids, proteins and carbohydrates present in the sample, which also affect the amount of reactions taking place, which alters the bio-crude yield and quality.

Chapter 7. Influence of formic acid on bio-crude quality

7.1. Introduction

Bio-crudes produced from hydrothermal liquefaction of micro algae that had undergone hydrothermal pre-treatment were investigated in the previous chapter. It was found that the bio-crudes produced from micro algae that had undergone pre-treatment require less upgrading than bio-crudes produced from raw algae.

Other methods for improving the bio-crude yield and quality that have been investigated in the literature are the addition of additives and catalysts during the liquefaction process. Watanabe et al. (2006) investigated the formation of oils from hydrothermal liquefaction of glucose with formic acid and a cobalt catalyst and found that the addition of the formic acid and catalyst, improved the yield of oil in comparison to the same experiments without additives. Ross et al. (2010) also undertook an investigation into the effect of hydrothermal processing of micro algae using alkali and organic acids and found that the addition of formic acid improved the yield and quality of the bio-crude produced.

Although additives and catalysts have been utilised during liquefaction, there has not been any previous works undertaken on the liquefaction of hydrothermally pre-treated micro algae with formic acid, therefore this will be investigated in this chapter.

The main aim of this chapter is to address objective 5 of this thesis, to investigate the influence of different process variables such as temperature, feedstock type and additives, on the quality of bio-crude produced. In this chapter, the main focus is on the addition of formic acid during hydrothermal liquefaction of the raw and pre-treated autotrophic *Chlorella vulgaris*. The main reason for focussing on just the *Chlorella vulgaris* is due to the *Ulva* not showing much difference in the bio-crudes from HTL of the three pre-treated algae. It was also chosen over the three other micro algae due to it being extensively studied in the

literature. The first half of the chapter focuses on the quality of bio-crude produced. The quality of the bio-crude is determined by the amount of nitrogen, oxygen and metals present in the bio-crude, the HHV and the amount of heavy molecular weight material in the bio-crude. The second half of the chapter focuses on the process waters from liquefaction of the autotrophic *Chlorella vulgaris* with the addition of formic acid, to investigate what is released into the process waters during the liquefaction process and to determine if these process waters can be used to produce platform chemicals or for cultivation of more algae.

7.2. Effect of formic acid on hydrothermal liquefaction of autotrophic *Chlorella vulgaris*

Due to the small quantities of sample, hydrothermal liquefaction was carried out in the 75ml parr reactor, with a feedstock, water and acid volume of 30ml at 350°C, with pressures of 120-140bar,with a residence time of 1 hour. Due to the addition of formic acid, the temperature that the liquefaction was carried out at, was reduced to 300°C instead of the 350°C that the previous runs were carried out at. The main reason for this was to prevent the pressure within the reactor from overshooting the pressure capabilities of the reactor as the formic acid acts as a hydrogen donor. The method is described in detail in section 3.5.4 of the methodology chapter. These conditions were chosen based on literature from Ross et al. (2010) who carried out liquefaction of autotrophic *Chlorella vulgaris* and *Spirulina platensis* using a variety of acidic and alkali catalysts.

7.2.1. Hydrothermal liquefaction of autotrophic *Chlorella* with the addition of formic acid

Hydrothermal liquefaction was firstly carried out on the raw autotrophic *Chlorella* with the addition of 1ml, 2ml and 3ml of formic acid, replacing the equivalent amount of distilled water in the liquefaction process (maintaining a constant liquid quantity of 27ml in total). From these initial tests it was decided that the addition of 2ml of formic acid is the quantity that will be investigated as it seemed to show the most difference from the preliminary analysis of the bio-crude,

therefore the autotrophic *Chlorella* pre-treated at 100, 150 and 200°C was hydrothermally liquefied with the addition of 2ml of formic acid.

Table 7-1 shows the yields of products from hydrothermal liquefaction of raw and pre-treated autotrophic *Chlorella vulgaris* with and without the addition of formic acid. Firstly raw autotrophic *Chlorella* was liquified with the addition of 1ml, 2ml and 3ml of formic acid replacing the same amount of distilled water. The autotrophic *Chlorella* pre-treated at 100, 150 and 200°C was then liquefied with the addition of 2ml of formic acid.

Table 7-1: Yields of bio-crude, char and aqueous phase from hydrothermal liquefaction of raw and pre-treated micro algae with and without the addition of formic acid

Pre-treatment temperature (°C)	Formic acid added (ml)	%		
		Bio-crude	Char	Aqueous phase*
Raw	0	28.0	5.0	67.0
100	0	40.0	6.3	53.7
150	0	25.6	8.0	66.4
200	0	41.6	16.7	41.7
Raw	1	24.3	4.0	71.7
Raw	2	24.0	3.7	72.3
Raw	3	30.0	2.7	67.3
100	2	28.0	3.0	69.0
150	2	31.0	5.3	63.7
200	2	55.3	7.3	37.3

*by difference

The results from the raw autotrophic *Chlorella* with 1, 2 and 3ml formic acid show that the bio-crude yield for the liquefaction with 1 and 2ml of formic acid are the same and the char and aqueous phase yields are also very similar. The addition of 3ml of formic acid to the raw *Chlorella* results in an increase in the bio-crude yield and decrease in the char and aqueous phase yield.

For the autotrophic *Chlorella* pre-treated at 100, 150 and 200°C, with the addition of 2ml of formic acid in the liquefaction process, the bio-crude yield is higher than for the raw *Chlorella* with 1, 2 and 3ml of formic acid. The bio-crude and char yields, of the pre-treated *Chlorella* with 2ml formic acid, increase with increasing pre-treatment temperature. This trend was also observed in a study by

Jazrawi et al. (2015) who undertook 2 stage hydrothermal liquefaction with formic acid and found that the addition of formic acid in the liquefaction stage results in higher bio-crude yields overall. The aqueous phase yield decreases with increasing pre-treatment temperature, however, the largest fraction of the process products is found in the aqueous phase. The % aqueous phase increases with the addition of formic acid for the raw *Chlorella* and the *Chlorella* pre-treated at 100°C but shows a decrease for the *Chlorella* pre-treated at 150 and 200°C. The increase in the aqueous phase suggests that water is being formed with the raw and lower temperature pre-treated algae.

It is also possible that the extra hydrogen supplied from the formic acid, favoured the conversion of organic matter in the algae into bio-crude, due to the stabilisation of the active intermediates formed during the reaction (Duan, P. et al., 2013). The addition of formic acid to the liquefaction process also results in the formic acid breaking down into CO and H_2 in the gas phase (Ross et al., 2010).

Comparison between the raw *Chlorella* and the pre-treated *Chlorella* hydrothermally liquefied in distilled water alone and also in distilled water with 2ml of formic acid is shown in Figure 7-1.

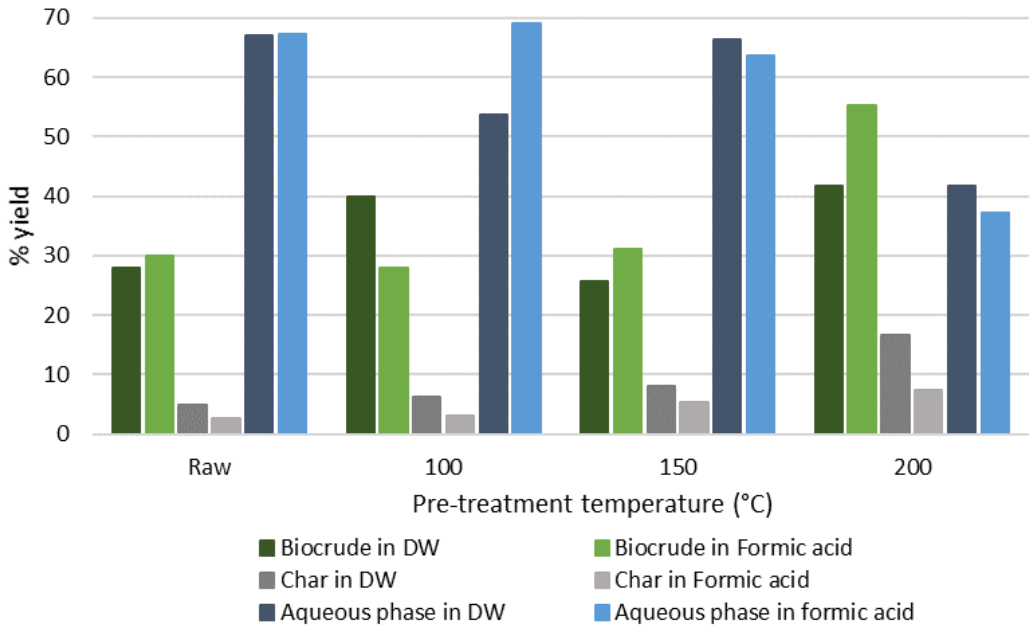


Figure 7-1: % bio-crude, char and aqueous phase yield from HTL of raw and pre-treated autotrophic *Chlorella* in distilled water and distilled water with 2ml formic acid

The % oil yield from the raw *Chlorella* and the *Chlorella* pre-treated at 100°C is higher when liquified in distilled water alone, whereas the raw *Chlorella* and the *Chlorella* pre-treated at 150 and 200°C give a higher yield with the addition of 2ml formic acid. This suggests that the affect of formic acid is different on the different biochemical components of the algae.

Chlorella pre-treated at 200°C and liquified using 2ml of formic acid with the distilled water gives the highest bio-crude yield overall. Less char is produced from liquefaction with 2ml of formic acid with the distilled water, which suggests that more material is being converted into bio-crude or released into the process waters.

It is noted that the liquefaction with the addition of formic acid was carried out at the lower temperature of 300°C instead of 350°C as it was with the liquefaction using only distilled water. This should be factored in when comparing the results, as there may be lower bio-crude yields due to the lower temperature.

7.2.2. Ultimate and proximate analysis of HTL bio-crude from autotrophic *Chlorella* with the addition of formic acid

Table 7-2 shows the ultimate and proximate analysis of the bio-crude from hydrothermal liquefaction of the autotrophic *Chlorella* with 1ml, 2ml and 3ml of formic acid added respectively, along with the autotrophic *Chlorella* pre-treated at 100, 150 and 200°C, with the addition of 2ml formic acid. Firstly, the raw *Chlorella* with the addition of 1ml, 2ml and 3ml of formic acid will be discussed and then the autotrophic *Chlorella* pre-treated at 100, 150 and 200°C, with the addition of 2ml formic acid.

From the proximate analysis of the raw autotrophic *Chlorella* with 1, 2 and 3ml formic acid, the moisture, ash and volatiles contents increase with the addition of increasing amounts of formic acid, which results in the fixed carbon content of the bio-crudes reducing from 8.7% to 5.8%, with the addition of increasing amounts of formic acid. The carbon content of the bio-crude reduces from 79.8% with 1ml formic acid to 77.4% with 2ml formic acid, then shows a considerable increase from the bio-crude with 2ml to the bio-crude with 3ml of formic acid (85.0%).

The hydrogen content of the three bio-crudes from the raw *Chlorella* are similar with only 0.5% difference between the highest and lowest content. The nitrogen content of the bio-crude from the raw *Chlorella* with 1, 2 and 3ml of formic acid is similar with 5.6, 5.7 and 5.4% contents respectively. The raw autotrophic *Chlorella* feedstock contains 9.27% (d.a.f.) of nitrogen. Therefore it shows that some nitrogen is being removed from the feedstock during liquefaction with the formic acid. However, there is little difference between the bio-crudes from the different amounts of formic acid added, which suggests that a catalyst (such as formic acid) may need to be present to further reduce the nitrogen in the bio-crude. The sulphur content differs for the bio-crude with the varying formic acid quantities, however the changes are quite small and under 1%. The oxygen content of the bio-crude increases from 5.7% with 1ml of formic acid to 7.9% with 2ml of formic acid. The bio-crude produced with 3ml of formic acid, has a considerably lower oxygen content of 0.4%. The higher heating value decreases from 38.9MJ/kg with 1ml formic acid to 37.7MJ/kg with 2ml of formic acid. The HHV then increases again to 41.8MJ/kg for the bio-crude with 3ml formic acid.

Although the differences in the proximate and ultimate analysis of the three bio-crudes produced from hydrothermal liquefaction with the addition of different amounts of formic acid (1, 2 and 3ml) are small, it is evident that the formic acid affects the quality of the bio-crudes produced.

From the proximate analysis of the bio-crudes produced from the *Chlorella* pre-treated at 100, 150 and 200°C, with the addition of 2ml of formic acid, in Table 7-2, it is shown that the moisture content decreases with increasing pre-treatment temperature. The ash content increases from 0.5% for the *Chlorella* pre-treated at 100°C, to 3.9% for the *Chlorella* pre-treated at 150°C but then decreases again to 0.6% for the *Chlorella* pre-treated at 200°C. The volatiles content of the bio-crude from the pre-treated *Chlorella* with 2ml of formic acid, decreases with increasing pre-treatment temperature. The fixed carbon content increases with increasing pre-treatment temperature.

Table 7-2: Ultimate and proximate analysis of HTL oils from autotrophic *Chlorella vulgaris* with formic acid

Pre-treatment temp (°C)	Formic acid (ml)	Proximate % (d.a.f.)				Ultimate % (d.a.f.)					HHV (MJ/kg^{-1})
		Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*	
Raw	1	2.6	0.3	91.3	8.7	79.8	8.8	5.6	0.2	5.7	38.9
Raw	2	3.1	1.9	92.9	7.1	77.4	8.6	5.7	0.3	7.9	37.7
Raw	3	5.2	6.3	94.2	5.8	85.0	9.1	5.4	0.1	0.4	41.8
100	2	2.3	0.5	95.0	5.0	80.6	9.2	5.3	0.0	4.9	39.9
150	2	1.1	3.9	94.8	5.2	78.8	9.7	4.9	0.0	6.6	39.9
200	2	0.6	2.7	92.1	7.9	78.0	9.5	5.6	0.0	6.9	39.3

*Oxygen by difference

The carbon content of the bio-crudes from the pre-treated *Chlorella* are quite similar to each other (78.0-80.6%) but are slightly higher in comparison to the raw *Chlorella* with 2ml of formic acid. The hydrogen content of the bio-crude from the pre-treated *Chlorella* are all similar with only 0.5% difference between the highest and lowest content. In comparison to the raw *Chlorella* with 2ml of formic acid which has a slightly lower hydrogen content. This suggests that some hydrogen is being added to the bio-crude from the formic acid. There is no sulphur present in the bio-crude from the pre-treated *Chlorella*. The oxygen content of the bio-crudes increases from 4.9% for the *Chlorella* pre-treated at 100°C to 6.9% for both the *Chlorella* pre-treated at 150 and 200°C. The oxygen content from the pre-treated *Chlorella* are lower than the bio-crude from the raw *Chlorella* with 2ml formic acid. In general, higher oxygen content is favoured for the production of biofuels, however, depending on the types of compounds containing the oxygen, higher oxygen content can be both positive and negative as some of the compounds are good lubricants, whereas others can be problematic as they can poison catalysts used for upgrading. The higher heating value of the bio-crude from the three pre-treated *Chlorella* are similar (39.3-39.9MJ/kg) and are slightly higher than the bio-crude from the raw *Chlorella* with 2ml formic acid (37.7MJ/kg).

Again, although the differences between the raw *Chlorella* with 2ml formic acid and the three pre-treated *Chlorella* with 2ml of formic acid are small, there are still differences shown. Overall, the bio-crudes from the pre-treated *Chlorella* with 2ml formic acid have a higher hydrogen content, and lower nitrogen content than the raw *Chlorella* with 1, 2 and 3ml formic acid, which suggests that the pre-treated *Chlorella* have better characteristics for use as biofuels than the raw *Chlorella*.

A comparison can also be made between the raw *Chlorella* and the pre-treated *Chlorella* hydrothermally liquefied in distilled water alone and also in distilled water with 2ml of formic acid. The ultimate and proximate analysis of the raw and pre-treated *Chlorella*, liquefied in distilled water alone is shown in Table 5-6.

Figure 7-2 shows the % moisture, ash and fixed carbon content of the bio-crudes from the liquefaction of the raw and pre-treated *Chlorella* in both distilled water and distilled water with the addition of 2ml formic acid. The moisture content of the two sets of data show different trends. For the bio-crudes from liquefaction in

distilled water alone, the moisture content is similar from the raw *Chlorella* and the *Chlorella* pre-treated at 100 and 200°C. Whereas for the bio-crude from liquefaction with distilled water with 2ml of formic acid, the moisture content shows a decrease with increasing pre-treatment temperature. For the bio-crude from liquefaction with distilled water alone, it is shown that the ash decreases from the raw to the 100 and 150°C but then is increases again for the bio-crude from pre-treatment at 200°C. For the bio-crudes liquified with formic acid, the ash content of the bio-crude decreases from the raw to the *Chlorella* pre-treated at 100°C, then increases significantly at 150°C and then decreases again for the *Chlorella* pre-treated at 200. The fixed carbon content of the *Chlorella* liquefied in distilled water alone shows an increase from the raw to the *Chlorella* pre-treated at 100, then decreases again at 150 and then increases again for the *Chlorella* pre-treated at 200. The bio-crudes from liquefaction with formic acid have significantly higher fixed carbon content than the bio-crudes from liquefaction in distilled water alone. This is not ideal as the higher the fixed carbon content the more likely an issue coking will be. There is a reduction in the fixed carbon content from the raw *Chlorella* to the *Chlorella* pre-treated at 100°C. The *Chlorella* pre-treated at 100 and 150°C are similar whereas the *Chlorella* pre-treated at 200°C is higher and has the highest fixed carbon content of all the bio-crudes. The differences between the moisture, ash and fixed carbon content are very evident from the data shown in Figure 7-2.

The volatiles content of the bio-crudes are quite similar for both the raw and pre-treated *Chlorella* in both liquefaction conditions. This is also the case for the carbon content.

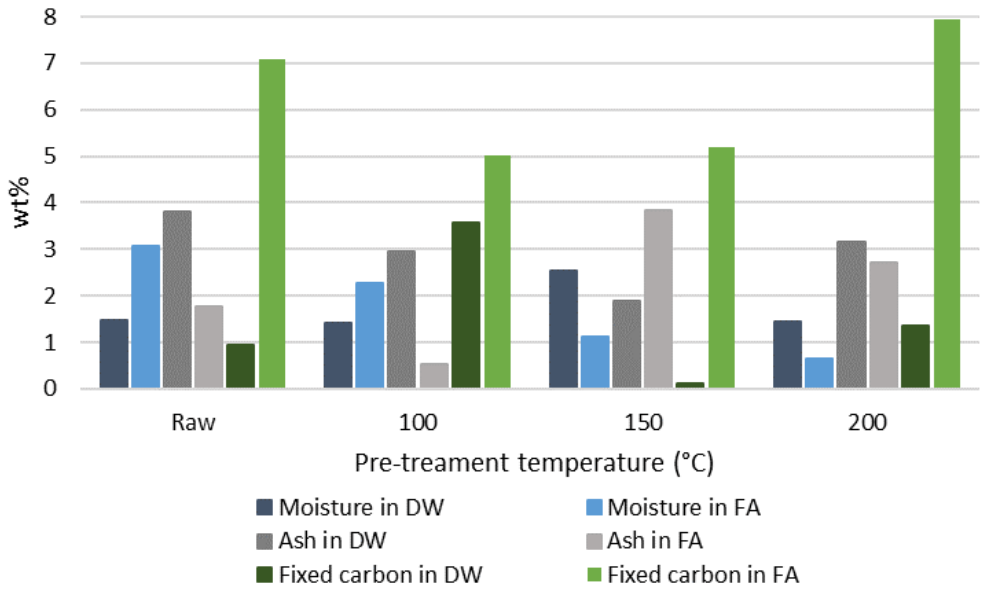


Figure 7-2: % moisture, ash and fixed carbon (d.a.f.) content of the bio-crudes from the raw and pre-treated autotrophic *Chlorella vulgaris* liquefied in distilled water and distilled water with 2ml formic acid

Figure 7-3 shows the % hydrogen and nitrogen content of the two sets of bio-crude from the different liquefaction conditions. The hydrogen content of the bio-crudes from liquefaction with distilled water alone show a decrease from the raw to the *Chlorella* pre-treated at 150°C and then a slight increase for the *Chlorella* at 200°C although this is still lower than the hydrogen content of the raw *Chlorella*. The bio-crudes from liquefaction with the formic acid show the opposite trend, with the hydrogen content increasing from the raw *Chlorella* to the *Chlorella* pre-treated at 150°C and then a slight decrease for the *Chlorella* at 200°C although this is still higher than the hydrogen content of the raw *Chlorella*. Both sets of bio-crudes show a decrease in the nitrogen content with increasing pre-treatment temperature except for the *Chlorella* pre-treated at 200 with 2ml formic acid, which is similar to the nitrogen content of the raw *Chlorella*. Overall, the hydrogen and nitrogen content of the bio-crudes from liquefaction with 2ml formic acid are slightly lower than for the bio-crude from distilled water alone.

