

CRANFIELD UNIVERSITY

ALEJANDRO MOURE ABELENDA

STUDY OF THE EFFECT OF THE ULTRASONIC  
PRETREATMENT ON THE HYDROTHERMAL LIQUEFACTION OF  
MICROALGAE

SCHOOL OF ENERGY, ENVIRONMENT, AND AGRIFOOD

MSc by RESEARCH

Supervisor: Dr Philip Longhurst and Dr Beatriz Fidalgo  
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## ABSTRACT

The depletion of the reserves of fossil fuel promotes the search for sustainable renewable sources of energy. Due to their similarities with the petroleum products, the biofuels represent a better alternative than other clean energies. The conversion process greatly depends on the biomass composition, which is a topic of debate, especially when it increases the price of the food. Therefore, third generation biofuels, derived from algae, are better accepted by society than using other raw materials. Due to the way of cultivating and the high moisture content of microalgae, hydrothermal liquefaction (HTL) is the transformation technology most suitable for this type of feedstock.

Even when the application of HTL to microalgae is quite recent, a lot of research is being done because of the good expectations for biofuel production. In this way, the best operating conditions have already been determined, they are the critical point of water (374 °C and 221 bar), and the research is focussing now in the improvement of the quality of the products and the upscaling to a continuous process. The most important product is the biocrude, which has a high content of oxygen and nitrogen. The use of hydrogen for the removal of these heteroatoms is one of the most investigated techniques. Especially important is the presence of nitrogen, as the large production of NO<sub>x</sub> upon combustion is banned.

The use of ultrasound as a pretreatment technology before the HTL of the microalgae slurry is intended to increase the yield of the biocrude while reducing the severity of the operating conditions. Also, milder conditions result in lower nitrogen content of the biocrude.

This thesis shows the results of the use of a sonication bath to disrupt the microalgae cells before being liquefied. Three different microalgae species were considered: *Nannochloropsis gaditana* (*N. gad.*), *Scenedesmus almeriensis* (*S. alm.*), and *Tetraselmis suecica* (*T. suec.*). The experiments were carried out in a tubular batch reactor without stirrer and with an electric heater.

It was found that the ultrasonic pretreatment does not affect the performance of the HTL of microalgae. The lack of influence of the pretreatment on the quantity and quality of the liquefaction products could be related to the fact that the microalgae samples were already disrupted during the drying process to prepare the powder biomass. Moreover, the poor performance of the sonication bath, in terms of achieving the microalgae cell breakage, is also considered as explanation. Therefore, the analysis of the ultrasonic equipment was done to understand the reason for its poor operation. The characteristics of the sonication equipment (configuration, ultrasonic power output, and energy frequency) were defined for future experiments.

Keywords:

Sonication bath, liquefaction, biocrude, Nannochloropsis, Scenedesmus, Tetraselmis

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## LIST OF ABBREVIATIONS

|                 |                                                    |
|-----------------|----------------------------------------------------|
| CHNS            | Elemental analysis                                 |
| daf%            | dry, ash free percentage                           |
| db%             | dry basis percentage                               |
| Dr TGA          | Derivative curve of the thermogravimetric analysis |
| EDX             | Energy-dispersive X-ray spectroscopy               |
| FA              | Fatty Acids                                        |
| FTIR            | Fourier Transform Infrared Spectroscopy            |
| GC-MS           | Gas Chromatography – Mass Spectroscopy             |
| HHV             | Higher heating value                               |
| HTL             | Hydrothermal liquefaction                          |
| Mtoe            | Megatonne of oil equivalent                        |
| <i>N. gad.</i>  | <i>Nannochloropsis gaditana</i>                    |
| <i>S. alm.</i>  | <i>Scenedesmus almeriensis</i>                     |
| SEM             | Scanning Electron Microscope                       |
| <i>T. suec.</i> | <i>Tetraselmis suecica</i>                         |
| TGA             | Thermogravimetric analysis                         |
| XRD             | Powder X-Ray Diffraction                           |



# 1 Introduction

This chapter describes the background for the present thesis. Firstly, the current status on the use of fossil fuels and the need for alternative biofuels is described. Secondly, the relevance of microalgae as feedstock for biofuel production is explained. Because of the high moisture content of the microalgae biomass, and other characteristics, the suitability of the hydrothermal liquefaction (HTL) as conversion technology is particularly considered.

At the end of this chapter is established the layout of this study, giving a brief description of the content of each chapter and how they contribute to address the aim and objectives of this investigation.

## 1.1 Fossil fuel dependence

Humankind has used some petroleum products since early times. The availability of high quality fuel has favoured the development of technology for the transformation and utilization of this energy source. The growth of the transportation sector is one of the most representative examples (Jones 2010).

Most technologies depend directly or indirectly on oil and, consequently, petroleum reserves have become a matter of national security (Klare 2005). The scarcity of oil resources has promoted the development of renewable energy systems non-dependent on fossil fuels. One of the main drawbacks for employing many of these alternative energy sources in the transportation sector is that they require the deployment of technologies other than the advanced and optimized fossil fuel-based technologies and infrastructures. Liquid biofuels constitute an exception within the portfolio of renewable energy source. Biofuels may present similar characteristics to fuels derived from crude oil (Chisti 2008), and be applicable to conventional engines with few modifications. Moreover, their production does not present fluctuations and, thereby, the energy supply is guaranteed together with the stability of the network (Szarka et al. 2013). This fact is good for the transportation sector for being one of the largest fuel consumers (Peterson et al. 2008). Figure 1 shows the energy requirements of the different sectors in the world between 1971 and 2013.

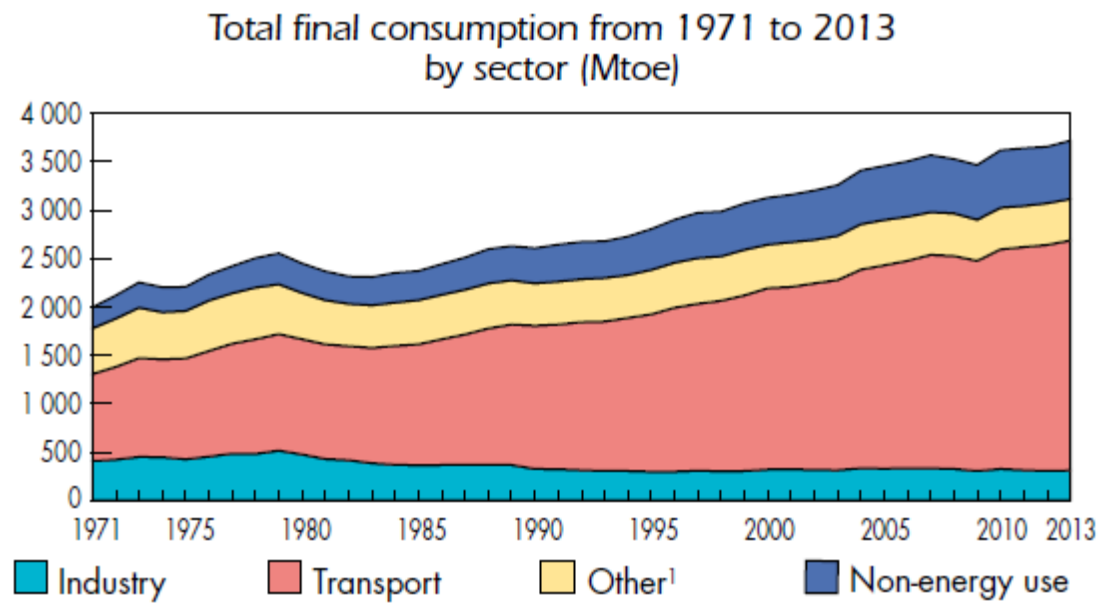
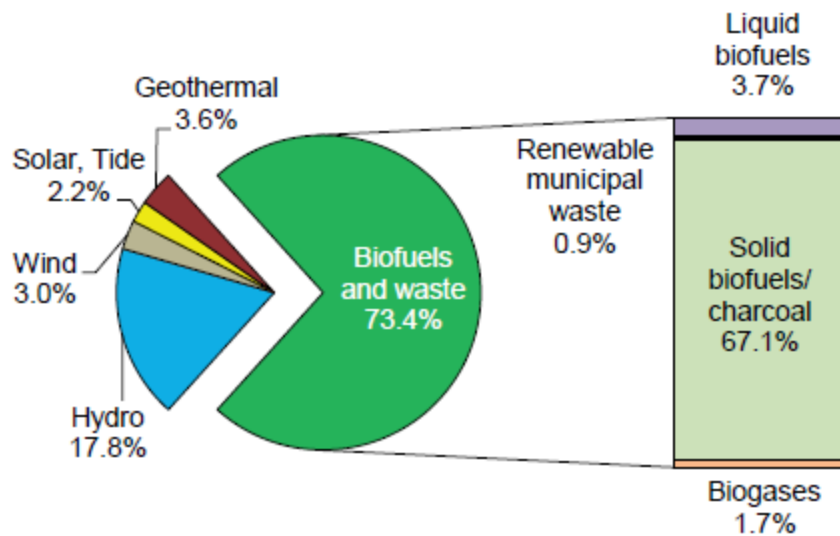


**Figure 1 – Energy consumption, in Megatonne of oil equivalent (Mtoe), by sector in the world between 1971 and 2013. Source: (IEA 2015a)**

## 1.2 Biofuels

Biofuels are the largest contributor to the total renewable energy generated. Around 73% of the total renewable energy consumed in the world comes from biofuels (Figure 2).

**Figure 2: 2013 product shares in world renewable energy supply**



Note: Totals in graphs might not add up due to rounding.

**Figure 2 – Product shares in world renewable energy supply during 2013 (IEA 2015b)**

### 1.2.1 Biofuel sources

The classification of biofuels depends on the feedstock. They are considered as first generation biofuels when they are produced from food crops, second generation are generally originated from lignocellulosic material and third generation are obtained from aquatic autotrophic organism such algae. A more detailed classification is present in the website of the European biofuels technology platform (European biofuels technology platform 2015).

The feedstock for renewable fuel production is an important discussion topic. Renewable liquid fuels are highly questioned when they are produced from crops as they raise the price of the food (Chisti 2008). In fact, Europe wants to meet the target of 10% for renewables in transport fuels by 2020: up to 7 % of first generation biofuels and the remaining 3% coming from multiple alternatives such as advanced biofuels, i.e. second and third generation biofuels (European biofuels technology platform 2015).

### **1.2.1.1 Microalgae as feedstock for biofuels**

Microalgae are photosynthetic microorganism “with a thallus not differentiated into roots, stem and leaves” (Richmond 2008). Although the term microalgae particularly refers to eukaryotic cells, many authors have also included the cyanobacteria in this classification (Mutanda et al. 2011). In this work, the term microalgae is used to refer to all photosynthetic microorganism.

Microalgae are a highly diversified group of microorganisms (Barsanti & Gualtieri 2005). Therefore, depending on the species, they are adapted to a specific environment and present different composition.

#### **1.2.1.1.1 Applications**

Microalgae are considered a non-food source material (third generation biofuel source), even when they have been eaten since early times in the oriental cultures (Fujiwara-Arasaki et al. 1984). Moreover, currently they are highly appreciated for their good properties and composition, and they are sold as nutraceuticals and food supplements (Guedes et al. 2011). On the other hand, if they are cultivated in wastewater as bioremediator (Becker 1994), they cannot be used as food; however, even in this case it could be possible to think about their use as animal feed (Markou & Nerantzis 2013). Therefore, the use of microalgae as food, although it is less commented, is also questionable.

Microalgae are considered a source of advanced biofuels because: (a) their impacts, both in the environment and in the food prices, are much lower than the first generation biofuel sources (Gouveia. 2011) and, (b) they uptake CO<sub>2</sub> and nutrients extending the carbon available to produce biofuels (European biofuels technology platform 2015).

The most important characteristics of microalgae to produce biofuels are their high growth rate and lipids content, and their capacity to be cultivated in different environments (Clarens et al. 2010). In terms of satisfying the global demand for energy, microalgae have the highest biomass/lipids productivity (Khan et al. 2009). Table 1 offers a comparison of microalgae with other sources of biodiesel. Furthermore, microalgae have other advantages that

makes them be considered by the scientific community as a sustainable solution (Chisti 2007).

**Table 1 – Suitability of biodiesel sources to satisfy the demand (data from (Chisti 2007))**

| Crop        | Oil yield/(L/ha) |
|-------------|------------------|
| Corn        | 172              |
| Soybean     | 446              |
| Canola      | 1,190            |
| Jatropha    | 1,892            |
| Coconut     | 2,689            |
| Oil palm    | 5,950            |
| Microalgae† | 136,900          |
| Microalgae‡ | 58,700           |

†70 wt.% oil in biomass

‡30 wt.% oil in biomass

#### 1.2.1.1.2 Cell structure and energy for processing

The cell structure and geometry play an essential role in the processing of microalgae. A lower surface-to-volume ratio implies higher shear strength to break the cell (Smith-baedorf 2012). The wall of the cell is especially important during the disruption and extraction of the inner compounds (Cooney et al. 2009). Therefore, the cell wall directly affects the efficiency of the whole process. In fact, the energy required for biofuel production from microalgae is mainly derived from, in addition to the drying, the disruption and extraction operations. The use of algae with thin cell wall reduces the energy in both processes (Razon & Tan 2011). In spite of the importance of the cell was for the downstream operations of microalgae, still there is no available standard method to measure the mechanical strength to break the wall (Smith-baedorf

2012). However, the characterization of the cell wall of some species has been done with acid and base hydrolysis (Montsant et al. 2001).

#### 1.2.1.1.3 Species, lipid content and growth rate

The composition of microalgae varies widely. Table 2 shows the biochemical composition of some microalgae.

**Table 2 – Composition of some widely used microalgae species in dry basis percentage (db%) (Becker 1994)**

| Microalgae                  | Protein | Carbohydrates | Lipids  | Nucleic acid |
|-----------------------------|---------|---------------|---------|--------------|
| <i>Scenedesmus obliquus</i> | 50 – 56 | 10 – 17       | 12 – 14 | 3 – 6        |
| <i>Chlorella vulgaris</i>   | 51 – 58 | 12 – 17       | 14 – 22 | 4 – 5        |
| <i>Dunaliella salina</i>    | 57      | 32            | 6       | -            |
| <i>Spirulina platensis</i>  | 46 – 63 | 8 – 14        | 4 – 9   | 2 – 5        |

The microorganism which contains more than 20 wt.% of lipids are designed as oleaginous species (Karatay & Dönmez 2011).

The synthesis of lipids in microalgae requires a large amount of metabolic energy (Huang et al. 2010) and, for this reason, the species with a high lipid content present lower biomass productivities than the other species. Lee et al. (Lee et al. 2010) showed that the biomass productivity of *Botryococcus sp.* – which is a high lipid content microalgae (about 40 db% lipids (Sakamoto et al. 2012)) considered by many as the best option for biofuel production for not having all their lipids located inside the cell (Lee et al. 2012) – had about half of the biomass productivity of *C. vulgaris* and *Scenedesmus sp.*, which have a much lower lipid content (see Table 2). However, *Botryococcus sp.* presented similar lipid productivity to the other species evaluated in their study. The lipid and biomass productivities of a high-lipid-content/low-growth-rate species, *Botryococcus sp.*, and low-lipid-content/high-growth rate species, *C. vulgaris* and *Scenedesmus sp.* are shown in Table 3.

**Table 3 – Comparison of high lipid content and fast growth rate species done by Lee et al. (Lee et al. 2010)**

| <b>Parameter</b>                                               | <b><i>Botryococcus</i><br/><i>sp.</i></b>                | <b><i>C. vulgaris</i></b>                                | <b><i>Scenedesmus</i><br/><i>sp.</i></b>                 |
|----------------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|
| <b>Description</b>                                             | High lipid content species with low biomass productivity | Low lipid content species with high biomass productivity | Low lipid content species with high biomass productivity |
| <b>Lipid content/(mg L<sup>-1</sup>)</b>                       | 160.3                                                    | 77.9                                                     | 66.5                                                     |
| <b>Biomass productivity/(mg L<sup>-1</sup> d<sup>-1</sup>)</b> | 35.7                                                     | 74.2                                                     | 71.4                                                     |
| <b>Lipid productivity/(mg L<sup>-1</sup> d<sup>-1</sup>)</b>   | 11.5                                                     | 11.1                                                     | 9.5                                                      |

Rodolfi et al. (Rodolfi et al. 2009) identified *Nannochloropsis sp.* as the best combination between biomass productivity and the lipid content. They screened 21 marine species, included *Nannochloropsis*, and 9 freshwater species, like *Chlorella vulgaris* and *Scenedesmus*. However, *Botryococcus* were not considered for their study. The cultivation conditions of these species were different from the experiments of (Lee et al. 2010) and the values obtained for this three species are shown in Table 4.

Table 4 – Screening of microalgae species done by (Rodolfi et al. 2009)

| Parameter                                                      | <i>Nannochloropsis</i><br><i>sp.</i> | <i>C. vulgaris</i> | <i>Scenedesmus sp.</i> |
|----------------------------------------------------------------|--------------------------------------|--------------------|------------------------|
| Lipid content/(db%)                                            | 29.6                                 | 18.4               | 19.6                   |
| Biomass productivity/<br>(mg L <sup>-1</sup> d <sup>-1</sup> ) | 210                                  | 200                | 210                    |
| Lipid productivity/<br>(mg L <sup>-1</sup> d <sup>-1</sup> )   | 61                                   | 36.9               | 40.8                   |

It is worth mentioning that the quality of biofuels is determined by the structure of the compounds that constitute them. Knothe (Knothe 2008) found that the palmitic, stearic, oleic and linolenic acids give good properties to the biodiesel. Therefore, using biomass with a good fatty acids profile is essential for the optimization of the quality of the biodiesel (Lee et al. 2010). This is why microalgae are better than other raw material for biofuel production (Miao & Wu 2004). Particularly, the presence of oleic acid gives good properties to the biodiesel (Rashid et al. 2008). For this reason, the quality of the biodiesel obtained with the lipids of *Botryococcus braunii* is very similar to the diesel fuel, due to the high fraction of oleic acid – about 55% of the lipids (Mathumitha 2010).

### 1.2.2 Biofuel technologies

There are many types of biofuel technologies but special attention has received the production of bioethanol and biodiesel because both present similar characteristics to petroleum-derived transport fuels. Although the microalgal biomass can be used as feedstock in almost all conversion process (Figure 2), it is especially suitable for the liquefaction or gasification process because they

do not require complete drying of the biomass. Nevertheless, microalgae have been traditionally used as feedstock for biodiesel production due to their high lipid content (oil extraction and conversion via transesterification).

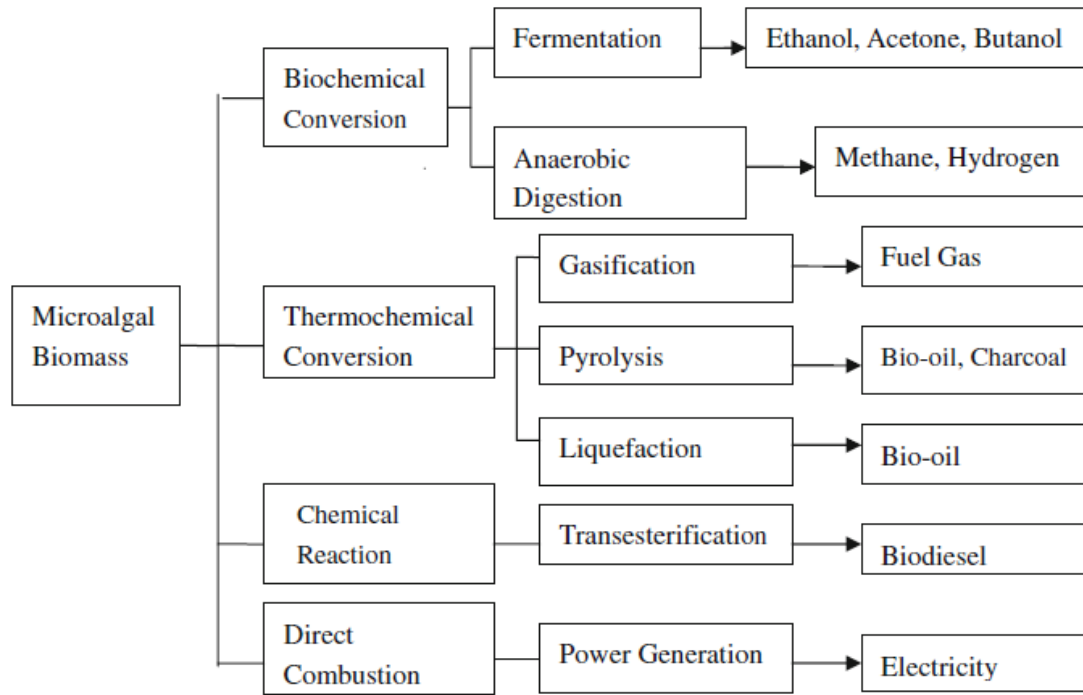


**Figure 3 – Processes for energy production with microalgae (Wang et al. 2008)**

On the other hand, biodiesel from microalgae seems to hardly achieve a positive energy balance due to the amount of energy required for the harvesting and drying (Lardon et al. 2009). Generally, the presence of water increases the energy consumption of the biofuel production process. For example, in the case of bioethanol, Johnson (Johnson 2006) reported that the distillation and drying consume 50 % of the total energy needs. The energy requirements for these two steps are mainly derived from the volatilization of the water (Peterson et al. 2008). The energy distribution of the production of bioethanol from corn grain is broken down in Figure 4.

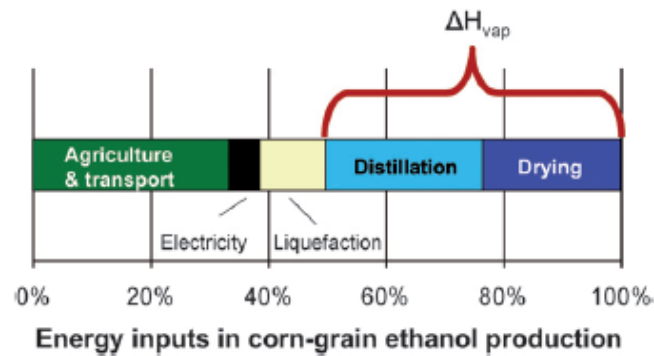


**Figure 4 – Energy distribution in corn-grain ethanol production (Peterson et al. 2008)**

### 1.3 Hydrothermal liquefaction

HTL is a technology that converts the wet biomass to a liquid fuel, also called biocrude. The process uses the subcritical water (from 175 to 375 °C and 100 to 250 bar (López Barreiro, Prins, et al. 2013)) as solvent/catalyst to decompose the biomass in different products. In addition to the biocrude, other phases are formed in the process (solid, gas and aqueous phases). However, the main product of the process is the bio-oil. On the other hand, in order to achieve a profitable liquefaction process, it is necessary to find uses for the other products. Generally, the solid residue is being considered as fertilizer due to its high ash content; moreover, as the water phase contains nitrogen and organic compounds, the recirculation to the heterotrophic cultivation of microalgae has been tested (Biller et al. 2012). Finally, under the liquefaction conditions, the gas contains mainly CO<sub>2</sub> (Brown et al. 2010), which means that its use in the cultivation of microalgae also could be developed.

Nitrogen, used as a fertilizer in the cultivation of the microalgae, represents an important cost of the cultivation and, therefore, it has a high impact on the biofuel production cost. Thereby, the fate of this element is especially important. The ideal operation would be with all the nitrogen ending up both in the aqueous phase and in the solid residue. This is because it allows to close the nitrogen cycle and using this element again as fertilizer. However, it is considered that having high nitrogen content in the water phase is more

important than in the solid residue, as the latter could imply a low conversion of the algae biomass.

### 1.3.1 Reaction mechanism

Although the catalytic effect and roles of the water in subcritical conditions has already been studied (Akiya & Savage 2002), the reaction mechanism in the liquefaction of microalgae has not been elucidated yet. Several authors agree that at the conditions nearby to the critical point (374 °C and 221 bar), the water became nonpolar due to its low permittivity (14.07 F m<sup>-1</sup> at 350 °C and 200 bar), and it is able to dissolve organic molecules. Furthermore, the ionic product of water increases two orders of magnitude (being 10<sup>-12</sup> instead 10<sup>-14</sup> at 25 °C); as a result, the H<sup>+</sup> and OH<sup>-</sup> ions can catalyse acid and base reactions (López Barreiro, Prins, et al. 2013).

The competition between hydrolysis and repolymerization reactions is being considered as explanation of the reaction mechanism (Zou et al. 2010). While the hydrolysis and decomposition reactions make possible the dissolution in water of the less hydrophobic part of the molecules of proteins, lipids and carbohydrates; the repolymerization contribute to the formation of cyclic compounds and large molecules which contribute to increase the molecular weight of the biocrude. This implies that all the biochemical components (lipids, protein and carbohydrates) of microalgae, contribute to the formation of the biocrude. Moreover, several authors consider the formation of biocrude from algaenans, which is another component of the microalgae (Rodríguez et al. 1999).