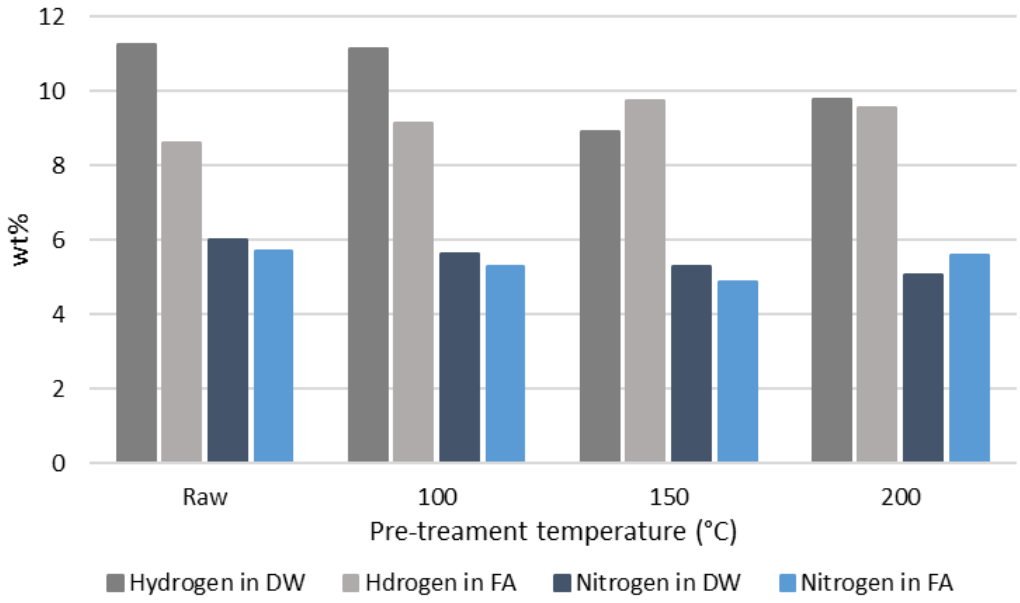


Figure 7-3: % hydrogen and nitrogen content of the bio-crudes from the raw and pre-treated autotrophic *Chlorella vulgaris* liquefied in distilled water and distilled water with 2ml formic acid

Although there are noticeable differences in the composition of the bio-crude from processing in both distilled water and distilled water with formic acid, there is also a difference in the hydrothermal liquefaction temperature, which could also contribute to these differences.

7.2.3. GC-MS analysis of HTL oils from autotrophic *Chlorella* with formic acid

Table 7-3 shows the GC-MS analysis of HTL oils from autotrophic *Chlorella* with the addition of varying amounts of formic acid. The nitrogen compounds that were calibrated for have not been detected in the HTL oils produced with the addition of formic acid. This is also the case for the cyclopentanones and alkanes, with only pentadecane being detected in very small quantities in the HTL oils from the autotrophic *Chlorella* pre-treated at 150 and 200°C with 2ml of formic acid.

Table 7-3: GC-MS of HTL oils from autotrophic *Chlorella* with formic acid

	Formic acid added (ml)		1	2	3	2	2	2
	Pre-treatment temp (°C)		Raw autotrophic			100	150	200
Compounds (mg/l)	**Nitrogen**		ND	ND	ND	ND	ND	ND
	Cyclopentanones		ND	ND	ND	ND	ND	ND
	Alkanes	Pentadecane	ND	ND	ND	ND	5.5	4.5
		Hexadecane	ND	ND	ND	ND	ND	ND
	Phenols	Phenol	229.6	226.4	219.3	226.7	225.2	221.6
		p-Cresol	117.5	110.8	106.7	111.0	110.1	108.8
		4-Ethylphenol	65.4	76.3	64.5	75.0	74.8	50.5
		2,3-Dimethylphenol	ND	ND	ND	ND	ND	ND
		2,6-Dimethoxyphenol	ND	ND	ND	ND	ND	ND
	Fatty acids & glycerol	Hexadecanoic acid	2222.5	2142.2	2347.8	2354.3	2378.4	2423.9
		Oleic acid	961.6	961.5	978.1	978.3	992.3	972.0
		Glycerol	2390.3	2420.4	2384.9	2392.2	2395.0	2405.1

All of the HTL oils produced with the addition of formic acid contain phenolics, with phenol being the most abundant. There is not much difference in the quantity of phenol present between the six different HTL oils. The HTL oil from the raw autotrophic *Chlorella* in distilled water shows a phenol content of 279mg/l. The HTL oil from autotrophic *Chlorella* pre-treated at 150°C, liquified in just distilled water, shows a phenol content of 243mg/l. This is a decrease in the HTL oils from the raw to the autotrophic *Chlorella* pre-treated at 150°C. There is also a decrease in the HTL oil for the autotrophic *Chlorella* pre-treated at 150°C with the addition of 2ml formic acid in comparison to the counterpart with no formic acid added.

The hexadecenoic acid content of the HTL oils from the autotrophic *Chlorella* pre-treated at 100, 150 and 200°C,with the addition of 2ml of formic acid, is similar for the three different pre-treatment temperatures with the 200°C sample showing the highest quantity. There is a difference between the raw autotrophic *Chlorella* with 2ml of formic acid and the pre-treated *Chlorella* with 2ml of formic acid as the HTL oil from the raw *Chlorella* has a lower hexadecenoic acid content than the HTL oils from the pre-treated *Chlorella*. The oleic acid content is very similar for all of the HTL oils produced with the addition of formic acid and does not show much difference between the raw and pre-treated *Chlorella* with and without the addition of formic acid. The glycerol content of the HTL oils produced with the addition of formic acid is also similar for all of the oils, with the *Chlorella*

pre-treated at 150°C with addition of formic acid giving a slightly lower quantity than the *Chlorella* pre-treated at 150°C without addition of formic acid.

Overall, the *Chlorella* pre-treated at 150°C with 2ml of formic acid has the highest quantity of the detected compounds and therefore suggested more of the *Chlorella* is being converted into phenolics, fatty acids and glycerol during liquefaction with the *Chlorella* pre-treated at this temperature with 2ml of formic acid.

7.2.4. Pentane fractionation of HTL oils produced from autotrophic *Chlorella* with formic acid

As with the HTL oils from the raw and pre-treated *Chlorella* in distilled water, the HTL oils produced in the presence of formic acid were also fractionated using pentane to determine how much high molecular weight material is produced and how this compares to the bio-crudes produced in distilled water alone. Table 7-4 shows the percentage of pentane soluble and insoluble material present in the oils from hydrothermal liquefaction with the addition of formic acid.

Table 7-4: Pentane fractionation of HTL oils from autotrophic *Chlorella vulgaris* with formic acid

	ml	mg	%	
Sample	**Formic acid added**	**Original Oil**	**Pentane Insoluble**	**Pentane Soluble**
Raw	1	105.2	39.4	59.4
Raw	2	105.0	46.3	52.8
Raw	3	97.4	48.5	47.9
100	2	113.5	50.2	49.2
150	2	105.1	53.6	46.2
200	2	161.4	30.7	69.1

The HTL oil produced from the raw autotrophic *Chlorella*, with the addition of 1, 2 and 3ml of formic acid, have slightly more of the pentane soluble fraction than the pentane insoluble fraction. The pentane soluble percentage decreases with increasing addition of formic acid for the oils from the raw autotrophic *Chlorella*. The oil from the raw autotrophic *Chlorella* produced with 3ml of formic acid has very similar percentage of both the pentane soluble and insoluble fractions. The

HTL oil from the autotrophic *Chlorella* pre-treated at 100°C and 150°C have very similar percentages of both pentane fractions, however the HTL oil from the *Chlorella* pre-treated at 200°C has a much higher percentage of the pentane soluble fraction. These results show that the HTL oil produced from *Chlorella* pre-treated at 150°C with 2ml of formic acid, has the highest percentage of pentane insoluble material and therefore has more heavy molecular weight material than the other oils.

A comparison can also be made between the raw *Chlorella* and the pre-treated *Chlorella* hydrothermally liquefied in distilled water alone and also in distilled water with 2ml of formic acid, which is shown in Figure 7-4. It is shown that the addition of formic acid increases the percentage of the pentane soluble fraction and decreases the percentage of the pentane insoluble fraction for the bio-crude from the raw *Chlorella* and the *Chlorella* pre-treated at 100°C, with the opposite trend for the *Chlorella* pre-treated at 150 and 200°C. This suggests that the addition of formic acid is contributing towards producing more heavy molecular weight material from the bio-crudes produced from the *Chlorella* pre-treated at the higher temperatures of 150 and 200°C. These results suggest that pre-treating the *Chlorella* at higher temperatures has an effect on the molecular weight distribution of the bio-crudes produced with the addition of 2ml formic acid.

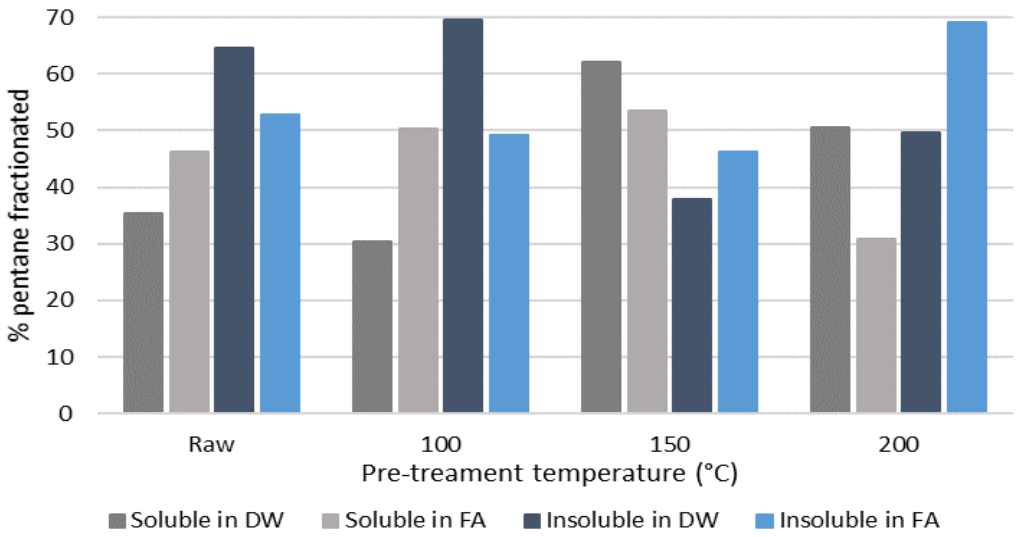


Figure 7-4: % pentane soluble and insoluble fractions of bio-crude from HTL of raw and pre-treated autotrophic *Chlorella* in distilled water and distilled water with 2ml formic acid

7.2.5. Ultimate and proximate analysis of the pentane fractions from HTL oils of autotrophic *Chlorella* with the addition of formic acid

Table 7-5 shows the ultimate and proximate analysis of the pentane fractions from the bio-crudes from hydrothermal liquefaction of autotrophic *Chlorella* with formic acid.

From the proximate analysis of the insoluble fractions, the moisture content of the raw *Chlorella* with 1ml of formic acid is the highest at 0.9%. The moisture content decrease with the addition of more formic acid for the raw *Chlorella*, however for the pre-treated algae, the moisture content increases with increasing pre-treatment temperature. However, as the moisture content is <1% it is not significant. The ash content of the raw autotrophic *Chlorella* with 1, 2 and 3ml of formic acid added respectively, increases with increasing amounts of formic acid added to the HTL process. Of all the samples, the *Chlorella* pre-treated at 100°C has the highest ash content at 4.6%. The *Chlorella* pre-treated at 150° and 200°C, have very similar ash contents.

The volatiles content is the highest in the insoluble fractions which where liquefied using 2ml of formic acid. There is a noticeable difference in the volatiles content between the raw *Chlorella* with 1ml, 2ml and 3ml of formic acid added, whereas there is little difference (<0.3%) between the pre-treated *Chlorella* with 2ml of formic acid. The fixed carbon content of the raw *Chlorella* with 1ml of formic acid is the highest at 20.5%. There is a little difference (<1.2%) between the remainder of the insoluble fractions, with the lowest being the raw *Chlorella* with 2ml of formic acid.

Table 7-5: Ultimate and proximate of pentane fractions from HTL oils of autotrophic algae with formic acid

Pentane fraction	Sample	Formic acid added (ml)	Proximate % (d.b.)				Ultimate % (d.a.f.)					HHV (MJ/kg^{-1})
			Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*	
Insoluble	Raw	1	0.9	3.7	75.7	20.5	75.9	4.1	6.1	0.8	13.2	29.3
	Raw	2	0.2	4.1	79.5	16.4	75.5	4.5	5.7	0.8	13.5	29.8
	Raw	3	0.1	4.5	77.9	17.6	76.1	4.9	5.6	0.9	12.6	28.5
	100	2	0.0	4.6	78.9	16.5	76.2	5.0	5.7	0.9	12.2	27.4
	150	2	0.1	3.5	79.2	17.3	74.9	3.8	5.7	1.0	14.6	27.6
	200	2	0.2	3.6	78.8	17.6	76.1	3.9	5.9	0.7	13.4	25.8
Soluble	Raw	1	0.2	2.0	96.3	1.7	79.2	2.2	3.4	0.0	15.2	27.0
	Raw	2	0.4	1.3	98.6	0.1	78.9	1.5	3.3	0.0	16.3	25.0
	Raw	3	0.4	1.8	97.7	0.5	78.4	1.9	3.0	0.0	16.7	27.2
	100	2	0.5	0.8	98.8	0.4	77.2	0.9	3.1	0.0	18.9	25.0
	150	2	0.4	1.7	97.5	0.8	77.5	1.9	3.0	0.0	17.6	26.7
	200	2	0.5	0.6	98.6	0.8	77.4	0.6	2.9	0.0	19.1	24.7

*Oxygen by difference

The carbon content of the raw *Chlorella* with 1ml, 2ml and 3ml of formic acid are very similar. This is also true of the pre-treated *Chlorella* with 2ml of formic acid, with the *Chlorella* pre-treated at 150°C having a slightly lower carbon content, of 74.9%, than the rest of the samples. The hydrogen content of the raw *Chlorella* with 1ml, 2ml and 3ml of formic acid added, increases with the increase in the amount of formic acid added to the HTL process. The pre-treated algae at 100°C has the highest hydrogen content of 5.0%, which is very similar to the raw *Chlorella* with 3ml of formic acid at 4.9%. The *Chlorella* pre-treated at 150°C and 200°C are very similar but are lower than the other samples at 3.8 and 3.9% respectively. The sulphur content of the three raw *Chlorella* samples is almost the same for all three samples. The sulphur content increases for the *Chlorella* pre-treated at 100°C and 150°C, although it is the lowest at 0.6% for the *Chlorella* pre-treated at 200°C. As they are all <1% these differences are not significant. The oxygen content of the raw *Chlorella* with 1ml and 2ml of formic acid are very similar, however the raw *Chlorella* with 3ml of formic acid has a lower oxygen content of 12.6%. The *Chlorella* pre-treated at 150°C has the highest oxygen content of 14.6%. The HHV of the insoluble fraction from the raw *Chlorella* with 1ml, 2ml and 3ml of formic acid are all comparable (28.5-29.8MJ/kg). The pre-treated *Chlorella* with 2ml of formic acid also have comparable higher heating values but are lower than the values for the raw *Chlorella* (25.8-27.6MJ/kg).

There is little difference in the proximate and ultimate analysis of the soluble fractions from the different liquefaction conditions. The soluble fractions of the bio-crudes from HTL are different to the insoluble fractions. They contain similar amounts of moisture, <1% which is negligible. The ash content is also lower for the soluble fractions, which suggests that the metals and other inorganics are concentrated in the insoluble fraction. The volatiles content are significantly higher (>16%) than the insoluble fractions, whereas the fixed carbon content is significantly lower at less than 2% for the bio-crude with the highest content. This is expected as the fixed carbon content is high molecular weight material and therefore would not solubilise easily in pentane. The higher ash and fixed carbon and lower volatiles content in the insoluble fractions was expected. The carbon and oxygen contents of the soluble fraction are higher than the insoluble fractions, whereas the hydrogen, nitrogen and sulphur contents, along with the HHV, are lower.

7.3. Composition of process waters from HTL of autotrophic *Chlorella* with the addition of formic acid

The process waters from liquefaction of the raw and pre-treated *Chlorella* using distilled water with formic acid were also analysed by GC-MS, UV-vis and XRF to determine what components are released from the algae into the process waters, to investigate if these process waters can be used to produce platform chemicals or for cultivation of more algae.

7.3.1. GC-MS analysis of process waters from HTL with formic acid

The process waters from liquefaction using distilled water with formic acid are also analysed using an Shimadzu QP2010E GC-MS. The method used is described in section 3.7.5.2. Formic acid, lactic acid and the glucose, fructose, ribose and mannose where analysed for, using the HPLC method described in section 3.7.4.

Table 7-6 shows the total compounds, analysed for using GC-MS, in process waters from hydrothermal liquefaction of raw *Chlorella* with 1, 2 and 3ml of formic acid along with the *Chlorella* pre-treated at 100, 150 and 200°C with 2ml of formic acid. Additional data of the individual compounds can be found in Appendix 5.

Table 7-6: Compounds in the process waters from HTL of autotrophic *Chlorella vulgaris* with the addition of formic acid

	Formic acid added (ml)	**1**	**2**	**3**	**2**	**2**	**2**
	Pre-treatment temp (°C)	**Raw autotrophic**			**100**	**150**	**200**
Compounds mg/l	Acids	5931	8441	5982	1758	1917	5903
	Nitrogen compounds	2752	2806	2399	2343	2291	0
	Cyclopentanones	161	94	33	54	47	53
	Phenols	47	49	50	46	57	50
	Sugars	0	262	4638	93	296	479

For the raw *Chlorella* with 1, 2 and 3ml of formic acid, the acids present in the highest quantity are formic, lactic and acetic acid. The formic acid is only present for the process waters from the raw *Chlorella* with 3ml formic acid at 2529mg/l and for the *Chlorella* pre-treated at 200°C with 2ml formic acid at 2744mg/l. This may not have been formed but may be left over from the addition of the formic acid to the process. Lactic acid is the most abundant acid present in the process waters, with 3956, 6614 and 1720 mg/l for the raw *Chlorella* with 1, 2 and 3ml formic acid respectively. It is also only present in the process water from the *Chlorella* pre-treated at 200°C with 2ml formic acid at 1628 mg/l. All three of the process waters from the raw *Chlorella* have similar amounts of acetic acid at ~1000mg/l. The process waters from the pre-treated *Chlorella* with 2ml formic acid have a slightly lower amount of acetic acid, with the *Chlorella* pre-treated at 200°C having the lowest amount at 712 mg/l. The other notable acids present in the process waters for both the raw and pre-treated *Chlorella* are: isovaleric, 3-methyl-Pentanoic, 4-methyl-Pentanoic, levulinic, succinic and hydrocinnamic acids.

Of the nitrogen compounds tested for, the 3-Hydroxypyridine monoacetate is the only compound that there is a significant amount of present for both the raw *Chlorella* with 1, 2 and 3ml formic acid and the pre-treated *Chlorella* with 2ml formic acid. There was no 3-Hydroxypyridine monoacetate detected in the *Chlorella* pre-treated at 200°C with 2ml formic acid.

The cyclopentanones and phenols in the process waters are present in low quantities and similar amounts in the process waters from both the raw and pre-treated *Chlorella*.

The sugars are present in varying quantities for the different process waters. There were no sugars detected in the process water from the raw *Chlorella* with 1ml of formic acid. There is also no sugars apart from fructose detected for the raw *Chlorella* with 2ml formic acid (261mg/l) and the *Chlorella* pre-treated at 100°C (93mg/l). All four sugars are present in the process water from the raw *Chlorella* with 3ml formic acid. The glucose is present in the highest quantity at 3321mg/l, with significant amounts of ribose and mannose present too, at ~500mg/l. The process waters from *Chlorella* pre-treated at 150°C contains small quantities of glucose and fructose at 212 and 82mg/l and the process water from *Chlorella* pre-treated at 200°C also contains small quantities of glucose and mannose.

Overall, the quantities of the compounds present in the process waters from the raw and pre-treated *Chlorella* are similar with only a few differences.

A comparison of the compounds present in the process waters from HTL of the raw and pre-treated algae using distilled water alone and also distilled water with 2ml of formic acid are shown in Figure 7-5.