Although some research has been done, no industrial facility is operating nowadays using microalgae as feedstock. The batch experiments done in the lab with volumes between 1 – 100 mL do not offer reliable data to design an industrial scale continuous process (López Barreiro, Prins, et al. 2013). The use of continuous plants to assess the HTL of microalgae is more useful to scale-up this technology; it brings to mind other problems such as the pumpability of the slurry which are not considered when doing batch experiments (Jazrawi et al. 2013).

### 1.3.2 Operating conditions

The key parameters for the optimization of the process are the yield and composition of the biocrude. The yield calculation depends on the authors; the approach followed in this thesis is to consider the yield as the weight percentage of biocrude with regard to the amount of dry biomass used as feedstock; this definition of the yield is widely used in the literature (López Barreiro, Prins, et al. 2013). Besides, the composition of the biocrude is equally important; the biocrude contains heteroatoms such as oxygen and nitrogen. Special attention has been given to the nitrogen as this element can prohibit the use of the biocrude due to the amount of  $\text{NO}_x$  generated upon combustion (Jazrawi et al. 2013).

The best conditions (375 °C and 5 min of reaction time), that promote the highest yield, also give the highest nitrogen content in the biocrude (Garcia Alba et al. 2012) (see Figure 9). This is because, the severe conditions promote the conversion of the protein and carbohydrates in biocrude, in addition to the lipids, and therefore, the nitrogen is present in some of the oily compounds. At mild conditions (175 °C), the biocrude is mainly formed from the lipids of the microalgae. Furthermore, the use of soft conditions to carry out the liquefaction results in an increase of the  $\text{N}_2$  in the gas (Brown et al. 2010), resulting in a low nitrogen in the biocrude. On the other hand, the high temperature and long reaction time also have a positive effect on the composition of biocrude, as decarboxylation to form  $\text{CO}_2$  is one of the reactions that takes place at subcritical conditions (Garcia Alba et al. 2012). This means that, more severe conditions, in addition to increase the yield, reduce the oxygen content (Garcia Alba et al. 2012).

It is worth noting that the oxygen content of the biocrude obtained by means of HTL of microalgae (10 wt.%) is about half of the biofuel produced by pyrolysis of other biomass such as wood (~30 wt.%) (Miao & Wu 2004).

As said above (Figure 4), the HTL process is ideal for processing wet biomass, such as microalgae, because it reduces the energy in harvesting and drying the microalgae (Brown et al. 2010). According to Xu et al. (Xu et al. 2011),

microalgae concentrations of 5 – 20 wt.%, which are the concentrations most often used for HTL, can be obtained using only 5 % of the energy necessary for total thermal drying. Furthermore, the hydrothermal technologies, such as liquefaction and gasification, avoid the vaporization of water and therefore, they process the biomass more efficiently (Peterson et al. 2008).

Table 5 offers a comparison between the HTL and the biodiesel and bioethanol production processes.

**Table 5 – Comparison of HTL, transesterification and fermentation processes (adapted from (Hammerschmidt et al. 2011))**

| <b>Category</b>                            | <b>HTL</b>                       | <b>Transesteri-<br/>fication</b> | <b>Fermentation</b>               |
|--------------------------------------------|----------------------------------|----------------------------------|-----------------------------------|
| <b>Type of conversion</b>                  | Thermo-chemical                  | Chemical                         | Biochemical                       |
| <b>Product</b>                             | Biocrude                         | Biodiesel                        | Bioethanol                        |
| <b>Feed</b>                                | Biomass of different composition | Oily biomass                     | Sugar and starched material       |
| <b>Influence in the food market prices</b> | No                               | Yes                              | Yes                               |
| <b>Typical residence time</b>              | 10 min                           | 45 min                           | 72 – 96 h, can be as long as 18 d |
| <b>Process temperature</b>                 | <sup>a</sup> 280 – 370 °C        | <45 °C                           | <90 °C                            |
| <b>Process pressure</b>                    | <sup>a</sup> 100 – 250 bar       | 1 bar                            | 1 bar                             |
| <b>Stage of development</b>                | R&D work                         | Widely used                      | Widely used                       |

<sup>a</sup> Data from (Toor et al. 2011)

### 1.3.3 Upgrading of biocrude

The high nitrogen and oxygen content of the biocrude make it necessary to use upgrading technologies, such as hydrotreating, in order to obtain a bio-oil free of heteroatoms. Furthermore, in spite of being an energy dense liquid (~35 MJ/kg), the boiling point of the major part of the biocrude (>80 wt.%) is above 250 °C; thereby, their application to the transport sector is hardly achievable. Torri et al. (Torri et al. 2012) performed the thermogravimetric analysis (TGA) of the biocrude obtained from 8 wt.% slurry concentration of *Desmodesnus sp.* at 375 °C and 5 min; they found that only 18 wt.% of the biocrude had a boiling point below 200 °C. Hydrocracking has been proposed by (Zhu et al. 2013) in order to reduce the boiling point of the biocrude. In their study, 85 wt.% of the upgraded bio-oil is in the diesel fuel boiling range (119 – 412 °C).

The use of the upgrading technologies increases significantly the cost of biofuel production (Zhu et al. 2013).

### 1.3.4 Profitable operation

Many authors have proposed the idea of a biorefinery to reduce the bio-oil production cost. The extraction of high-added value compounds prior to HTL results in a profitable biofuel production (Garcia Alba et al. 2012). Some lipids, carbohydrates, proteins, and pigments from microalgae are considered as nutraceutical products with a high price (Markou & Nerantzis 2013).

Although the lipids from microalgae contribute to improve the yield and properties of the biocrude, and are themselves a source of biofuel, in the case of strains with a lipid content lower than 33 wt.% db, the amount of bio-oil produced from the lipid-extracted algae (LEA) – generated mainly from proteins and carbohydrates – is higher than the hydrocarbons obtained from the hydrotreated lipids (Figure 5 from (Zhu et al. 2013)). This could be the case of *Nannochloropsis sp.*, which present less than 33 db% of lipids and the simple extraction of its oil for biofuel production, lead to waste the other microalgae components, which in this case would contribute more to the biocrude than the lipids. This marine microalgae species has been used by many researchers as

a whole feedstock in their batch experiments (Brown et al. 2010). Nonetheless, the use of *Nannochloropsis sp.* as biofuel source is not without opposition of a part of the food industry, which sells the eicosapentaenoic acid (EPA) from *Nannochloropsis* as a food supplement (Adarme-Vega et al. 2014).

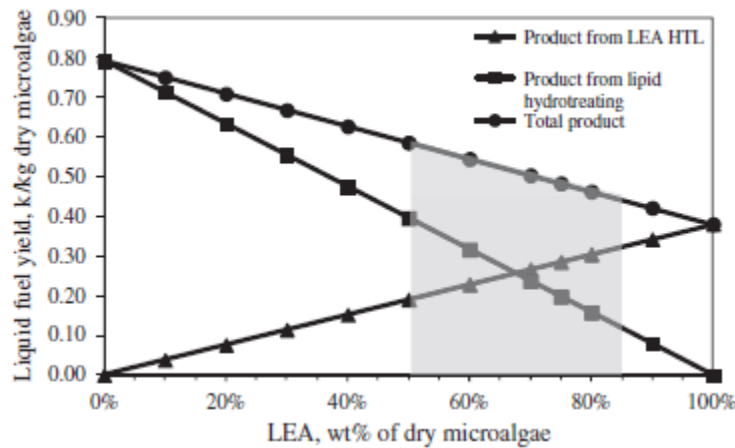


**Figure 5 – Liquid fuel yield from different microalgae components (Zhu et al. 2013). The grey area represents the typical LEA content of the microalgae.**

### 1.3.5 Homogeneous and heterogeneous catalyst

The high ash content of some microalgae species, such as *Nannochloropsis sp.* – which contains about 26.4 wt.% of ash according to Biller & Ross (Biller & Ross 2011) – includes metals that could play a catalytic role during HTL (Biller & Ross 2011).

The use of catalysts in HTL, both homogeneous and heterogeneous, has been investigated in a way to improve the quantity and quality of biocrude. With regard to the homogeneous catalyst, Biller & Ross (Biller & Ross 2011) conclude that the use of  $\text{Na}_2\text{CO}_3$  lead to a better conversion of the carbohydrates while the lipids and proteins have higher contribution to the biocrude when the liquefaction takes place without catalyst (only with water). On the other hand, an increment of the amount of  $\text{H}_2$  during the supercritical water gasification (SCWG), can be obtained by adding potassium compounds, such as KOH or  $\text{K}_2\text{CO}_3$  (Peterson et al. 2008). Although the presence of hydrogen is more important during the liquefaction of coal (Behrendt et al. 2008), for the lack

of this element in the raw material and its requirement to form liquid hydrocarbons, in the case of microalgae, the hydrogen availability is also very important to create a reducing atmosphere (Duan & Savage 2011b), which avoids the cyclization, aromatization, and condensation. Therefore, a good availability of hydrogen reduces the amount of large molecules and promotes the formation of smaller ones – more volatiles, with the subsequent molecular weight reduction of the biocrude (Duan & Savage 2011b) – in addition to the removal of heteroatoms such as nitrogen, oxygen and sulphur to form  $\text{NH}_3$  (Elliott et al. 2013),  $\text{H}_2\text{O}$  (Vasilakos & Austgen 1985), and  $\text{SH}_2$  (Stenberg & Nowok 1986), respectively (Du et al. 2013).

It is worth noting that the use of inorganic bases enhance the saponification reactions, which results in a decrease in the bio-crude yield and an increase in the solid yield (Biller & Ross 2011).

Generally, the potassium salts offer better results than sodium salts for the conversion of woody biomass:  $\text{K}_2\text{CO}_3 > \text{KOH} > \text{NaCO}_3 > \text{NaOH}$  (Toor et al. 2011). In fact, the potassium catalysts are widely used as in hydrothermal reactions, both liquefaction and gasification (Jazrawi et al. 2013). The  $\text{K}_2\text{CO}_3$  is the homogeneous catalyst employed in the CatLiq® process, which utilize a range of second generation biomass as feedstock (Nielsen et al. 2012).

Although it seems that there is an agreement in the best homogeneous catalyst –  $\text{Na}_2\text{CO}_3$  or  $\text{K}_2\text{CO}_3$ , depending on the feedstock – the heterogeneous catalyst has not been well established yet. The use of heterogeneous catalyst has been suggested by many researchers as a way to reduce the nitrogen, or even the oxygen, in the biocrude as they increase the availability of hydrogen during the liquefaction (Duan & Savage 2011b).

#### **1.4 Aim and objectives of this MSc by research thesis**

The aim of this thesis is to determine the influence of the sonication pretreatment of the slurry on the performance of the HTL of microalgae. In order to achieve this goal the following objectives were defined:

1. Assessing the influence of the composition of microalgae, using different species, in the sonication pretreatment and in the HTL.
2. Understanding of the experimental and analytical equipment until being confident for the HTL of pretreated microalgae.
3. Applying the procedures available in the literature for the characterization of the HTL products.

For the first objective, it was necessary to characterise the three microalgae species used in the experiments: *Nannochloropsis gaditana* (*N. gad.*), *Scenedesmus almeriensis* (*S. alm.*), and *Tetraselmis suecica* (*T.. suec.*). The level of characterization is in accordance with the literature. Once the different composition of the species is established, it would be possible to understand the differences in the pretreatment and in the products of the HTL.

A number of experiments of HTL of untreated microalgae were done in order to understand the experimental and analytical equipment. Furthermore, these experiments served to use the procedures for the characterization of the products. All the yields were determined, however the chemical analyses were only performed for the biocrude.

## 1.5 Structure of the thesis

This thesis is organized into 7 chapters with the following content:

1. Introduction: The background of the study is explained, i.e. why the HTL of microalgae is the topic of broad and current interest.
2. Hypothesis development: The chemistry of the HTL of microalgae is analysed and a theoretical reaction mechanism is proposed. Based on the mechanism, a number of improvements of this conversion technology are identified. Finally, the aim and objectives of this work are described.
3. Materials and methods: Present the equipment used to obtain the empirical data together with the procedures for the analysis of the biomass and to carry out the liquefaction experiments.

4. Biomass characterization: The most important parameters of the three microalgae species are established. A comparison is done and the potentially best species for HTL is selected.
5. HTL of untreated microalgae: The performance of the high-pressure reactor is evaluated. A number of modifications in its configuration are considered before doing the HTL of pretreated microalgae.
6. HTL of pretreated microalgae: The liquefaction of microalgae is done after pretreating the slurry in a sonication bath. Analyses of the liquefaction products and of the sonication bath are presented in this chapter.
7. Conclusions and future work: The conclusions of this study are gathered together and the objectives that could not be addressed in this thesis are considered for future work.

The appendices contain the explanation to some calculations together with the data of the analysis of microalgal biomass and the biocrude.



## 2 Hypothesis development

This chapter explains the development and reasoning for the concept of the ultrasonic pretreatment of microalgae slurry before the HTL. A theoretical reaction model was developed after doing a literature review of the HTL of microalgae. Once the chemistry of the process was understood, four different improvements of the HTL of microalgae were conceived: (a) use of ultrasounds as pretreatment of the slurry in the HTL of microalgae, (b) use hydrogen donor solvent during the liquefaction, (c) recirculation of the biocrude, and (d) upgrading of the biocrude with subcritical water. Due to the lack of time, only one enhancement could be investigated and the others remained as proposed for future work.

Below are stated the base of the theoretical reaction model and the improvements of the HTL of microalgae, giving more details about the sonication of the microalgae slurry before the liquefaction as it is the topic that is covered in this thesis.

### 2.1 Development of the theoretical model

An initial model for the reaction mechanism of HTL of microalgae was conceived after doing a literature review; the schematic representation of the mass transfer between the phases is presented in Figure 6.

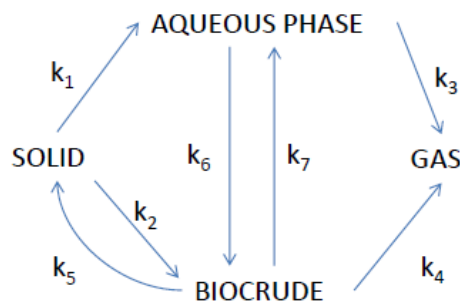


**Figure 6 – Qualitative reaction model of the HTL of microalgae. The  $k$  values represent the kinetic constants of the model:  $k_1 > k_2 > k_3 > k_4 > k_5 > k_6 > k_7$ . (Modified from Joseph Valdez (Valdez 2013)).**

The model was based on the literature review of HTL. Special relevance was the work of Joseph Valdez (Valdez 2013) who performed kinetic studies of HTL on three microalgae species – *Nannochloropsis sp.*, *Chlorella protothecoides*, and *Scenedesmus sp.* –, bacteria, and yeast. The main difference is that Joseph Valdez (Valdez 2013) consider separately the light and the heavy biocrudes, while in Figure 6 the biocrude is considered as a single phase in favour of a simpler explanation. This simplification is supported by many publications which consider the biocrude as a single phase (López Barreiro, Zamalloa, et al. 2013; Biller & Ross 2011; Brown et al. 2010). The level of knowledge developed on the reaction mechanism only allows a qualitative comparison of the kinetic constants to be made:  $k_1 > k_2 > k_3 > k_4 > k_5 > k_6 > K_7$ .

### 2.1.1 Stepwise mechanism

The model consists of a stepwise mechanism. Although in reality, all steps of the mechanism could take place simultaneously, the explanation offered here, makes relevant the importance of one of the reactions at each time allowing an easier and more useful explanation. The most representative phases are:

1. Cell disruption: Thermolysis
2. Chemical reaction
  - a. Decomposition
  - b. Repolymerization

This is the first work that seriously considers the lysing of the cell as part of the liquefaction mechanism of microalgae. On the other hand, the literature focuses on the chemical reactions, mainly decomposition and repolymerization, but there is not available a deep explanation about the molecular interaction (López Barreiro, Prins, et al. 2013)

Moreover, the model was not only thought of as a simple explanation of how the liquefaction occurs but to optimize the performance of the HTL reactor. In this way, both the influence of each step in the yield and composition of the biocrude are described.

The mechanism presented here is explained from the macroscale point of view. The role of the functional groups in the molecules has not been covered. Information about the reaction mechanism on the atomic scale can be found in the work of Torri et al. (Torri et al. 2012).

#### **2.1.1.1 1<sup>st</sup> phase: Thermolysis**

The cell disintegration was considered responsible for the results obtained in the HTL of microalgae. This first phase was conceived after considering the results of Garcia et al. (Garcia Alba et al. 2012), among other authors. In their study, they show photographs where the algal cells (*Desmodesmus sp.*) can be seen after the liquefaction: (a) at different temperatures, when they are processed during 5 min and; (b) at different residence times, when they are liquefied at 200 °C. In the first case (Figure 7), the cells only start to lose their initial shape at temperatures higher than 250 °C. On the hand, only the cells converted at 200 °C during 60 min or longer are destroyed and clumped together (Figure 8).

This means that the conditions used to do the liquefaction are not severe enough to destroy the cell wall, and it is the first evidence of the cell disruption step in the liquefaction. Furthermore, more evidence of the cell lysing role in the liquefaction, are the yield and composition of the biocrude obtained (Figure 9): In the same work of Garcia et al. (Garcia Alba et al. 2012), using 5 min of reaction time, the biocrude yields reported are 8.6 and 49.4 daf% at 175 and 375 °C, respectively. With regard to the nitrogen content of the biocrude, it changes from 0.4 wt.% at 175 °C, to 6.3 wt.% at 375 °C. Note that the yields are expressed in daf% while nitrogen content is given in wt.%; this is because for the calculation of the yields the amount of the product was divided by the daf amount of microalgae used as feedstock while the amount of nitrogen found in the biocrude was divided by the total mass of the sample (biocrude). The variation in the yield and composition can be easily explained by means of the cell disruption hypothesis: Only a complete breakage of the cell is achieved at 375 °C, which implies that all the lipids were extracted and the conversions of some of the proteins – which increase the nitrogen content – and carbohydrates

were possible. Besides, at 175 °C, the grade of cell breakage is very low; therefore, few lipids were extracted and low conversions of protein – resulting in low nitrogen content – and carbohydrate were achieved. Also, the authors of the paper point out that the composition of the biocrude obtained at low temperature resembles the composition of the lipids in *Desmodemus sp.* Torri et al. (Torri et al. 2012) explains that the “extraction phenomenon” is the main crude oil formatting mechanism in less severe conditions than 200 °C for 60 min or 250 °C for 5 min of reaction time.

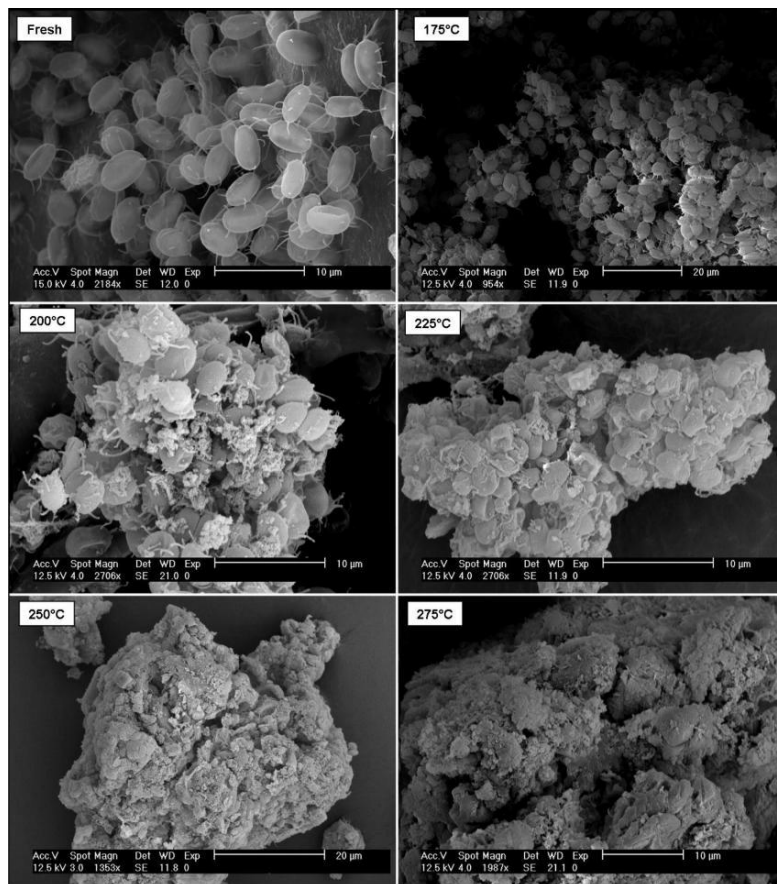


**Figure 7 – Scanning electron microscope (SEM) images of the solid residue obtained from the HTL of 7 – 8 wt.% (daf) *Desmodemus sp.* during 5 minutes at different temperatures (Garcia Alba et al. 2012)**

According to the model, the cell disruption step represents a bottleneck that determines the quantity and quality of the final products due to, like in any other chemical reaction, the availability of the reagents – in this case, the biochemical

components of the microalgae cells – define the interaction between the molecules and determine the final products.

At this point, the results from the HTL of microalgae can be discussed in terms of micro- and macro-mixing (Giridhar & Krishnaiah 1993; Lehwald et al. 2012). Even when the reactor is well stirred (good macromixing), the micromixing determines the final results. Thorough micromixing is only achieved when the microalgae cells are completely disintegrated.

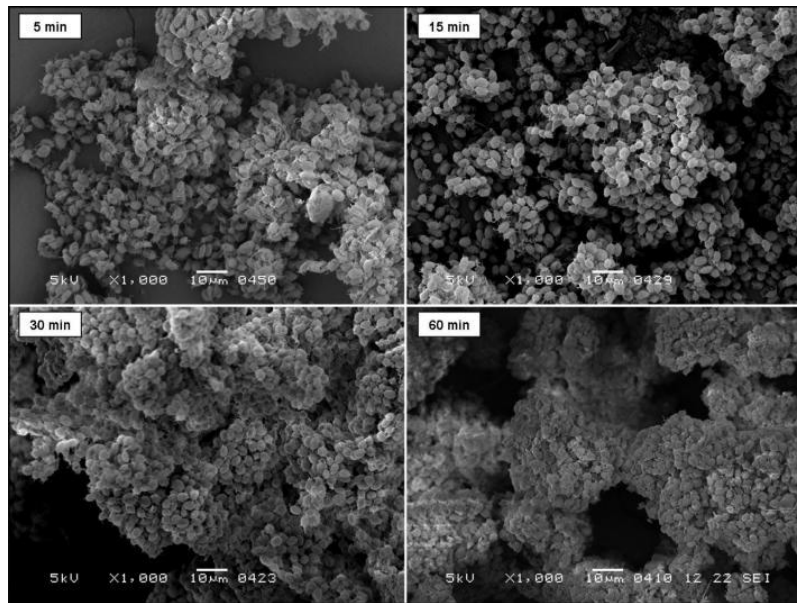


**Figure 8 - SEM images of the solid residue obtained from the HTL of 7 – 8 wt.% (daf) *Desmodesmus* sp. at 200 °C for different reaction times (Garcia Alba et al. 2012).**

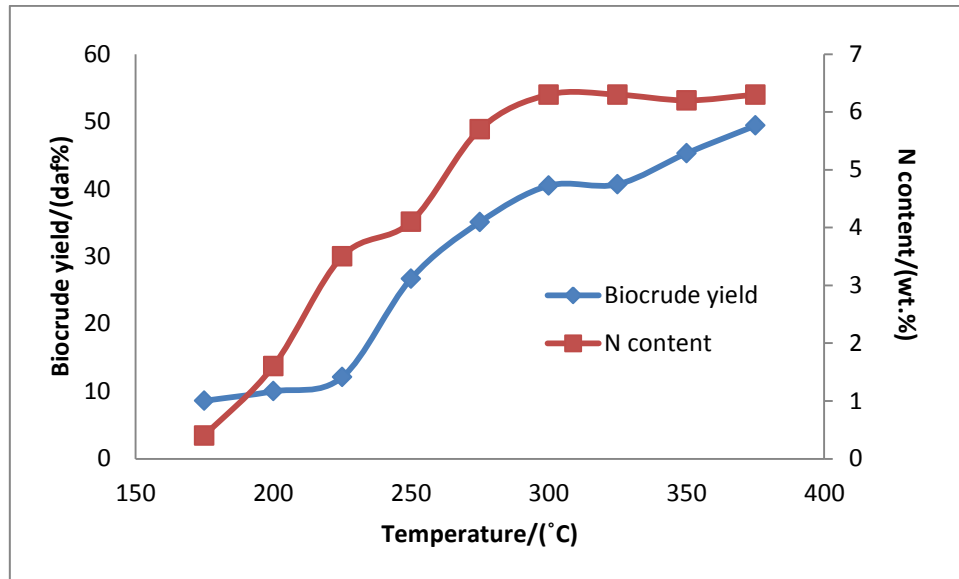


Figure 9 – Yield and nitrogen content of the biocrude obtained from the HTL of *Desmodesmus sp.* during 5 minutes at different temperatures (data from (Garcia Alba et al. 2012)).