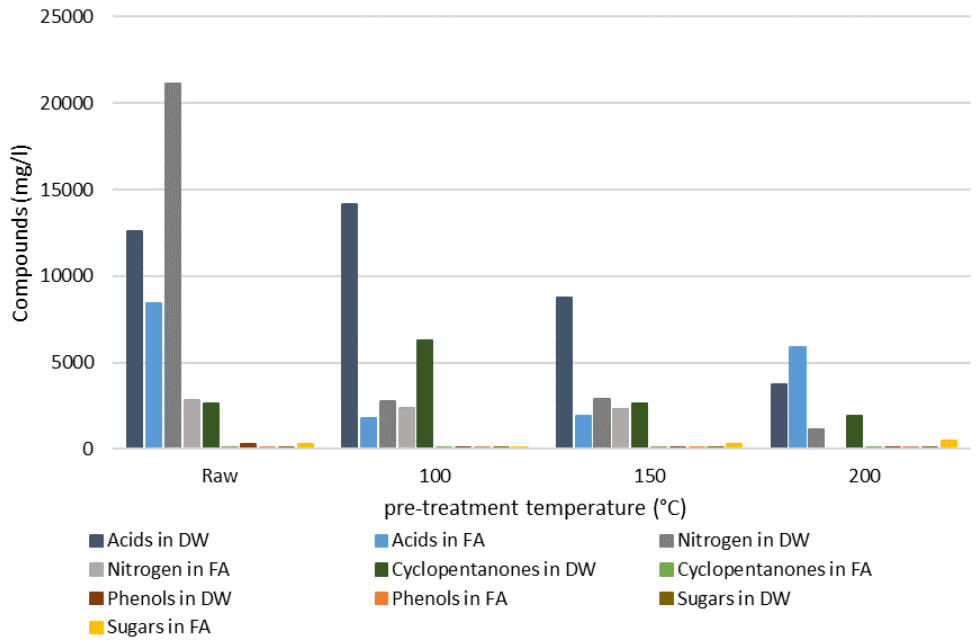


Figure 7-5: Comparison of the compounds in the process waters from HTL with both distilled water and 2ml of formic acid

For the raw *Chlorella*, the acids, cyclopentanones and phenols show a small decrease when processed with the addition of formic acid. There is a significant difference between nitrogen content of the two processing waters, with the addition of formic acid resulting in much less nitrogen in the process waters. For the *Chlorella* pre-treated at 100°C there is a significant difference between the two processing waters, with the addition of formic acid resulting in a much lower acids and cyclopentanones content. There is little difference between the nitrogen, phenols and sugars content from the two processing waters. The *Chlorella* pre-treated at 150°C, also shows a significant difference between the acids and cyclopentanones, but on a smaller scale than for the *Chlorella* pre-treated at 100°C. Again, there is little difference between the nitrogen, phenols and sugars content from the two

processing waters. The process water from the *Chlorella* pre-treated at 200°C shows a different trend to the other pre-treatment temperatures. There is a small reduction in the nitrogen and cyclopentanones when processed with the addition of formic acid, whereas the acids and sugars show an increase.

A study by Srokol, Zbigniew et al. (2004) carried out hydrothermal liquefaction of glucose under similar conditions to this study and found that formic, acetic, lactic and acrylic acid were present in the process waters. These are polar organics which easily dissolve into the aqueous phase and therefore do not contribute to the bio-crude yield. The aldehydes and other aromatic structures are precursors of larger hydrocarbons which make up the bio-crude fraction. This is also what is shown in the results in this chapter, as there is formic, acetic, lactic and acrylic acid present in the process waters from liquefaction of the algae and aldehydes present in the bio-crudes.

7.3.2. Organic carbon, nitrogen and phosphate content of process water from HTL with formic acid

Table 7-7 shows the total organic carbon, nitrogen and ammonium, along with the phosphate and orthophosphate content of the process waters from hydrothermal liquefaction of the raw *Chlorella* with 1, 2 and 3ml formic acid and pre-treated *Chlorella* with 2ml formic acid.

The total organic carbon content of the process waters from the raw *Chlorella* with 1, 2 and 3ml show an increase with increasing amount of formic acid added. The raw *Chlorella* with 2 and 3ml have very similar TOC contents of 9281 and 9340mg/l respectively, whereas the raw *Chlorella* with 1ml of formic acid is significantly lower at 1323mg/l. The TOC content of the pre-treated *Chlorella* with 2ml of formic acid is significantly higher than for the raw *Chlorella* with 1, 2 and ml of formic acid. The *Chlorella* pre-treated at 100°C shows a TOC content of 8725mg/l. This is doubled to 16637mg/l for the *Chlorella* pre-treated at 150°C and increases again to 19774 for the *Chlorella* pre-treated at 200°C.

The total nitrogen content of the process waters from the raw *Chlorella* with 1, 2 and 3ml are very low with less than 112mg/l for all three samples. Of this, a very small amount is ammonium for the raw *Chlorella* with 1ml and 3ml of formic

acid at 7.9 and 5.0mg/l respectively, whereas the raw *Chlorella* with 2ml of formic acid shows approximately half of the total nitrogen is ammonium (38.2mg/l). The remainder of the nitrogen is organic nitrogen which makes up the majority of the nitrogen present in the process waters for the raw *Chlorella*.

The total nitrogen content of the process waters from the pre-treated *Chlorella* with 2ml of formic acid are significantly higher than for the raw *Chlorella*. The reason for this may be due to the increase in the ratio of proteins in the pre-treated *Chlorella* in comparison to the raw *Chlorella*, and also the interactions between with the formic acid which is causing more nitrogen to be released into the process waters. The *Chlorella* pre-treated at 100 and 200°C contain very similar amounts of nitrogen at 5920 and 5940mg/l respectively. The *Chlorella* pre-treated at 150°C contains less than these at 3700mg/l but is still significantly higher than the raw *Chlorella*. Of the total nitrogen content, the ammonium makes up only a small percentage. This may be due to the volatility of the ammonium, which may have evaporated and the ammonium that is still present is in the form of salts that are dissolved in the water. The ammonium content shows an increase with increasing pre-treatment temperature. The *Chlorella* pre-treated at 100 and 150°C are similar at 404 and 574mg/l respectively, whereas the *Chlorella* pre-treated at 200°C shows a significant increase to 1416mg/l. The remainder of the total nitrogen is made up of the organic nitrogen which is still quite high (>3000mg/l).

Table 7-7: Total organic carbon, nitrogen, ammonium, orthophosphate and phosphate content of process waters from HTL of autotrophic *Chlorella vulgaris* with the addition of formic acid

		mg/l						
Pre-treatment temperature (°C)	Formic acid (ml)	Total Organic Carbon	Total Nitrogen	Ammonium	Organic nitrogen	Total Phosphate	Ortho-phosphate	Organic Phosphate
Raw	1	1323.8	112.0	7.9	104.1	2.4	2.1	0.3
Raw	2	9281.2	84.2	38.2	46.0	2.0	1.9	0.1
Raw	3	9340.1	94.9	5.0	89.9	0.9	0.4	0.6
100	2	8725.3	5920.0	404.0	5516.0	105.0	70.0	35.0
150	2	16637.1	3700.0	574.0	3126.0	640.0	540.0	100.0
200	2	19774.4	5940.0	1416.0	4524.0	460.0	360.0	100.0

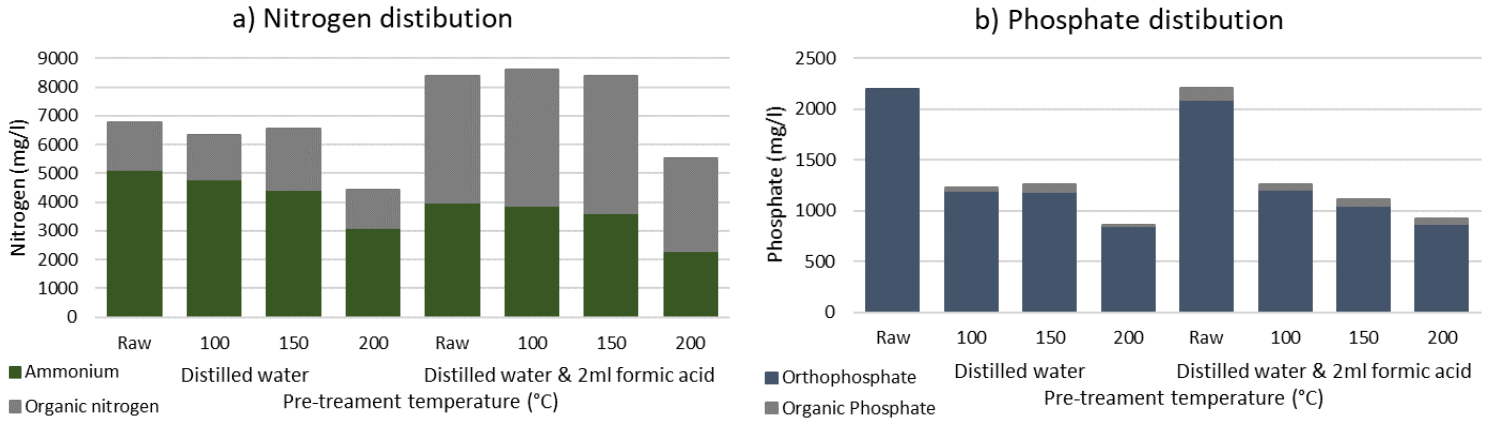


Figure 7-6: a) Nitrogen and b) Phosphate distribution in process waters from HTL of autotrophic *Chlorella vulgaris* with and without formic acid

The total phosphate content of the raw *Chlorella* with 1, 2 and 3ml are very low and contain <2.4mg/l. The total phosphate is higher at 105, 640 and 460mg/l for the *Chlorella* pre-treated at 100, 150 and 200°C respectively. The orthophosphate makes up the larger fraction of the total phosphate content.

There is a big difference between the process waters from the raw *Chlorella* pre-treated with 2ml formic acid and the pre-treated *Chlorella* with 2ml formic acid. The addition of formic acid alters the pH of the processing waters and therefore could be affecting the phosphate content in the process waters too.

A comparison of the nitrogen and phosphate distribution of the process waters from the raw and pre-treated *Chlorella* liquefied with 2ml formic acid and the raw and pre-treated *Chlorella* liquefied with distilled water alone is shown in Figure 7-6. The process waters from liquefaction with distilled water alone contains more ammonium than organic nitrogen, than the process waters from liquefaction with the addition of 2ml of formic acid. This suggests that the addition of formic acid is interacting with the nitrogen compounds and producing more ammonium resulting in more nitrogen being released into the process waters overall. For the phosphate it is shown that the majority of the phosphate is orthophosphate and there is only a little amount of organic phosphate present in both sets of process waters. The process waters from liquefaction with 2ml formic acid have a slightly higher organic phosphate content than the process waters from liquefaction with distilled water alone.

7.3.3. Metal analysis of the process waters from HTL with formic acid

Metal analysis of the process waters was carried out using XRF following the method described in section 3.7.6. Table 7-8 shows the XRF of the process waters from hydrothermal liquefaction of the raw *Chlorella* with 1, 2 and 3ml of formic acid and the pre-treated *Chlorella* with 2ml of formic acid. Although XRF covers a larger range of metals, only the ones present in the process waters are shown.

Table 7-8: XRF of process waters from HTL of autotrophic *Chlorella vulgaris* with the addition of formic acid

		ppm					
Pre-treatment temp (°C)		**Raw autotrophic**			**100**	**150**	**200**
	Formic acid (ml)	**1**	**2**	**3**	**2**	**2**	**2**
Metals (mg/l)	Na	ND	ND	ND	ND	ND	ND
	Mg	443	ND	ND	362	ND	ND
	Al	ND	ND	90	ND	148	ND
	Si	170	151	169	198	164	162
	P	2158	2400	2330	1776	1652	1413
	S	243	247	230	204	206	147
	Cl	259	241	334	134	111	ND
	K	509	567	579	192	170	146
	Ca	182	216	207	221	227	226
	Fe	18	ND	ND	21	ND	21
	Br	ND	ND	ND	ND	ND	ND
	Sr	ND	ND	ND	ND	ND	ND

There is no sodium, bromine or strontium detected in any of the process waters using XRF. Magnesium is only present in the raw *Chlorella* with 1ml of formic acid at 443ppm and the *Chlorella* pre-treated at 100°C with 2ml of formic acid at 362ppm. Aluminium is only present in the process waters from the raw *Chlorella* with 3ml formic acid and the *Chlorella* pre-treated at 150°C with 2ml formic acid. The silicon content of all the process waters is quite similar. The phosphorous content of the process waters from the raw *Chlorella* are higher than the for the pre-treated *Chlorella*. This may be due to the low phosphate content in the process waters from the raw *Chlorella* which suggests that less of the phosphorous is phosphate. The phosphorous and sulphur content shows a decrease with increasing pre-treatment temperature for the process waters from the pre-treated *Chlorella* with 2ml formic acid. The raw *Chlorella* with 2ml formic acid has the highest phosphorous and sulphur content at 2400 and 247ppm respectively. The chlorine and potassium are similar to one another, with a decrease from the raw with 1ml formic acid to the raw with 2ml formic acid and then an increase for the raw *Chlorella* with 3ml formic acid which contains the highest quantity of chlorine at 334 and potassium at 597ppm. There is a decrease with increasing pre-treatment temperature for the process waters from the pre-treated *Chlorella* with 2ml formic acid for both the chlorine and potassium. Iron has not been detected in all the process waters and the ones that do contain iron, only have very small quantities detected.

Figure 7-7 shows a comparison of the metals detected in the process waters from HTL with distilled water alone and with distilled water with the addition of formic acid. There is no Na detected in any of the process waters apart from for the *Chlorella* pre-treated at 100 liquefied in DW, which has a significant amount present. The Mg content is higher in the DW process waters in comparison to the FA process waters, apart from for the *Chlorella* pre-treated at 200°C, which doesn't show any detected. The remainder of the metals are present in similar amounts in both the DW process waters and FA process waters.

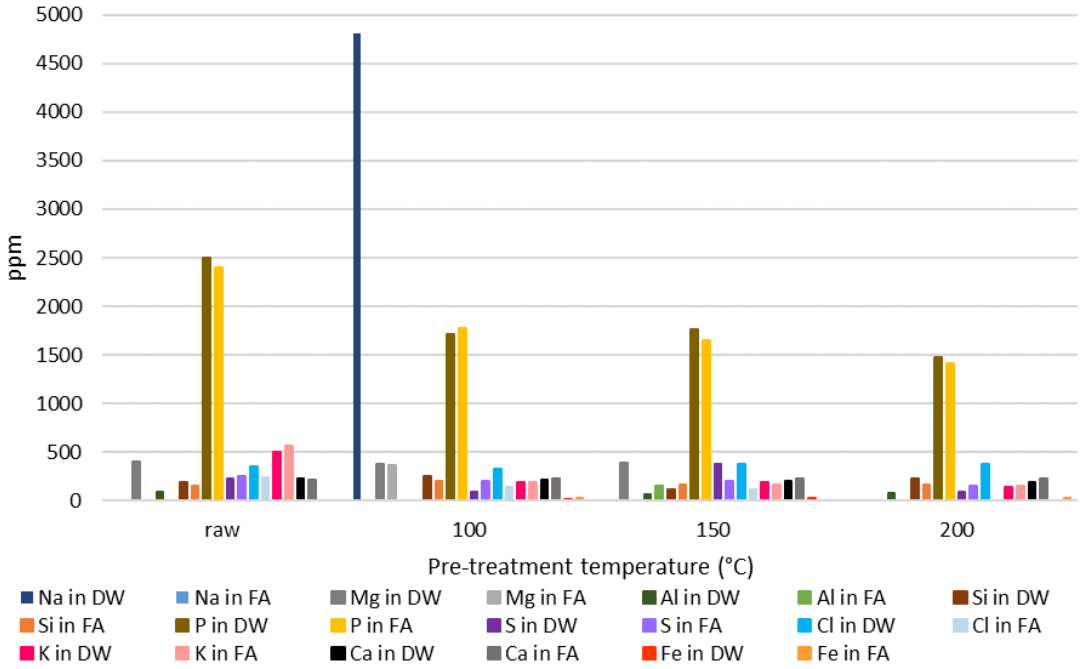


Figure 7-7: Comparison of the metals detected in the process waters from HTL with both distilled water and with the addition of 2ml of formic acid

7.4. Conclusion

Hydrothermal liquefaction of the raw *Chlorella* with the addition of 1, 2 and 3ml of formic acid and hydrothermal liquefaction of the pre-treated *Chlorella* with the addition of 2ml formic acid showed that the bio-crude from the pre-treated *Chlorella* was of better quality for upgrading into biofuels than the bio-crude from the raw *Chlorella* due to some organics and inorganics being removed during pre-treatment.

Comparison of the hydrothermal liquefaction of the raw *Chlorella* with the addition of 1, 2 and 3ml of formic acid and hydrothermal liquefaction of the pre-treated *Chlorella* with the addition of 2ml formic acid found that the *Chlorella* pre-treated at 200°C with 2ml of formic acid had the highest bio-crude yield and the raw *Chlorella* with 2ml of formic acid had the lowest bio-crude yield.

Analysis of the bio-crudes from the 6 different liquefaction combinations found that the pre-treated *Chlorella* with 2ml of formic acid showed the highest hydrogen and lowest nitrogen contents, which are favourable characteristics for the bio-crudes to be upgraded into usable chemicals and biofuels. The bio-crude from the *Chlorella* pre-treated at 150°C with 2ml of formic acid contains the highest levels of phenolics, fatty acids and glycerol, although the other five bio-crudes are not too dis-similar.

Solvent fractionation of the bio-crudes from HTL found that there was a higher amount of pentane insoluble in the bio-crudes from the pre-treated *Chlorella* with the addition of 2ml formic acid, suggesting that more heavy molecular weight material is produced.

Analysis of the process waters from liquefaction of the raw *Chlorella* with the addition of 1, 2 and 3ml of formic acid and hydrothermal liquefaction of the pre-treated *Chlorella* with the addition of 2ml formic acid found that the process water from the pre-treated *Chlorella* with 2ml of formic acid, contained significantly higher quantities of TOC, nitrogen and phosphate than the process waters from the raw *Chlorella*.

Comparison of the bio-crudes from the raw and pre-treated *Chlorella* with 2ml formic acid against the raw and pre-treated *Chlorella* in distilled water alone found that the bio-crude yield was higher for the raw *Chlorella* and the *Chlorella* pre-treated at 100 and 150°C in distilled water alone, whereas the *Chlorella* pre-treated at 200°C with 2ml formic acid showed the highest bio-crude yield of the six bio-crudes. The addition of formic acid also resulted in the increase of fixed carbon content and reduction in the hydrogen content of the bio-crudes. Comparison of the process waters of the raw and pre-treated *Chlorella* with 2ml formic acid against the raw and pre-treated *Chlorella* in distilled water alone showed that the addition of formic acid showed an increase in the organic nitrogen present in the process waters, which suggests that the addition of formic acid is enhancing the release of nitrogen

into the process waters from the *Chlorella* and in turn reducing the amount that is present in the bio-crude.

Overall, the data in this chapter shows that although the addition of formic acid made slight differences to the yield of bio-crude and the quality of the bio-crude produced, the differences were not significant enough to warrant the use of formic acid in further liquefaction experiments. The main reason that there is not much difference with the addition of formic acid may be due to the formic acid affecting the pH, which effects the processing conditions and in turn has a big affect on the fixed carbon content of the bio-crude, which is a major component of producing higher molecular weight material. From this work it is suggested that an alkali additive such as potassium hydroxide may have had a more positive affect on the bio-crude yield and quality as it would make the processing conditions closer to neutral or slightly alkali as there are acids released from the algae during processing which make the processing conditions acidic. Further work is required, taking into account the pH of the processing conditions and the use of acidic or alkali hydrogen donors.

Chapter 8. Recycling of process waters and nutrient recovery

8.1. Introduction

Hydrothermal processing, be it at low temperatures (carbonisation) or higher temperatures (liquefaction), facilitates the release of organics and inorganics into the process waters (Biller and Ross, 2012). This results in the process waters being rich in nutrients, such as phosphate and ammonium, along with salts and metals. These nutrient rich process waters have the potential to be cleaned using carbon adsorbents, to remove the nutrients and allow them to be recycled (Takaya et al., 2016). There is limited literature on the re-cycling and re-use of the aqueous phase from hydrothermal processing of algae. Of the literature that does cover this, the focus is mainly on re-using the aqueous phase for cultivation. This chapter investigates the potential of recycling from hydrothermal pre-treatment into hydrothermal processing both as the aqueous phase is received and also after cleaning using carbon adsorbents.

Carbon adsorbents are heterogenous structures comprised of carbonised organic and inorganic matter. They can be sorbed with volatiles and have functional groups of nitrogen, sulphur and oxygen on the surface, but these are speciality adsorbents. The carbon adsorbents are produced from thermochemical processes such as pyrolysis, gasification and hydrothermal carbonisation, with pyrolysis being the most commonly used and established method. Variations in the processing parameters of these processes such as temperature, heating rate, residence time and pressure, result in different proportions of chars, aqueous and gaseous products.