## 2<sup>nd</sup> phase: Chemical reaction

### Decomposition

The decomposition of the algae biochemical compounds results in the presence of low molecular weight molecules in the aqueous, oily and gas phases. The decomposition stage can be subdivided into three steps:

1. **Chemical degradation:** This represents the first decomposition step in which the algae cells are attacked by the ions of the medium. In the case the microalgae slurry consists only of algae in water, these ions would be  $H^+$  and  $OH^-$ , and the acid and base reactions are generally known as *hydrolysis* (López Barreiro, Prins, et al. 2013). The presence of an organic solvent instead of water, or together with water, would promote another type of degradation reaction.

A considerable reduction in the size of the molecules is achieved in this first step.

2. **Phase formation:** The medium size molecules formed are more susceptible to be solubilized in the medium. This substep is generally explained in the literature considering that the water became apolar as a

result of a drop in the dielectric constant in subcritical conditions (Torri et al. 2012). They further explain that the subcritical water is able to dissolve the small parts of the organic molecules.

The presence of an organic solvent would increase the amount of the organic molecules present in the oily phase. In the case of absence of such organic liquid, the lipids from microalgae are the first compounds to contribute to the oil phase formation.

3. **Decomposition:** The final reduction in the molecular weight of the molecules takes place when they are solvated in the aqueous or oily phase. Peterson et al. (Peterson et al. 2008) described the free-radical reactions as a possible pathway in the water phase. This agrees with the explanation given by López et al. (López Barreiro, Prins, et al. 2013) about the cracking of the biocrude molecules under supercritical water conditions. However, the later do not specify the phase where the reaction takes place. It is in this substep, when the structures of the fatty acids are affected because of their good stability do not undergo any transformation during the chemical degradation stage. In this case, only the acyl part of the fatty acids (FA), resulting from the removal of the hydroxyl groups, remains in the bio-oil (Brown et al. 2010). Nevertheless, it is important to highlight that some of the FA do not suffer any transformation during HTL. In fact, Torri et al. (Torri et al. 2012) claims that the concentration of total FA – which includes the free fatty acids and the bounded fatty acids – change from more than 60 wt.% in the lipid extract at 25 °C of *Desmodesmus sp.*, to about 5 – 28 wt.% in the HTL oils. Furthermore, the severity of the thermal treatment, defined in terms of temperature and residence time, has an effect in the amounts of bounded and free FA. Generally, higher temperature and longer residence times promotes the formation of free FA with the consequent reduction of the amount of the bounded ones. Therefore, as percentages of the total FA present in the HTL oil, the amount of free FA changes from 42 wt.% at 200 °C and 60 min, to 71 – 72 wt.% at 375 °C and 5 min

(Torri et al. 2012). Further discussion about the conversion of the lipids during the HTL can be found in the work of Torri et al. (Torri et al. 2012).

Although the transference between phases is possible, especially towards the gas phase via further decomposition of the molecules (such as decarboxylation, to form  $\text{CO}_2$ ), it is unlikely to happen between the organic and water phase (Figure 6). The author of the thesis assumes that most of the intermediates of these phases remain in the same phase or contribute to the gas phase.

### **Repolymerization**

This stage describes the formation of the high molecular weight compounds via repolymerization, cyclization and condensation. The repolymerization and cyclization would mainly take place in the oil phase while the condensation could occur both in the water and in the organic phase. Several references introduce the condensation reactions as a way to explain the formation of oil and char (Agrawal & Chakraborty 2013; Peterson et al. 2008). The model presented here considers the condensation as the mass transfer from the aqueous phase to the oil, and the transfer of larger molecules from the bio-oil to the solid (Figure 6). The increase of the molecular weight of the oil compounds can be done through two pathways: (a) via repolymerization, for the formation of large acyclic molecules and; (b) via cyclization for the formation of cyclic compounds. However, the formation of large acyclic molecules in the biocrude via repolymerization is quite unlikely. In fact, the acyl part of the fatty acids together with some unconverted of these acids are the main acyclic compounds (Brown et al. 2010). The compounds derived from carbohydrates and proteins are most of them cyclic compounds. According to Toor et al. (Toor et al. 2011), they are forming from intermediates presented in the water phase.

### **Implications of the model: Predictions**

Considering the model proposed, there are two ways to improve the operation of the HTL reactor: (a) pretreatment of the microalgae slurry, and (b) recirculation of the biocrude.

### Pretreatment of the microalgae slurry

If the model is applied to the operation of a plug flow reactor (PFR), which is the type of reactor that has already been implemented in a pilot study of HTL of microalgae (Jazrawi et al. 2013), it is possible to see that the disruption of the microalgae slurry directly affects the length of the reactor as this variable is defined by a specific conversion of the feedstock.

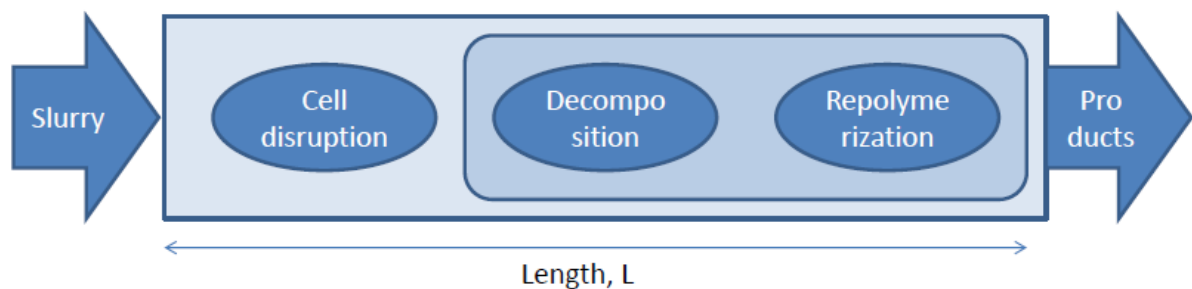


**Figure 10 – Stepwise mechanistic model of HTL of microalgae in a PFR.**

The length of the tubular reactor, or its volume, determines both the constructing and operating costs. The three stages of the biocrude production are presented inside the reactor (Figure 10); however, it is expected a significant reduction of the reactor length if the cell breakage step is done prior to the introduction of the microalgae slurry in the reactor.

There are several cell lysing technologies developed for the lipid extraction of microalgae (Lee et al. 2012). The application of these technologies to the HTL process must be assessed to determine how they affect the overall cost of the biofuel production and the quality of the biocrude. It is expected that the utilization of a cell breakage technology prior to the HTL reactor offers the following benefits:

1. Reduction of the volume and operation costs of the reactor necessary to achieve a certain degree of conversion of the microalgae slurry.
2. Improvement in the yield and composition of the biocrude, for two reasons:
  - a. Although thermolysis requires less energy, the mechanical cell disruption technologies are more effective in breaking the cells

(McMillan et al. 2013). The grade of cell disintegration determines the availability of the biochemical reagents from the microalgae structure and, therefore, is directly involved in the yield and composition of the biocrude. A good micromixing is necessary for the optimum operation of the reactor.

- b. The use of less severe conditions – as high temperature and long residence times are not required to ensure thermolysis – results in a lower nitrogen content in the biocrude, due to the mild conditions do not promote the presence of nitrogen in the oil.

Sonication and microwave processing are cell disruption technologies which could be applicable to the HTL of microalgae as a pretreatment. This is because of their low energy consumption and easy scalability (Lee et al. 2012).

### **Recirculation of the biocrude**

After sorting out the bottleneck of the cell disintegration, the next obstacle is the mass transfer between phases. According to the theoretical reaction model, the most important reactions forming molecules that contribute to increase the biocrude (repolymerization and cyclization) take place in the organic phase. Therefore, the presence of an initial oil phase reduces the attack of the water ions to the organic molecules, which imply that fewer molecules undergo hydrolysis reactions and end in the water or gas phase. As result, the presence of an initial organic phase promotes the biocrude formation and it is expected an increment of the biocrude yield. This agrees with the results of Ross et al. (Ross et al. 2010), who reported that the highest yields are obtained using organic acids. In their study, they tested KOH, Na<sub>2</sub>CO<sub>3</sub>, acetic and formic acid.

The use of an organic solvent to carry out the liquefaction increases the cost of production. Additionally, the solvent must be in liquid state during the HTL and preferably have similar properties to the biocrude. The best option is the recirculation of the biocrude, as the major part of the biocrude (more than 50 wt.%) has a boiling point higher than 280 °C (Torri et al. 2012).

The recirculation of the biocrude has never been tested in the HTL of microalgae, however, it is a common operating procedure in mature liquefaction processes such as PERC (Pittsburgh Energy Research Centre) (Toor et al. 2011) and CatLiq® (Nielsen et al. 2012).

## 2.2 Improvements to the model

The model proposed requires the incorporation of the following aspects, all related to the catalysis of the process:

1. Catalytic role of water: The literature suggests that the water ions ( $H^+$  and  $OH^-$ ) play a catalytic role in a greater extent than what is considered in the proposed model. This is because the current model prioritises the resistance of the mass transfer between phases, rather than consider the catalytic effect of water and its apolar state under subcritical conditions. The results presented in the literature, support the initial hypothesis in some way, as they report some compounds in the biocrude which did not go through the water phase at all (such as the free fatty acids present in the final biocrude) or whose reaction must be carried out in the oil phase (the presence of the acyl chain of the fatty acids in the final biocrude is indicative that the dehydroxylation reaction took place in the oil phase). On the other hand, Biller & Ross (Biller & Ross 2011) reported higher biocrude yield when the HTL of microalgae takes place in water rather than in  $Na_2CO_3$  or  $HCOOH$ . The catalytic role of the inorganic bases has not been covered by the mechanistic model yet. With regard to the formic acid, the model can explain the lower yields as the  $HCOOH$  do not really contribute to the organic phase formation because of its low boiling point and different structure from the biocrude.
2. Role of homogeneous and heterogeneous catalyst: The use of catalysts during the HTL has been investigated as a way to improve the quality and quantity of the biocrude. It would be necessary to review more references in order to fully understand how the catalyst modifies the reaction conditions. Although there is no consensus about the best homogeneous catalyst, both the  $Na_2CO_3$  and  $KOH$  are the catalysts most

amply used for the algae liquefaction (Ross et al. 2010). With regard to the heterogeneous catalysts, the research has focussed in testing hydrogen promoting catalysts during the HTL of microalgae (Duan & Savage 2011b; Biller et al. 2011).

3. Catalytic effect of the ash present in the microalgae: The metals which form the ash contained in the microalgae has been reported to play a catalytic/inhibitory effect in processes such as combustion (López-González et al. 2014) and HTL of microalgae (Biller & Ross 2011). Particularly, the potassium present in the ash of microalgae ameliorates the liquefaction operation (Jazrawi et al. 2013). Generally, the potassium ions are known for their positive effects on the thermal conversion of the biomass (Hammerschmidt et al. 2011). This explains that the KOH and  $K_2CO_3$  are catalysts used in well-developed HTL processes such as the HTU® (Karagöz et al. 2006) and the CatLiq® (Nielsen et al. 2012).

## **2.3 Ultrasonic pretreatment of hydrothermal liquefaction of microalgae**

This is the first study in evaluating the sonication as pretreatment of HTL of microalgae.

### **2.3.1 Background**

Carrying out the liquefaction under mild conditions (175 – 250 °C) promotes a low nitrogen content (0.4 wt.% at 175 °C) of the biocrude. The nitrogen content of the biocrude is the main inconvenient of the biocrude obtained from microalgae. Actually, the use of the biocrude can be prohibited due to the formation of  $NO_x$  upon combustion (Jazrawi et al. 2013). On the other hand, it is important to highlight that work at severe conditions (300 – 375 °C) result in a higher biocrude yield and lower oxygen content (Garcia Alba et al. 2012).

A comparison of different liquefaction conditions is shown in Table 6. The performance of the HTL of microalgae is evaluated in terms of yield and composition of the biocrude.

**Table 6 – Comparison of mild and severe conditions used in the HTL of 7 – 8 daf% slurry of *Desmodesmus* sp. during 5 min (data from (Garcia Alba et al. 2012))**

| Parameter               | Mild conditions | Severe conditions |
|-------------------------|-----------------|-------------------|
| Temperature/(°C)        | 225             | 375               |
| Biocrude yield/(wt.%)   | 12.1            | 49.4              |
| Nitrogen content/(wt.%) | 3.5             | 6.3               |
| Oxygen content/(wt.%)   | 13.6            | 10.5              |

The low yield obtained at mild conditions is a consequence of the poor cell disintegration. Therefore, the cell disruption prior to the liquefaction must be evaluated as a potential way of increasing the yield and improving the composition. In order to achieve these results, it would be necessary to increase the amount of nitrogen in water while decreasing the amount of carbon in the same phase.

One of the objectives of the experiment was to find a direct relation between the sonication treatment and the biocrude yield. In this way, the methodology developed by Gerde et al. (Gerde et al. 2012) was applied, to find a relation between the sonication treatment and the amount of lipids extracted from the microalgal cells. This means that similar sonication energies were used as pretreatment and instead of measuring the lipids extracted from microalgae, the yields of the HTL products were determined.

### **2.3.2 Cell breakage technologies**

Although there are a large number of cell breakage technologies – bead milling, high pressure homogenization, ultrasounds, osmotic shock, microwave processing, acid treatment, etc. (Lee et al. 2012) – only sonication and microwave processing seems appropriate to be used as a pretreatment technology in the production of a cheap commodity such as biofuel. Generally,

the efficiency of the cell disruption depends on the energy consumption of the equipment. Although the bead mill has the highest efficiency (Halim et al. 2012), it also has an energy requirement prohibitive for a biofuel production process. A comparison done by Lee et al. (Lee et al. 2012) shows that the technology that requires less energy is sonication (132 MJ/kg algae), followed by microwave processing (140-420 MJ/kg algae), bead milling (504 MJ/kg algae), and high pressure homogenization (529 MJ/kg algae).

The use of microwaves has been evaluated by Biller et al. (Biller et al. 2013). However, in their study, they focus on the use of microwaves as a way to carry out the liquefaction, instead of using it as pretreatment technology. They also offer some results of treating the algae powder with microwaves prior to the liquefaction. Although this could be considered in some way as a pretreatment of the biomass, it would be preferred to apply the same treatment to the microalgae slurry instead. The presence of water during the pretreatment can be considered as a minor detail, but it offers a more realistic data for a continuous plant operating with microalgae slurry. Biller et al. (Biller et al. 2013) found that the biocrude yield greatly increases with the application of microwaves as pretreatment of the algae powder when they perform the liquefaction at 350 °C during 15 min. In addition to the increase in the amount of biocrude, they reported a decrease in the nitrogen content, up to 0.6 wt.%, for the liquefaction of *Pseudochoricystis ellipsoidea*.

Although the use of ultrasounds has been considered as a way to enhance the conversion of biomass to biofuel, its application in HTL of microalgae has not been considered yet (Luo et al. 2014). The use of sonication technology has been amply studied as a lipid extraction method for microalgae (Bermúdez Menéndez et al. 2014).

### **2.3.3 Physical principles of sonication cell disruption**

The disruption of the cell structures with sonication is due to two mechanisms: cavitation and acoustic streaming (Gerde et al. 2012).

The generation of microbubbles in the medium is known as cavitation (Suslick & Flannigan 2008). These microbubbles expand and contract until they implode and, as result, the shock wave originated damage the cell structures (Montalbo-Lomboy et al. 2010). On the other hand, the acoustic streaming promotes the mixture of the solution (Khanal et al. 2007).

Moreover, it was reported that sonication increases the permeability of the cells, so the extraction of some of the intracellular compounds is possible even before the complete disintegration of the cell wall (Azencott et al. 2007).

#### **2.3.4 Advantages of sonication over other cell breakage technologies**

Sonication represents a non-invasive method to increase the extraction of the intracellular compounds. This represents an advantage with regard to other pre-treatment technologies because sonication avoids the addition of beads, like in the case of bead milling, and chemicals, such as inorganic salts, in the case of osmotic shock. In addition to a much pure products, this fact is also economically favourable due to there is no need to use and remove such additives.

Although in the case of a high lipid strains, bead milling and microwave processing are more efficient techniques for lipid extraction, when strains of lower lipid content are used, there is no a significant difference between the efficiency of the methods (Lee et al. 2010).

Similar to bead milling, there is an increase in the temperature of the medium during the operation. However, Gerde et al. (Gerde et al. 2012) proves that the cell disruption is not significantly affected by the temperature rise. A temperature control system must be used in the case of treating thermally degradable compounds, like in the case of protein extraction from microalgae (Gerde et al. 2012). If the ultrasounds are used as a pre-treatment of HTL, not only there is no negative effect from the increase of temperature but also it reduces the heat duties of the heater system.

This pre-treatment technology can be easily scaled-up for continuous operation (Harrison 1991).

### **2.3.5 Optimization**

The optimization of the ultrasonic-assisted cell disruption technique is based on the minimization of (a) the energy required to achieve the maximum extractability of the intracellular compounds and, (b) the production of free radicals which can affect the composition of the products via oxidation (Gerde et al. 2012).

The value found by Gerde et al. (Gerde et al. 2012), which has the best balance between the lipids extracted and the energy required, was approximately 800 J/10mL slurry. They report that this value is independent of the biomass concentration in the range studied (0.1 – 4 wt.%). Considering the threshold value of 800 J/10mL for a slurry concentration of 4 wt.%, the energy used in the sonication treatment would be 2 MJ/kg algae. This value is much lower than the value of 132MJ/kg algae reported by Lee et al. (Lee et al. 2012) and, it is even lower than the energy density of microalgae (20 MJ/kg, (Brown et al. 2010)).

It is very important to keep the energy consumption in the pretreatment as low as possible because most of the energy budget for the biofuel production is required for the cultivation and other operations of the downstream processing.

## **2.4 Further development of the process**

In addition to the pretreatment of the microalgae slurry before the liquefaction or the recirculation of the biocrude, other ameliorations of the liquefaction of microalgae were found from the literature:

### **2.4.1 In situ hydrogen production during HTL of microalgae as upgrading technology**

#### **2.4.1.1 Background**

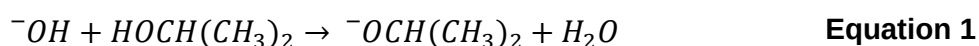
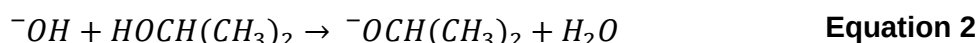
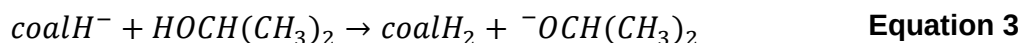
The use of heterogeneous catalyst as a way of improving the performance of the liquefaction has been widely cited in the literature. There are several works related to this topic (Duan & Savage 2011b; Biller et al. 2011), nonetheless,

most of the solid catalysts that were used, are well known for their activity in the hydrogenation processes (Pd/C, Pt/C, Ru/C, Ni/SiO<sub>2</sub>-Al<sub>2</sub>O<sub>3</sub>, CoMo/ $\gamma$ -Al<sub>2</sub>O<sub>3</sub> and zeolite (Duan & Savage 2011b)). This means that the research has focussed on the use of hydrogen during the liquefaction to get better results.

The homogeneous catalysts offer better results than the heterogeneous ones (Ross et al. 2010). For example, the tetralin (1,2,3,4-tetrahydronaphthalene) is a reputed hydrogen donor solvent used in the coal liquefaction (Vasilakos & Austgen 1985), which has been tested in the HTL of *Spirulina*, giving satisfactory results (Matsui et al. 1997). Only the biocrude yield was reported by Matsui et al., and not data of the nitrogen or oxygen content of the biocrude were presented. It is worth noting that tetralin has a boiling point of 207 °C and is insoluble in water. This could increase the resistance of mass transfer between the phases of the system with microalgae, reducing its hydrogen donation effect.

#### 2.4.1.2 Hydrogen donor: Isopropanol

In this context, it was proposed a hydrogen donor solvent which is miscible with water. Although the 2-propanol has already been tested in the liquefaction of coal and cellulose (Vasilakos & Austgen 1985) (Equation 1 to Equation 3), it has not been used yet in the liquefaction of microalgae.



It is expected that if the hydrogen donor solvent is miscible with water, the removal of the heteroatoms will be greater.

It is important to highlight that the isopropanol does not act alone in the hydrogen donation. As appears in Equation 1, it requires the presence of OH<sup>-</sup> ions to start the reaction series. The hydroxide anion is a natural constituent of the subcritical water under the hydrothermal conditions. However, the use of

KOH increases the availability of  $\text{OH}^-$ , which results in an enhancement of the hydrogen donation (Vasilakos & Austgen 1985).

#### **2.4.2 Upgrading biocrude with subcritical water**

Some experiments were developed for the recirculation of the biocrude (the data is not shown in this thesis) and the results obtained is that the nitrogen content of the water phase increases significantly when the biocrude is recirculated. Therefore, it can be thought the use of subcritical water to reduce the content of heteroatoms in the biocrude. Similar ideas are starting to be investigated for the upgrading of the biocrude (Duan & Savage 2011).

### 3 Materials and methods

According to the Aim and objectives of this MSc by research thesis, the Biomass characterization and the familiarization with the experimental and analytical equipment (HTL of untreated microalgae) were done before carrying out the HTL of pretreated microalgae, as shown in Figure 11.



**Figure 11 – Methodology flow chart**

#### 3.1 Characterization of microalgae

The cultivation conditions of the three microalgae species are unknown as they were not specified by the supplier. However the microalgae were completely characterized in terms of all the parameters stated in the relevant literature (Biller & Ross 2011; Brown et al. 2010; López Barreiro, Zamalloa, et al. 2013; Bi & He 2013).

Three microalgae species were used in this thesis: *Nannochloropsis gaditana* (*N. gad.*), *Scenedesmus almeriensis* (*S. alm.*), and *Tetraselmis suecica* (*T. suec.*). The three of them have been widely tested for biofuel production (Valdez & Savage 2013; Harman-Ware et al. 2013; Singh & Gu 2010). The selection of these samples was driven by the fact that the three samples present differences in composition which allows to assess the effects of feedstock composition on the products of the liquefaction. The three species were provided by the Estación Experimental Las Palmerillas (Fundación CAJAMAR) in Spain as a dried powder. The slurry was prepared by mixing the powder biomass with deionised water to achieve a concentration of 7 – 8 db wt%. This concentration falls in the range of 5 – 20 db wt.% which is considered suitable for the HTL of microalgae (Garcia Alba et al. 2012). No time for

hydration was allowed since the slurry was charged to the reactor straight after being made.

Table 9 summarizes the characterization techniques used in this work. It is worth noting that, even when the yields of all the phases obtained in the liquefaction were determined, only the biocrude phase has been chemically analysed as it is the most valuable product. Ideally, all the phases should be characterized with the purpose of determining the carbon distribution to assess the performance of the liquefaction rather than use only the information provided by the yields of the phases.

**Table 7 – Characterization techniques applied to feedstock and/or products**

| <b>Analysis</b>                                                                 | <b>Equipment</b>                                                                         | <b>Application</b>                |
|---------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-----------------------------------|
| Moisture analysis                                                               | HR83 alogen moisture analyser (Mettler Toledo)                                           | Biomass                           |
| Ash content                                                                     | Muffle furnace                                                                           | Biomass                           |
| High heating value (HHV)                                                        | Parr 6300 Bomb Calorimeter                                                               | Biomass                           |
| Thermogravimetric analysis (TGA)                                                | DTG-60 Shimadzu (Japan)                                                                  | Biomass                           |
| Powder X-Ray Diffraction (XRD)                                                  | Powder X-ray diffractometer, D8 Advance, Bruker AXS Gmbh (Germany)                       | Biomass & Solid residue           |
| Scanning Electron Microscope (SEM) – Energy-Dispersive X-ray spectroscopy (EDX) | FE Scanning Electron Microscopy with EDS-EBSD System Quanta 200 F, FEI (The Netherlands) | Biomass & Solid residue           |
| Fourier transform infrared (FTIR) spectroscopy                                  | Thermo, Nicolet 8700 (USA)                                                               | Biomass, Solid residue & Biocrude |
| Gas Chromatography – Mass Spectroscopy (GC-MS)                                  | GC-MS, Agilent 7890 B                                                                    | Biomass (lipids) & Biocrude       |
| Ultimate analysis (CHNS)                                                        | Vario Micro Cube Elemental Analyser & XP6 microbalance (Mettler Toledo)                  | Biomass & Biocrude                |

### **3.1.1 Moisture and Ash content**

Moisture content (M) in the biomass samples was determined in an HR83 alogen moisture analyser by following a standard procedure (Toledo 2016). A muffle furnace was used to determine ash content (A) following the method described in ASTM D 3174. These two are the parameters most widely reported in the proximate analysis of microalgae (Duan & Savage 2011b; Anastasakis & Ross 2011; Jazrawi et al. 2013; Biller et al. 2013; Jazrawi et al. 2015).