As a comparison of the products from hydrothermal pre-treatment and the products from hydrothermal liquefaction from both the *Chlorella vulgaris* and the *Ulva lactuca* were undertaken in Chapter 4 and Chapter 5 respectively, this chapter focuses on the process waters from both the hydrothermal pre-treatment and liquefaction stages for both algae.

This chapter aims to address objectives 3 and 5 of this thesis, to investigate the composition of the process waters for the whole experimental procedure, from carbonisation to liquefaction, along with the potential for nutrient recovery and recycling using carbon adsorbents.

The first part of this chapter discusses the production and use of carbon adsorbents to remove problematic components released into the process waters during hydrothermal pre-treatment. Nutrients released into the process waters during the hydrothermal process are also recovered using the carbon adsorbents.

The second part of this chapter investigates the potential of recycling the process waters from the hydrothermal pre-treatment stage, after they have been cleaned by the carbon adsorbents, into the hydrothermal liquefaction stage to investigate the effect of the different aqueous phases on the quality and yield of bio-crude produced.

8.2. Recovery of nutrients from process waters

Bio-chars were considered as a method to remove contaminants and recover nutrients from the process waters as the process waters are rich in products released from the algae during hydrothermal processing. There are a number of reuse options for the aqueous phase from either the pre-treatment or liquefaction steps. These include: recovery of energy via anaerobic digestion or recycling of the process waters to enhance conversion or extraction of useful chemicals. Each of these approaches can be used once the process waters have been treated to remove problematic components. The process waters can also be recycled and used for cultivation of algae, however the ratio of nutrients such as phosphate in the process waters is not ideal for cultivation and requires adjusting. As phosphorous is a finite resource, it is important to be able to recover this from the process waters and reuse it. The removal of nitrogen compounds is also important, firstly from the algae to improve the bio-crude quality and secondly from the process waters to prevent inhibition of growth during cultivation of algae when the process waters are recycled. Bio-chars are well known to adsorb ammonium and phosphate as well as heavy metals. There is also potential for the bio-char to remove higher molecular weight organic macromolecules from the process waters. Extensive work has

previously been performed at the University of Leeds, on the post modification of bio-char to enhance their ability to remove nutrients such as phosphate and ammonia/ammonium. One such modification involves the impregnation of magnesium onto the biochar. This has previously been shown by Takaya et al. (2016) to enhance the adsorption of phosphate and ammonia.

8.2.1. Carbon adsorbents

In this work, the carbon adsorbents are produced from oak wood chips modified by the addition of magnesium chloride and then pyrolysed at 600°C using the method in section 3.6. These conditions were used as previous work by Takaya et al. (2016) showed that they allow the highest uptake of nitrogen and phosphate from process waters from hydrothermal processing of various biomass feedstocks, suggesting that the bio-chars could also be used for this work.

Table 8-1 shows the ultimate and proximate analysis of the unmodified and Mg modified oak bio-char. There are clear differences between proximate analysis of the unmodified oak bio-char and the Mg modified oak bio-char. The reason for this is as the addition of Mg has changed the ratio of the elements from proximate and ultimate analysis.

Table 8-1: Ultimate and proximate analysis of unmodified and Mg modified oak bio-char

		%	Oak unmodified	Mg modified oak
Proximate	(a.r.)	Moisture	9.6	5.8
	(d.b.)	Ash	14.8	29.9
		Volatiles	10.2	11.3
		Fixed Carbon	75.0	58.8
Ultimate	(d.a.f.)	C	83.0	76.7
		H	15.1	4.6
		N	0.4	0.5
		S	0.0	0.0
		O*	1.5	18.2
		HHV (MJ/kg)	49.7	30.3

*Oxygen by difference

The ash content of the Mg bio-char is over double of the unmodified oak, this is due to the added magnesium which forms part of the ash content. The volatiles content are quite similar for both chars. The fixed carbon content of the Mg bio-char is lower than the unmodified char. The ultimate analysis of the bio-chars shows that both chars have a similar carbon content, with the unmodified char being slightly higher. The hydrogen content of the Mg char is significantly lower than the unmodified char. The nitrogen content of both chars are very similar and ≤0.5%. There is no sulphur detected for either sample. The oxygen content of the Mg modified char is significantly higher than the unmodified oak char. This suggests that the oxygen has incorporated into the char structure making it more polar, which would therefore attract polar compounds such as nitrogen, phosphate and metal compounds.

These differences show that the addition of magnesium chloride to the oak is producing bio-chars with difference characteristics than to the unmodified oak. The modified bio-chars are now used to clean the process waters by passing the process waters from hydrothermal pre-treatment through. This is done to investigate the adsorption abilities of the bio-char.

8.2.2. Adsorption of process waters from hydrothermal pre-treatment using Mg modified bio-char

The process waters from hydrothermal pre-treatment are rich in organics, inorganics and nutrients, released from the algae. These process waters are passed through the modified bio-chars to investigate if the bio-chars can remove problematic components and recover nutrients. Adsorption tests were carried out using the magnesium modified bio-chars and the process waters from hydrothermal pre-treatment of *Chlorella vulgaris* and *Ulva lactuca*, using the method described in section 3.6.1.

The total organic carbon, total nitrogen, total ammonium, orthophosphate and phosphate content was analysed for the process waters from hydrothermal pre-treatment of both the *Chlorella vulgaris* and *Ulva lactuca* at the three different temperatures (100, 150 and 200°C) and the process waters from hydrothermal pre-treatment after passing through Mg bio-chars. The additional data can be found in Appendix 6

Figure 8-1 shows the TOC content of the process waters from the *Chlorella* and the *Ulva* at the three different temperatures (100, 150 and 200°C). and the process waters from hydrothermal pre-treatment after passing through Mg bio-chars. For the autotrophic *Chlorella*, the data shows a significant decrease in the TOC content of the process waters at 100°C, whereas the process waters at 150 and 200°C show a much smaller difference before and after passing through the Mg bio-chars. The process waters from the *Ulva* show a small difference between the process waters before and after passing through the Mg bio-chars. The results from both the *Chlorella* and the *Ulva* show that the Mg bio-chars are removing organic carbon from the process waters.

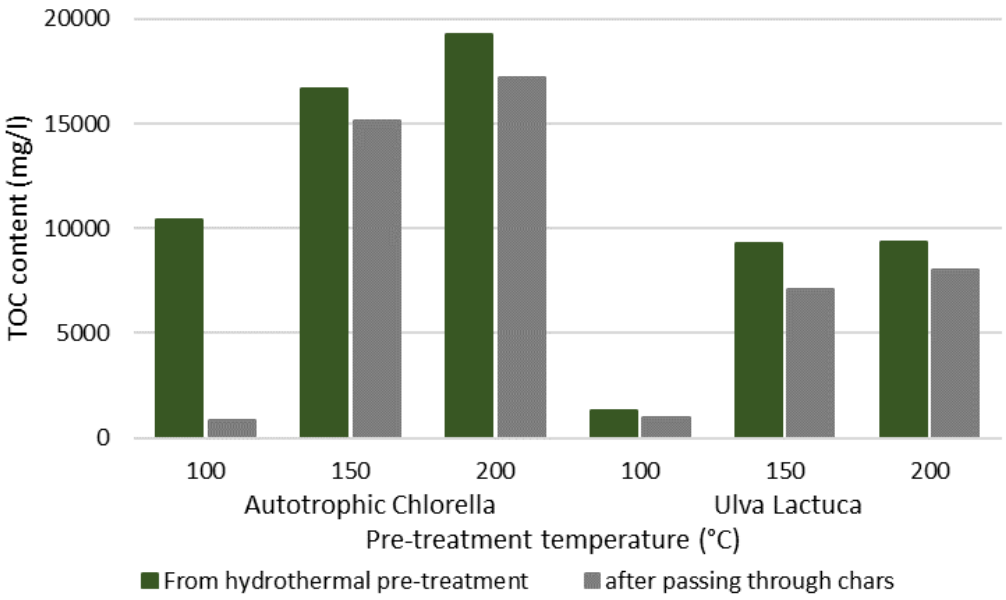


Figure 8-1: TOC content of process waters from hydrothermal pre-treatment and after passing through Mg bio-chars for autotrophic *Chlorella* and *Ulva lactuca*

Figure 8-2 shows a) nitrogen content and phosphate content, of the process waters from the *Chlorella* from hydrothermal pre-treatment and after passing through the Mg bio-chars. Overall there is a reduction in the total nitrogen content of the process water after passing through the Mg bio-chars, with the process water at 100°C showing the biggest reduction. There is an increase in the amount of ammonium present in all three process water samples after passing through the Mg

bio-chars, however, there is a reduction in the organic nitrogen content, which in turn reduces the total nitrogen content of the process waters.

For the phosphate content of the process waters, the total phosphate content increases with increasing pre-treatment temperature, with the majority of the phosphate consisting as orthophosphate. After passing through the Mg bio-chars, the total phosphate content of process waters has been significantly reduced, with the majority of that being orthophosphate.

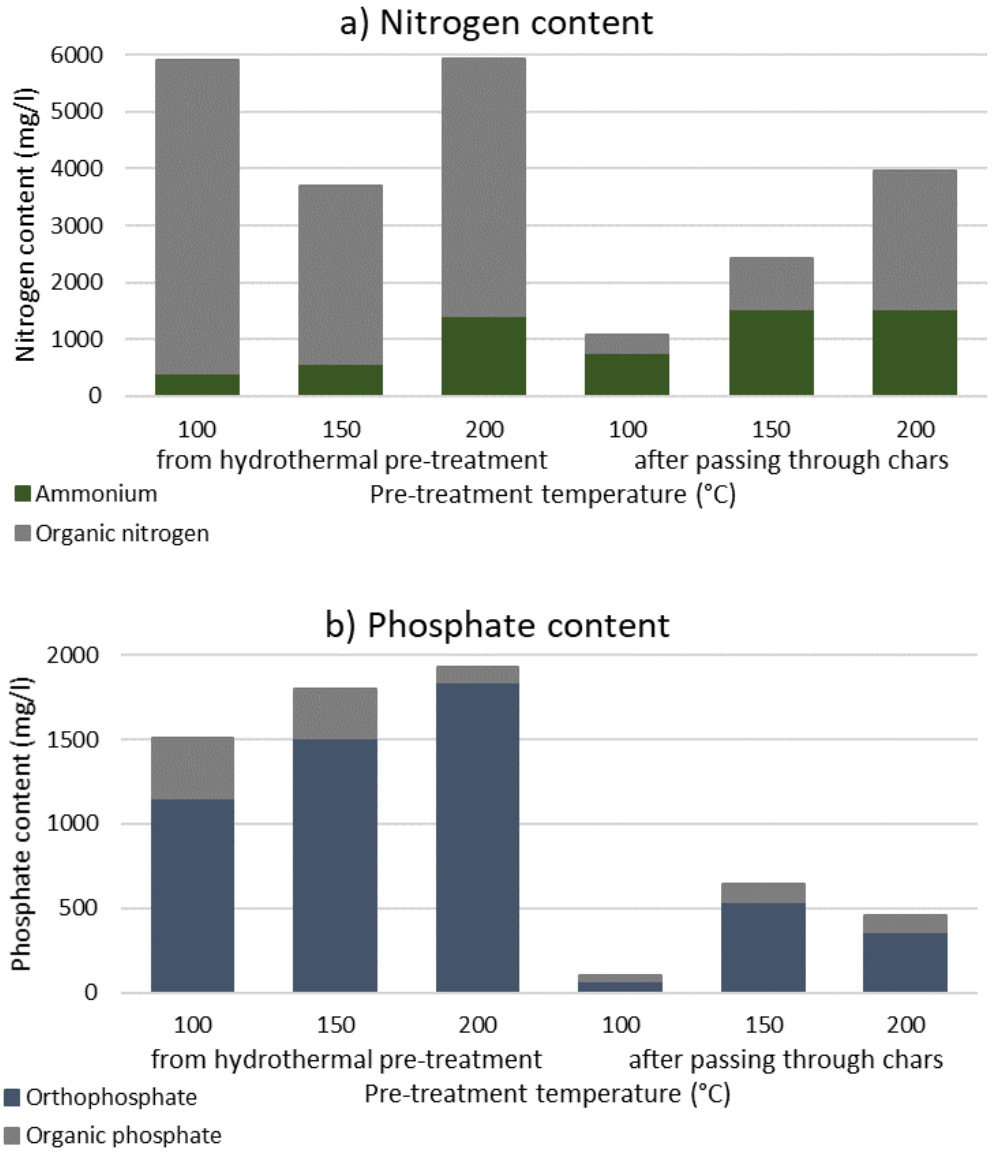


Figure 8-2: a)Nitrogen content and b) Phosphate content of process waters from hydrothermal pre-treatment and after passing through Mg bio-chars for autotrophic *Chlorella*

Figure 8-3 shows a) nitrogen content and phosphate content, of the process waters from the *Ulva* from hydrothermal pre-treatment and after passing through the Mg bio-chars. The total nitrogen and phosphate contents of the process waters from pre-treatment are quite low. This is due to the low initial protein content of the raw *Ulva*. There is a reduction in the nitrogen and phosphate in the process waters after passing through the Mg bio-chars, which shows that they are being removed from the process waters by the chars.

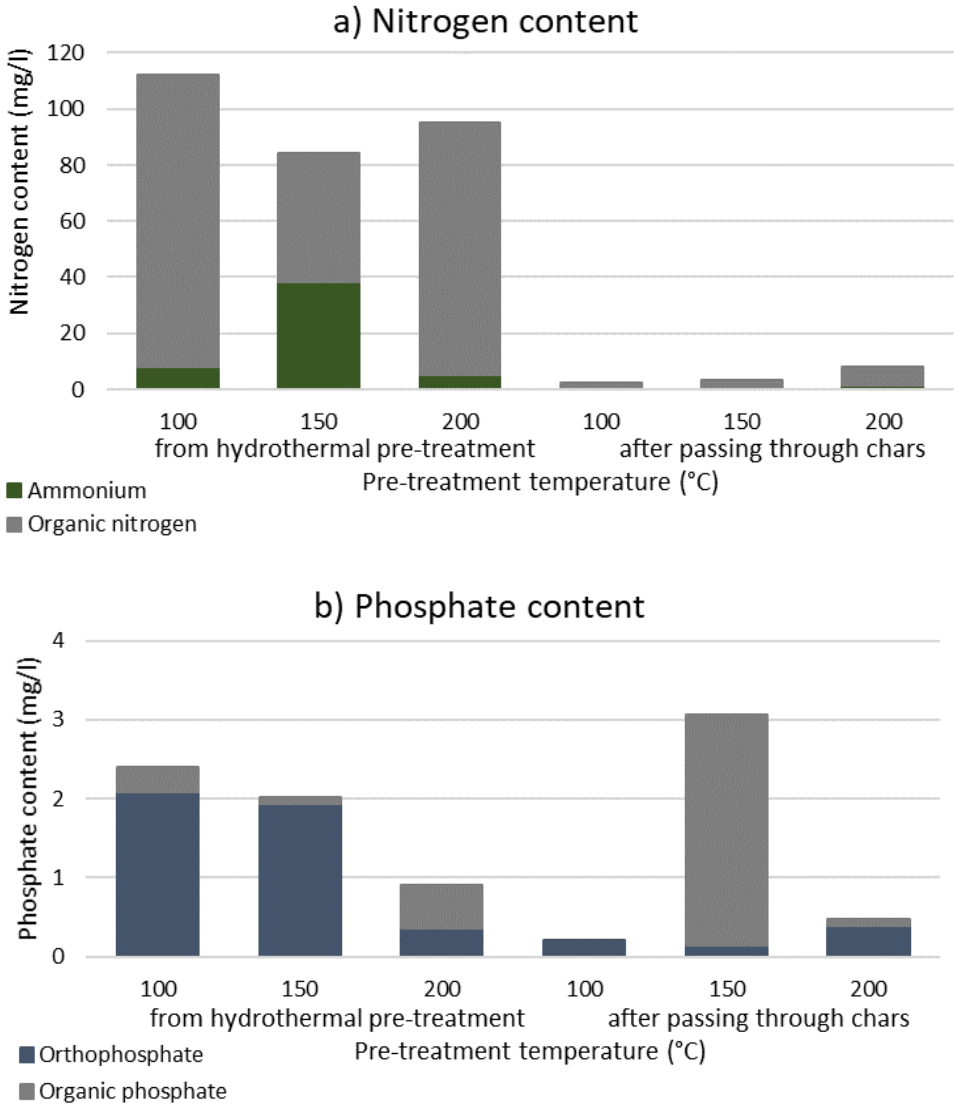


Figure 8-3: a)Nitrogen content and b) Phosphate content of process waters from hydrothermal pre-treatment and after passing through Mg bio-chars for *Ulva lactuca*

Table 8-2 shows the compound classes in the process waters after hydrothermal pre-treatment at 100°C, 150°C and 200°C and after passing through the Mg char, for both the *Chlorella* and *Ulva*, analysed for using an Agilent 7890B GC-MS. The method used is described in section 3.7.5.2. Formic acid, lactic acid and the glucose, fructose, ribose and mannose where analysed for, using the HPLC method described in section 3.7.4. Additional data of the individual compounds can be found in Appendix 7.

Table 8-2: Total compounds from GC-MS analysis of process waters from *Chlorella* and *Ulva* from hydrothermal pre-treatment and after passing through Mg bio-chars

		Autotrophic *Chlorella*			***Ulva lactuca***		
Compounds (mg/l)		**100**	**150**	**200**	**100**	**150**	**200**
From hydrothermal pre-treatment	Acids	11021.0	13648.9	14334.1	197.0	683.7	7057.8
	Nitrogen	1194.2	1540.9	5965.0	1227.0	868.2	1122.6
	Cyclopentanones	176.4	204.6	434.7	182.6	216.4	459.2
	Phenols	0.0	0.0	0.6	0.0	0.0	4.5
	Sugars	0.9	2.7	2.5	0.8	0.6	0.2
After passing through chars	Acids	7167.6	13402.7	18704.0	4860.9	2366.5	7068.6
	Nitrogen	1089.0	1186.5	4393.1	1296.0	892.4	1132.8
	Cyclopentanones	181.4	198.8	396.3	211.7	197.6	454.5
	Phenols	0.0	0.0	0.0	0.0	0.0	1.3
	Sugars	0.0	0.0	0.0	0.0	0.2	0.1

The data from the process waters from hydrothermal pre-treatment of the autotrophic *Chlorella* and *Ulva lactuca*, have previously been discussed in section 4.3.3. In summary, it was stated that the notable acids in the process waters are acetic, levulinic and succinic acid, all of which are present in much higher quantities than the other acids that were analysed for. The nitrogen compounds present in the process waters appear to remain as organic compounds such as nitrogen heterocycles, pyrazines and pyridines, with ionic nitrogen (nitrate, nitrite, ammonia) making up only a relatively small portion of the extracted nitrogen. There are low levels of cyclopentanones, phenols and sugars present in the process waters from hydrothermal pre-treatment for both the *Chlorella* and *Ulva*.

The acids present in the process waters show a reduction for the *Chlorella* pre-treated at 100 and 150°C but show an increase for the *Chlorella* at 200°C and

for the *Ulva* pre-treated at all three temperatures. The acetic acid content increases in the process waters for both the *Chlorella* and *Ulva*, after they have passed through the bio-chars. The levulinic acid content decreases in the process after passing through the bio-chars for the *Chlorella*. However, for the *Ulva*, there is a significant increase in levulinic acid in the process waters from hydrothermal pre-treatment at 100°C, whereas the process waters from hydrothermal pre-treatment at 150 and 200°C show little difference in the levulinic content before and after passing through the chars. The succinic acid content of the process waters after passing through the bio-chars, decrease for the *Chlorella* pre-treated at 100 and 150°C, but shows little difference in the process waters at 200°C. The *Ulva* shows a significant increase in the succinic acid content of the process waters after passing through the bio-chars at 100°C and a slight increase for the process waters at 150°C, whereas the process water at 200°C shows little difference before and after passing through the bio-char. The increase in the acids in the process waters may be due to leaching from the chars that are being used to clean the process waters.

The data also shows that there is a reduction in the nitrogen content of the process waters from the *Chlorella*, however there is not much evidence that the nitrogen content in the *Ulva* samples is being affected. This is also the case for the cyclopentanones, phenols and sugars. However this does not correlate with the total and organic nitrogen reported in Table 8-2. This may be due to the sensitivity of the GC-MS and the UV-vis, which may not detect all of the nitrogen present.

Table 8-3 shows the XRF analysis of the process waters after hydrothermal pre-treatment and after passing through Mg char, for both the *Chlorella* and *Ulva*. The method used is described in section 3.7.6. The data from the process water from hydrothermal pre-treatment of the autotrophic *Chlorella* and *Ulva lactuca*, have previously been discussed in section 4.3.3.