### **3.1.2 Ultimate analysis**

A Vario Micro Cube Elemental Analyser & XP6 microbalance was used to determine the C, H, N and S content of microalgae. The O content was determined by difference. The analysis was carried out by a designated operator. The data obtained from this equipment are the percentages of the elements present in the biomass volatilized during the analysis. It is important to highlight that the percentage is calculated based on the total weight of the sample (Table 28), so ash is included, even when these inorganic compounds are not quantified with this method. As the yields of the products are expressed in db, it is interesting to show the ultimate analysis also on the same basis (Table 14). In the literature, the most common way of presenting the data of the ultimate analysis of microalgae samples are both in db (López Barreiro, Zamalloa, et al. 2013), and in daf basis (Ross et al. 2010).

In order to do the calculation to get the weight percentages in db (Appendix B), it is necessary to know the amount of moisture and ash in the samples. Both values (moisture and ash) were determined in the proximate analysis. The detailed calculation of db percentages is shown in Appendix B.

### **3.1.3 Calorific value**

This value was directly measured with a Parr calorimetric bomb. The analysis was carried out by a designated operator. Higher Heating Values obtained in this work were compared to typical HHV of the microalgae biomass obtained from the literature.

### 3.1.4 Biochemical composition

The FTIR analyses were done with a Nicolet 8700 FT-IR spectrometer and the sample powder was diluted with KBr. Spectra were collected from 4000 to 400  $\text{cm}^{-1}$  (Balagurumurthy et al. 2015). The analysis was carried out by a designated operator.

The results of the FTIR have served to identify characteristic functional groups present in the biomass and to compare, qualitatively and quantitatively, the biochemical composition of the three microalgae species according to the procedure described by Meng et al. (Meng et al. 2014). For the quantitative analysis (Table 12), the protein content has been calculated taking into account the nitrogen present in the biomass (from the Ultimate analysis) and considering a nitrogen-to-protein conversion factor of 4.78 (Lourenço et al. 2004). The amount of carbohydrates and lipids were determined using the correlations given by (Meng et al. 2014). The results obtained from these correlations are given in dry weight percentage (Table 13); however, this study expresses the results in total weight percentage by considering also the amount of ash and moisture (Table 12). Although the quantitative analysis is not considered very accurate, this methodology is a strong tool for the qualitative comparison of microalgae species (Meng et al. 2014).

### 3.1.5 Characterization of lipids

The lipids are considered the most valuable fraction of the organic compounds present in the biomass for the biofuel industry. This is why microalgae are on the top of the biomass sources, for having a very high content of lipids (Gouveia. 2011). Among the lipidic compounds, the determination of the fatty acids (FA) is especially important as these compounds affect the composition of the biocrude obtained after the liquefaction (Biller et al. 2011). The extraction of the lipids was done following procedure described by Bligh & Dyer (Bligh & Dyer 1959) using a mixture of chloroform with methanol. The GC-MS was used for the characterization of the lipids. Only the compounds with a quality of identification higher than 90% were considered for further analysis. The FA identified were compared with literature references (Hita Peña et al. 2015).

To carry out the GC-MS analyses of the lipids and the biocrude the equipment of was used. The carrier gas was He and column flow rate was  $1\text{ mL min}^{-1}$ . A HP-1 column ( $25\text{ m} \times 0.32\text{ mm} \times 0.17\text{ }\mu\text{m}$ ) was used for the separation. An oven isothermal program was set at  $50\text{ }^{\circ}\text{C}$  for 2 min, followed by a heating rate of  $5\text{ }^{\circ}\text{C/min}$  to  $260\text{ }^{\circ}\text{C}$  for 5 min. The injected volume was  $0.4\text{ }\mu\text{L}$  in a splitless mode (Balagurumurthy et al. 2015). The analysis was carried out by a designated operator.

### 3.1.6 Pyrolytic behaviour

A DTG-60 Shimadzu was used to do the TGA of the microalgae samples. The analysis was carried out by a designated operator. The TGA was applied in an attempt to resolve the decomposition profile of the biomass. On the other hand, the derivative curve of the thermogravimetric analysis (DrTGA) was used to determine the optimum temperature to carry out the experiments of HTL. The conditions of the TGA are shown in .

**Table 8 – Conditions of the TGA of three microalgae species**

|                  |                                  |
|------------------|----------------------------------|
| Atmosphere       | Nitrogen                         |
| Flow rate        | 100 ml/min                       |
| Cell             | Alumina                          |
| Temperature rate | $10\text{ }^{\circ}\text{C/min}$ |
| Hold temperature | $900\text{ }^{\circ}\text{C}$    |
| Hold time        | 0 min                            |

### 3.1.7 Ash analysis

The analysis of the ash present in the microalgae was done using two techniques:

- The Energy-dispersive X-ray (EDX) spectroscopy was done with FE Scanning Electron Microscopy with EDS-EBSD System Quanta 200 F. The analysis was carried out by a designated operator. The EDX does a chemical characterization of a particular point the biomass samples considering some of the atoms present in the ash. This characterization cannot be considered representative of the whole sample but gives an idea about some of the elements present in the ash.
- The Powder X-Ray Diffraction (XRD) gives an idea about the crystalline structure of the biomass. The organic material in the biomass has amorphous structure; this means that the crystalline material in the microalgae can be only related to their ash content.

The powder XRD patterns of the microalgae samples were recorded with a Bruker D8 advance X-ray diffractometer () fitted with a Lynx eye high-speed strip detector and a Cu K $\alpha$  radiation source. Diffraction patterns in the 2–80° region are collected with a 0.04 step size and 2 seconds of step time (Singh et al. 2013). The analysis was carried out by a designated operator.

### **3.1.8 Scanning electron microscope**

The SEM images were taken with the equipment of using a tungsten filament doped with LaB<sub>6</sub> as an X-ray source, fitted with an ETD (Everhart Thornley Detector), which works as a secondary electron detector. The sample for SEM analysis was dispersed on a carbon coated adhesive followed by gold coating (Balagurumurthy et al. 2015). The analysis was carried out by a designated operator. Prior to the analysis the samples were grinded with a mortar and a pestle.

The photographs of the biomass obtained from SEM could be used to compare with pictures of the solid residue after the HTL in order to gather more information about how the liquefaction took place. Due to the time constraint, no SEM pictures of the solid residue were taken; however some photographs of the microalgae are shown in Appendix A.

### 3.2 HTL of untreated microalgae

These experiments were performed with the purpose of training with all the experimental and analytical equipment. Furthermore, they were used to:

- a. Assess the feasibility of the calculation of the yield of all the phases.
- b. Determine the influence of the duration of the heating-up stage in the HTL.
- c. Determine the influence of the biomass composition on the HTL.
- d. Learn the procedures for the characterization of the products of the liquefaction, especially the biocrude.

#### 3.2.1 High pressure reactor

The tubular reactor used to carry out the HTL experiments (Figure 12 to Figure 14) has the following characteristics (Singh et al. 2014):

- Total reactor volume of 35 mL: This volume quantifies only the main body of the reactor, without considering the tubes used to connect the pressure gauge and the purge valves at the top of the reactor (Figure 12). It is important to mention that these tubes connected to the main body were only used in the HTL of untreated microalgae. Both the removal of these tubes and the use of a lower amount of slurry to carry out the liquefaction, were modifications in the procedure done after the HTL of untreated microalgae.

The reason to remove the pressure gauge and purge valves was to get a better performance of the reactor; a lot of slurry remained unconverted in these zones of the reactor due to the fact that there is no heat for being outside of the furnace. In this way, only in the HTL of untreated microalgae the air present in the headspace was purged by using nitrogen which remained at atmospheric pressure when the experiment started. On the other hand, in the HTL of pretreated microalgae the reactor was closed at the top using a plug.

Generally, the working volume of the reactors is about half of the total volume of the reactor. The volume of slurry charged in the reactor for the HTL of untreated microalgae was 20 mL. Nevertheless, for the same

reason of having unconverted slurry in the upper part of the reactor, in the HTL of pretreated microalgae only 15 – 12 mL slurry were used. This modification improves the accuracy of the calculation of the gas yield.

- Temperature and pressure directly measured: The thermowell is in the lower part while the pressure measurement is done with a pressure gauge placed at the top of the reactor. As described in the previous bullet point, the pressure gauge was only used in the HTL of untreated microalgae. The pressure range of the manometer is up to 250 bar with intervals of 5 bar, so this gauge is considered inaccurate to measure pressures lower than 50 bar, which is usually the remaining pressure after the liquefaction. The values of pressure measured in the HTL of untreated microalgae help to estimate the pressure in the HTL of pretreated microalgae; while it is true that the pressure would be slightly higher due to the reduction of the volume of the headspace of the reactor.
- Electric heater with PID controller (Figure 13 and Figure 14): The operation of the reactor is defined by the parameters that are introduced to the PID controller. The controller is capable of managing up to 8 stages defined by temperature and time; however, only two stages were considered: the heat-up and the reaction.
- Purging and gas sample collection. These accessories were only used in the HTL of untreated microalgae.



**Figure 12 – Tubular system with pressure gauge and valves**



**Figure 13 – HTL reactor with temperature controller**



**Figure 14 – Electric furnace**

Further discussion of the reactor is presented in section 5.1 of Limitations of the reactor.

### 3.3 HTL of pretreated microalgae

The aim of these experiments was to determine the influence of the sonication pretreatment of the slurry on the performance of the HTL of microalgae. The objectives of the pretreatment experiments were:

- a. Identification of the optimum range of ultrasonic energy to treat the microalgae slurry.
- b. Assessment of the influence of the pretreatment on the HTL products; paying special attention to the biocrude
- c. Determination of the influence of the pretreatment on the microalgae species.

#### 3.3.1 Sonication equipment

The Elmasonic P 60 H was used to disrupt the microalgae cells both in the lipid extraction and in the HTL of pretreated microalgae. The common application of this ultrasonic unit is for cleaning laboratory glassware. Nevertheless, according to the data sheet of this unit, the equipment has more power than standard ultrasonic cleaning units so the equipment could be valid for the microalgae cell disruption.

The operation can be done at two different frequencies, 37 and 80 kHz. According to Luo *et al.* (Luo *et al.* 2014), high frequency ultrasound (1 – 3 MHz) can be used to harvest microalgae. For microalgae cell disruption high intensity (lower frequency) ultrasound signals are preferred as this prevents intracellular products undergoing degradation (Chisti & Moo-Young 1986; Geciova *et al.* 2002): 20 kHz is the most commonly used frequency to treat the microalgae for this reason (Luo *et al.* 2014). references to other low frequencies and power conditions that have been applied for microalgae disruption.

**Table 9 – Frequencies and ultrasonic power output of the ultrasonic equipment used for microalgae cell disruption**

| Frequency/(KHz) | Power/(W) | Reference                       |
|-----------------|-----------|---------------------------------|
| 10              | -         | (Lee et al. 2010)               |
| 19.5            | 100       | (Bermúdez Menendez et al. 2014) |
| 20              | †400      | (Mitra et al. 2012)             |
| 20              | †2200     | (Gerde et al. 2012)             |
| 21.5            | 100       | (Bermúdez Menéndez et al. 2014) |
| 23              | -         | (Smith-baedorf 2012)            |
| 25              | -         | (Lee et al. 2012)               |
| 40              | †1 – 130  | (Halim et al. 2012)             |

† Maximum ultrasonic power output

The experiments were done with 37 kHz as this frequency is similar to the values in the literature. The specifications of the sonication equipment are presented in .

**Table 10 – Specifications of the sonication equipment used in the HTL of pretreated microalgae**

| Parameter                      | Sonication bath  |
|--------------------------------|------------------|
| Model                          | Elmasonic P 60 H |
| Frequency/(kHz)                | 37 / 80          |
| Ultrasonic peak output/(W)     | 720 / 560        |
| Ultrasonic effective power/(W) | 110 / 90         |
| Volume of the water bath/(L)   | 5.8              |

With regard to the ultrasonic power, other authors report only the maximum output of the equipment (Table 9). The maximum ultrasonic power output specified for the sonication bath is 560 – 720 W. It is important to highlight that this value is somehow meaningless for the comparison of the experiments as the relevant value is the effective ultrasonic power that reaches the microalgae sample. A calculation is proposed for the effective power of the equipment by measuring the increase in temperature of the sample, which is the same for the whole water bath, and considering the heat transfer equation (Equation 4):

$$\dot{Q} = \frac{m \times C_p \times \Delta T}{t} \quad \text{Equation 4}$$

Where:

$\dot{Q}$  is the heat transfer per unit of time, W

m is the mass of the water bath, which is 2 kg

$C_p$  is the calorific value of water. It was considered a constant value of 4185.5 J/kg °C

$\Delta T$  is the increase of the temperature, °C

t is the duration of the pretreatment, s

Only sonication pretreatments lasting 30 minutes were considered for the calculation of the effective ultrasonic power. This is because the  $C_p$  of the water depends on the temperature (Kuroki et al. 2001). A total of 13 measurements were noted (see Appendix A); the average of the calculations was 107.75 W with a standard deviation of 10.95 W.

It is worth noting that the increase in temperature of the sample (and thus the bath temperature) is not a unique consequence of the effect of the ultrasounds on the sample; for instance, the horn which produces the ultrasounds release heat during the operation and in some equipment this transducer is cooled with water (Sonics & Materials 2006). Therefore, even considering only this small portion of the total ultrasonic output, it is not possible to ensure that all this energy reaches the sample in the sonication bath. Generally, the equipment reported in the literature was intended for microalgae cell disruption so their configuration ensures that almost all the ultrasonic peak output affect the microalgae sample (see section 6.3 Analysis of the sonication equipment). Consequently, despite the calculated value of the ultrasonic effective power of the sonication bath match with the values of the ultrasonic power output of the equipment used by other authors (Table 9), chances are the actual effective power of the Elmasonic equipment is lower than the value reported by other authors.

Further discussion of the sonicator is presented in section 6.3 of Analysis of the sonication equipment.

### **3.4 Conditions**

A comparison of the experimental conditions of the liquefaction experiments is shown in Table 11.

Table 11 – Conditions of the HTL experiments

| Experiments                     | Vol<br>l/(mL) | Slurry<br>conc.<br>l/(wt.%<br>db) | Heat-<br>up<br>l/(min) | Reaction<br>time<br>l/(min) | Temp<br>l/(°C) | Pres<br>l/(bar) |
|---------------------------------|---------------|-----------------------------------|------------------------|-----------------------------|----------------|-----------------|
| HTL of untreated<br>microalgae  | 20            | 7 – 8                             | <sup>a</sup>           | 30                          | 300            | 90 –<br>100     |
| HTL of pretreated<br>microalgae | 15<br>12      | - 7 – 8                           | 15                     | 30                          | 250            | 35 –<br>45      |

<sup>a</sup> The duration of the heating-up stage was not constant for all the experiments as this was one of the parameters investigated in the HTL of untreated microalgae.

In the case of the HTL of pretreated microalgae, the slurry was sonicated prior to be charged in the reactor with energies up to 572.4 J/g slurry, depending on the sonication pretreatment. The range of ultrasonic energies are in accordance with the results of (Gerde et al. 2012) which used up to 250 J/g slurry to determine the energy which maximizes the cell disruption. They used concentrations of microalgae slurry up to 4 wt.% of two different species *Schizochytrium limacium* and *Chlamydomonas reinhardtii*.

### 3.5 Sample recovery procedure

Figure 15 exhibits the sample recovery procedure. After the reaction, the reactor was cooled down by quenching in water at room temperature for 10 minute and the products were recovered for analysis. The gas yield was calculated using two methodologies in the HTL of untreated microalgae: (a) considering the remaining pressure in the reactor after the liquefaction (Biller & Ross 2011) and, (b) by weight difference of the full reactor before and after the reaction without the gas system (see Figure 12) (Miao & Wu 2004). Even when both procedures were adopted from the literature, it was considered that using the second procedure (b) the accuracy of the results obtained is better than using the analogical pressure gauge. For this reason, only the calculation of the

gas yield based on procedure (b) was applied in the HTL of pretreated microalgae. In case of using the procedure (a), a typical gas composition (35% CO<sub>2</sub>, 35% N<sub>2</sub>, and 30% H<sub>2</sub>) was considered to compute the mass of gas formed during the liquefaction with the ideal gas law (Biller & Ross 2011).

Dichloromethane (DCM) and water were used to clean the reactor. This is the solvent most widely used for the extraction of the biocrude in the experiments of HTL of microalgae (López Barreiro, Prins, et al. 2013). The amount of DCM and water used for completely cleaning the reactor was determined empirically; this means that both solvent and water were used until ensuring that the eluent from the reactor was totally clean. The amount of DCM used was 50 mL with 25 mL of water. The solids were removed from the total mixture via vacuum filtration. The filter cake was dried in an oven at 105 °C during 24 hours before the calculation of the yield of the solid residue according to Equation 5.

A separation funnel was used to split up the filtrate into aqueous and biocrude phase. The water phase was further extracted with 40 mL DCM. The yield was the only parameter determined for the water. Typically the yields of the water phase reported in the literature were calculated by difference after knowing the yields of the other phases (Biller & Ross 2011). The same approach has been followed in this thesis.

For the complete removal of the moisture, the organic phase was mixed and passed through a filter containing sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>). The removal of the DCM was done in a rotary evaporator using a pre-weighed round bottom (RB) flask. After the separation, the RB flask with the biocrude was left in a vacuum oven under -0.6 to -0.5 barg at 40 °C for about 12 hours to ensure that there was no solvent inside the RB before the calculation of the yield by weighing the RB with the biocrude. At this point, the biocrude was completely stuck to the walls of the RB, so its recovery required the use of DCM. It was intended to use the smallest amount of DCM as possible so the samples can be used for analysis. In this way, the mixture solvent-biocrude could be used directly or transferred to centrifuge tubes which were placed again under the same conditions inside the vacuum oven for solvent removal. The analytical

techniques considered for the characterization of the biocrude were: FTIR, GC-MS, CHNS.

The calculation of the db% yields was done according to Equation 5 which takes into account the mass of each phase ( $m_i$ ) and the mass of dry microalgae ( $m_{\text{microalgae}}$ ) initially loaded into the reactor:

$$Yield(wt. \%, db) = (m_i / m_{\text{microalgae}}) \times 100 \quad \text{Equation 5}$$

Some authors (López Barreiro, Zamalloa, et al. 2013) only consider the organic mass of each product in order to do the calculation of the yield. It is worth mentioning that the fate of the ash initially present in the microalgae is not only the solid residue (Anastasakis & Ross 2011); this means that the calculation of the organic fraction of each product should be directly determined.

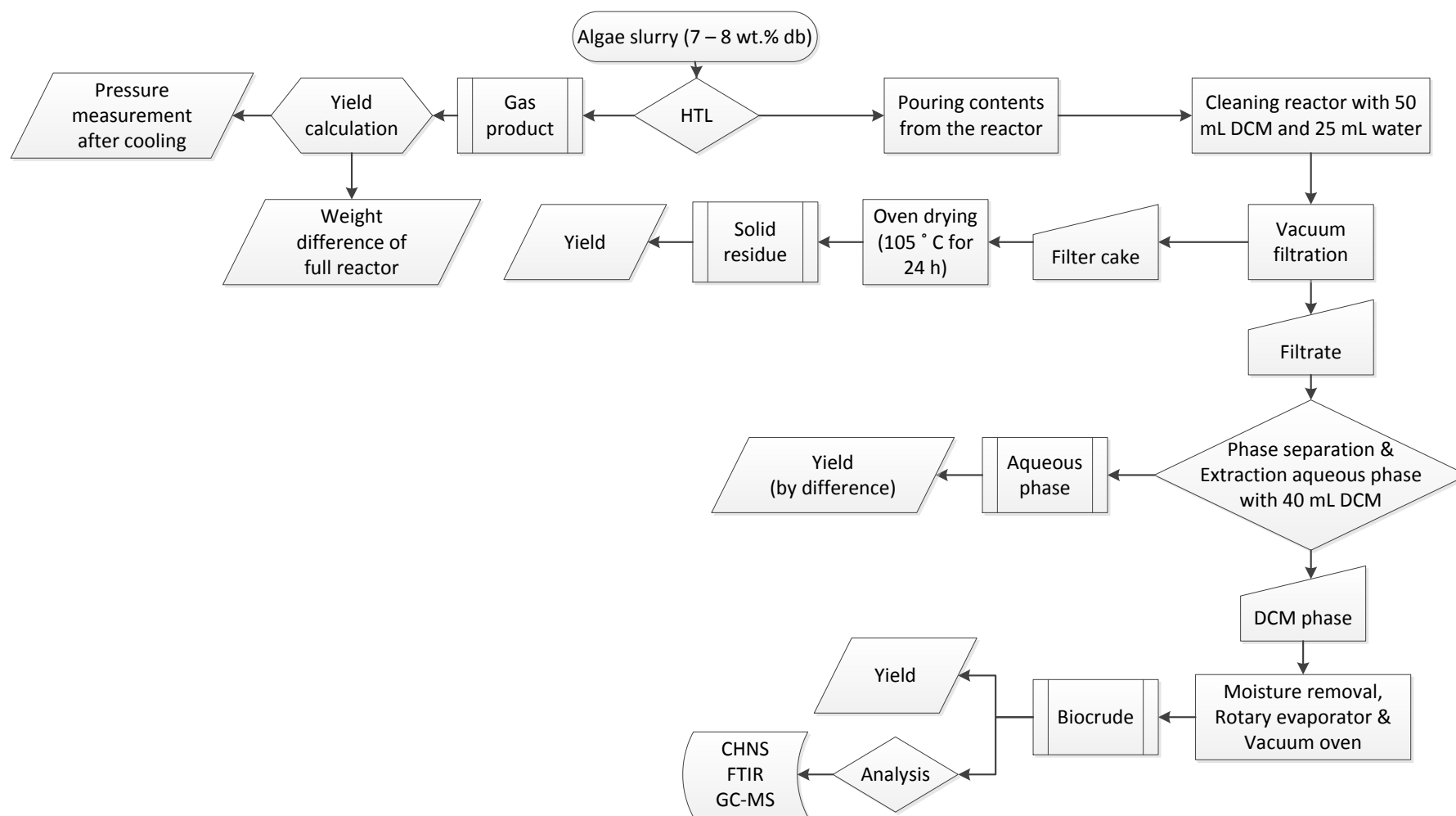


Figure 15 – Products workup procedure (modified from (Garcia Alba et al. 2012))

## 4 Biomass characterization

The establishment of the properties of the biomass used as feedstock is necessary to properly assess how the liquefaction took place. For this reason, the determination of the parameters of the microalgae used in the HTL experiments was firstly addressed. The level of characterization of the microalgae agrees with the related literature (Bi & He 2013; Vardon et al. 2011). This chapter presents the discussion of the results of the composition (moisture and ash content, biochemical composition, ultimate analysis, lipids and ash characterization, and SEM photographs), and thermal properties (calorific value, and TGA) of microalgae. The most important conclusions of this chapter and how they support the other chapters of the thesis are compiled at the end.

### 4.1 Moisture, ash, and biochemical composition

Table 12 summarizes the moisture, ash, lipid, carbohydrate, and protein contents in wet basis of the three microalgae species used in this work. These are the parameters usually reported in published work on HTL of microalgae (Vardon et al. 2011).

**Table 12 – Moisture, ash, and biochemical composition of the three microalgae species in wt.% wet basis**

| wt.%            | Moisture | Ash   | Lipid <sup>a</sup> | Carbohydrate <sup>a</sup> | Protein <sup>b</sup> |
|-----------------|----------|-------|--------------------|---------------------------|----------------------|
| <i>N. gad.</i>  | 11.42    | 29.25 | 25.22              | 10.91                     | 23.20                |
| <i>S. alm.</i>  | 10.55    | 16.65 | 28.17              | 15.24                     | 29.40                |
| <i>T. suec.</i> | 10.93    | 33.04 | 24.13              | 12.98                     | 18.92                |

<sup>a</sup> Data obtained by FTIR analysis, following the procedure of (Meng et al. 2014)

<sup>b</sup> N from ultimate analysis and nitrogen-to-protein conversion factor of 4.78 (Lourenço et al. 2004).

The main difference in the composition of the three species is the ash content, which is related to the conditions used for the cultivation microalgae. In this way, the marine species, *Nannochloropsis* and *Tetraselmis*, have higher ash

content than the freshwater species, *Scenedesmus*. In general, the values obtained for lipids, protein, and carbohydrate are acceptable taking into account the wide range of these components reported in the literature (Becker 2007).

Table 13 offers a comparison of the ash, lipids, and protein content in dry basis with the data of López et al. (López Barreiro, Zamalloa, et al. 2013) for the same microalgae species.

**Table 13 – Ash, lipid and protein composition of the three microalgae species compared to data from López et al. (López Barreiro, Zamalloa, et al. 2013) in wt.% dry basis**

| Db %            | Ash              |                 |                  | Lipids |                  |                  | Protein |      |
|-----------------|------------------|-----------------|------------------|--------|------------------|------------------|---------|------|
| Source          | Lit <sup>a</sup> | MF <sup>b</sup> | TGA <sup>c</sup> | Lit    | Ind <sup>d</sup> | Dir <sup>e</sup> | Lit     | Ind  |
| <i>N. gad.</i>  | 11.8             | 33.0            | 18.5             | 25.1   | 28.5             | 13.4             | 43.9    | 26.2 |
| <i>S. alm.</i>  | 12.0             | 18.6            | 21.4             | 21.8   | 31.5             | 6.7              | 51.7    | 32.9 |
| <i>T. suec.</i> | 15.9             | 37.1            | 23.8             | 19.5   | 27.1             | 10.1             | 43.6    | 21.4 |

<sup>a</sup> Lit – Literature data from López et al. (López Barreiro, Zamalloa, et al. 2013)

<sup>b</sup> MF – ash content obtained experimentally in the muffle furnace

<sup>c</sup> TGA – ash content obtained experimentally in the TGA (see section 4.5 of Pyrolytic behaviour and ash characterization). The analysis took place at 10 ° C/min and the last temperature was 900 °C held for 0 min.

<sup>d</sup> Ind – Indirect determination. Lipid content: from FTIR data and (Meng et al. 2014) correlation. Protein content: N from ultimate analysis and nitrogen-to-protein conversion factor of 4.78 (Lourenço et al. 2004).

<sup>e</sup> Dir - Direct determination of the lipid content of the microalgae using the method described by Bligh & Dyer (Bligh & Dyer 1959)

The difference in the values could be related to the different conditions used for the cultivation of microalgae. The deviation of the values of lipids and protein is explained by the use of different methods for determination of these components of the organic material of microalgae; the accuracy of the data determined indirectly is lower than the values reported by López et al. (López

Barreiro, Zamalloa, et al. 2013). The application of the procedure of (Bligh & Dyer 1959) to directly determine the amount of lipids present in the microalgae led to low values compared to the data available in the literature (Becker 2007). The reason is the loss of lipids during the application of the procedure. In this way, it would be necessary to repeat the methodology using a larger sample of biomass in order to reduce the influence of the lipid losses in the final result.

For the calculation of the values of the ash in both cases the values were determined using oven dried samples at 105 °C for 24 hours, however López et al. (López Barreiro, Zamalloa, et al. 2013) firstly washed the biomass to remove the water soluble inorganics and later dried the samples prior to use 550 °C for 5 hours under oxidizing conditions. Besides, the values of this study were obtained at 575 °C for 3 hours using air as the atmosphere. The lower ash content reported by López et al. (López Barreiro, Zamalloa, et al. 2013) is related to two facts: (a) the longer stay in the oven and, (b) the atmosphere of the muffle furnace. The latter is only described by López Barreiro et al. (López Barreiro, Zamalloa, et al. 2013) as oxidizing conditions, which can go from normal air to pure oxygen. According to López et al. (López Barreiro, Zamalloa, et al. 2013) the reason for the washing of the biomass was to avoid the overestimation of the organic matter of the microalgae. They say that this inorganic matter is made of water soluble salts which are stable at 105 °C but not at 550 °C, so it cannot be considered as ash (López Barreiro, Zamalloa, et al. 2013). The TGAs performed to the three microalgae species (Figure 17) also show a residue lower than the values of ash calculated with the muffle furnace (Table 13). TGA ash content data were discarded as a standard procedure was not followed. Nevertheless, these results prove that the ash calculated with the muffle furnace was overestimated because of not burning all of the the organic material, leaving some fixed carbon in the samples. Despite that the TGA ash is lower than the muffle furnace value, it is still higher than the values reported by López et al. (López Barreiro, Zamalloa, et al. 2013); this fact could be associated with the removal of some of the ash during the washing step, which is contrary to the procedure of López et al. (López Barreiro, Zamalloa, et al. 2013).

## 4.2 Ultimate analysis

The outputs of the elemental analyser are the percentages of the elements present in the biomass which have been volatilized during the analysis. It is important to highlight that the percentages given by this equipment are based on the total weight of the sample (Table 28), so ash and moisture is included, even when the components of the ash are not quantified in this method. Generally, the ultimate analysis of the biomass is given in dry basis (Zhu et al. 2013). In this way, the values directly obtained from the elemental analyser (Table 28) were converted to dry basis (Table 14) by discounting the amount of moisture in the biomass. For this purpose, the data of moisture content calculated in the proximate analysis (Table 12) was used. The calculation of these values is shown in Appendix B.

For the sake of comparison, Table 14 includes results of Barreiro et al. (López Barreiro, Zamalloa, et al. 2013), which have done the elemental analysis of the same microalgae species used in this study. The main differences are the values of the ash content and consequently the oxygen as it is calculated by difference. The reason for the deviation in the ash content was explained in the results of the Moisture, ash, and biochemical composition.

Table 14 – Comparison of elemental analysis of microalgae in db with the results of Barreiro et al. (López Barreiro, Zamalloa, et al. 2013)

| Db %            | N   |        | C    |        | C:N ratio |        | H   |        | S   |        | †O  |        | Ash  |        |
|-----------------|-----|--------|------|--------|-----------|--------|-----|--------|-----|--------|-----|--------|------|--------|
| Source          | Lit | Thesis | Lit  | Thesis | Lit       | Thesis | Lit | Thesis | Lit | Thesis | Lit | Thesis | Lit  | Thesis |
| <i>N. gad.</i>  | 6.9 | 7.70   | 51.0 | 41.92  | 7.39      | 5.44   | 6.6 | 5.64   | 0.4 | 0.74   | Nr  | 11.01  | 11.8 | 33.02  |
| <i>S. alm.</i>  | 6.8 | 8.23   | 50.6 | 47.61  | 7.44      | 5.78   | 6.4 | 6.94   | 0.4 | 0.67   | Nr  | 17.93  | 12.0 | 18.62  |
| <i>T. suec.</i> | 6.3 | 5.86   | 45.0 | 36.15  | 7.14      | 6.17   | 5.9 | 5.08   | 1.1 | 1.10   | Nr  | 14.72  | 15.9 | 37.09  |
| Mean            |     |        |      |        | 7.33      | 5.80   |     |        |     |        |     |        |      |        |
| St. deviation   |     |        |      |        | 0.16      | 0.36   |     |        |     |        |     |        |      |        |

†Calculated by difference

Lit – Values taken from López et al (López Barreiro, Zamalloa, et al. 2013)

Nr –Not reported

The carbon to nitrogen ratio (C:N) can be used for comparison of different microalgae species (Ho et al. 2003). The C:N ratios are relatively constant and any deviation is representative of a change in the biochemical composition of microalgae (Ho et al. 2003). There is an average C:N ratio of  $7.33 \pm 0.16$ , for the microalgae species from the literature, and  $5.80 \pm 0.36$ , for the samples used in this thesis. These results agree with the biochemical composition (Table 14) in two ways: (a) the small standard deviations of the constant values of the C:N ratios prove the similarities in the biochemical composition of the microalgae used in the literature and in this thesis separately, and (b) the different constant values of the C:N ratio for the species used by the López et al (López Barreiro, Zamalloa, et al. 2013) and used for this thesis explain the dissimilitude of the biochemical composition of the species.

### 4.3 Calorific value

Generally, the HHV is determined using correlations such as Dulong's Equation 6 (Duan & Savage 2011b).

$$HHV(MJ/kg) = 0.338C + 1.428(H - O/8) + 0.095S \quad \text{Equation 6}$$

Additionally, in this thesis the HHV was determined with a Parr calorimetric bomb (see Table 7). The calorific values of the microalgae obtained with both methods are very similar and comprised within the range of the data present in the literature (Biller & Ross 2011). Also, the trend of the HHV agrees with the ash content of microalgae (Table 12); the more ash present in the microalgae, the lower the HHV of the biomass.

**Table 15 – Calorific value of the raw microalgae**

| HHV/(MJ/kg)     | Parr bomb | <sup>a</sup> Dulong |
|-----------------|-----------|---------------------|
| <i>N. gad.</i>  | 17.07     | 18.01               |
| <i>S. alm.</i>  | 19.33     | 20.46               |
| <i>T. suec.</i> | 13.60     | 15.09               |

<sup>a</sup> Note that the application of the Dulong's equation should be done with the elemental analysis in total weight percentage (Table 28)

#### 4.4 Lipid characterization

The lipids isolated with the procedure of Bligh & Dyer (Bligh & Dyer 1959) for the calculation of the lipid content of microalgae (Table 12) were analysed by GC-MS. The characterization of the lipids allows the comparison of the compounds present in the microalgae. Furthermore, it helps to evaluate the performance of the liquefaction after concluding the transformations that undergo those molecules. The lipid content of the microalgae species is exhibited in Table 16, with special emphasis on the FA content. The analysis of the results of the GC-MS was done at two levels: (a) selection of the compounds present in the lipids with a quality of identification higher than 90%, (b) comparison and characterization of the FA present in the three microalgae species.

In general, the number of compounds identified in the lipids of the three microalgae was smaller (Table 16) than the number of compounds present in the biocrude (Table 22) which imply a complex chemistry of the HTL of microalgae. There are only 6 compounds commonly present in the lipids of the three microalgae: 1-Nonadecene, (1-methyldodecyl)-Benzene, n-Hexadecanoic acid (Palmitic acid), Phytol, (9Z,12Z)-9,12-Octadecadienoic acid (linoleic acid), and Cyclotetracosane. The main differences in the lipids of the three microalgae are the FA present in each of them (Table 17).

Table 16 - Characterization of the lipids of the three microalgae with GC-MS

| Lipids          | <sup>a</sup> Identified | <sup>b</sup> Considered | <sup>c</sup> FA | <sup>d</sup> %Area | <sup>e</sup> %F.A. | <sup>f</sup> Nanno | <sup>f</sup> Scen | <sup>f</sup> Tetra |
|-----------------|-------------------------|-------------------------|-----------------|--------------------|--------------------|--------------------|-------------------|--------------------|
| <i>N. gad.</i>  | 20                      | 16                      | 2               | 88.55              | 11.35              | 3                  | 5                 | 2                  |
| <i>S. alm.</i>  | 30                      | 21                      | 5               | 93.24              | 56.16              | 5                  | 9                 | 1                  |
| <i>T. suec.</i> | 23                      | 15                      | 4               | 84.37              | 12.39              | 2                  | 1                 | 6                  |

<sup>a</sup> number of compounds identified with GC-MS in the lipids

<sup>b</sup> number of compounds having a quality of identification equal or higher than 90%

<sup>c</sup> number of fatty acids present in the lipids with a quality of identification equal or higher than 90%

<sup>d</sup> percentage of the total ion chromatogram peak area of the compounds considered

<sup>e</sup> percentage of the total ion chromatogram peak area of the fatty acids considered

<sup>f</sup> number of considered compounds shared only with this species

For example, in the case of the lipids of *Nannochloropsis*, 20 were the total number of compounds identified, but only 16 compounds were considered for having a quality of identification higher or equal to 90%. These 16 compounds represent the 88.55% of the total ion chromatograph peak area. There are only 2 FA with a quality of identification equal or higher than 90%, which represents 11.35% of the total ion chromatograph peak area. *Nannochloropsis* has 6 lipidic compounds in common with all the species, 5 in common only with *Scenedesmus*, 2 in common only with *Tetraselmis*, and 3 lipidic compounds which are not present in the lipids of any other species.

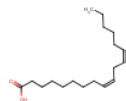
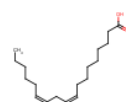
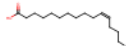
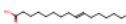
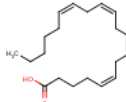
The number of FA present in the lipids of the microalgae is considered low, taking into account the results of Hita (Hita Peña et al. 2015) who found 10 different fatty acids in *Nannochloropsis gaditana*. This disagreement could be attributed to the differences in the procedures used both for lipid extraction and for identification of the FA with the GC-MS: with regard to the lipid extraction,

the procedure of Bligh & Dyer (Bligh & Dyer 1959) requires a mixture of chloroform with methanol while Hita et al. (Hita Peña et al. 2015) have used KOH, ethanol, and water to do the extraction. On the other hand, the results of this thesis were obtained with a GC-MS equipment, and Hita et al. (Hita Peña et al. 2015) used a GC-FID (gas chromatography flame ionization detector). This means that different columns were used. The utilization of different GC equipment possibly explains the difference in the amount of fatty acid detected. It is considered that GC-FID provides more accurate data because of the calibration with fatty acid methyl esters before analysing the samples. The reason for using the GC-MS in this thesis was that the FAs were not the only targeted compounds of the analysis; instead, the identification of all compounds present in the lipids of microalgae was attempted.

In spite of the low number of FAs identified, in the case of *Scenedesmus* they represent more than 50% of the total ion chromatogram (TIC) peak area. The differences in the amount of FAs of the microalgae is justified by their different genus and the cultivation conditions (Volkman et al. 1989).

The analysis of the amount of FA in microalgae is a common procedure in case the lipids are used for biodiesel production. The FA contained in the microalgae is considered optimum for such utilization (Demirbas & Fatih Demirbas 2011). For the purpose of this study, it is only necessary to know whether the FAs undergo any conversion in order to evaluate how the liquefaction took place. In the HTL of microalgae the properties of the biocrude is starting to be investigated (Duan & Savage 2011c); however, no research has been done related to the dependence of the fatty acids on the biocrude yet.

**Table 17 – Characterization of the fatty acids present in the lipids of the three microalgae species**

| Fatty acids                                         |      | Species  | % Area | Formula                                        | Structure                                                                             |                                                                                     |
|-----------------------------------------------------|------|----------|--------|------------------------------------------------|---------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| n-Hexadecanoic acid<br>(Palmitic acid)              | acid | N. gad.  | 5.3641 | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> |    |                                                                                     |
|                                                     |      | S. alm.  | 33.846 |                                                |                                                                                       |                                                                                     |
|                                                     |      | T. suec. | 3.8617 |                                                |                                                                                       |                                                                                     |
| 9,12-Octadecadienoic acid<br>(Z,Z)- (Linoleic acid) | acid | N. gad.  | 5.991  | C <sub>18</sub> H <sub>32</sub> O <sub>2</sub> |    |                                                                                     |
|                                                     |      | S. alm.  | 7.7985 |                                                |                                                                                       |                                                                                     |
|                                                     |      | T. suec  | 2.3474 |                                                |                                                                                       |  |
| Hexadecenoic acid, Z-11-                            |      | S. alm.  | 5.2705 | C <sub>16</sub> H <sub>30</sub> O <sub>2</sub> |   |                                                                                     |
|                                                     |      |          |        |                                                |                                                                                       |                                                                                     |
|                                                     |      |          |        |                                                |                                                                                       |                                                                                     |
| Palmitoleic acid                                    |      | S. alm.  | 0.8517 | C <sub>16</sub> H <sub>30</sub> O <sub>2</sub> |  |                                                                                     |
| Oleic Acid                                          |      | S. alm.  | 8.3925 | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub> |  |                                                                                     |
| Arachidonic acid                                    |      | T. suec. | 3.8976 | C <sub>20</sub> H <sub>32</sub> O <sub>2</sub> |  |                                                                                     |
|                                                     |      |          |        |                                                |  |                                                                                     |

| Fatty acids                                                           | Species         | % Area | Formula                                        | Structure                                                                           |
|-----------------------------------------------------------------------|-----------------|--------|------------------------------------------------|-------------------------------------------------------------------------------------|
| 9,12,15-Octadecatrienoic acid,<br>(Z,Z,Z)- ( $\alpha$ -Linoleic acid) | <i>T. suec.</i> | 2.2871 | C <sub>18</sub> H <sub>30</sub> O <sub>2</sub> |  |

#### 4.5 Pyrolytic behaviour and ash characterization

Even when the pyrolytic behaviour of microalgae is not especially important for HTL, because of the presence of water, it helps to understand in which range of temperature the degradation of the biomass is faster. Figure 16 and Figure 17 shows the percentage and rate of weight loss of each microalgae species. Furthermore, considering the catalytic role that the ash contained in microalgae (Jazrawi et al. 2013) may play during the thermal degradation of the biomass it is interesting to establish the composition of these trace metals. Table 18 presents the atoms detected in the biomass samples with the EDX spectroscopy, with the exception of hydrogen which cannot be detected with this technique (Stojilovic 2012). Besides, the nature of the ash in the microalgae was also analysed by XRD (see Appendix C).

As in most of the TGA of the microalgae it is possible to define three zones: the first stage (I), going up to 285 °C, in which the weight loss is associated with water and light volatile compounds; the second stage (II), from 244 to 394 °C, corresponding with the main pyrolysis process. The decomposition of most of the organic compounds takes place in this stage. In the case of microalgae, these compounds are mainly protein, carbohydrates and lipids. Finally, the third stage (III) going from 334 to 900 °C, where the carbonaceous material decomposed at a very slow rate (Shuping et al. 2010). In the case of *Nannochloropsis* and *Tetraselmis*, the decomposition taking place at a temperature close to 900 °C is related to their high content of lignin (Bi & He 2013).

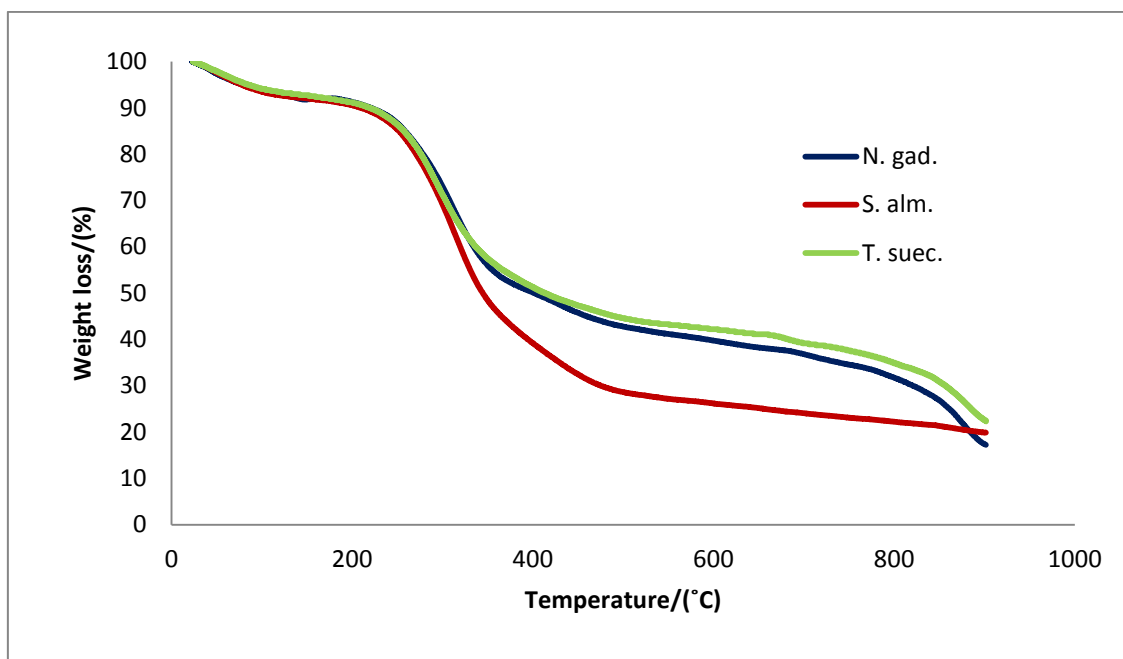


Figure 16 – TGA curves of three microalgae species

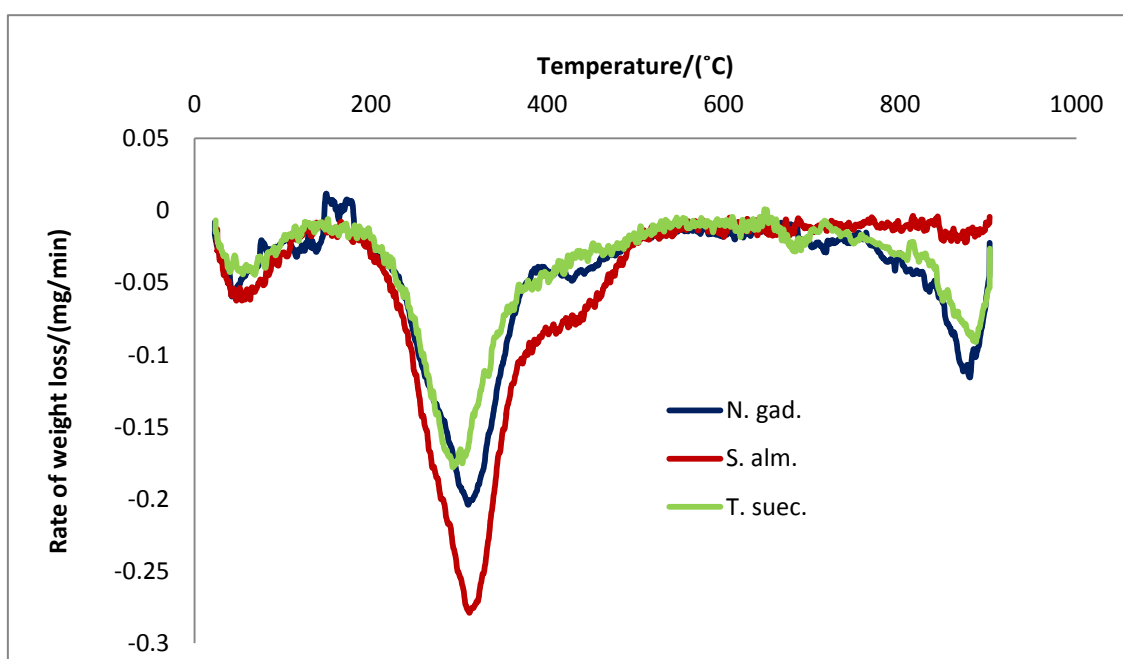


Figure 17 – DrTGA curves of three microalgae species

The TGA shows that the decomposition of the different macromolecules forming the microalgae is simultaneous. It would be very interesting if the protein, carbohydrates, and lipids decompose independently as one of the problems of the biocrude is the high nitrogen content. The broad peaks in the DrTGA (rate of

weight loss) mean that the decomposition is slow and not at a single temperature.

It is not possible to quantify the individual components in the three biomass samples, the TGA proves the similarities in the composition of *Nannochloropsis* and *Tetraselmis*. Both species present similar ash content, which is about twice the ash in *Scenedesmus* (Table 12). This fact is connected with the cultivation method of the microalgae; while *Nannochloropsis* and *Tetraselmis* are marine species which require salt water to grow, *Scenedesmus* needs fresh water for growing. Accordingly the number elements present in the ash of *Scenedesmus* is much lower than the other two species; it is important to highlight that both Na and Cl are not present in the ash of *Scenedesmus*, which proves that the EDX technique is working.

Potassium is considered a good catalyst for reactions in hydrothermal media (Jazrawi et al. 2013). This concentration of this element is much higher in the marine species.

The ash obtained from TGA was also recorded for comparison purpose with ash content obtained in the muffle furnace (Table 13).

As all microalgae present some ash, the XRD results (Appendix C) should show some crystalline structure for the three species. Many diffraction peaks are present in the XRD spectrum of *Nannochloropsis* and *Tetraselmis*, nonetheless in the spectrum of *Scenedesmus* is difficult to differentiate the peaks. The reason is the low amount of crystal lattices present in the sample of *Scenedesmus*; these crystals can only be found in the ash because the organic material has an amorphous structure. The XRD results prove the different composition of the ash present in the three species.

**Table 18 – Chemical characterization of the three microalgae species with EDX spectroscopy.**

| <b>Element/(wt.%)</b> | <b><i>Nannochloropsis</i></b> | <b><i>Scenedesmus</i></b> | <b><i>Tetraselmis</i></b> |
|-----------------------|-------------------------------|---------------------------|---------------------------|
| <b>C</b>              | 61.53                         | 59.27                     | 52.07                     |
| <b>N</b>              | 7.40                          | 11.85                     | 12.14                     |
| <b>O</b>              | 10.68                         | 26.56                     | 25                        |
| <b>Na</b>             | 8.49                          | -                         | 4.41                      |
| <b>Al</b>             | 0.37                          | -                         | -                         |
| <b>P</b>              | 0.53                          | -                         | 0.82                      |
| <b>S</b>              | 0.58                          | -                         | 0.7                       |
| <b>Cl</b>             | 9.60                          | -                         | 2.71                      |
| <b>K</b>              | 0.81                          | 0.37                      | 0.69                      |
| <b>Mo</b>             | -                             | 0.58                      | -                         |
| <b>Ca</b>             | -                             | 1.37                      | 1.47                      |
| <b>Total</b>          | 99.99                         | 100                       | 100.01                    |

#### 4.6 SEM images

Because of the similarities between *Scenedesmus* and *Desmodesmus* (Johnson et al. 2007) since they belong to the same class of green algae (Chlorophyceae), it is possible to compare the first picture of Figure 7 of *Desmodesmus* with the Figure 19 of *Scenedesmus*. Only one single cell of *Scenedesmus* can be identified with similar structure to *Desmodesmus*; this is because of the grinding done before taking the SEM pictures for sample preparation. The grinding destroys the microalgae cells. It would be highly recommended to do the SEM analysis to the raw sample to check the initial

state of the cells. It is possible that the cells were disrupted during the drying process.

On the other hand, the photographs of *Nannochloropsis* and *Tetraselmis* show more particles due to the presence of salt for being marine species. This agrees with the results of the EDX analysis (Table 18); therefore considering the high temperature and pressure used to do the HTL, *Scenedesmus* is preferred since it gives fewer corrosion issues.