Table 8-3: XRF analysis of process waters from hydrothermal pre-treatment, before and after passing through bio-char

Process water	Sample	Pre-treatment temperature (°C)	ppm											
			Na	Mg	Al	Si	P	S	Cl	K	Ca	Fe	Br	Sr
Before passing through chars	autotrophic *Chlorella*	100	ND	627	120	125	1905	122	256	432	362	ND	ND	ND
		150	ND	ND	133	100	1970	197	240	405	235	ND	ND	ND
		200	ND	370	97	113	2172	334	197	416	298	16	ND	ND
	Ulva lactuca	100	13790	2315	163	158	1093	3103	5341	1749	764	ND	33	9
		150	ND	1720	ND	278	1147	3284	5174	1801	2259	ND	33	ND
		200	ND	2000	93	135	1084	2316	5623	2065	588	24	45	10
After passing through chars	autotrophic *Chlorella*	100	ND	157	92	119	969	ND	474	ND	461	ND	ND	ND
		150	ND	ND	119	121	1052	ND	201	ND	286	16	ND	ND
		200	ND	ND	60	71	1076	ND	496	28	438	ND	ND	ND
	Ulva lactuca	100	ND	676	117	169	1037	61	1139	49	261	ND	ND	ND
		150	ND	ND	146	143	1015	84	1114	60	223	ND	ND	ND
		200	ND	521	ND	102	1001	55	1094	43	243	ND	ND	ND

For the process waters from the *Chlorella*, by passing through the bio-chars, the Mg, Al, P, S, K and Fe content decreases. The Si content is similar in both instances. There is an increase in the Cl and Ca content after passing through the process waters through the bio-chars. The process waters from *Ulva* show a decrease for the Mg, Si, S, Cl, K, Ca, Fe, Br and Sr contents after passing through the bio-char. The reduction of S and Cl could result in a reduction of corrosion and the formation of chlorinated compounds in the final fuel. For the P content, the content is similar in both sets of process waters. There is no Na detected in the process waters apart from for the *Ulva* from pre-treatment at 100°C. The reason for this may be due to the unreliability of the XRF method for detecting Na, which may be due to the Na being the lightest element that can be detected by XRF (Brouwer, 2003). The decrease in the metal content of the process waters shows that bio-chars are trapping the metals from the process waters. Alternatively, atomic absorption spectroscopy would be a better suited method which is commonly used for the detection of Na.

Overall, the bio-chars seems to be removing more of the metals than the organic compounds discussed in the previous section. This is also beneficial as it results in the process waters containing less metals and therefore being less problematic when further utilised. Further investigation into the removal of the metals is required. As there are significant differences shown between the process waters with and without passing through the bio-char, analysis of the bio-char was carried out to determine if there was any notable differences to the bio-char.

Table 8-4 shows the ultimate and proximate analysis of the Mg bio-chars after the process waters have been passed through them. The bio-chars from passing through the process water from both the *Chlorella* and *Ulva* show that the ash and moisture content of the bio-char has decreased.

Table 8-4: Ultimate and proximate of Mg chars after passing process waters through

		Pre-treatment temperature (°C)	(a.r.)	Proximate (d.b.)			Ultimate (d.a.f.)				
			Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*
	Mg char	Raw	5.8	29.9	11.3	58.9	76.7	4.6	0.5	0.0	18.2
After char treatment	Auto *Chlorella*	100	3.0	23.9	16.3	59.8	87.5	2.8	0.6	0.0	9.1
		150	2.4	19.3	14.7	66.0	83.5	2.7	0.5	0.0	13.3
		200	3.2	21.0	16.4	62.6	82.9	2.7	0.6	0.0	13.8
	Ulva lactuca	100	2.9	22.8	15.3	61.9	82.5	2.7	0.5	0.0	14.3
		150	2.5	22.2	13.9	63.9	82.7	2.7	0.5	0.0	14.1
		200	2.5	22.9	13.6	63.4	83.5	2.7	0.6	0.0	13.3

*Oxygen by difference

The volatiles and fixed carbon content of the bio-char has increased. This may be due to some of the organic carbon in the process waters being adsorbed on to the chars, which also correlates with the data in Table 8-2, which shows a decrease in the TOC content of the process waters after passing through the bio-chars. From the ultimate analysis, the carbon content has increased (again this relates to the TOC content), whereas the hydrogen content has decreased. The nitrogen content does not differ much from the original bio-char content. The oxygen content of bio-char has decreased which may be due to the acids leaching from the bio-chars into the process waters. The HHV is similar to the original bio-char. Overall, the ultimate and proximate analysis do not show much difference between the original modified bio-char and the bio-chars after passing the process waters through.

8.3. Recycling of process waters from hydrothermal pre-treatment into hydrothermal liquefaction

Once the process waters from hydrothermal pre-treatment had been passed through the Mg bio-char, they were then considered for the hydrothermal liquefaction stage. The main purpose of using these 'cleaned' process waters is to investigate a closed loop system where the process waters are cleaned using bio-chars and re-used within the cycle, either in the liquefaction stage, for cultivation or to produce something of use (such as being put into anaerobic digestion to produce methane or hydrogen).

Hydrothermal liquefaction was carried out using three different types of water; distilled (DW), process waters from hydrothermal pre-treatment (PW) and process waters from hydrothermal pre-treatment after passing through bio-char (CW), for both the autotrophic *Chlorella vulgaris* and *Ulva lactuca.* Liquefaction was carried out at 350°C using the 25ml bomblet reactors, as described in section 3.5.3 of the methodology chapter.

8.3.1. Yields from hydrothermal liquefaction with different types of processing waters

The yields of bio-crude from hydrothermal liquefaction of the autotrophic *Chlorella* and *Ulva lactuca* with the three different types of process waters (DW, PW and CW) are shown in Table 8-5.

Table 8-5: % bio-crude yields from HTL using different processing waters

Type of algae	Pre-treatment temperature (°C)	Bio-crude (%)		
		Distilled water	Process water	Cleaned water
Auto *Chlorella*	Raw	28.7		
	100	37.0	42.3	39.7
	150	41.3	49.9	44.2
	200	48.8	58.2	52.4
Ulva lactuca	Raw	10.2		
	100	16.4	16.4	17.8
	150	12.8	11.6	15.9
	200	12.0	20.1	20.9

For the autotrophic *Chlorella*, the % bio-crude yield increases from the DW to the PW and then decreases slightly for the CW, but is still higher than the DW yields. This suggests that the Mg bio-char is removing components of the process waters which interact during liquefaction, thus resulting in less interactions and lower bio-crude yield. For the *Ulva lactuca*, there is no difference between the yield from the DW and the PW for the *Ulva* pre-treated at 100°C, but there is a slight increase for the CW. For the *Ulva* pre-treated at 150°C there is a decrease in the bio-crude yield from the DW to the PW, but then an increase for the CW. For the *Ulva* pre-treated at 200°C, there is an increase of the bio-crude yield from the DW to the PW, however there is no difference between the bio-crude yield from the PW and CW.

Overall, using the PW enhances the bio-crude yield for the *Chlorella* but has little effect on the *Ulva*, apart from with the *Ulva* pre-treated at 200°C, which enhances the bio-crude yield.

8.3.2. Analysis of bio-crudes from HTL with different processing waters

Table 8-6 shows the ultimate and proximate analysis of the bio-crudes from hydrothermal liquefaction of both the autotrophic *Chlorella* and *Ulva*, with distilled water (DW), the recycled process waters from hydrothermal pre-treatment (PW) and the cleaned waters (CW) after passing through the Mg bio-char. The bio-crudes for both the autotrophic *Chlorella* and *Ulva* in distilled water, have been discussed previously in section 5.4.

The bio-crudes produced using process waters recycled from hydrothermal pre-treatment and the cleaned process waters, have different characteristics from the bio-crudes produced using distilled water. For the *Chlorella* the moisture content of the bio-crudes from the 100 and 200°C solid residues in PW is higher than the 150°C solid residue which decreases in the PW and CW apart from for the 200°C bio-crude. For the ash content of the bio-crudes produced from the 100 and 150°C solid residue in PW is higher than in DW but shows a decreases for the 200°C solid residue in PW. The ash content then decreases for all three bio-crudes in CW with the bio-crudes from the 100 and 200°C solid residues showing lower ash content than in the DW. The volatiles content increases in the bio-crudes from the 100 and 200°C solid residues but decreases for the 150°C in PW. The volatiles content of the bio-crudes in CW are again higher for the bio-crudes from the 100 and 200°C solid residues but decreases for the 150°C in comparison to the bio-crudes from DW. The fixed carbon content of the bio-crude from the 100 and 200°C solid residues in PW are considerably lower than for their DW counterparts. The 150°C solid residue shows a slight increase in PW but decreases slightly in the CW.

There is a decrease in the carbon content of the bio-crudes in PW and CW, in comparison to the bio-crudes in DW. The bio-crudes in PW and CW show an increase in the hydrogen and nitrogen content. The bio-crudes from DW, PW and CW have very similar sulphur contents. The oxygen content shows a decrease in the bio-crudes from the 100 and 200°C solid residues in PW, but an increase in the 150°C solid residue, whereas the bio-crudes from CW show an increase for the 100 and 200°C solid residues but an decrease in the 150°C. The HHV of the bio-crudes in DW, PW and CW are very similar with the exception of the bio-crude from the 100°C solid residues in CW which is lower.

Table 8-6: Ultimate and proximate analysis of bio-crudes from HTL of micro and macro algae with distilled water, recycled process water and cleaned process water

Algae	Process water	Pre-treatment temperature (°C)	Proximate (d.b.)				Ultimate (d.a.f.)					HHV (MJ/kg^{-1})
			Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*	
autotrophic *Chlorella*	Distilled (DW)	Raw	1.5	3.9	95.3	0.9	73.6	10.8	5.6	0.0	9.9	39.3
		100	1.4	3.0	93.4	3.6	77.4	9.1	5.6	0.2	7.8	38.3
		150	2.6	1.9	98.0	0.1	78.1	10.1	5.2	0.1	6.6	40.1
		200	1.5	3.2	95.5	1.3	77.8	9.7	5.0	0.1	7.5	39.4
	Process water (PW)	100	1.8	4.5	95.0	0.5	77.4	10.4	6.0	0.1	6.1	40.4
		150	1.8	3.2	96.5	0.4	75.9	10.3	5.8	0.2	7.8	39.6
		200	1.9	2.1	97.6	0.3	76.8	10.3	5.6	0.0	7.2	40.0
	Cleaned process water (CW)	100	3.0	11.5	86.5	2.1	65.0	7.7	3.6	0.7	23.1	30.2
		150	2.1	2.6	96.3	1.1	75.0	9.6	4.2	0.1	11.1	37.9
		200	3.3	3.6	94.9	1.6	78.8	9.3	4.4	0.0	7.5	39.1
Ulva lactuca	Distilled (DW)	Raw	6.2	18.3	80.8	0.9	66.5	8.1	3.5	3.3	18.6	40.2
		100	1.2	5.1	92.5	2.4	79.6	10.8	3.5	0.0	6.1	41.8
		150	2.4	6.9	91.8	1.4	79.6	10.8	4.5	0.0	5.1	41.9
		200	1.7	5.4	84.2	10.2	78.7	8.7	4.5	0.2	8.0	38.2
	Process water (PW)	100	1.4	2.6	96.9	0.5	72.7	9.5	6.1	0.4	11.3	36.9
		150	1.4	2.9	96.8	0.3	77.2	10.3	6.0	0.2	6.4	40.2
		200	2.1	2.8	96.4	0.8	76.9	9.7	5.7	0.0	7.7	39.1
	Cleaned process water (CW)	100	3.3	12.1	86.7	1.2	56.1	7.3	3.2	0.5	33.0	25.4
		150	3.5	13.3	86.1	0.6	68.8	9.3	4.0	0.0	17.9	34.4
		200	4.4	7.5	81.9	8.1	82.2	10.5	5.2	0.0	2.2	42.6

*Oxygen by difference

The bio-crudes produced from *Ulva* in PW and CW differ from the bio-crude produced in DW. From the proximate analysis it is shown that the moisture content of the bio-crudes from PW and CW are higher than the bio-crudes from DW, with the exception of the 150°C solid residue in PW which decreases. The bio-crudes from PW show a decrease in the moisture content, whereas the bio-crudes from CW show a significant increase in comparison to the bio-crudes from DW, but are still lower than the raw *Ulva* in DW. The bio-crudes from PW show an increase in the volatiles content, whereas the bio-crudes from the CW show a decrease, however are still higher than the raw *Ulva* in DW. The fixed carbon content of the bio-crudes from the 100 and 150°C solid residue show a slight decrease in PW, with the 200°C solid residue showing a significant decrease in PW compared to in DW. The fixed carbon content of the bio-crudes from CW are slightly lower than the DW.

From the ultimate analysis of the bio-crudes from the *Ulva*, the carbon content of the bio-crudes from the 100 and 150°C solid residues show a significant decrease from DW to PW and CW, with the bio-crude from the *Ulva* pre-treated at 100°C in CW having the lowest content. The hydrogen content of the bio-crudes from all three process waters are similar with the *Ulva* pre-treated at 100°C in CW having the lowest content. The nitrogen content of the bio-crudes from PW are slightly higher than the bio-crudes from DW, but are lower for the bio-crudes from CW, which suggests that some nitrogen is being removed by the Mg bio-chars or is contributing to the nitrogen in the bio-crude. The sulphur contents of the bio-crudes from all three process waters are similar and show a decrease in comparison to the raw *Ulva* in DW. With the values being so low, the sulphur can be considered negligible. The oxygen content increase for the bio-crudes from PW and again for the bio-crudes from CW, with the exception of the *Ulva* pre-treated at 100°C in CW which has the lowest oxygen content overall. The HHV decreases for the bio-crudes from both PW and CW in comparison to the DW.

Overall, there is not a big difference between the bio-crude quality for the *Chlorella* and *Ulva* using the different processing waters, however, based on the data in Table 8-6 it can be assumed that the bio-crudes with the most desirable characteristics were produced from the autotrophic *Chlorella* using PW.

8.3.3. Analysis of process waters from hydrothermal liquefaction using different processing waters

Table 8-7 shows the analysis of the process waters from hydrothermal liquefaction of the autotrophic *Chlorella* and *Ulva* using DW, PW and CW. The TOC content of the process waters from the *Chlorella* shows an increase from the DW to the PW and then to the CW. For the TOC from the *Ulva*, the process water from HTL of the *Ulva* pre-treated at 100°C decreases from DW to PW and then increases again for CW. For the *Ulva* pre-treated at 150°C, the DW increases to the PW and then decreases again for the CW. For the *Ulva* pre-treated at 200°C, the TOC increase from the DW to the PW to the CW.

For the *Chlorella*, the total phosphate content of the process waters increases from the DW to the PW but then shows a decrease from PW to CW. The majority of the total phosphate is orthophosphate which results in the same trend as the total phosphate content in the process waters from HTL. For the *Ulva*, the total phosphate content is quite low and is similar for all three processing waters. The majority of the total phosphate is organic, with very little orthophosphate present. From the results, it can be shown that for the *Chlorella* the CW at 100°C and for the *Ulva* the DW at 100°C, are the best processing waters to use if trying to reduce the phosphate content in the bio-crudes.

The total nitrogen content of the process waters from the *Chlorella*, increases from the DW to the PW and then reduces slightly for the CW HTL process waters. The ammonium and organic nitrogen content are similar quantities, which suggests that the reactions taking place during HTL do not favour either type of nitrogen. The results show increases from the DW to PW and then decrease for the CW for both the ammonium and organic nitrogen content. For the *Ulva*, the total nitrogen content is quite low, however it stills shows a slight increase from the DW to the PW and then a slight decrease from the PW to the CW. The majority of the total nitrogen is organic, with very little ammonium present. From the results, it can be shown that for the *Chlorella* the DW at 200°C and for the *Ulva* the CW at 200°C, are the best processing waters to use if trying to reduce the nitrogen content in the bio-crudes.

Table 8-7: Analysis of process waters from HTL of *Chlorella* and *Ulva* with different processing waters

Algae	Process water	Pre-treatment temperature (°C)	mg/l						
			Total Organic Carbon	Total Nitrogen	Ammonium	Organic Nitrogen	Total Phosphate	Orthophosphate	Organic Phosphate
autotrophic *Chlorella*	Distilled (DW)	Raw	14235.4	2350.0	2310.0	40.0	7400.0	3900.0	3500.0
		100	13600.3	1180.0	1150.0	30.0	6800.0	3740.0	3060.0
		150	14192.2	1150.0	1150.0	0.0	6820.0	3640.0	3180.0
		200	9538.3	700.0	700.0	0.0	4500.0	2380.0	2120.0
	Process water (PW)	100	16542.6	2260.0	2190.0	70.0	9050.0	5180.0	3870.0
		150	16635.7	2400.0	2370.0	30.0	9600.0	5780.0	3820.0
		200	14927.1	2300.0	2200.0	100.0	8900.0	4900.0	4000.0
	Cleaned process water (CW)	100	14841.2	68.9	52.3	16.6	7860.0	4520.0	3340.0
		150	16933.8	440.0	440.0	0.0	8900.0	5260.0	3640.0
		200	15892.6	870.0	840.0	30.0	8650.0	5280.0	3370.0
Ulva lactuca	Distilled (DW)	Raw	6660.0	57.2	5.9	51.3	126.0	8.5	117.5
		100	8537.1	55.5	5.2	50.3	80.6	6.0	74.6
		150	6250.2	61.4	1.7	59.7	120.0	1.6	118.4
		200	3360.9	55.6	0.6	55.0	128.0	38.4	89.6
	Process water (PW)	100	5775.6	57.4	3.4	54.0	131.0	0.0	131.0
		150	8722.0	57.2	2.2	55.0	147.0	4.1	142.9
		200	6167.2	59.8	0.4	59.4	159.0	4.1	154.9
	Cleaned process water (CW)	100	8221.4	61.5	3.3	58.2	130.0	0.3	129.7
		150	7750.9	54.0	3.4	50.6	135.0	0.2	134.8
		200	7480.4	53.8	0.1	53.7	49.2	26.2	23.0

Previous works by Madsen, R. B. et al. (2016), on process waters from hydrothermal liquefaction of micro algae, have found that the nitrogen content in the process water from HTL depends on the protein content of the algae. A study by Gai, C. et al. (2015) states that algae with higher protein content results in higher TN and ammonium content in the process waters. Another study by Li et al. (2017) shows that, algae with low protein content results in lower TN and ammonium content. In most instances, over half of the nitrogen in the algae is released into the process waters during HTL.

Table 8-8 and Table 8-9 show the results of the GC-MS analysis of the process waters from hydrothermal liquefaction of the *Chlorella* and *Ulva* with the three different types of processing water which were analysed using an Agilent 7890B GC-MS following the method described in section 3.7.5.2. Formic acid, lactic acid and the glucose, fructose, ribose and mannose where analysed for, using the HPLC method described in section 3.7.4. Additional data of the individual compounds for both the micro and macro algae can be found in Appendix 8 and 9, respectively.

The data in Table 8-8 shows the total acids, nitrogen and cyclopentanones content of the process waters from HTL of the raw and pre-treated *Chlorella* with DW, PW and CW. The total acid content increases from the 100°C to the 150°C but then reduces again in the 200°C in DW. The acids in PW and CW show a similar trend to one another with a decrease from the 100°C to the 150°C but then increase in the 200°C. The notable acids in the process waters are acetic, Isovaleric, levulinic, succinic and glutaric acid, all of which are present in much higher quantities than the other acids that were analysed for. The acetic acid content differs for the *Chlorella* in DW, PW and CW. The raw *Chlorella* in DW has the lowest acetic acid content at 3091.9mg/l. For the *Chlorella* pre-treated at 100°C, in DW, the acetic acid content is higher than that of the raw. The PW also shows an increase from the raw and DW. There is not much difference between the PW and CW, but there is still an increase. For the *Chlorella* pre-treated at 150°C, in DW, the acetic acid content is higher than that of the raw, and 100°C *Chlorella*. This decreases for the PW and then increases again for the CW, but is still lower than DW. For the *Chlorella* pre-treated at 200°C, in DW, the acetic acid content is higher than that of the raw, and 100°C *Chlorella*, but lower than the 150°C *Chlorella*. The acetic acid content decreases significantly

for the PW and then increases again for the CW but is still lower than the PW. For the Isovaleric acid content, the 100°C *Chlorella* has the highest content in DW, whereas for the 150 and 200°C *Chlorella*, the isovaleric content is highest in the CW.

Table 8-8: GC-MS of process waters after hydrothermal liquefaction of micro algae in distilled water, recycled process waters from pre-treatment and recycled waters from pre-treatment passed through chars

		Pre-treatment temperature (°C)			
Compounds (mg/l)		**Raw**	**100**	**150**	**200**
Distilled water (DW)	Acids	12594.9	29321.7	87857.0	56202.7
	Nitrogen	21114.4	19142.3	16948.9	2614.9
	Cyclopentanones	2642.0	2631.1	2327.8	1958.8
	Phenols	305.0	366.5	372.9	62.2
	Sugars	1.4	2.4	0.8	0.5
Recycled process waters (PW)	Acids	-	55768.8	19241.5	37853.1
	Nitrogen	-	21767.3	32396.1	18747.9
	Cyclopentanones	-	2820.5	3484.8	2303.6
	Phenols	-	493.6	350.4	334.5
	Sugars	-	3.6	0.6	0.7
Process waters after char (CW)	Acids	-	58932.3	48084.3	56056.6
	Nitrogen	-	19535.2	31419.4	13097.9
	Cyclopentanones	-	3254.6	4233.6	2511.1
	Phenols	-	369.0	389.0	417.7
	Sugars	-	0.8	3.4	1.1

The remaining notable acids are present in lower quantities than the acetic acid but are still significantly higher than the remainder of the acids that were analysed for. The levulinic acid content of the raw and pre-treated *Chlorella* in DW is very similar. The results for the pre-treated *Chlorella* in PW and CW are also similar apart from for the *Chlorella* pre-treated at 150°C which is lower. The succinic acid content of the process waters from HTL show that the pre-treated *Chlorella* in DW have the lowest content, with the results from the PW and CW

being higher and similar to the raw *Chlorella* in DW. The Glutaric acid content of the raw *Chlorella* in DW is the highest, whereas for the pre-treated *Chlorella* in DW, PW and CW the glutaric acid content is significantly lower. Overall, it seems as though the acids are concentrating in the CW. This may be due to them being leached from the bio-chars during the HTL process.

The total nitrogen content shows a decrease with increasing pre-treatment temperature for the DW, whereas the PW and CW show an increase from100°C to the 150°C but then reduces again in the 200°C. There are three notable nitrogen compounds present in the process waters from HTL with the different processing waters; pyrazine, methyl-pyrazine and pyrrolidinone. The pyrazine and methyl-pyrazine are present in very similar quantities. For the *Chlorella* in the DW, the raw has the highest content. The *Chlorella* pre-treated at 100 and 150°C have similar amounts present in the DW process waters, whereas the *Chlorella* pre-treated at 200°C is significantly lower. The *Chlorella* pre-treated at 100°C has a similar content in the PW and CW. The *Chlorella* pre-treated at 150°C significantly increases for the PW which is similar for the CW. The *Chlorella* pre-treated at 200°C shows a significant increase from the DW to the PW and then a slight decrease for the CW but is still lower than the raw *Chlorella* in DW. The pyrrolidinone content of the process waters from HTL increase from DW to the PW which are similar to the CW.

There are a lot of nitrogenous compounds presence in the process waters, which could have been produced by the degradation of the proteins and their amino acid components in the algae during HTL. The organic acids present in the process waters could be produced from the degradation of the carbohydrate fraction of the algae during HTL. These results are also confirmed by the findings from a study by Maddi et al. (2016) who investigated the composition of the process waters from HTL of micro algae using GC-MS and found similar amounts of the compounds in the process waters.

The cyclopentanones show little variation between the three different pre-treatment temperatures and the three different processing waters. Of the cyclopentanone compounds, the 3-methyl- 2-Cyclopenten-1-one is present in a notably higher quantity than the other cyclopentanone compounds. The pre-treated *Chlorella* shows an increase from the DW to the PW and then to the CW.

There are low levels of phenols and sugars detected in the process waters after HTL with the three different processing waters (DW, PW and CW).

Overall, the results in Table 8-8, show that there are significant differences in the amount of acids in the three different processing waters, but little difference in the other compounds detected with the *Chlorella* in DW having the lowest contents for all of the compounds. Of the three types of processing waters after HTL, the DW contain the least amount of compounds whereas the PW contains the highest quantity of compounds and the CW falls in the middle of the DW and PW. This suggests that the bio-chars are removing some of the compounds during cleaning of the process waters. As the DW contains the least amount of compounds in the process water after liquefaction, the results suggest that pre-treatment and subsequent use of the bio-chars (as a feedstock for HTL) results in more material being released into the process waters, which would in turn result in cleaner bio-crude from liquefaction with the PW and CW processing waters.

Table 8-9 shows the results of the GC-MS analysis of the process waters from hydrothermal liquefaction of the *Ulva* with the three different types of processing waters.

The total acid content decreases with increasing pre-treatment temperature for the DW and increases with increasing pre-treatment temperature for the PW, whereas the CW shows a decrease from the 100°C to the 150°C but then increases again for the 200°C. Again, the notable acids in the process waters are acetic, Isovaleric, levulinic, succinic and glutaric acid, all of which are present in much higher quantities than the other acids that were analysed for. These four acids follow a similar trend in terms of the content, with a decrease from DW to PW and then increase for the CW which contain the highest content.

The total nitrogen content shows an increase from the 100°C to the 150°C and then decreases again for the 200°C for the DW and PW whereas the CW shows an increase with increasing pre-treatment temperature. The cyclopentanones show a decrease with increasing temperature for all three processing waters. There are three notable nitrogen compounds present in the process waters from HTL with the different processing waters; pyrazine, methyl-pyrazine and pyrrolidinone. For these three nitrogen compounds, the raw *Ulva* in DW has the highest content with the pre-treated *Ulva* in the three different processing waters being significantly lower.

Table 8-9: GC-MS of process waters after hydrothermal liquefaction of macro algae in distilled water, recycled process waters from pre-treatment and recycled waters from pre-treatment passed through chars

Compounds (mg/l)		Pre-treatment temperature (°C)			
		Raw	**100**	**150**	**200**
Distilled water (DW)	Acids	12594.9	14176.7	8772.4	3736.9
	Nitrogen	21114.4	2775.7	2873.4	1102.5
	Cyclopentanones	2642.0	6589.5	2737.5	1958.8
	Phenols	305.0	107.9	85.8	62.2
	Sugars	0.0	0.0	0.3	0.3
Recycled process waters (PW)	Acids	-	10238.9	13037.5	15908.5
	Nitrogen	-	2079.1	2598.1	1773.4
	Cyclopentanones	-	5472.1	3295.0	4290.3
	Phenols	-	104.7	93.3	98.7
	Sugars	-	0.3	5.6	0.3
Process waters after char (CW)	Acids	-	15443.4	12577.5	13841.1
	Nitrogen	-	3809.2	2832.9	1936.8
	Cyclopentanones	-	5938.3	4970.2	4528.7
	Phenols	-	95.2	108.9	94.5
	Sugars	-	0.3	6.3	0.2

Of the cyclopentanones compounds, the 2-methyl- 2-Cyclopenten-1-one and 3-methyl- 2-Cyclopenten-1-one are present in notably higher quantities than the other cyclopentanones compounds. For both the 2-methyl- 2-Cyclopenten-1-one and 3-methyl- 2-Cyclopenten-1-one, the raw *Ulva* in DW contains the lowest content, with the pre-treated *Ulva* in DW showing higher content than the raw in DW. The PW and CW are also higher than the raw *Ulva* in DW, with the CW containing the most 3-methyl- 2-Cyclopenten-1-one.

There are low levels of phenols and sugars detected in the process waters after HTL of the *Ulva* with the three different processing waters (DW, PW and CW).

Overall, the results for the *Ulva* in Table 8-9 follow similar trends to the results in Table 8-8 for the *Chlorella* with significant differences in the acids for the three different processing waters, but little difference in the other compounds, with

the *Ulva* in DW having the highest content of compounds in the process water. Again, of the three types of processing waters after HTL, the CW contain the lowest quantity of compounds, which again suggests that the Mg bio-chars are removing some compounds from the process waters. The results for the *Ulva* also show the same as the *Chlorella* with the DW resulting in the highest quantity of compounds in the process waters after liquefaction, which again suggests pre-treatment and subsequent use of the bio-chars (from hydrothermal pre-treatment as a feedstock for HTL) results in more material being released into the process waters, which would in turn result in cleaner bio-crude from liquefaction with the PW and CW processing waters.

XRF of the process waters was also undertaken and is shown in Table 8-10. For the process waters from HTL of the *Chlorella*, there are definitive differences between the three different process waters. There is an increase for the Si, P, Cl, K and Fe from the DW to the PW but then a decrease for the CW. The three pre-treated *Chlorella* show an increase from DW, to PW to CW for the S content. There is little or no difference between the three different process waters for the Al and Ca content.

For the process waters from the *Ulva*, there is an increase for the Mg, Al, Si, S, Ca and Br from the DW to the PW but then a decrease for the CW. All three temperature algal solid residues show an increase from DW, to PW to CW for the Cl, K content. The P content decreases from DW to PW but increase in CW. All three temperature algal solid residues show a decrease from DW, to PW to CW for the Fe content and the Sr content is similar for all three process waters.

For the *Chlorella*, the results show that most of the metals present follow a similar trend, with the quantity reducing in the PW, but then increasing again for the CW. This shows that the hydrothermal pre-treatment is releasing metals into the process waters and then the bio-chars are adsorbing some of the metals when the process waters are passed through the bio-chars, but are not adsorbing them all. This also correlates with the ash content of the bio-crudes from liquefaction using the different processing waters shown in Table 8-6. The data for the *Chlorella* shows an increase in the bio-crudes from liquefaction with the PW but then a decrease for the bio-crudes in CW.

Table 8-10: XRF analysis of process waters from HTL of *Chlorella* and *Ulva* in different process waters

Process water	Sample	mg/l											
		Na	Mg	Al	Si	P	S	Cl	K	Ca	Fe	Br	Sr
Distilled water (DW)	Raw	ND	ND	125	155	2268	170	290	489	211	ND	ND	ND
	Chlorella 100	ND	ND	109	164	1659	172	ND	224	200	13	ND	ND
	Chlorella 150	ND	ND	ND	158	1638	166	ND	216	211	16	ND	ND
	Chlorella 200	ND	ND	ND	147	1295	306	ND	126	213	ND	ND	ND
	Raw	ND	ND	726	96	157	1037	1642	6929	2559	564	ND	ND
	Ulva 100	ND	ND	78	91	1042	639	1892	968	794	14	ND	ND
	Ulva 150	ND	ND	ND	141	1045	534	1995	761	952	18	ND	ND
	Ulva 200	ND	ND	100	152	1036	239	618	345	723	16	ND	ND
From hydrothermal pre-treatment (PW)	*Chlorella* 100	ND	ND	130	194	2358	306	323	578	199	ND	ND	ND
	Chlorella 150	ND	367	67	177	2409	323	385	624	219	21	ND	367
	Chlorella 200	ND	130	ND	203	2396	236	293	572	211	23	ND	130
	Ulva 100	ND	842	80	150	987	1267	3951	1632	880	ND	ND	842
	Ulva 150	ND	543	151	1024	1012	8094	2967	846	ND	ND	ND	543
	Ulva 200	9789	680	120	263	1083	709	5958	2359	550	17	9789	680
From hydrothermal pre-treatment after passing through char (CW)	*Chlorella* 100	ND	ND	ND	203	1108	881	2247	810	217	ND	ND	ND
	Chlorella 150	ND	ND	82	175	1157	756	1517	621	236	13	ND	ND
	Chlorella 200	ND	ND	ND	153	1426	428	1474	556	188	20	ND	ND
	Ulva 100	ND	827	70	98	1044	641	3829	1552	721	ND	ND	827
	Ulva 150	ND	833	65	125	1037	969	7647	2424	719	ND	ND	833
	Ulva 200	5596	623	ND	183	1018	765	6370	2238	654	ND	5596	623

For the *Ulva* the results show that most of the metals present follow a similar trend, with the quantity reducing in the PW and then decreasing again for the CW. This shows that the hydrothermal pre-treatment is releasing metals into the process waters and then the bio-chars are adsorbing some of the metals when the process waters are passed through the bio-chars, but are not adsorbing as much as for the *Chlorella*, which may be due to the higher initial ash content of the *Ulva*, that could be resulting in saturation of the bio-char thus resulting in less metals being removed from the process waters. This also correlates with the ash content of the bio-crudes from liquefaction using the different processing waters shown in Table 8-6. The bio-crude analysis shows an increase in the ash content of the bio-crudes from liquefaction with the PW and CW in comparison to the bio-crude from DW.

The fate of phosphorous from the algae, in the HTL process, depends on the initial phosphorous and metal content of the algae. Algae with lower levels of Ca, Mg, Cu, Fe and Zn resulting in the majority of the P (>85%) being transferred into the process waters from HTL (Bagnoud-Velásquez et al., 2015; Valdez et al., 2012). Algae with a higher content of Ca, Mg, and Fe results in less P (<30%) being released into the process waters during HTL (Jena, U. et al., 2011). For the process waters from HTL of the *Chlorella*, the P content increases for the PW process waters but then shows a decrease for the CW process waters, which is lower than the content of the DW process waters. For the process waters from the *Ulva*, there is very little difference between the P content of the three different HTL process waters. This may be due to the low initial P content and high metal content of the *Ulva*.

Further investigation of the process waters from HTL was not undertaken in this work, however there are other studies which investigate the use of them. One such study is by Erkelens et al. (2015) who investigated the potential of cultivating *Tetraselmis sp*. using the process waters from HTL after they had been passed through activated carbon.

8.4. Conclusions

The purpose of the work in this chapter was to investigate if passing process waters through Mg modified bio-char could ‘clean’ the process waters and improve

the quality of the bio-crude when subsequently processed using the cleaned process waters for liquefaction of both raw and pre-treated autotrophic *Chlorella* and *Ulva lactuca*.

During hydrothermal pre-treatment, the quantity of the biochemical components are altered depending on the severity of the hydrothermal pre-treatment temperature, which in turn results in more material being released into the process waters. The cleaning of the process waters using the Mg bio-char results in some of the organic and inorganic material in the process waters being adsorbed.

The comparison of the process waters from hydrothermal pre-treatment at 100, 150 and 200°C before and after passing through the Mg modified bio-chars determined that the bio-chars were removing TOC, total nitrogen, ammonium, organic nitrogen, total phosphate, orthophosphate and organic phosphate from the process waters. From the GC-MS analysis of the process waters from hydrothermal pre-treatment, before and after passing through the Mg bio-chars, it is shown that there is little difference in the nitrogen, cyclopentanones, phenols and sugars content, however the acids show some difference. The XRF data showed that the magnesium, silicon, potassium, phosphate and iron contents of the process waters from both algae decreased after passing through the bio-chars.

Hydrothermal liquefaction of the raw and pre-treated *Chlorella* and *Ulva* with the three processing waters (distilled, process water from pre-treatment and cleaned process water from pre-treatment) resulted in varying bio-crude yields. HTL of the *Chlorella* in PW produced the highest bio-crude yield whereas as the CW produced the highest yield for the *Ulva*. There is little difference between the bio-crude quality from liquefaction with the three different processing waters.

Comparison of the three process waters after hydrothermal liquefaction using the three different processing waters (distilled, process water from pre-treatment and cleaned process water from pre-treatment) determined that the PW process waters contained more TOC, total phosphate (of which the majority was orthophosphate) and total nitrogen. The CW process waters have lower levels of TOC, TN and TP in comparison to the PW, but are similar to the DW content except for the TP which shows a significant reduction. From the GC-MS analysis of the process waters from hydrothermal liquefaction with the three processing waters, for the *Chlorella*, it is shown that the acids and cyclopentanones content is the highest in the CW

processing waters, whereas the PW processing waters contain the highest nitrogen contents. For the *Ulva*, the CW processing waters contain the highest content of acids, nitrogen and furanic compounds. The phenols and sugars are present in similar quantities in all three processing waters for the *Chlorella* and again for the *Ulva*. The XRF data shows that the majority of the metals follow a similar trend with lower values in the PW than the DW but higher content in the CW.

Overall, the use of the Mg bio-chars results in organics and inorganics being extracted from the process waters after hydrothermal pre-treatment. The use of the three different processing waters during liquefaction of the *Chlorella* shows that the PW contains the most TOC, phosphate and nitrogen for the processing waters whereas the *Ulva* shows that the CW contains the highest TOC and phosphate, however the PW contains the most nitrogen. Of the three processing waters, the PW gives the highest bio-crude yield for the *Chlorella*, whereas the CW gives the highest for the *Ulva*. Although the yields are higher, the quality of the bio-crude is not necessarily better. The best processing waters for the bio-crudes with the more desirable characteristics are DW for the *Chlorella* and PW for the *Ulva*. Although the bio-char does clean the process waters, it doesn't make much difference to the quality of the bio-crude produced.

Chapter 9. Conclusion and Future Work

9.1. Summary

The introduction of this thesis identifies the increasing need for alternative energy sources due to the depletion of fossil fuels and their effect on climate change. Biofuel production from various biomass has become an established method for alternative fuels. In particular, algae has been identified as a feedstock of great interest for third generation biofuels due to their rapid growth and lipid accumulation rates, although the cost of cultivation is still a drawback. Another main drawback of producing biofuels from algae is the high nitrogen and phosphate content of the bio-oil. This requires upgrading of the bio-oil from algae to make the bio-oil into a usable fuel. Hydrothermal pre-treatment has been identified as a step that can be implemented before conversion, which can result in bio-oil of an improved quality, which requires less upgrading than bio-oil from direct conversion of the algae.

The overall aim of this project was to develop approaches for improving the quality and yields of biofuels derived from algae. Hydrothermal pre-treatment was implemented to remove problematic components from the algal feedstocks (e.g. salts, nitrogen and other heteroatoms) before conversion. An assessment was made on the influence of pre-treatment on subsequent downstream processing of the algae and also on approaches to remove and recover valuable nutrients from the algae in a form which allows its reuse. A range of algae with different biochemical content including phototrophic and heterotrophic micro algae and macro algae were considered. Three conversion routes were investigated; pyrolysis, solvent extraction and hydrothermal liquefaction, which produced bio-oils of varying quality, with the bio-oils from hydrothermal liquefaction showing the most difference between the raw and pre-treated algae. This resulted in hydrothermal liquefaction becoming the focussed upon conversion method throughout the thesis.

9.2. Review of objectives

This section reviews the objectives set out in the introduction chapter and discusses to what extent they have been achieved.

The first objective was to conduct a literature review of previous work on pre-treatment and conversion of algae into biofuels. The review of the literature in Chapter 2 revealed that there has been a lot of work recently carried out on conversion of both raw and pre-treated algae, with various pre-treatment techniques used, however hydrothermal pre-treatment was rarely used.

The second objective was to investigate the influence of hydrothermal processing for pre-treatment of both micro and macro algae. This was conducted at three different temperatures to determine the appropriateness of the products. This was covered in chapter 4 with particular focus placed on the yields of products, the fate of heteroatoms, the fate of biochemical components and the fate of mineral content in the process. In the first section of Chapter 4 the raw algae was characterised. It was found that of the 5 different algae, the *Chlorella vulgaris* contained the highest lipid content and the *Ulva* contained the lowest. In the second section of the chapter, a comparison of common micro algae (autotrophic *Chlorella vulgaris*) and macro algae (*Ulva lactuca*) was undertaken. It was found that yields of the solid algal residue from the autotrophic *Chlorella* were significantly higher than the yields from the *Ulva*. It was also found that hydrothermal pre-treatment lowers the inorganics and ash content of both algae, resulting in a higher energy dense solid residue. It was decided that further hydrothermal pre-treatment would be carried out on the three other micro algae; heterotrophic *Chlorella vulgaris*, *Spirulina platensis* and *Chlorogloeopsis fritschii*, at 150°C as initial tests showed the most difference between the autotrophic *Chlorella* and *Ulva* at this temperature, which is investigated in the last section of Chapter 4. Again, it was found that the solid algal residue has a higher energy density than the raw algae and problematic components, such as inorganics, were released into the process waters. It was necessary to establish the pre-treatment temperatures in the beginning of the thesis for use throughout the remainder of the experiments.

Overall, the second objective was met as different pre-treatment temperatures were tested for a variety of algae. The biochemical content of the raw

and pre-treated algae differed after pre-treatment. Furthermore, a comparison of the pre-treatment of these algae has not been undertaken previously.

The third objective was to investigate the potential of nutrient recovery and recycling from the process waters from hydrothermal pre-treatment of the autotrophic *Chlorella* and *Ulva* (from the second objective). This was covered in the first section of Chapter 8. Mg modified bio-chars were produced based on findings from previous work in the literature which found that Mg modified bio-chars had the ability to remove problematic components from hydrothermal process waters from various feedstocks. The process waters from Chapter 4 were analysed before and after passing through the Mg modified bio-chars to determine if organic carbon, ammonium, organic nitrogen, orthophosphate and organic phosphate were being removed. It was found that the Mg bio-chars were removing all of these components from the process waters. GC-MS analysis was also undertaken on the process waters before and after passing through the Mg bio-chars and found that there was a noticeable difference in the acids content, whereas there was little difference in the nitrogens, cyclopentanones, phenols and sugars contents. XRF analysis of the process waters also showed that metals such as Mg, silicon, potassium, phosphate and iron were being removed by the Mg bio-char.