Additionally, the SEM images of the raw microalgae could be used to compare with the SEM photographs of the solid residue. This will help to further understand the liquefaction process. Due to the lack of time, it was not possible to take pictures of the solid residue obtained after the liquefaction.

The different structure of the biomass samples is shown in the Figure 18 to Figure 20. The presence of more particles in the photographs of *Nannochloropsis* and *Tetraselmis* is related with the presence of salt for being marine species.

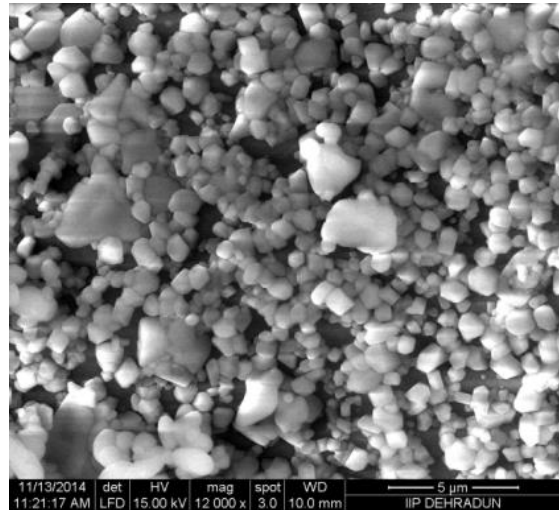


**Figure 18 - *Nannochloropsis* (12000x)**

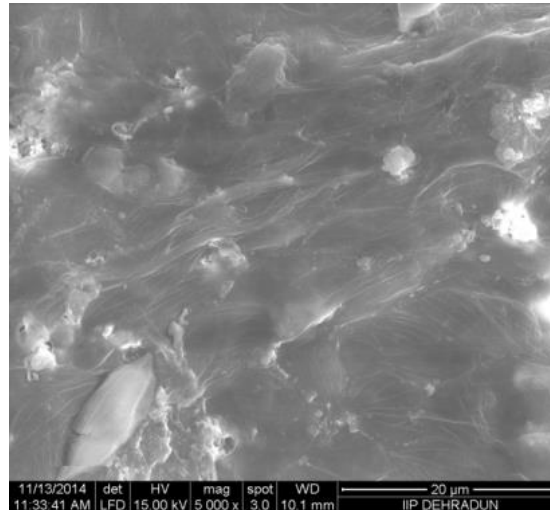


**Figure 19 - *Scenedesmus* (5000x)**

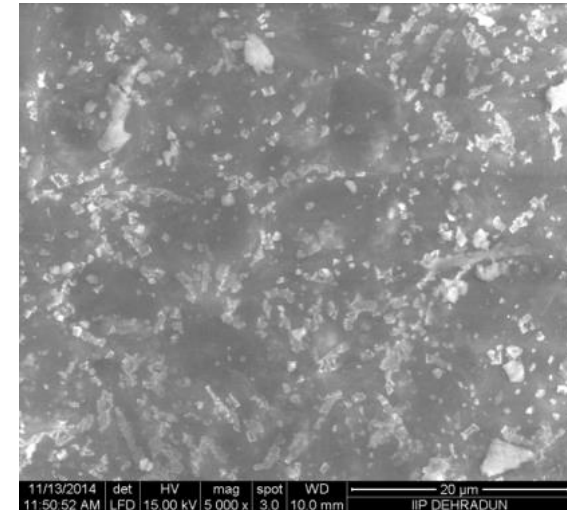


**Figure 20 - *Tetraselmis* (5000x)**

## 4.7 Summary

The study of the properties of the biomass is necessary to assess the performance of the liquefaction and to understand how the changes in the composition affect the HTL process, which will be treated in more detail in the following chapters (Chapters 5 and 6). Taking into account the data presented in this chapter, *Scenedesmus* is the most adequate species for biofuel production, at least in terms of the bio-oil yield, for the following reasons:

- Regardless of the catalytic role that the ash in microalgae may have in the thermal degradation of the biomass (Table 18), the lower content of inorganic material in the cells of *Scenedesmus* (Table 12, about half of the amount of ash content of the other two species) derive in a higher calorific value (Table 15).
- Lower lignin content and faster pyrolysis (Figure 16 and Figure 17).
- Less corrosion problems for being a fresh water species.

Also, the benefit of the HTL will be also given by the composition of the biocrude. Again, the higher content of FA of *Scenedesmus* (Table 16) could lead to a biofuel with better properties after the HTL of the biomass. On the other hand, *Scenedesmus* contains the largest amount of nitrogen (Table 14) which is undesired for HTL application. In any case, the quality of the biocrude should be directly determined because of the complex chemistry of the HTL (Torri et al. 2012).

Since the aim of this thesis is to evaluate the influence of the pretreatment of the microalgae slurry in the liquefaction it would be highly recommended to evaluate the initial state of the microalgae via optical microscopy. As the biomass samples were obtained as dried powder, it is possible that the cells were disrupted during the drying process. In such case, the pretreatment would not have any effect in the HTL. The grinding done for sample preparation before taking the SEM photographs destroyed most of the cells, in this way, it is possible to identify only one cell in good state from *Scenedesmus* in Figure 19.

## 5 HTL of untreated microalgae

This chapter discusses the results obtained in the HTL of untreated microalgae done to understand the reactor and to apply the procedures for the characterization of the products of the liquefaction. It was important to get confident with the equipment and the procedures before starting the HTL of pretreated microalgae. Some modifications were applied to the reactor (Figure 12 to Figure 14) after these initial experiments in order to improve the operation.

All these experiments were carried out with a working volume of 20 mL, 5 – 7 daf wt.% of slurry concentration, 30 min of reaction time, 300 °C, and 9 to 10 MPa of autogenous pressure

The results of the experiments are presented in two blocks: quantitative and qualitative analysis. The calculation of the yields was done according to the procedure described in section 3.5 Sample recovery procedure; the values obtained are compared with the data available in the literature. With regard to the qualitative analysis, only the characterization of the biocrude was done by means of CHNS, FTIR, and GC-MS analysis; this is because the biocrude is the most important product obtained in the liquefaction. Ideally, the characterization of all the fractions would be done if the time allowed it.

### 5.1 Limitations of the reactor

The conditions used for carrying the HTL experiments are very determined by the features of the reactor:

- Heating system: It is necessary to consider a heating rate of 10 °C/min for the heat-up stage, otherwise a temperature overshooting occurs in the reaction stage (Figure 21). This means that for some time the temperature in the reactor will be much higher than the desired temperature. By using 10 °C/min during the heat-up, it is ensured the desired temperature during all the reaction stage. Nevertheless, the heat-up stage can be considerably long, which means that the liquefaction is taking place not only during the reaction stage but also during the heat-up (see the discussion of the Products yield in the HTL of untreated

microalgae). This is especially important considering that short residence times (1 – 10 min) are the best conditions to carry out the HTL of microalgae (Garcia Alba et al. 2012).

In most of the experiments it was necessary to work at higher heating rates in order to have the desired residence time. An example of shooting is shown in ; the operation consists in 15 min of heat-up to get 250 °C from room temperature (25 °C), which means a heating rate of 15 °C/min, and a reaction time of 30 min. Both the shootings of temperature and pressure are equally important. It may look greater the increase of pressure due to the axis scale. In the case of temperature shooting, the maximum value is 274 °C. On the other hand, the maximum value of pressure is 44 bar, which is significantly higher than the 30 bar which is present at 250 °C.

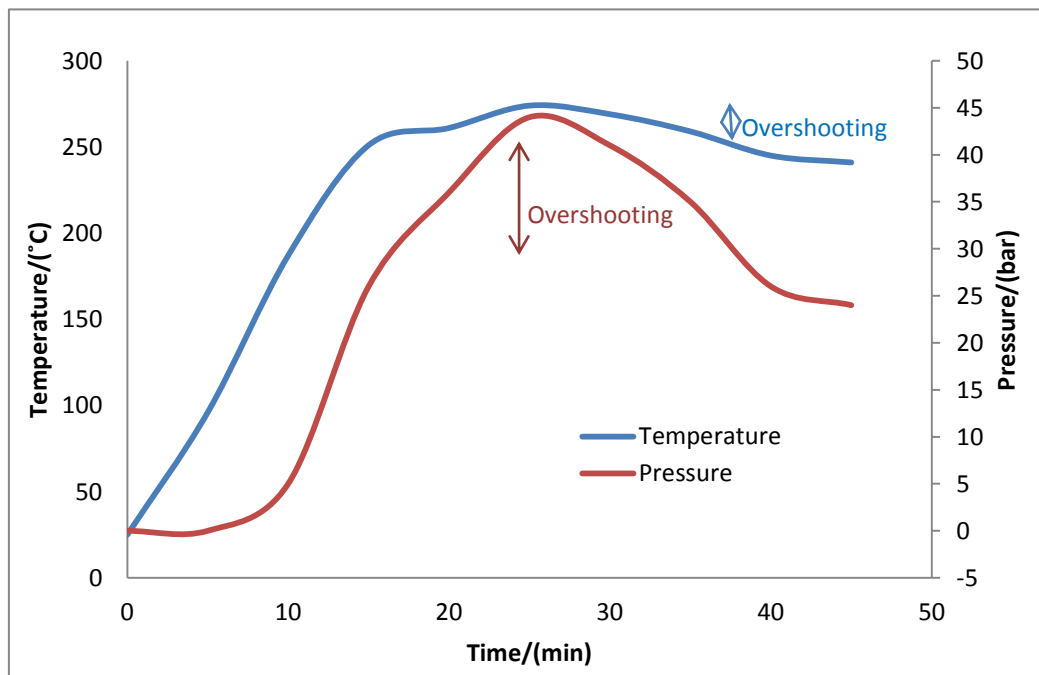


**Figure 21 – Shooting of temperature and pressure during the HTL of 8 wt.% db Nannochloropsis at 250 °C: Heat-up time of 15 min and reaction time of 30 min.**

- Safety of the system: The tubular system is made of Swagelok tubing and fittings (Swagelok 2016). It would be better to place the reactor inside a safety cabinet taking into account the high pressures used to

carry out the experiments and that there is no relief valve or rupture disc in the setup. It has been proven experimentally that working at temperatures above 300 °C with a pressure around 150 bar some fumes are released from the reactor. At these conditions, the failure of any of the reactor components results in an explosion. In this way, a temperature of 250 °C was used for being conditions for HTL of microalgae with a moderate pressure, in spite of the liquefaction being worse than at higher temperatures (up to 374 °C) (López Barreiro, Zamalloa, et al. 2013; Torri et al. 2012).

- Pressure measurement: One of the methods for the calculation of the gas yield is to measure the pressure in the reactor once it has been cooled down, assuming a composition of the gas of 35% CO<sub>2</sub>, 35% N<sub>2</sub>, and 30% H<sub>2</sub> (Biller & Ross 2011). The range of the pressure gauge (up to 250 bar) does not allow to measure accurately pressures lower than 5 bar, which are the typical pressures in the system after the reaction. This means that the calculation of the mass of gas generated should be done by measuring the weight loss of the full reactor before and after the reaction. Once the reactor is cooled down to room temperature, it is assumed that all the water vapour has been condensed; therefore, the weight difference of the full reactor before and after the liquefaction can be only associated with the gas formation. Before weighting the reactor, the gas is vented. The calculation of the gas yield in db is done with equation Equation 5.

## 5.2 Products yield

The calculation of the yields of the products can be done in different basis; the most popular are the dry basis, and daf (López Barreiro, Prins, et al. 2013). The yields in this thesis are given in dry basis because the distribution of the ash in the products was not determined in order to do the calculation in daf basis (Anastasakis & Ross 2011).

Table 19 shows the db yields of the phases obtained in the liquefaction experiments as function of the microalgae species and the heat-up time. In

general, the yields obtained are a bit lower compared to the literature references; this is because some of the slurry moves to the top of the reactor during the liquefaction (Figure 12 to Figure 14) where there is no heat. The main modification done in the reactor after these experiments was the removal of the pressure gauge and the valves for purging and gas sampling which are placed at the top of the reactor. In this way, the reactor was sealed at the top by means of a plug.

**Table 19 – Yields in wt.% db of the HTL of untreated microalgae done at 300 °C for 30 min with a 7 – 8 wt.% db slurry**

| No | Species         | Heatup/(min) | Biocrude | <sup>a</sup> Gas | Solid | <sup>b</sup> Aqueous |
|----|-----------------|--------------|----------|------------------|-------|----------------------|
| 1  | <i>N. gad.</i>  | 20           | 20.88    | 19.17<br>[9.60]  | 12.42 | 47.53                |
| 2  | <i>N. gad.</i>  | 50           | 22.01    | 17.29            | 5.08  | 55.62                |
| 3  | <i>N. gad.</i>  | 60           | 23.71    | 20.23<br>[29.35] | 3.95  | 52.11                |
| 4  | <i>S. alm.</i>  | 20           | 26.27    | 13.70            | 9.50  | 50.53                |
| 5  | <i>T. suec.</i> | 10           | 17.96    | 19.06<br>[25.26] | 14.03 | 48.94                |
| 6  | <i>T. suec.</i> | 20           | 20.28    | 13.76            | 12.35 | 53.61                |

<sup>a</sup> Values calculated using the remaining pressure of the reaction after the liquefaction and equation Equation 5 [values determined utilizing the weight difference of the full reactor (without the gas system, Figure 12) before and after the liquefaction, in accordance with equation Equation 5].

<sup>b</sup> Determined by difference

Brown et al. (Brown et al. 2010) reported an oil yield of ~32 db wt.% in the liquefaction of *Nannochloropsis* at 300 °C for 60 min. The higher oil yield is because they used a concentration between 5 and 15 daf wt.% slurry. It has been proven that the biocrude yield is directly related to the concentration of

microalgae slurry used as feedstock, especially in the range of 10 to 20 daf wt.% (Jena et al. 2011). Moreover, Brown et al. (Brown et al. 2010) have done the HTL for 60 min, and the conversion of the biomass is higher at larger residence times, with the highest biocrude yield at 60 min (Jena et al. 2011).

There is a clear difference in the calculation of the gas yield with the two methods. Valdez et al. (Valdez et al. 2011) reported gas yields of ~7 db wt.% in the liquefaction of 20 – 25 wt.% solids of *Nannochloropsis* for 60 min. This value is lower than the values obtained with Equation 5. In the case of the values of the gas yield determined by weight difference, the overestimation was due to the use of the gas system (see Figure 12) at the top of the reactor; some slurry moved at this point of the reactor due to the high pressures during the liquefaction and it was considered as gas generated. Besides, due to the low accuracy of the pressure gauge at low pressures (<5 bar) the calculation using the weight difference is preferred for future experiments.

The same authors (Valdez et al. 2011) report a solid yield of approximately 4 db wt.%. This result is very similar to the third experiment of Table 19 which was done with the same conditions. The yield of the water phase was calculated by difference. As discussed above, the results of Table 19 match with the data of the literature, at least quantitatively. The qualitative acceptance of the biocrude is discussed in the following section of Characterization of the biocrude.

The experiments have helped to understand that it is necessary to reduce as much as possible the heating-up stage as the biocrude yield increases and the solid residue decreases when the heat-up is longer (experiments 1, 2, and 3 of Table 19). This means that the liquefaction occurs at undesired conditions. The assessment of the influence of the species in the process was done with the experiments of 20 min of heat-up time (experiments 1, 4, and 6 of Table 19). The highest biocrude yield was obtained with *Scenedesmus* followed by *Nannochloropsis* and *Tetraselmis*. Furthermore, the best conversion of the biomass is again obtained with *Scenedesmus* as they give the lowest solid residue at the end of the liquefaction and, both *Nannochloropsis* and *Tetraselmis* present the similar conversion. The different pyrolytic behaviours of

the microalgae species shown in the TGA (Figure 17) agree with the results of the experiments 1, 4, and 6 of Table 19: while *Nannochloropsis* and *Tetraselmis* present similar behaviour, the thermal degradation of *Scenedesmus* differs from the other two species. This could be related to the different ash content and composition of the organic material.

### **5.3 Characterization of the biocrude**

The biocrude was characterized via Elemental, FTIR, and GC-MS analyses to compare its composition with the results available in the literature.

#### **5.3.1 Elemental analysis biocrude**

The values of the CHNS analysis agree with the data present in the literature (Garcia Alba et al. 2012). They are presented in total weight percentage because the contents of ash and moisture in the biocrude are unknown; furthermore, this is the most common way of expressing the ultimate analysis of the biocrude (López Barreiro, Zamalloa, et al. 2013; Biller & Ross 2011; Garcia Alba et al. 2012). It is important to highlight the high nitrogen content of the biocrudes. Data of the nitrogen content of the biocrude reported in the literature is shown in Table 20.

Table 20 – Nitrogen content of the biocrude reported in the literature

| Reference                               | Sp.               | Temp/(°C) | Time/(min) | Slurry/(wt.%, daf) | N/(wt.%) |
|-----------------------------------------|-------------------|-----------|------------|--------------------|----------|
| (López Barreiro, Zamalloa, et al. 2013) | <i>N. gad.</i>    | 250       | 5          | 5 – 7              | 3.7      |
|                                         | <i>N. gad.</i>    | 375       | 5          | 5 – 7              | 5.2      |
|                                         | <i>S. alm.</i>    | 250       | 5          | 5 – 7              | 4.8      |
|                                         | <i>S. alm.</i>    | 375       | 5          | 5 – 7              | 6.1      |
|                                         | <i>T. suec.</i>   | 250       | 5          | 5 – 7              | 4.1      |
|                                         | <i>T. suec.</i>   | 375       | 5          | 5 – 7              | 6.1      |
| (Biller et al. 2011)                    | <i>Nanno. sp.</i> | 350       | 60         | <sup>a</sup> 10    | 4.1      |

<sup>a</sup> Value given in total wt.%

Table 21 - Ultimate analysis of the biocrude of experiments No 1 (*N. gad.*), 4 (*S. alm.*), and 6 (*T. suec.*) of Table 19 done at 300 °C for 30 min with a 7 – 8 wt.% db slurry. The values are total wt.% because the contents of ash and moisture of the biocrude are unknown.

| No Exp | Species         | N    | C     | H    | S    | <sup>a</sup> O | <sup>b</sup> HHV/(MJ/kg) |
|--------|-----------------|------|-------|------|------|----------------|--------------------------|
| 1      | <i>N. gad.</i>  | 6.01 | 73.59 | 8.49 | 0.69 | 11.21          | 35.06                    |
| 4      | <i>S. alm.</i>  | 6.37 | 72.86 | 8.36 | 0.92 | 11.48          | 34.60                    |
| 6      | <i>T. suec.</i> | 6.75 | 72.23 | 7.75 | 0.97 | 12.29          | 33.38                    |

<sup>a</sup> Determined by difference

<sup>b</sup> Calculated with Dulong's Equation 6

Generally, the oxygen content of the bio-oil obtained with HTL (approximately 15 wt.% of oxygen) is about half of that from the pyrolysis oil (between 30 to 40 wt.% of oxygen) (Xiu & Shahbazi 2012; Mullen & Boateng 2008; Vardon et al.

2011). Besides, the biocrude from microalgae has lower oxygen content than using other types of biomass such as wood (Miao et al. 2004). In spite of the low oxygen contents obtained, they are still significantly higher than the oxygen present in fossil fuel, which is around 1 wt.% (Miao et al. 2004); therefore, the use of hydrotreatment to remove these heteroatoms is required (Elliott et al. 2013).

The HHVs of the biocrudes are in the range described in the literature (Duan et al. 2013), between 32 and 36 MJ/kg. The calorific value can rise significantly, up to 44 KJ/kg, after the use of hydrotreatment (Duan & Savage 2011).

### **5.3.2 FTIR biocrude**

The similarities of the biocrudes from the experiments 2, 3, and 4 of Table 19, in terms of functional groups, were determined with FTIR spectroscopy. The data was plotted in Figure 22 without the necessity of giving an additional value to the trend line of the results of some microalgae because of the different noise of the results.

The results from the FTIR analysis show that the three biocrudes present the same functional groups. Four zones were identified: (A at  $710 - 860\text{ cm}^{-1}$ ) related with substituted benzenes, (B at  $1475$  &  $1600\text{ cm}^{-1}$ ) indicating the presence of aromatic and olefin carbon assignments, (C at  $1600 - 1680\text{ cm}^{-1}$ ) both for N-H bending and C=O stretching vibration, and (D at  $2840 - 3000\text{ cm}^{-1}$ ) –CH<sub>3</sub> and –CH<sub>2</sub>– groups. The peak attribution and interpretation are based on previous studies (Duan & Savage 2011a; Vardon et al. 2011; Duan & Savage 2011c).

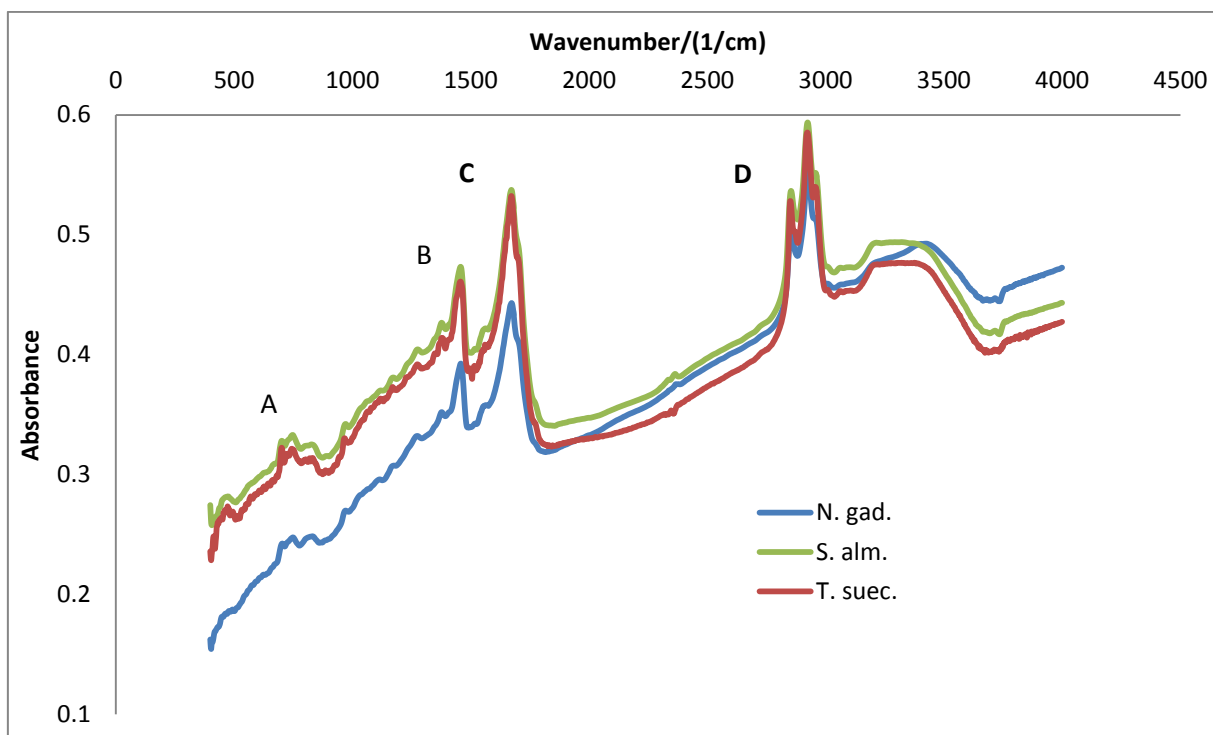


**Figure 22 – FTIR of the biocrude of experiments No 1 (*N. gad.*), 4 (*S. alm.*), and 6 (*T. suec.*) of Table 19**

The concentration of the molecules can be calculated with the results of FTIR using the Lambert Beer law (Volkamer et al. 2005), i.e. the higher the absorption peak, the more concentration of that molecules. Despite the different noise of the trend lines, it is possible to realize about the shorter peak at 1600 - 1680  $\text{cm}^{-1}$  (C) in the case of the biocrude of Nanno agrees with lower nitrogen content shown in the results of the Elemental analysis biocrude.

### 5.3.3 GC-MS biocrude

The degrees of complexity of the biocrudes were stated with the use of GC-MS. As per the limitations of this technique to identify the compounds, only the compounds with a quality of identification higher than 90% were considered. According to the FTIR biocrude, the bio-oil from the three microalgae are similar in terms of functional groups present in the samples. There are 7 compounds that were determined in all the biocrudes with a probability of identification higher or equal to 90%: 4-methylphenol (p-cresol), 4-methyl quinoline, cyclotetradecane, 2,4-bis(1,1-dimethylethyl)-phenol, 1-nonadecene, n-

hexadecanoic acid (palmitic acid), and (Z)-9-Tricosene. On the other hand, the large number of compounds present proves the high complexity of the biocrudes.