Overall, the third objective was met as there is a significant difference between the process waters before and after passing through the Mg bio-chars, with problematic components such as nitrogen, phosphate and metals being removed from the process waters.

Once it was established that hydrothermal pre-treatment was improving the composition of the algal feedstock, suitable conversion methods for the production of oils, from the solid algal residues from hydrothermal processing, were investigated in Objective 4. Pyrolysis, solvent extraction and hydrothermal liquefaction were the conversion methods investigated which were chosen based on their suitability for wet feedstock. For the purpose of determining which conversion method was the most suitable, a comparison was again undertaken of the autotrophic *Chlorella* and *Ulva lactuca* with both the raw and pre-treated algae. It was found that for all three of the conversion methods *Chlorella* produced the highest oil yield. The quality of the oils from pyrolysis and solvent extraction were of a lower yield and quality than those from hydrothermal liquefaction. This is due to the whole

algae being converted during liquefaction, whereas there was solid residue remaining after conversion by both pyrolysis and solvent extraction. There is also significantly less ash and fixed carbon in the bio-crude from liquefaction in comparison to the oils from pyrolysis and solvent extraction. From this analysis it was evident that upgrading was required to convert the bio-oils into useable fuels.

A calculation of the amount of hydrogen required to upgrade the bio-oils from the three conversion routes was undertaken in the final section of Chapter 5. Per g of oil, the solvent extracted oils require the most hydrogen, whereas per kg of algal feedstock, the bio-crude from HTL requires the most hydrogen. Although the amount of hydrogen required for upgrading the bio-crude from HTL is higher than the oils from the other two conversion routes, it still contains less inorganics and fixed carbon. Therefore, from this comparison it was decided that further investigations in the thesis would be undertaken with conversion by hydrothermal liquefaction and with micro algae.

Overall, the fourth objective was met, as hydrothermal liquefaction was chosen as a conversion method, based on the criteria set out in Chapter 5. However there is still scope to improve the investigation including a number of factors such as the type of pyrolysis used (fast/slow), the type of solvent used during solvent extraction and the type of reactor and heating/cooling rate of the hydrothermal liquefaction.

Objective 5 investigated the influence of different process variables such as temperature, feedstock type and additives on hydrothermal liquefaction of the algae, by comparing the raw and pre-treated micro algae and is split across chapters 6 and 7.

Chapter 6 investigates hydrothermal liquefaction of the four micro algae with varying biochemical composition for both the raw algae and pre-treated at 150°C. Liquefaction was carried out at 350°C in small bomblet reactors. The bio-crudes from the pre-treated algae were found to have lower fixed carbon, hydrogen and nitrogen contents, which suggests that less upgrading is required. Pentane fractionation of the bio-crudes showed that the pre-treated algae contained more pentane solubles than the bio-crude from the raw algae, which suggests pre-treatment is allowing more material to be broken down into lighter molecular weight material during liquefaction.

Chapter 7 investigates the influence of the addition of formic acid to the hydrothermal liquefaction process, with focus on just the autotrophic *Chlorella vulgaris*. The addition of the formic acid required adjustments to be made to the liquefaction temperature to keep within the confines of the reactor capabilities and was decreased to 300°C. 1, 2 and 3 ml of formic acid was added to the liquefaction of the raw *Chlorella*. It was found that the addition of 3ml formic acid obtained the highest bio-crude yield. 2ml of formic acid was also added to the liquefaction of the *Chlorella* pre-treated at 100, 150 and 200°C. It was found that the yield of bio-crude produced from both raw and pre-treated *Chlorella*, increased in comparison to liquefaction in distilled water alone, except for the *Chlorella* pre-treated at 100°C. Overall, the bio-crudes from the pre-treated *Chlorella* with 2ml formic acid have a higher hydrogen content, and lower nitrogen content than the raw *Chlorella* with 1, 2 and 3ml formic acid, which suggests that the pre-treated *Chlorella* have better characteristics for use as biofuels than the raw *Chlorella*. Pentane fractionation of the bio-crudes showed a higher amount of the pentane insoluble fraction with the addition of the formic acid, which suggests that more heavy molecular weight material is produced due to the addition of the formic acid. The addition of potassium hydroxide instead of formic acid may have given better results.

Overall, objective 5 was met across the two chapters, as the variables that were investigated showed that the yields and quality of the bio-crudes from liquefaction changed and were improved.

Objective 6 was to investigate the potential of using various process waters during hydrothermal liquefaction. This was covered in Chapter 8, using a comparison of both autotropic *Chlorella* and *Ulva lactuca*. A review of the literature found that bio-chars could be used as adsorbents for stripping out components from process waters. Based on this search, it was decided that bio-chars modified with magnesium would be used to ‘clean’ the process waters from hydrothermal pre-treatment. The Mg modified bio-chars were shown to adsorb some of the organic and inorganic material from the process waters such as the nitrogen, phosphate, organic carbon and metals. Thus, resulting in ‘cleaned’ process waters which have the potential to be reused.

A comparison was then conducted for hydrothermal liquefaction of the raw and pre-treated algae with three different processing waters; distilled water, process

water from hydrothermal pre-treatment and cleaned process water from hydrothermal pre-treatment. The results from this comparison were mixed and did not show a clear benefit of using bio-chars to clean the process waters. The objective was met as the different processing waters were utilised in the liquefaction process, and there were some improvements to the yields and quality of the bio-crude, although there was no clear trend for both the micro and macro algae.

The overall aim was met through the objectives set out in the introduction, as some improvements were made to the yield and quality of the bio-crudes produced from algae. However, there are some improvements that could be made to the way the experiments were conducted.

9.3. Limitations

There are some limitations to the work which should be noted and adapted for future research.

The first is the range of types of algae used. Although 5 different algae were used, they may not have been the most representative of the different types of algae available or be the correct type to be utilised for liquid biofuels. There was also a limitation based on the quantity of the algae available for this work, which limited the amount and types of experiments undertaken.

There were three different types of hydrothermal reactors used throughout the thesis, all of which had different heating and cooling rates. This affects the quantity of the different product fractions and also has an effect on the quality of the products produced as further reactions could be taking place whilst the reactor is slowly cooling down.

There were two GC-MS analysers used in this work, as mentioned in the methodology chapter. Different products were analysed on the different analyser and some figures were much lower than expected. Therefore, the results from the GC-MS analysis may not be accurate and would need to be repeated, but with the time constraints and the global situation with COVID-19 it was not possible to do this.

Although there are some limitations to the work, there is the possibility to reduce these by carrying out further work.

9.4. Future work

A continuing interest throughout the thesis has been the utilisation of the process waters. Although the process waters were recycled from the hydrothermal pre-treatment stage and re-used in the hydrothermal liquefaction stage there is still the issue of what happens to the process waters next. One approach that could be taken is the continuous recycling of the process waters between the hydrothermal pre-treatment stage and the hydrothermal liquefaction step, however, this would require a lot of cleaning. Another approach would be to utilise the process waters for cultivation of the algae or into other processes such as anaerobic digestion. This would also reduce the cost of disposing of the excess process waters.

The Mg bio-chars utilised in this study were chosen based on their functionality for adsorbing nitrogen and phosphate. Although the Mg bio-chars adsorbed certain materials out of the process waters, alternative carbon adsorbents with different functionalities could be investigated, which could vary the material removed from the process waters and hence alter the characteristics of the process waters. The addition of bio-chars to the liquefaction process could also be investigated as previous work shows that the bio-char works as a catalyst and alters the ratio of high and low molecular weight material in the oil.

Another area of interest is the addition of additives to the hydrothermal liquefaction process. Although the addition of formic acid was investigated in this study, there are other additives that could be used in its place. For example, potassium hydroxide would make the algal feedstock slurry more base instead of acidic.

The most persistent challenge in the process was the analysis of the oils. As the oils consist of high molecular weight material, the chromatography techniques employed in this thesis may not have been well suited for their analysis. Alternative chromatography techniques that could be employed are High Temperature Gas Chromatography (HTGC) (Philp et al., 2004) or LC-MS (Ito et al., 2013) which could improve the results. Other alternative techniques could also be employed for analysing the oils such as NMR (Sarpal et al., 2016) and Xray absorption near edge structure (XANES) analysis (Mitra-Kirtley et al., 1993).

Finally, a socio-techno--economic assessment of the process is recommended to investigate the life cycle of the process, including the amount of energy required for the process. An investigation into the feasibility of scaling the process up and also of applying to an existing bio-refinery is also recommended.

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Appendix 1 –Cultivation trial

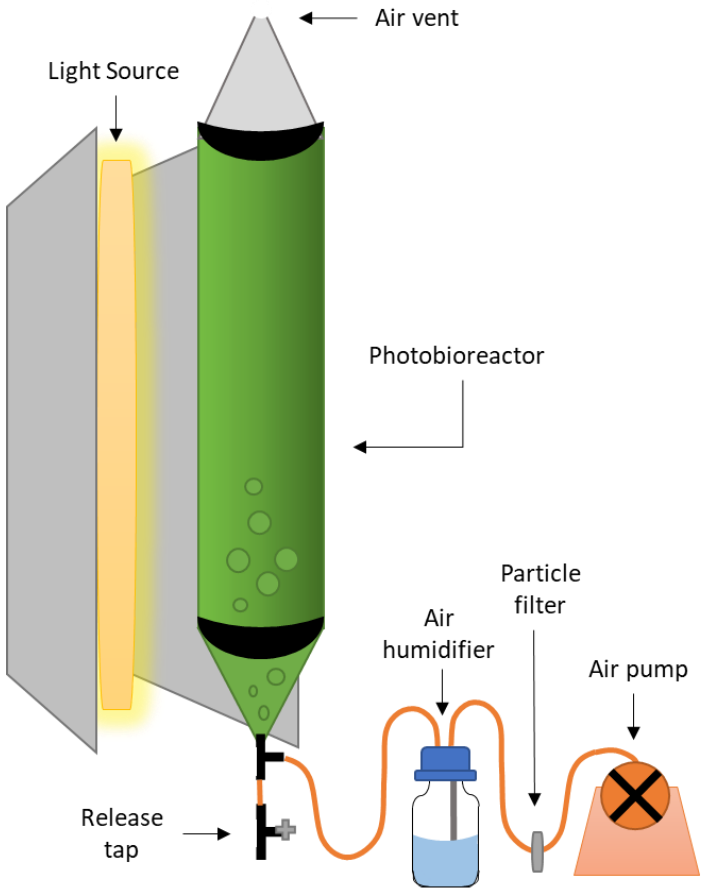
An axenic strain of freshwater *Chlorella vulgaris* (strain 211/11B) was obtained from the Culture Collection of Algae and Protozoa (SAMS Research Services Ltd, Scottish Marine Institute, Oban, Scotland) and cultivated autotrophically. The growth media used to cultivate the *Chlorella vulgaris* was bold's basal medium (BBM) and is outlined in table below. To create the BBM, 10ml of stocks 1-6 and 1ml of stocks 7-10 were added to a 1 litre duran flask and made up to 1 litre with either distilled or deionised water.

Bold's Basal Medium for cultivation of *Chlorella vulgaris*

Stocks	Nutrients	g per litre
1	$NaNO_3$	25
2	$MgSO_4.7H_2O$	7.5
3	NaCl	2.5
4	K_2HPO_4	7.5
5	KH_2PO_4	17.5
6	$CaCl_2.2H_2O$	2.5
7 Trace elements solution (autoclave to dissolve)	$ZnSO_4.7H_2O$	8.82
	$MnCl_2.4H_2O$	1.44
	MoO_3	0.71
	$CuSO_4.6H_2O$	1.57
	$Co(NO_3)_2.6H_2O$	0.49
8	H_3BO_3	11.42
9	EDTA	50.0
	KOH	31.0
10	$FeSO_4.7H_2O$	4.98
	H_2SO_4 (conc)	1.0

The cultures were firstly cultivated in 300ml conical flasks on a shaker bed inside an incubator on a 12 hour light and 12 hour dark cycle, over a 1 week period. At the end of the 1 week period, the high density of cells was transferred to

centrifuge tubes, spun down into a pellet and the spent media removed. The pelleted cells were transferred to a 2 litre photobioreactor with fresh media. This set-up is shown in the figure below. The same conditions were used for the photobioreactors with the bubbling of air through the photobioreactors added. The same process was employed to retrieve the cells from the photobioreactors. The retrieved cells were re-seeded into photobioreactors to continue the cultivation process.



Set-up of the micro algae cultivation equipment at the University of Leeds

20g of glucose was added to one of the photobioreactors to attempt to change the growth parameters from autotrophic to mixotrophic and eventually heterotrophic. This was added in excess to make sure there was enough carbon present. However, once the glucose was added, the contents of the photobioreactors did not grow as expected. The reason for this is as it seems as though the sample became contaminated and there was some bacterial growth. Therefore, the heterotrophic *Chlorella vulgaris* used was supplied by the University of Dakota instead

Appendix 2 – Compounds in process waters after hydrothermal pre-treatment of macro algae (*Ulva lactuca*) and autotrophic micro algae (*Chlorella vulgaris*) at 100, 150 and 200°C

		mg/l					
	Compound	**Macro**			**Micro**		
		100°C	**150°C**	**200°C**	**100°C**	**150°C**	**200°C**
Acids	Formic acid	ND	1.44	ND	ND	0.2	ND
	Lactic acid	ND	ND	ND	2.5	1.5	7.6
	Acetic acid	ND	1.7	705.5	274.7	126.0	6155.9
	Butyric acid	ND	ND	3.2	1.0	0.5	53.4
	Crotonic acid	ND	ND	1.0	1.3	0.8	ND
	Isovaleric acid	ND	ND	ND	0.9	0.8	44.5
	Pent-4-enoic acid	6.0	4.9	4.1	9.2	8.9	25.0
	Valeric acid	ND	35.7	19.7	73.2	6.3	43.2
	3-methyl-Pentanoic acid	ND	ND	ND	0.6	ND	15.5
	4-methyl-Pentanoic acid	ND	ND	ND	2.6	1.0	32.6
	Hex-5-enoic acid	3.0	3.0	24.1	11.9	12.4	41.3
	Malonic acid	13.6	17.5	15.9	23.5	21.1	16.1
	2-methyl-pent-2-enone acid	25.3	14.8	14.4	34.2	39.1	7.4
	Methyl Malonic acid	5.3	25.8	16.2	5.4	4.6	280.4
	Levulinic acid	53.2	169.6	763.9	6394.7	8104.7	1086.8
	Fumaric acid	3.5	14.0	302.0	11.5	39.7	186.6
	Succinic acid	13.5	189.0	4648.5	3965.9	5028.2	5989.1
	Benzoic acid	ND	3.5	8.4	1.9	4.8	4.4
	Glutaric acid	32.8	102.9	418.5	123.5	143.4	184.4
	Phenyl- Acetic acid	8.7	9.5	10.9	14.0	17.2	37.3
	Adipic acid	21.3	18.9	21.1	19.7	23.4	23.8
	Hydrocinnamic acid	10.8	9.3	10.2	9.1	10.5	44.5
	Propanetricarboxylic acid	ND	62.1	70.4	39.7	53.6	54.5
Nitrogen compounds	Pyrazine	52.2	45.8	42.6	40.3	27.9	2261.7
	Pyrazine, methyl-	107.5	93.3	212.2	118.3	133.3	2265.1
	2,5-dimethyl-Pyrazine	7.3	6.3	8.6	6.6	7.5	26.3
	Ethyl-Pyrazine	9.8	8.2	12.4	9.3	10.2	80.2
	Trimethyl- Pyrazine	9.3	8.7	16.5	9.5	11.1	87.5
	Pyrrolidinone	893.2	577.1	692.6	669.3	877.8	710.8
	3-Hydroxypyridine monoacetate	147.6	128.9	137.9	124.2	152.9	212.7
	L-proline	ND	ND	ND	216.7	320.4	320.7
Cyclopentanones	Cyclopentanone	5.5	24.6	23.9	26.0	29.8	26.5
	2-methyl-2-Cyclopenten-1-one	29.5	25.6	29.6	26.0	29.1	106.9
	3-methyl-2-Cyclopenten-1-one	101.4	110.0	355.8	85.7	100.4	251.3
	2,3-dimethyl-2-Cyclopenten-1-one	46.1	56.3	50.0	38.7	45.2	50.0

Phenols	Phenol	ND	ND	3.7	ND	ND	0.6
	p-Cresol	ND	ND	0.9	ND	ND	ND
Sugars	Glucose	0.1	0.3	ND	0.3	2.0	ND
	Fructose	0.7	0.3	0.2	0.6	0.7	0.3
	Ribose	ND	ND	ND	ND	ND	2.2
	Mannose	ND	ND	ND	ND	ND	ND

Appendix 3 - Total organic carbon, nitrogen, ammonium, orthophosphate and phosphate content of process waters from pre-treatment of macro and micro algae at 100, 150 and 200°C

		mg/l						
Type of algae	Pre-treatment temperature (°C)	Total Organic Carbon	Total Nitrogen	Ammonium	Organic nitrogen	Total Phosphate	Ortho-phosphate	Organic Phosphate
Ulva lactuca	100	1323.8	112.0	7.9	104.1	2.4	2.1	0.3
	150	9281.2	84.2	38.2	46.0	2.0	1.9	0.1
	200	9340.1	94.9	5.0	89.9	0.9	0.4	0.6
autotrophic *Chlorella*	100	8725.3	5920.0	404.0	5516.0	105.0	70.0	35.0
	150	16637.1	3700.0	574.0	3126.0	640.0	540.0	100.0
	200	19774.4	5940.0	1416.0	4524.0	460.0	360.0	100.0

Appendix 4 - Proximate and Ultimate analysis of the HTL oils of the raw and pre-treated micro algae

	Type of algae	Proximate % (d.b.)				Ultimate % (d.a.f.)					HHV (MJ/kg^{-1})
		Moisture	Ash	Volatiles	Fixed Carbon	C	H	N	S	O*	
Raw	Auto *Chlorella*	1.5	3.9	95.3	0.9	77.7	11.4	5.9	0.0	4.9	42.2
	Hetero *Chlorella*	3.4	6.9	82.6	10.4	85.5	7.6	5.4	0.1	1.5	39.7
	Spirulina platensis	4.0	0.5	88.4	11.1	79.4	0.5	6.9	0.6	12.6	26.1
	Chlorogloeopsis fritschii	3.8	1.7	84.2	14.1	80.2	1.8	6.8	0.6	10.6	28.5
Pre-treated	Auto *Chlorella*	2.6	1.9	98.0	0.1	74.7	9.6	4.9	0.1	10.7	37.8
	Hetero *Chlorella*	2.2	2.0	92.2	5.8	79.6	0.8	3.8	0.1	15.6	26.1
	Spirulina platensis	5.9	0.4	89.5	10.0	80.3	0.5	6.8	0.6	11.8	26.4
	Chlorogloeopsis fritschii	4.4	2.1	85.1	12.8	81.0	2.4	7.0	0.4	9.2	29.7

*Oxygen by difference

Appendix 5 - Compounds in the process waters from GC-MS analysis from HTL of autotrophic Chlorella vulgaris with the addition of formic acid