**Table 22 – Comparison of the three biocrudes obtained in the experiments No 1 (*N. gad.*), 4 (*S. alm.*), and 6 (*T. suec.*) of Table 19 via GC-MS analysis**

| Biocrude        | <sup>a</sup> Total | <sup>b</sup> Identified | <sup>c</sup> Considered | <sup>d</sup> %Considered | <sup>e</sup> Nanno | Tetra | Scen |
|-----------------|--------------------|-------------------------|-------------------------|--------------------------|--------------------|-------|------|
| <i>N. gad.</i>  | 138                | 26                      | 14                      | 28.81                    | 2                  | 5     | 0    |
| <i>S. alm.</i>  | 105                | 27                      | 17                      | 30.26                    | 5                  | 1     | 4    |
| <i>T. suec.</i> | 97                 | 23                      | 15                      | 21.28                    | 0                  | 4     | 4    |

<sup>a</sup> Total number of compounds identified

<sup>b</sup> Number of compounds with a quality of identification higher or equal to 90 %

<sup>c</sup> Compounds with a probability of identification higher or equal to 90% in all the biocrudes in which they appear.

<sup>d</sup> Percentage of the area of the considered compounds relative to the total ion chromatogram (TIC) peak area.

<sup>e</sup> Number of compounds identified in the biocrude of the other species.

For example, were identified in the biocrude of *Nannochloropsis* 138 compounds, but only 26 compounds have a probability of identification higher or equal to 90% in that specific sample of biocrude. Assuming that the composition of the biocrude does not depend too much on the composition of microalgae (as all the species present lipid, carbohydrates and protein in a similar range) special value is given to the compounds which have 90% identification probability in all the biocrudes where they appear. This is a clear proof that these compounds are in the biocrudes and also serve to check the resemblances among the bio-oils. The bio-oil of *Nannochloropsis* has 7 compounds in common with the bio-oil of the other two species, 5 in common with *Tetraselmis*' bio-oil, none in common with *Scenedesmus*' biocrude, and 2 compounds which are not present in the biocrude of any other species.

The presence of some compounds in the bio-oils which have been previously identified in the lipids of the microalgae, as is the case of 1-nonadecene and palmitic acid, means that the conditions used to carry out the liquefaction are not severe enough.

The high amount of nitrogen compounds detected agrees with the CHNS and FTIR analyses. A total number of 82 different nitrogen compounds are in the biocrudes with a probability of identification higher or equal to 90%. Of them, 22 compounds are nitrogen heterocycles, 9 amides, and 2 amines.

Summarize, the results of GM-MS show the high complexity of the biocrudes and their resemblance; in spite of the different composition of the microalgae, some compounds appear in all the biocrudes.

## 5.4 Summary

These experiments have served to identify the performance of the reactor. Two main modifications were applied after the experiments: (a) the removal of the gas system at the top of the reactor, and (b) reduce the temperature of the reactor to avoid the failure of the reactor.

The gas system at the top of the reactor, i.e. the pressure gauge and the gas purging and sampling valves, were swapped with a plug in order to avoid the losses of slurry during the liquefaction. Basically, if the slurry moves to the top of the reactor it remains unconverted because of the lower temperature in the parts of the reactor that are outside of the furnace (Figure 12 to Figure 14). By replacing the parts at the top of the reactor with a plug, the volume of headspace was reduced and all the volume of the reactor is exposed to high temperature. However, these modifications have an intrinsic risk associated with them as it is not possible to measure the pressure during the liquefaction, which can rise up to 100 bar when at 300 °C.

The second modification was taken for safety reasons. Some leaks in the reactor were observed when using 300 °C as the reacting temperature. The autogenous pressure inside the reactor can lead to the failure of the components and the subsequent explosion. In this way, in the HTL of pretreated

microalgae, the temperature was 250 °C, which corresponds with a pressure of 35 to 45 bar.

Also, the procedures for the characterization of the products were successfully applied. The results of these HTL of untreated microalgae prove that *Scenedesmus* is the best species for the liquefaction, as they give both the highest biocrude yield and conversion due to their lower ash content. The compositions of the biocrudes are very similar in the cases, so this factor would not make any difference when taking the decision of the best liquefaction performance, as in all the cases would be necessary to use upgrading techniques to agree with the specifications of the biofuel use. Nevertheless, this helps to understand the chemistry of the HTL; some of the final products could be fixed independently of the microalgae composition.

Considering the corrosion that may take place in the reactor, the use of a fresh water species such as *Scenedesmus* is also recommended for having less ash content.



## 6 HTL of pretreated microalgae

The use of ultrasounds as pretreatment of the microalgae slurry before the liquefaction was tested. No research has been conducted on the application of cell disruption technologies to the microalgae slurry before the liquefaction. Only the use of microwaves to algae powder has been assessed (Biller et al. 2013); however, it is considered that the cell lysing technology should be applied directly to the microalgae slurry before the HTL. Initially, it was expected an improvement in the performance of the liquefaction, i.e. improvement in the yield and composition of the products, based on the hypothesis of better availability of the compounds during the reaction.

The pretreatment experiments were done with a working volume of 15 – 12 mL, 5 – 7 daf wt.% of slurry concentration, 30 min of reaction time, 250 °C, and 35 to 45 bar of autogenous pressure. The slurry was sonicated prior to being charged to the reactor. The ultrasonic energies used are in accordance with the results of (Gerde et al. 2012): from 0 to 575 J/g slurry.

In this chapter, the influence of the pretreatment on the performance of the liquefaction of microalgae is discussed based on the results of the characterization of the products. The analysis of the products was done at two levels: (a) quantitative, calculating the yield of each phase; and (b) qualitative, using FTIR and GC-MS to assess the influence of the ultrasonic pretreatment energy on the composition of the biocrude. As the results differ from what it is expected, a deep analysis of the sonication equipment was done. Finally, the most relevant points of this chapter are gathered together as the outcome of the chapter.

### 6.1 Products yield

Figure 23 to Figure 25 show the qualitative analysis of the products of the liquefaction; the yields of the products are shown for the different experiments done with different sonication energies as pretreatment. Only in one experiment, denoted as “separate”, the pretreatment was not applied and the microalgae biomass and the water were charged separately into the reactor.

The quantitative analysis of the products of the liquefaction (Figure 23 to Figure 25) does not show any influence of the pretreatment on the liquefaction of the three species. In order to obtain reliable results, each sonication condition was repeated 3 to 4 times. The error bars that appear in Figure 23 to Figure 25 represent the standard deviation. In general, the variation of the yields is in the range of the error bars.

Gerde et al. (Gerde et al. 2012) found that the energy which maximizes the cell disruption was 80 J/g slurry, independently on the microalgae species and the slurry concentration. They calculate the amount of cell breakage by measuring the amount of intracellular compounds released to the medium. The idea behind using ultrasounds as liquefaction pretreatment is to disrupt the algae so there is a better mixing and interaction of the compounds during the liquefaction. In this way, it was expected that the influence of the ultrasounds in yield of the HTL products follows a similar trend of the results of Gerde et al. (Gerde et al. 2012).

The lack of trend in Figure 23 to Figure 25 could be related to the fact that the sonication equipment used is not intended for the disruption of the microalgae cell. In this way, the equipment does not present the proper configuration and power output to effectively produce the breakage of the microalgae cells (see section 6.3 of Analysis of the sonication equipment). In order to prove that the equipment employed was not applicable for the lysing of microalgae, the microalgae slurry was sonicated before carrying out the TGA with the dried biomass. This allows us to understand whether the use of the pretreatment affects the pyrolytic behaviour of microalgae or not.

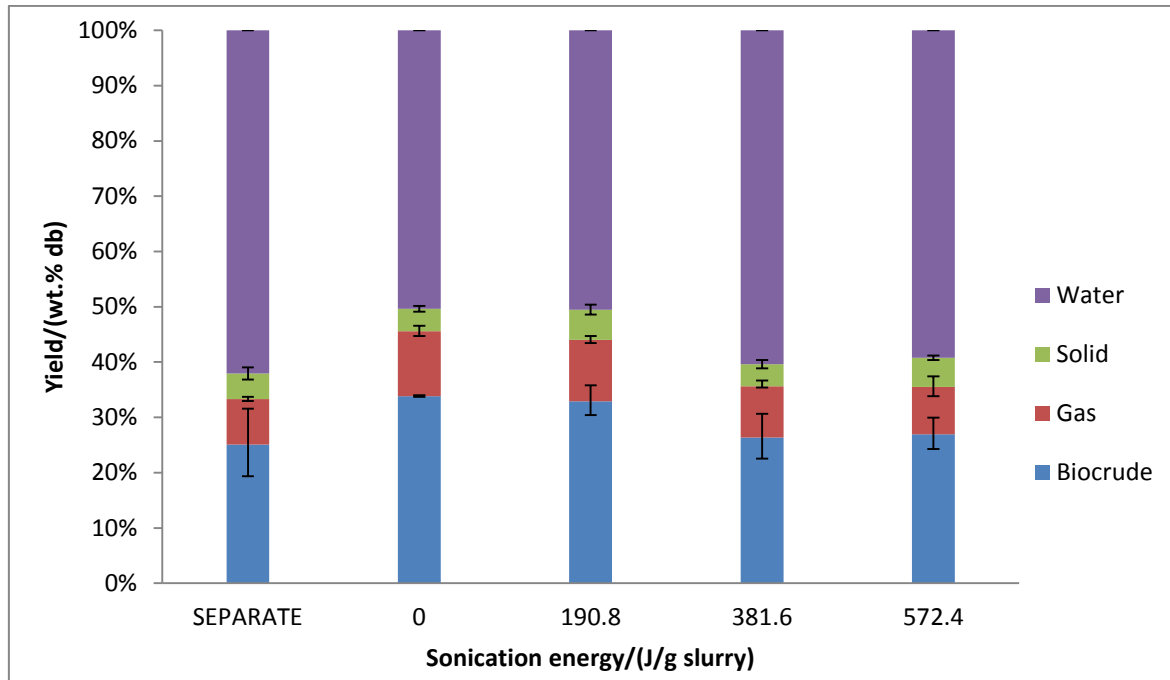


**Figure 23 – Influence of sonication pretreatment on product yields (wt.% db) obtained from HTL of *Nannochloropsis* at 250 °C for 30 min. For all of the experiments the slurry was prepared outside of the reactor with the exception of the experiment denoted as “SEPARATE” in which the microalgae biomass and the water were charged separately into the reactor without applying any pretreatment.**

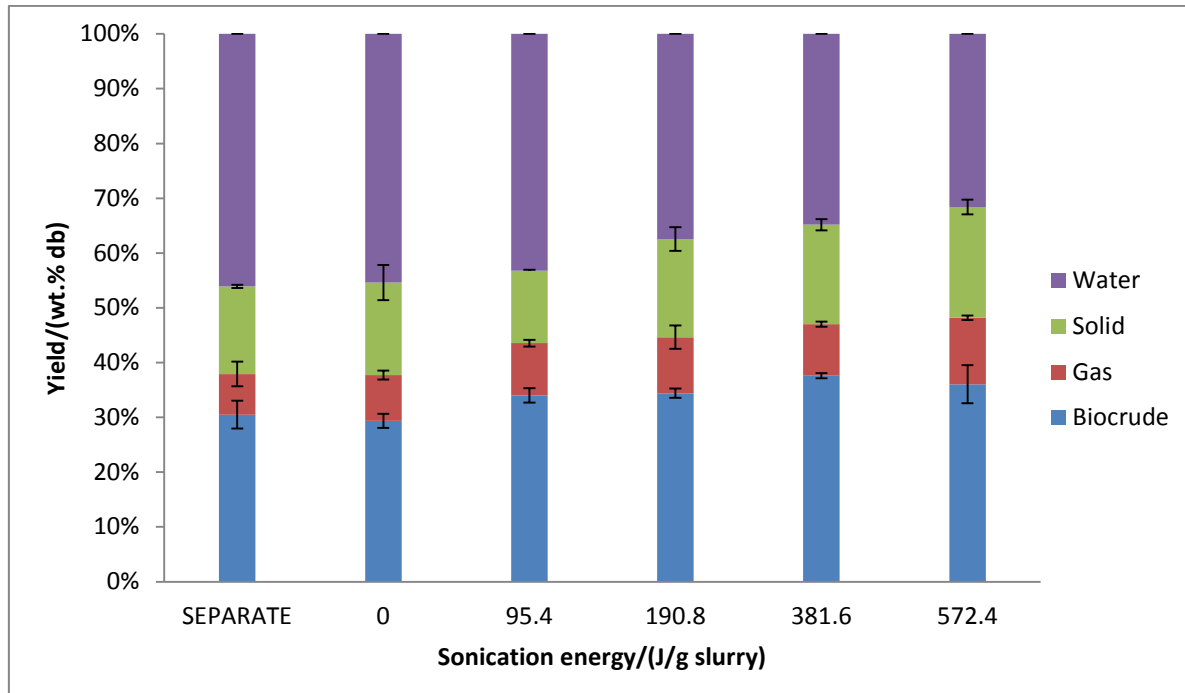


**Figure 24 – Influence of sonication pretreatment on product yields (wt.% db) obtained from HTL of *Scenedesmus* at 250 °C for 30 min. For all of the experiments the slurry was prepared outside of the reactor with the exception of the experiment denoted as “SEPARATE” in which the microalgae biomass and the water were charged separately into the reactor without applying any pretreatment.**

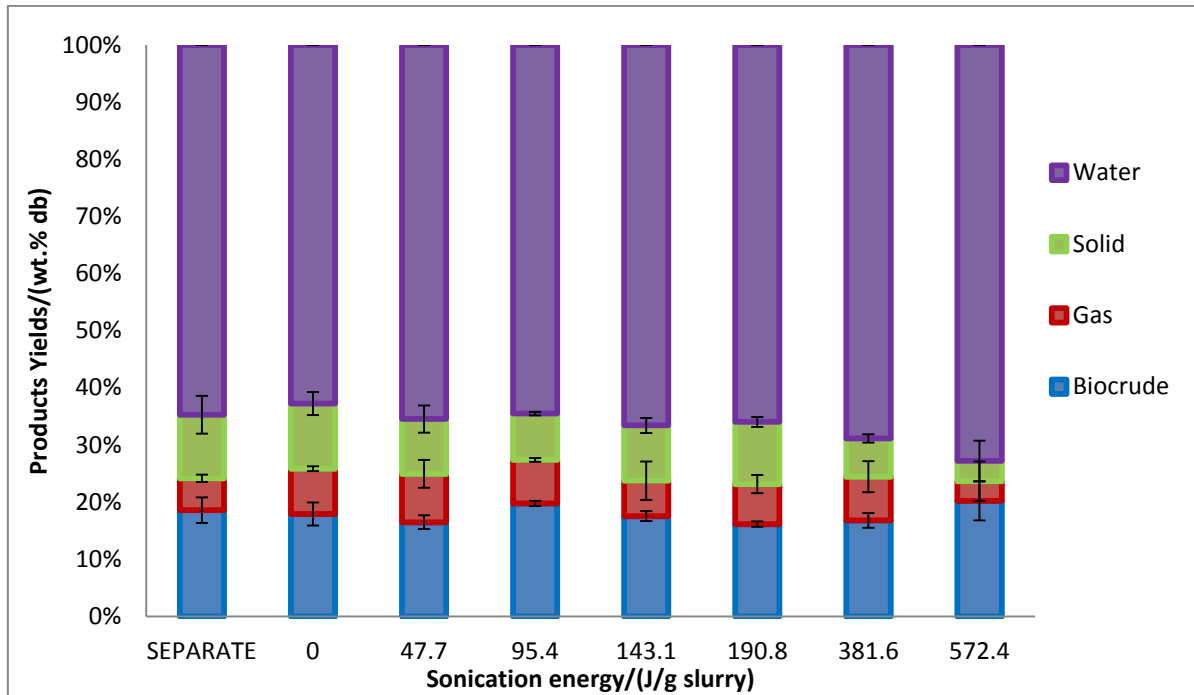


**Figure 25 – Influence of sonication pretreatment on product yields (wt.% db) obtained from HTL of *Tetraselmis* at 250 °C for 30 min. For all of the experiments the slurry was prepared outside of the reactor with the exception of the experiment denoted as “SEPARATE” in which the microalgae biomass and the water were charged separately into the reactor without applying any pretreatment.**

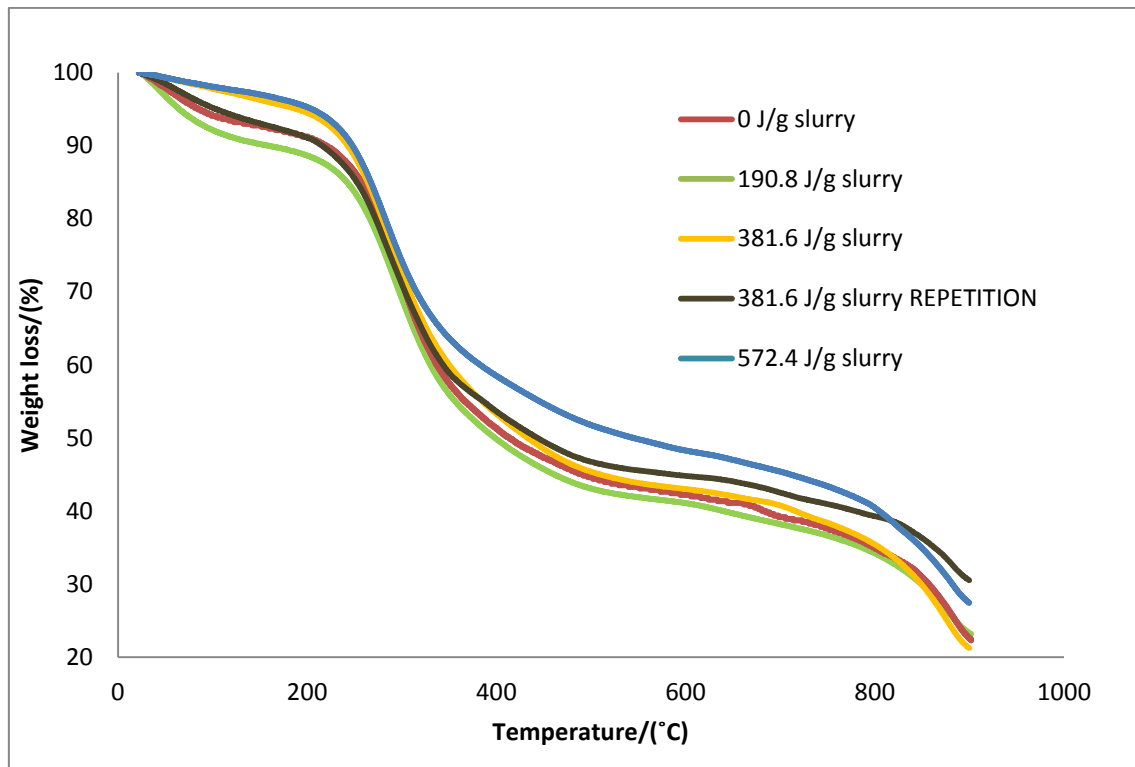


Figure 26 – TGA of *Tetraselmis* using different sonication energies as pretreatment

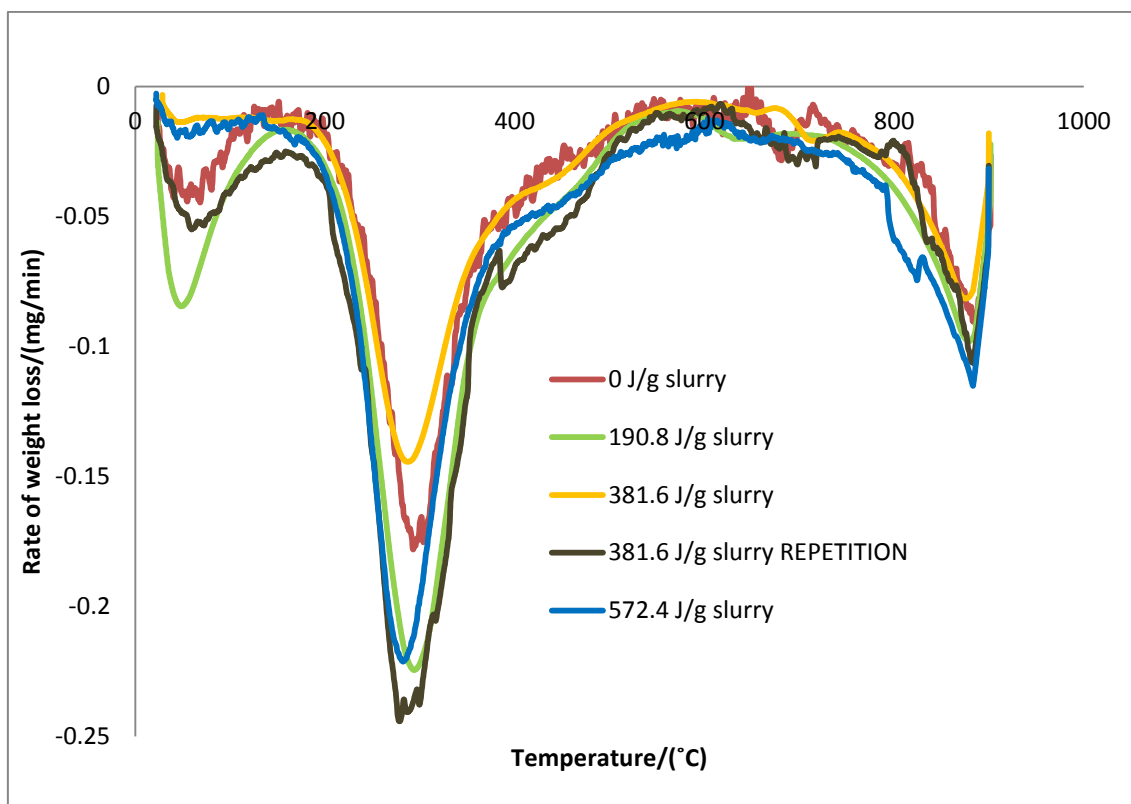


**Figure 27 – DrTGA of *Tetraselmis* using different sonication energies as pretreatment**

The TGA of the microalgae sonicated at different energies (Figure 27) shows that the amount of biomass degraded during the pyrolysis is not affected by the energy used in the pretreatment (Table 23): The TGA directly determines the percentage of biomass weight loss with the increase of temperature; the conditions used to carry out the analysis are shown in Table 8. The two experiments were carried out using 381.6 J/g slurry as sonication energy and the variation results obtained covers the full range of the results obtained using different ultrasonic energies. The same conclusion can be obtained for the speed of degradation (Table 24). According to the TGA results (Figure 27), the pretreatment of microalgae with the sonication equipment of does not affect the pyrolytic behaviour of microalgae. It would be interesting to repeat the TGA in order to get reproducible results and make this conclusion more reliable.

**Table 23 – Percentage of *Tetraselmis* degraded in the TGA when using different sonication energies as pretreatment**

| Sonication<br>energy/(J/g slurry) | % Biomass degraded |               |            |
|-----------------------------------|--------------------|---------------|------------|
|                                   | Average            | St. deviation | N measures |
| 0                                 | 77.68              | 0             | 1          |
| 190.8                             | 76.8               | 0             | 1          |
| 381.6                             | 74.10              | 6.55          | 2          |
| 572.4                             | 72.53              | 0             | 1          |

**Table 24 – Rate of weight loss at the main pyrolysis step of *Tetraselmis* during the TGA when using different sonication energies as pretreatment**

| Sonication<br>energy/(J/g slurry) | Rate of weight loss/(mg/min) |               |            |
|-----------------------------------|------------------------------|---------------|------------|
|                                   | Average                      | St. deviation | N measures |
| 0                                 | -0.1778                      | 0             | 1          |
| 190.8                             | -0.2243                      | 0             | 1          |
| 381.6                             | -0.1941                      | 0.0704        | 2          |
| 572.4                             | -0.2212                      | 0             | 1          |

The TGA of *Nannochloropsis* and *Scenedemus* at different sonication energies are shown in Appendix D.

On the other hand, it looks like there is a decrease in the temperature at which the maximum rate of weight loss happens. The temperature maximums are shown in Table 25. It may be not enough to actually note a change during HTL. It would be necessary more data to determine the reasons of the reduction of the maximum temperature.

Other possible explanation to the lack of trend in Figure 23 to Figure 25 is that the microalgae cells were disrupted during the drying process. In such case, it is

not possible to make any variation in the microalgae cell structure by means of any cell disruption technology; therefore no influence of the pretreatment could be observed in the performance of the HTL of microalgae.

**Table 25 – Decrease of temperature at which the maximum rate of weight loss of *Tetraselmis* biomass takes place**

| Sonication/(J/g slurry)       | Max temp/(°C) | Max rate weight loss/(mg/min) |
|-------------------------------|---------------|-------------------------------|
| 0                             | 293.02        | -0.1781                       |
| 190.8                         | 293.82        | -.02245                       |
| 381.6                         | 287.57        | -0.1444                       |
| 381.6 REPETITION <sup>a</sup> | 278.34        | -0.2442                       |
| 572.4                         | 282.22        | -0.2213                       |

<sup>a</sup> The TGA of *Tetraselmis* was repeated with the samples of 381.6 J/g slurry to check the reproducibility of the results.