	Formic acid added (ml)	1	2	3	2	2	2
	Pre-treatment temp (°C)	Raw autotrophic			100	150	200
	Compounds (mg/l)						
Acids	Formic acid	ND	ND	2529.9	ND	ND	2744.3
	Lactic acid	3956.2	6614.2	1720.0	ND	ND	1628.9
	Acetic acid	1111.5	1022.7	999.2	916.5	916.8	712.3
	Butyric acid	23.9	21.5	28.9	19.3	17.7	17.4
	Crotonic acid	1.3	ND	ND	ND	2.9	ND
	Isovaleric acid	230.6	187.8	155.7	200.4	217.6	253.4
	Pent-4-enoic acid	ND	ND	ND	ND	ND	ND
	Valeric acid	10.2	10.4	7.3	10.6	12.5	12.8
	3-methyl-Pentanoic acid	159.0	138.6	117.3	169.1	176.0	129.1
	4-methyl-Pentanoic acid	191.0	173.8	125.6	155.3	233.4	144.5
	Hex-5-enoic acid	20.4	ND	ND	ND	5.2	ND
	Malonic acid	ND	ND	ND	ND	ND	ND
	2-methyl-pent-2-enone acid	ND	ND	ND	ND	ND	ND
	Methyl Malonic acid	ND	ND	ND	ND	ND	ND
	Levulinic acid	48.3	20.6	96.1	106.7	116.0	75.5
	Fumaric acid	ND	ND	ND	ND	ND	ND
	Succinic acid	107.9	130.5	122.3	100.9	131.9	102.2
	Benzoic acid	ND	ND	ND	ND	ND	8.2
	Glutaric acid	ND	39.6	ND	ND	ND	ND
	Phenyl- Acetic acid	ND	ND	ND	ND	ND	ND
	Adipic acid	ND	ND	ND	ND	ND	ND
	Hydrocinnamic acid	70.8	81.3	79.8	79.6	87.5	73.9
	Propanetricarboxylic acid	ND	ND	ND	ND	ND	ND
Nitrogen compounds	Pyrazine	3.5	ND	ND	ND	ND	ND
	Pyrazine, methyl-	8.2	6.0	ND	ND	ND	ND
	2,5-dimethyl-Pyrazine	16.8	ND	ND	ND	ND	ND
	Ethyl-Pyrazine	2.6	ND	ND	ND	ND	ND
	Trimethyl- Pyrazine	ND	ND	ND	ND	ND	ND
	Pyrrolidinone	ND	ND	ND	ND	ND	ND
	3-Hydroxypyridine monoacetate	2721.1	2799.5	2399.0	2343.3	2291.2	ND
	L-proline	ND	ND	ND	ND	ND	ND
Cyclopentanones	Cyclopentanone	24.9	11.6	6.4	9.6	6.4	13.8
	2-methyl-2-Cyclopenten-1-one	67.9	25.2	11.0	26.0	22.5	17.2
	3-methyl-2-Cyclopenten-1-one	42.3	38.1	ND	ND	ND	ND
	2,3-dimethyl-2-Cyclopenten-1-one	25.7	19.3	15.4	18.3	17.7	22.1
P	Phenol	31.2	32.1	32.5	29.8	36.7	31.4

	p-Cresol	16.0	16.6	17.6	16.2	20.1	18.5
Sugars	Glucose	ND	ND	3321.6	ND	212.9	119.4
	Fructose	ND	261.7	159.3	93.4	82.6	ND
	Ribose	ND	ND	587.1	ND	ND	ND
	Mannose	ND	ND	569.8	ND	ND	359.5

Appendix 6 - Analysis of process waters after hydrothermal pre-treatment and after passing through Mg bio-chars

Type of algae	Passing through chars	Pre-treatment temperature (°C)	mg/l						
			Total Organic Carbon	Total Nitrogen	Ammonium	Organic Nitrogen	Total Phosphate	Orthophosphate	Organic Phosphate
autotrophic *Chlorella*	Before	100	10380.2	5920.0	404.0	5516.0	1150.0	1510.0	360.0
		150	16637.1	3700.0	574.0	3126.0	1510.0	1800.0	290.0
		200	19274.3	5940.0	1416.0	4524.0	1840.0	1930.0	90.0
	After	100	866.8	1068.0	772.0	296.0	70.0	105.0	35.0
		150	15141.5	2420.0	1534.0	886.0	540.0	640.0	100.0
		200	17193.0	3960.0	1538.0	2422.0	360.0	460.0	100.0
Ulva lactuca	Before	100	1323.8	112.0	7.9	104.1	2.1	2.4	0.3
		150	9281.2	84.2	38.2	46.0	1.9	2.0	0.1
		200	9340.1	94.9	5.0	89.9	0.4	0.9	0.6
	After	100	967.3	2.1	0.1	2.0	0.2	0.2	0.0
		150	7079.4	3.5	0.4	3.1	0.1	3.1	2.9
		200	7989.9	8.0	1.2	6.8	0.4	0.5	0.1

Appendix 7 - GC-MS analysis of process waters from *Chlorella* and *Ulva* from hydrothermal pre-treatment and after passing through chars

	Compound	From hydrothermal pre-treatment before treatment through char (mg/l)						After passing through chars (mg/l)					
		autotrophic *Chlorella*			*Ulva lactuca*			autotrophic *Chlorella*			*Ulva lactuca*		
		100	150	200	100	150	200	100	150	200	100	150	200
Acids	Formic acid	ND	0.2	ND	ND	1.4	ND	ND	ND	ND	ND	ND	ND
	Lactic acid	2.5	1.5	7.6	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Acetic acid	274.7	126.0	6155.9	ND	1.7	705.5	800.5	2039.6	10514.8	553.9	1604.0	823.1
	Butyric acid	1.0	0.5	53.4	ND	ND	3.2	2.4	11.0	69.7	7.4	ND	5.1
	Crotonic acid	1.3	0.8	0.0	ND	ND	1.0	1.5	3.6	2.2	1.4	3.0	0.9
	Isovaleric acid	0.9	0.8	44.5	ND	ND	ND	ND	ND	55.8	1.1	ND	ND
	Pent-4-enoic acid	9.2	8.9	25.0	6.0	4.9	4.1	8.2	8.7	90.9	18.6	5.6	6.1
	Valeric acid	73.2	6.3	43.2	ND	35.7	19.7	61.0	107.9	41.0	4.9	25.3	18.5
	3-Methyl-,pentanoic acid	0.6	ND	15.5	ND	ND	ND	1.0	ND	15.1	0.6	ND	ND
	4-Methyl-pentanoic acid	2.6	1.0	32.6	ND	ND	ND	2.7	ND	31.7	1.9	ND	ND
	Hex-5-enoic acid	11.9	12.4	41.3	3.0	3.0	24.1	9.1	11.2	39.5	9.6	3.2	24.4
	Malonic acid	23.5	21.1	16.1	13.6	17.5	15.9	20.1	23.6	14.9	18.8	17.0	16.8
	2-Methylpent-2-enone acid	34.2	39.1	7.4	25.3	14.8	14.4	34.4	35.3	21.9	26.6	23.7	13.2
	Methyl Malonic acid	5.4	4.6	280.4	5.3	25.8	16.2	5.0	5.8	273.0	5.9	28.7	15.2
	Levulinic acid	6394.7	8104.7	1086.8	53.2	169.6	763.9	3758.4	6741.6	1053.5	2509.3	184.8	782.5
	Fumaric acid	11.5	39.7	186.6	3.5	14.0	302.0	3.8	8.3	172.5	4.0	11.9	282.4
	Succinic acid	3965.9	5028.2	5989.1	13.5	189.0	4648.5	2325.8	4180.0	5972.6	1543.6	322.6	4592.5
	Benzoic acid	1.9	4.8	4.4	ND	3.5	8.4	ND	1.9	5.5	1.5	2.6	7.8

	Glutaric acid	123.5	143.4	184.4	32.8	102.9	418.5	72.6	128.1	179.5	82.6	61.4	418.6
	phenyl- Acetic acid	14.0	17.2	37.3	8.7	9.5	10.9	11.3	15.4	35.1	12.6	7.7	11.6
	Adipic acid	19.7	23.4	23.8	21.3	18.9	21.1	20.3	22.1	21.8	27.6	19.5	21.9
	Hydrocinnamic acid	9.1	10.5	44.5	10.8	9.3	10.2	9.9	10.1	41.8	11.4	9.3	10.5
	Propanetricarboxylic acid	39.7	53.6	54.5	ND	62.1	70.4	19.5	48.3	51.3	17.3	36.3	17.5
Nitrogen compounds	Pyrazine	40.3	27.9	2261.7	52.2	45.8	42.6	42.2	31.2	1160.0	50.4	48.9	31.5
	Pyrazine, methyl-	118.3	133.3	2265.1	107.5	93.3	212.2	95.9	101.4	1895.6	108.1	94.8	196.2
	2,5-dimethyl-Pyrazine	6.6	7.5	26.3	7.3	6.3	8.6	6.7	6.9	23.8	7.1	6.3	8.9
	Ethyl-Pyrazine	9.3	10.2	80.2	9.8	8.2	12.4	8.6	8.9	67.9	9.7	8.4	11.7
	2,3-dimethyl-Pyrazine	9.5	11.1	87.5	9.3	8.7	16.5	9.0	9.9	79.7	9.4	8.5	15.9
	Trimethyl- Pyrazine	669.3	877.8	710.8	893.2	577.1	692.6	693.1	617.3	659.3	896.7	596.6	727.0
	Pyrrolidinone	124.2	152.9	212.7	147.6	128.9	137.9	131.1	138.8	191.3	148.2	128.9	141.7
	3-Hydroxypyridine monoacetate	216.7	320.4	320.7	ND	ND	ND	102.4	272.0	315.6	66.4	ND	ND
	L-proline	26.0	29.8	26.5	5.5	24.6	23.9	15.6	28.0	87.1	18.5	14.0	26.0
Cyclopentanones	Cyclopentanone	26.0	29.1	106.9	29.5	25.6	29.6	26.3	28.2	32.9	29.7	26.1	29.9
	2-methyl-2-Cyclopenten-1-one	85.7	100.4	251.3	101.4	110.0	355.8	98.7	99.4	230.8	117.3	105.3	347.2
	3-methyl- 2-Cyclopenten-1-one	38.7	45.2	50.0	46.1	56.3	50.0	40.8	43.3	45.6	46.3	52.2	51.4
	2,3-dimethyl- 2-Cyclopenten-1-one	ND	ND	0.6	ND	ND	3.7	ND	ND	ND	ND	ND	0.2
Phenols	Phenol	ND	ND	ND	ND	ND	0.9	ND	ND	ND	ND	ND	1.1
	p-Cresol	0.3	2.0	ND	0.1	0.3	ND	ND	ND	ND	ND	0.2	ND
Sugars	Glucose	0.6	0.7	0.3	0.7	0.3	0.2	ND	ND	ND	ND	ND	ND
	Fructose	ND	ND	2.2	ND	ND	ND	ND	ND	0.0	ND	ND	ND
	Ribose	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mannose	ND	0.2	ND	ND	1.4	ND	0.0	ND	ND	ND	ND	ND

Appendix 8 - GC-MS of process waters after hydrothermal liquefaction of micro algae in distilled water, recycled process waters from pre-treatment and recycled waters from pre-treatment passed through chars

Micro algae – Auto Chlorella		Distilled water (DW)				Recycled process waters (PW)			Process waters after char (CW)		
Compounds (mg/l)		Raw	100	150	200	100	150	200	100	150	200
Acids	Formic acid	ND	ND	1.4	ND	2.4	ND	ND	ND	ND	ND
	Lactic acid	ND	ND	1.7	ND	1.3	ND	ND	ND	ND	ND
	Acetic acid	3091.9	22289.3	77439.1	53374.1	45193.9	13513.5	32293.5	49260.1	33833.0	47705.0
	Butyric acid	9.6	615.0	1976.9	10.3	1151.6	126.7	109.7	1129.4	2817.4	302.5
	Crotonic acid	14.7	147.1	372.6	ND	290.6	99.7	87.2	255.4	643.3	239.9
	Isovaleric acid	216.2	1581.1	3637.2	7.2	2922.7	1079.9	667.2	2762.5	5526.6	1588.2
	Pent-4-enoic acid	24.2	24.6	19.0	20.8	29.4	29.0	25.1	22.4	30.8	36.8
	Valeric acid	68.5	65.4	63.8	25.1	124.2	88.2	102.2	79.2	103.0	160.0
	3-Methyl-,pentanoic acid	291.1	352.1	354.9	27.1	439.6	418.4	334.5	383.6	485.3	413.5
	4-Methyl-pentanoic acid	584.0	705.9	711.5	55.5	881.3	838.7	670.7	769.1	972.5	828.8
	Hex-5-enoic acid	64.9	80.5	101.7	31.7	211.3	107.0	184.1	92.3	129.6	344.4
	Malonic acid	16.8	105.8	16.4	12.7	25.4	18.6	23.1	16.9	18.7	32.4
	2-Methylpent-2-enone acid	12.2	52.0	4.1	8.4	12.6	10.1	8.0	14.6	14.1	12.5
	Methyl Malonic acid	351.8	221.5	150.4	162.7	349.0	302.0	277.5	336.5	380.4	347.0
	Levulinic acid	1353.6	1181.8	1059.2	1105.5	1342.2	187.8	1071.2	1292.2	201.9	1330.1
	Fumaric acid	14.8	12.1	12.3	16.9	12.4	11.8	9.9	13.5	12.7	10.4

	Succinic acid	1217.2	913.9	938.7	867.3	1416.0	1223.1	968.0	1340.8	1502.9	1329.6
	Benzoic acid	19.9	24.6	30.3	13.2	60.9	38.6	42.5	32.7	49.5	80.2
	Glutaric acid	4810.0	444.0	467.5	374.7	632.6	561.4	444.2	590.2	682.3	618.8
	phenyl- Acetic acid	127.2	146.8	149.3	20.3	207.1	179.1	166.0	176.7	226.8	237.6
	Adipic acid	37.5	60.5	44.7	17.2	66.9	53.6	55.9	47.3	59.4	52.1
	Hydrocinnamic acid	256.1	286.5	294.1	40.7	382.8	342.3	301.3	305.6	383.3	375.9
	Propanetricarboxylic acid	12.9	11.2	10.3	11.4	12.4	12.0	11.4	11.2	10.8	10.9
Nitrogen compounds	Pyrazine	7299.5	6230.8	5741.1	145.8	5047.9	10302.2	4138.7	5202.8	8703.5	1800.7
	methyl-Pyrazine	8112.6	6915.0	5955.7	232.4	7229.1	10394.0	5120.5	7102.3	10508.9	3083.8
	2,5-dimethyl-Pyrazine	130.1	102.3	88.4	8.9	130.7	170.3	96.6	129.4	186.8	71.7
	Ethyl-Pyrazine	300.2	280.2	233.0	11.0	282.8	396.2	190.1	255.8	409.2	105.4
	2,3-dimethyl-Pyrazine	135.0	103.0	87.4	ND	137.2	190.7	94.6	138.7	217.3	63.3
	Trimethyl- Pyrazine	124.3	153.7	139.1	12.0	173.9	147.1	137.0	145.8	168.2	167.2
	Pyrrolidinone	4865.8	5169.7	4540.1	2085.6	8618.4	10650.0	8793.8	6352.4	11098.7	7627.7
	3-Hydroxypyridine monoacetate	146.9	187.4	164.1	119.2	147.3	145.6	176.5	208.0	126.9	178.1
Cyclopentanones	Cyclopentanone	25.6	25.7	21.1	21.8	23.7	431.0	20.8	191.0	487.5	37.0
	2-methyl-2-Cyclopenten-1-one	687.0	607.9	541.3	417.6	607.9	653.0	499.6	671.1	787.5	478.1
	3-methyl- 2-Cyclopenten-1-one	1734.0	1803.9	1594.4	1360.9	1931.2	2136.8	1575.1	2147.5	2655.2	1755.2
	2,3-dimethyl- 2-Cyclopenten-1-one	195.3	193.6	170.9	158.6	257.6	264.1	208.1	245.0	303.4	240.9
Phenols	Phenol	192.2	215.9	213.7	54.9	293.6	213.5	202.1	215.7	227.1	250.7
	p-Cresol	112.9	150.6	159.1	7.3	200.1	136.8	132.4	153.3	161.9	167.0
Sugars	Glucose	1.0	1.3	0.6	0.3	3.3	0.5	ND	0.8	1.9	0.7
	Fructose	ND	0.5	0.2	0.2	0.1	0.1	0.2	ND	0.3	ND
	Ribose	ND	ND	ND	ND	0.3	ND	0.5	ND	0.4	0.4
	Mannose	0.4	0.6	ND	ND	ND	ND	ND	ND	0.9	ND

Appendix 9 - GC-MS of process waters after hydrothermal liquefaction of macro algae in distilled water, recycled process waters from pre-treatment and recycled waters from pre-treatment passed through chars

Macro algae – *Ulva lactuca*		Distilled water (DW)				Recycled process waters (PW)			Process waters after char (CW)		
Compounds (mg/l)		Raw	100	150	200	100	150	200	100	150	200
Acids	Formic acid	ND	ND	ND	0.1	ND	ND	ND	ND	ND	ND
	Lactic acid	ND	ND	16.6	8.2	18.6	28.0	18.6	17.4	18.2	15.0
	Acetic acid	3091.9	8081.3	4575.2	1366.5	5408.8	6859.6	9249.4	9144.6	6603.4	7037.0
	Butyric acid	9.6	67.0	41.9	10.3	31.3	35.0	68.2	34.3	46.4	26.1
	Crotonic acid	14.7	ND	ND	ND	ND	ND	0.1	0.3	ND	1.2
	Isovaleric acid	216.2	26.3	22.0	7.2	18.6	23.2	36.2	40.9	34.3	25.9
	Pent-4-enoic acid	24.2	270.8	22.4	20.8	20.6	19.3	442.7	486.4	28.1	24.3
	Valeric acid	68.5	39.9	27.3	25.1	33.2	38.3	41.9	39.9	43.8	49.8
	3-Methyl-,pentanoic acid	291.1	58.3	52.3	27.1	45.2	53.6	46.6	57.7	62.4	55.5
	4-Methyl-pentanoic acid	584.0	54.1	106.0	55.5	ND	0.6	49.5	5.5	62.9	0.6
	Hex-5-enoic acid	64.9	29.0	31.5	31.7	25.0	46.0	50.7	19.8	49.8	58.1
	Malonic acid	16.8	14.5	13.9	12.7	13.7	15.0	15.0	13.8	15.0	15.0
	2-Methylpent-2-enone acid	12.2	6.6	15.1	8.4	6.5	10.9	28.2	6.1	6.4	19.5
	Methyl Malonic acid	351.8	422.9	233.7	162.7	330.2	321.8	310.7	396.7	356.5	383.4
	Levulinic acid	1353.6	1616.8	906.9	639.0	1267.6	1235.5	1194.9	1516.8	1367.7	1465.4
	Fumaric acid	14.8	40.7	28.1	16.9	33.8	37.1	30.5	37.0	40.9	34.2
	Succinic acid	1217.2	2584.3	1900.8	867.3	2234.7	3326.1	3388.1	2791.9	2877.3	3547.0
	Benzoic acid	19.9	14.9	19.2	13.2	15.7	27.0	28.7	15.6	29.4	33.3
	Glutaric acid	4810.0	717.0	640.0	374.7	626.8	829.8	789.0	694.1	795.4	921.6
	phenyl- Acetic acid	127.2	25.6	22.5	20.3	17.8	19.9	20.7	24.0	24.1	25.8

	Adipic acid	37.5	20.6	18.7	17.2	19.5	20.8	20.1	19.3	20.8	19.2
	Hydrocinnamic acid	256.1	74.0	66.6	40.7	59.7	76.5	63.2	68.9	78.0	70.1
	Propanetricarboxylic acid	12.9	12.3	11.9	11.4	11.5	13.2	15.5	12.4	16.5	12.8
Nitrogen compounds	Pyrazine	7299.5	270.2	953.9	145.8	203.9	482.6	227.1	968.1	289.6	246.0
	methyl-Pyrazine	8112.6	1359.8	1071.7	232.4	860.4	1084.5	523.7	1632.3	1284.3	558.8
	2,5-dimethyl-Pyrazine	130.1	32.0	30.4	8.9	24.0	26.9	15.3	42.3	37.1	18.1
	Ethyl-Pyrazine	300.2	39.7	39.7	11.0	27.5	30.9	12.7	47.3	43.6	8.3
	2,3-dimethyl-Pyrazine	135.0	8.0	6.1	ND	0.9	5.2	ND	24.0	15.1	0.0
	Trimethyl- Pyrazine	124.3	59.1	41.3	12.0	42.3	40.8	25.7	77.2	60.9	28.8
	Pyrrolidinone	4865.8	869.4	602.9	573.1	772.3	801.2	829.1	891.9	950.0	931.8
	3-Hydroxypyridine monoacetate	146.9	137.6	127.3	119.2	147.7	126.0	140.0	126.1	152.4	144.9
Cyclopentanones	Cyclopentanone	25.6	254.5	23.4	21.8	212.8	20.7	415.6	456.3	469.2	231.9
	2-methyl-2-Cyclopenten-1-one	687.0	1208.6	714.5	417.6	923.2	651.7	684.8	1093.6	959.9	775.1
	3-methyl- 2-Cyclopenten-1-one	1734.0	4652.9	1753.9	1360.9	3948.1	2318.6	2856.5	3952.9	3139.8	3154.3
	2,3-dimethyl- 2-Cyclopenten-1-one	195.3	473.6	245.6	158.6	388.0	304.0	333.5	435.5	401.3	367.3
Phenols	Phenol	192.2	91.2	74.5	54.9	87.5	82.8	87.8	81.5	95.4	84.2
	p-Cresol	112.9	16.8	11.3	7.3	17.2	10.5	11.0	13.7	13.5	10.4
Sugars	Glucose	ND	ND	ND	0.1	ND	5.2	ND	ND	5.0	ND
	Fructose	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Ribose	ND	ND	0.3	0.2	0.3	0.4	0.3	0.3	0.4	0.2
	Galactose	ND	ND	ND	ND	ND	ND	ND	ND	0.9	ND