## 6.2 Qualitative analysis

The analytical techniques applicable to the biocrude which are cited in Table 7 represent different levels of characterization of the biocrude. Due to the lack of time, the biocrude was characterized only with FTIR and GC-MS. This is not a problem because, as it was explained in the section of Characterization of the biocrude of chapter 5, some information that these analyses provide are redundant.

### 6.2.1 FTIR biocrude

No variation was found in the FTIR spectra of the biocrudes of *Nannochloropsis* obtained at different sonication energies (Figure 28). This means that the pretreatment does not have any influence on the composition of the biocrude. It is important to mention the values of the FTIR matches in the level of absorbance, as it is shown in Figure 29; the different values of absorbance

present in Figure 28 were obtained by adding a constant value of absorbance (Table 26) in order to obtain the lines of FTIR at different absorbance levels.

**Table 26 – Added absorbance constant value to the FTIR trend line of the biocrude of *Nannochloropsis* obtained at different sonication energies**

| Pretreatment energy | Added absorbance constant value |
|---------------------|---------------------------------|
| Separated           | 0                               |
| 0 J/g slurry        | 0.3                             |
| 190.8 J/g slurry    | 0.6                             |
| 381.6 J/g slurry    | 0.9                             |
| 572.4 J/g slurry    | 1.2                             |

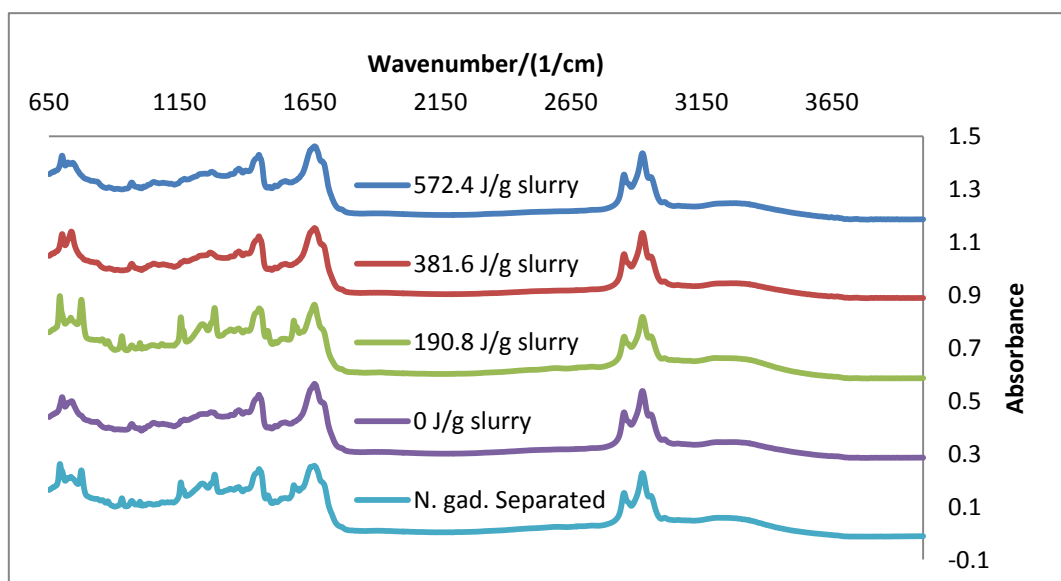


**Figure 28 – FTIR of the biocrude of *Nannochloropsis* using different sonication energies as pretreatment. “Separated” means that the microalgae biomass and the water have been fed separately into the reactor; therefore, the microalgae slurry has not suffered any pretreatment (0 J/g slurry).**

The results of the biocrude from *Scenedesmus* and *Tetraselmis* obtained with different sonication pretreatment energies are shown in Appendix E.

Furthermore, in accordance with the results of FTIR biocrude of the HTL of untreated microalgae, the FTIR spectra of the biocrudes from different microalgae are very similar (Figure 29). In spite of having identified the same peaks in the HTL of untreated microalgae carried out at 300 °C and in the HTL of pretreated microalgae done at 250 °C, the intensities are different (Figure 30).

To sum it up, considering that the microalgae samples were in good state before applying the pretreatment (as it can be observed an entire single cell of *Scenedesmus* in Figure 19) it is considered that the use of ultrasounds as pretreatment with the sonication bath does not make any change in the FTIR spectrum of the biocrude (Figure 28 and Figure 29). Nevertheless, the change in the conditions of the liquefaction, i.e. from 300 °C in the HTL of untreated microalgae to 250 °C in the HTL of pretreated microalgae, is a more important factor to change the composition of the biocrude as there is a significant change in the FTIR spectrum (Figure 30).

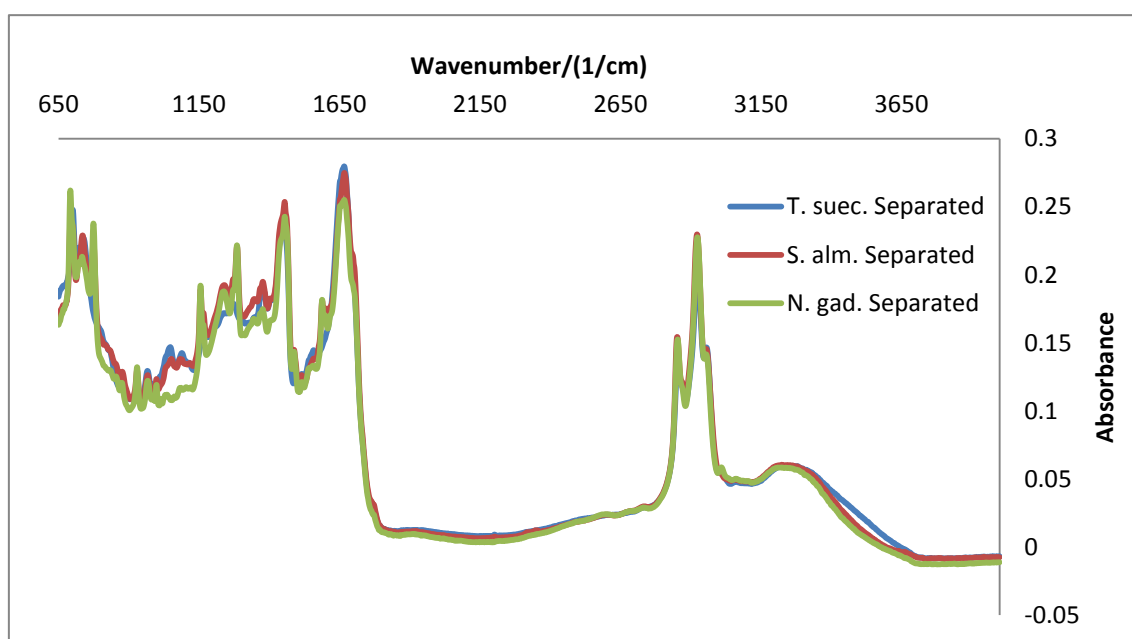


**Figure 29 – FTIR spectrum of the biocrude obtained from three different species charged separately in the reactor. This is the same data plotted in Figure 30: *N. gad.* 250, *S. alm.* 250, and *T. suec.* 250**

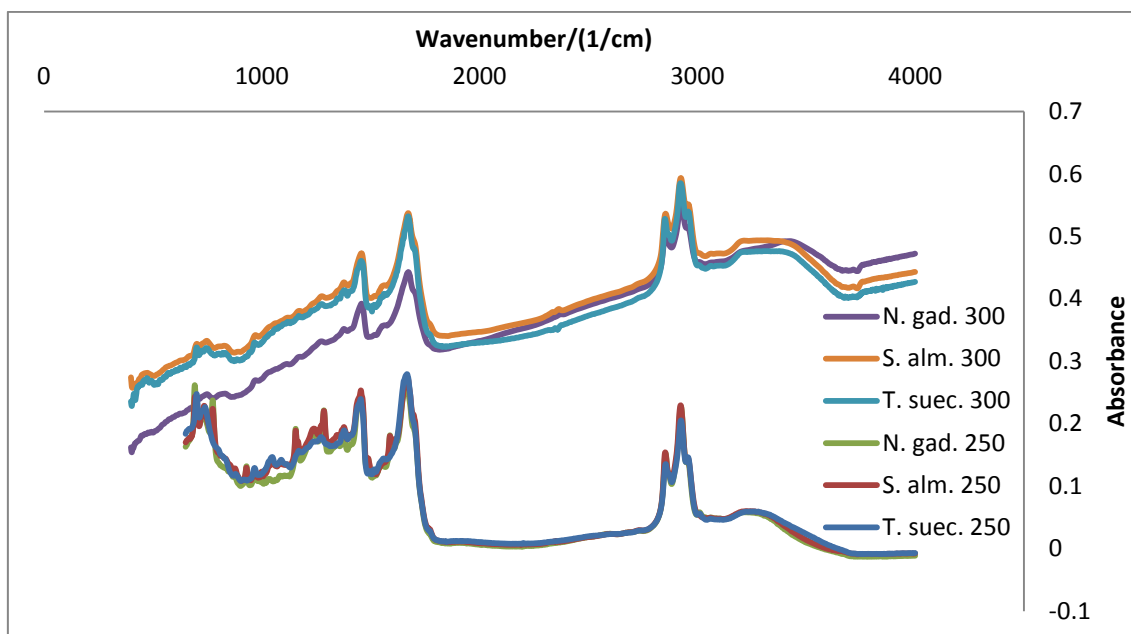


**Figure 30 – Comparison of the FTIR spectra of the biocrude of the HTL of untreated microalgae carried out at 300 °C (*N. gad.* 300, *S. alm.* 300, and *T. suec.* 300) and the HTL of pretreated microalgae done at 250 °C (*N. gad.* 250, *S. alm.* 250, and *T. suec.* 250). The latter is the same data plotted in Figure 29: *N. gad.* Separated, *S. alm.* Separated, and *T. suec.* Separated.**

### 6.2.2 GC-MS biocrude

Due to the lack of time, only selected samples of biocrude were analysed: one sample of *Nannochloropsis* charged separately in the reactor and four samples of *Tetraselmis* at 0, 143.1, 190.8, and 381.6 J/g slurry sonication energies. The results are shown in the Appendix F.

All the biocrudes present similar composition, independently of the sonication pretreatment or the microalgae species in the pretreatment. The most characteristic features are the low number of compounds identified, between 20 and 24, and the similarity of the composition of the biocrudes. It is important to highlight the presence of Palmitic acid in all the biocrudes and Linoleic acid only in the biocrude of *Nannochloropsis*.

The number of compounds identified is much lower than the HTL of untreated microalgae done at 300 °C (Table 22). In fact, the amount of compounds identified is more similar to the number of compounds present in the lipids of

microalgae (Table 16). It is generally recognised that the complexity of the biocrude increases with the temperature of the HTL (Torri et al. 2012). Again, both the ultrasonic pretreatment and the composition of the microalgae is less important than the temperature of the process to modify the composition of the biocrude.

### 6.3 Analysis of the sonication equipment

The Elmasonic P 60 H sonication equipment used to pretreat the microalgae slurry before the liquefaction has not been designed to disrupt the cells of the microalgae; instead, it was intended for other purposes which require less ultrasonic power output such as cleaning laboratory glassware. The main difference between the sonication baths and the ultrasonic cell disruption equipment is the configuration: while in the sonication bath the horn which produces the ultrasounds is applied to the whole water bath where the samples will be placed (Figure 31), in the case of the cell disruption equipment the transducer is directly in touch with the sample (Figure 32). The close contact between the horn and the microalgae sample is highly desired to ensure that all the ultrasounds generated affect the biomass.

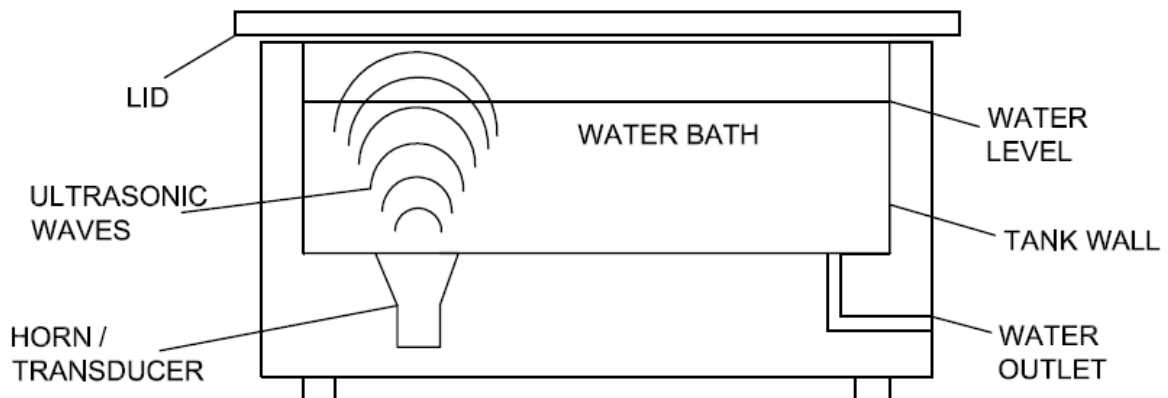


**Figure 31 – Scheme of the sonication bath. Elaborated from (S. Berliner 2010; UCE ultrasonics co. 2011)**

Lee et al. (Lee et al. 2010) used a sonicator (Sonic and Materials Inc., USA) at a resonance of 10 kHz for 5 min. These values are in the range of Elmasonic P

60 H equipment, however the configurations of the equipment are different (Figure 31 and Figure 32).

Sonics & Material Inc. offer a wide range of sonication equipment, some of them are specialised for the cell lysing, sample preparation, and homogenization (Sonics & Materials 2006). This is the type of sonicator that is supposed to be used for matters related to an efficient microalgae cell disruption.



**Figure 32 – Batch sonication equipment of Sonic and Materials Inc (Sonics & Materials 2006).**

The net power output reported in the data sheet of Sonics & Material Inc. equipment varies from 130 to 1500 W. The specifications of the sonicator bath (Table 10) are in the same range, nevertheless it is not possible to ensure that all this energy reaches the sample. The value of the effective ultrasonic output of the sonication bath was determined by measuring the increase of the temperature of the sample; this assumption has the problem that the increase in the temperature of the sample can be related to a number of reasons, not only to the cell disruption with ultrasounds, so the calculation of the sonication power for the sonication bath is not very reliable. For instance, the temperature of the sample can rise because of the heat released by the horn; that is why some sonication equipment have a cooling system around the transducer. The sonicator of Sonics & Materials Inc. introduces the sonication probe inside the sample ensuring that all the affects the sample in the first place. On the other

hand, the frequency of their equipment are low (15 – 40 kHz), which is optimum for cell disruption; similarly the Elamasonic P 60 H can work at 37 – 80 kHz (Table 10).

Gerde et al. (Gerde et al. 2012) have used a bench-scale Branson 2000 Series ultrasonic system (Branson Ultrasonics, Danbury, CT, USA) with the capacity to operate at a maximum power output of 2.2 kW and a frequency of 20 kHz. The horn was a standard 20 kHz half-wavelength catenoidal titanium horn with a flat 13 mm diameter face (gain 1:8). The cells were sonicated using 10 mL aliquots in customized glass centrifuge tubes (50 mL round bottom centrifuge tubes, 29mm O.D., Corning, Lowell, MA, USA) that were cut to a length of 68 mm. Again, the power output of their equipment is higher than the 560 – 720 W of the sonication bath.

## 6.4 Summary

The liquefaction of the microalgae slurry was done after pretreating the feedstock with ultrasounds using sonication energies up to 572.4 J/g slurry. The sonication energies used for the pretreatment were chosen based on the work of Gerde et al (Gerde et al. 2012), which used up to 250 J/g slurry to determine the energy which maximizes the cell disruption of microalgae. Even when they found that the optimum energy is 80 J/g slurry, the results of this thesis do not show any trend and the differences in the yields are a consequence of the variability of the results, determined as the standard deviation.

The same conclusion was obtained with the qualitative analysis of the biocrude: the pretreatment of the slurry does not affect the performance of the liquefaction. In fact, the temperature is the most important factor in determining the HTL products; the biocrudes from different species are very similar.

It is considered that the reason of having no influence of the ultrasonic pretreatment on the products of the liquefaction is the sonication bath used to disrupt the microalgae cells; this equipment is intended for cleaning laboratory glassware and not for microalgae cell disruption. The configuration of the machine does not ensure that all the ultrasonic power affect the structure of the

microalgae, therefore only a small fraction of the total power reaches the samples. Furthermore, the specifications of the sonication baths in terms of maximum power output and frequency, do not match with the equipment particularly designed for breaking the microalgae cells.

In this thesis is not presented enough evidence that the microalgae cells were in perfect state before applying the pretreatment. This means that, if the cell were already disrupted before applying the ultrasounds, the pretreatment would not be effective even when a specially designed equipment for microalgae cell disruption would be used. Only one entire cell of *Scenedesmus* could be found in the SEM pictures (Figure 19) consequence of the grinding done for the sample preparation.

In this way, the next step for testing the ultrasonic pretreatment of the microalgae before the HTL is to evaluate the efficiency of the lysing method through optical microscopy. If it is confirmed that the sonication bath is not breaking the algae cells, it would be necessary to procure an specially designed sonication equipment for microalgae cell disruption.

## 7 Conclusions and future work

The aim of this thesis was to determine the influence of the sonication pretreatment of the microalgae slurry on the performance of the liquefaction. This idea was proposed after doing a literature review and understanding the chemistry behind the HTL of the microalgae. To achieve a positive outcome, it was necessary to propose objectives in three different areas: biomass characterization, HTL of the microalgae, and ultrasounds applied to microalgae cell disruption.

The characterization of microalgae was necessary to understand the influence of the feedstock composition on the liquefaction. The parameters (moisture and ash content, biochemical composition, ultimate analysis, calorific value, lipid characterization, and pyrolytic behaviour) that were measured are reported in the relevant literature; some of them were determined indirectly which means that the values obtained are not very accurate. Particularly, the biochemical composition of the microalgae, which was established with the data of the FTIR, should be calculated again using chemical methods. Even when the procedure of Bligh & Dyer (Bligh & Dyer 1959) was used for the determination of the amount of lipids present in the microalgae, the values obtained were lower than the values reported in the literature (Becker 2007) due to the loss of lipids during the application of the procedure. In this way, it would be necessary to repeat the methodology using a larger sample of biomass in order to reduce the influence of the lipid losses in the final result. The most critical parameter identified for the microalgae utilization in HTL is the content and the composition of the ash. In this way, species with low ash content are preferred for HTL since they give better degradation and have less corrosive effect.

A number of HTL of untreated microalgae were performed to understand the experimental equipment and the influence of the biomass composition on the liquefaction. These experiments were performed at 300 °C, which implies about 100 bar of autogenous pressure inside the reactor; as the reactor is not inside of a safety cabinet, it was decided to do the HTL of pretreated microalgae at 250 °C in order to reduce the pressure to 40 bar inside the reactor. Another

conclusion obtained from these experiments was that it was necessary to remove the gas purge/sampling valves and the pressure gauge at the top of the reactor to avoid that some microalgae slurry remained unconverted in these parts of the reactor; this modification in the reactor led to an enhancement of the accuracy of yields calculation. According to the results obtained, *Scenedesmus* is the best species to use in HTL as it gives the highest biocrude yield and the best conversion of the biomass.

The understanding of the performance of the liquefaction was based on the yields of the products and the characterization of the biocrude via elemental analysis, FTIR, and GC-MS. Due to the lack of time, the elemental analysis was not applied in the HTL of pretreated microalgae. After a thorough analysis of the work done a number of deficiencies in the planning of the experiments were found: First of all, an evaluation of the initial state of the algae should be done. For this purpose, optical microscopy could be used. It is possible that the microalgae cell were disrupted during the drying process; in such case, the pretreatment would not have any effect. Also, after preparing the slurry, the rehydration time should be carefully considered since a different output for sonication and liquefaction can be obtained depending on the length of this phase. Finally, it is necessary a critical analysis of the applicability of the results of this thesis toward an industrial process of biofuel production in which dried algae would not be used. It is worth remembering that the HTL is considered very appropriate for microalgae because a completely drying is not required, however the experiments were done with dried algae.

The results of this thesis do not prove any relation between the sonication pretreatment and the HTL of microalgae. Despite the sonication bath is not suitable for microalgae cell disruption because it is intended for cleaning laboratory glassware, it is not possible to say that this equipment cannot be used for the pretreatment of algae because the initial state of the biomass was properly not checked. Only one entire cell of *Scenedesmus* could be found in the SEM pictures (Figure 19) consequence of the grinding done for the sample preparation. In this way, the next step for testing the ultrasonic pretreatment of

the microalgae before the HTL is to evaluate the efficiency of the lysing method through optical microscopy or any other method. If it is confirmed that the sonication bath is not breaking the algae cells, it would be necessary to procure an specially designed sonication equipment for microalgae cell disruption. The most important points to consider when selecting the sonication equipment are the configuration, the ultrasonic energy output and the frequency of the ultrasounds (20 kHz).

It also would be interesting to test microwaves as pretreatment technology before the HTL of microalgae. This cell breakage technology was also identified, together with ultrasounds, as applicable to the HTL of microalgae because of their easy scalability.

Other work proposed for the future are the other improvements found: (a) recirculation of the biocrude, (b) use of hydrogen donor solvents during the liquefaction, and (c) upgrading of the biocrude with subcritical water.



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## APPENDICES

### Appendix A Calculation of the ultrasonic power of the sonication bath

Table 27 – calculation of the ultrasonic power of the sonication bath

| Test No                   | Initial temp/(°C) | Final temp/(°C) | Time/(min) | Power/(W)     |
|---------------------------|-------------------|-----------------|------------|---------------|
| 1                         | 28                | 49              | 25         | 117.19        |
| 2                         | 18                | 41              | 30         | 106.96        |
| 3                         | 22                | 44              | 30         | 102.31        |
| 4                         | 18                | 44              | 30         | 120.91        |
| 5                         | 22                | 44              | 30         | 102.31        |
| 6                         | 27                | 48              | 30         | 97.66         |
| 7                         | 29                | 52              | 30         | 106.96        |
| 8                         | 30                | 54              | 30         | 111.61        |
| 9                         | 25                | 46              | 30         | 97.66         |
| 10                        | 26                | 51              | 30         | 116.26        |
| 11                        | 25                | 46              | 30         | 97.66         |
| 12                        | 37                | 65              | 30         | 130.22        |
| 13                        | 29                | 49              | 30         | 93.01         |
| <b>Average</b>            |                   |                 |            | <b>107.75</b> |
| <b>Standard deviation</b> |                   |                 |            | <b>10.95</b>  |



## Appendix B Calculation of ultimate analysis of microalgae biomass in dry basis

Table 28 shows the ultimate analysis (N, C, H, S, O) of the microalgae species in wet basis. While these data were directly collected from the elemental analyser (see section 1.1), the moisture and ash content were determined following the procedure described in the section 1.1.

**Table 28 – Ultimate analysis results of the microalgae biomass given in wet basis (wt.%) and directly obtained from the elemental analyser**

| Element/(wt.%)         | N    | C     | H    | S    | <sup>a</sup> O | M     | A     |
|------------------------|------|-------|------|------|----------------|-------|-------|
| <i>Nannochloropsis</i> | 6.80 | 37.13 | 6.27 | 0.66 | 49.15          | 11.42 | 29.25 |
| <i>Scenedesmus</i>     | 7.36 | 42.60 | 7.38 | 0.60 | 42.06          | 10.55 | 16.65 |
| <i>Tetraselmis</i>     | 5.22 | 32.20 | 5.74 | 0.98 | 55.86          | 10.93 | 33.04 |

<sup>a</sup> Determined by difference

The percentage of dry material in each microalgae was calculated by discounting the amount of moisture. In this way, the dry matter was 88.52, 89.45, and 89.07 wt.% of the total mass for *Nannochloropsis*, *Scenedesmus*, and *Tetraselmis*, respectively.

The values of H in Table 28 have a contribution from the dry biomass and from the moisture. Considering that the water contains 11 wt.% of H and 89 wt.% of O, the percentage of H coming from water is the result of multiplying the moisture content of each microalgae by 0.11. Then the H from corresponding to the dry biomass is obtained by difference of the total H and the H coming from water as summarized in Table 29.

**Table 29 – Allocation of H content of the wet biomass to the moisture and the dry biomass fractions (wt.%)**

| <b>Source</b>        | <b><i>Nannochloropsis</i></b> | <b><i>Scenedesmus</i></b> | <b><i>Tetraselmis</i></b> |
|----------------------|-------------------------------|---------------------------|---------------------------|
| <b>H water</b>       | 1.27                          | 1.17                      | 1.22                      |
| <b>H dry biomass</b> | 5.00                          | 6.21                      | 4.52                      |

After determination of the dry matter and the H corresponding to this dry matter, the normalization of the percentages of the elements was performed. The normalization was done by dividing the percentages of each element by the percentage of the dry sample and multiplying by 100.

The weight percentage of oxygen in db is calculated by difference by means of Equation 7.

$$O(\text{wt. \% db}) = 100 - (N + C + H + S + \text{Ash}) \quad \text{Equation 7}$$

Proximate analysis in dry basis of the microalgae is summarized in Table 14.

## Appendix C Powder XRD analysis of microalgae

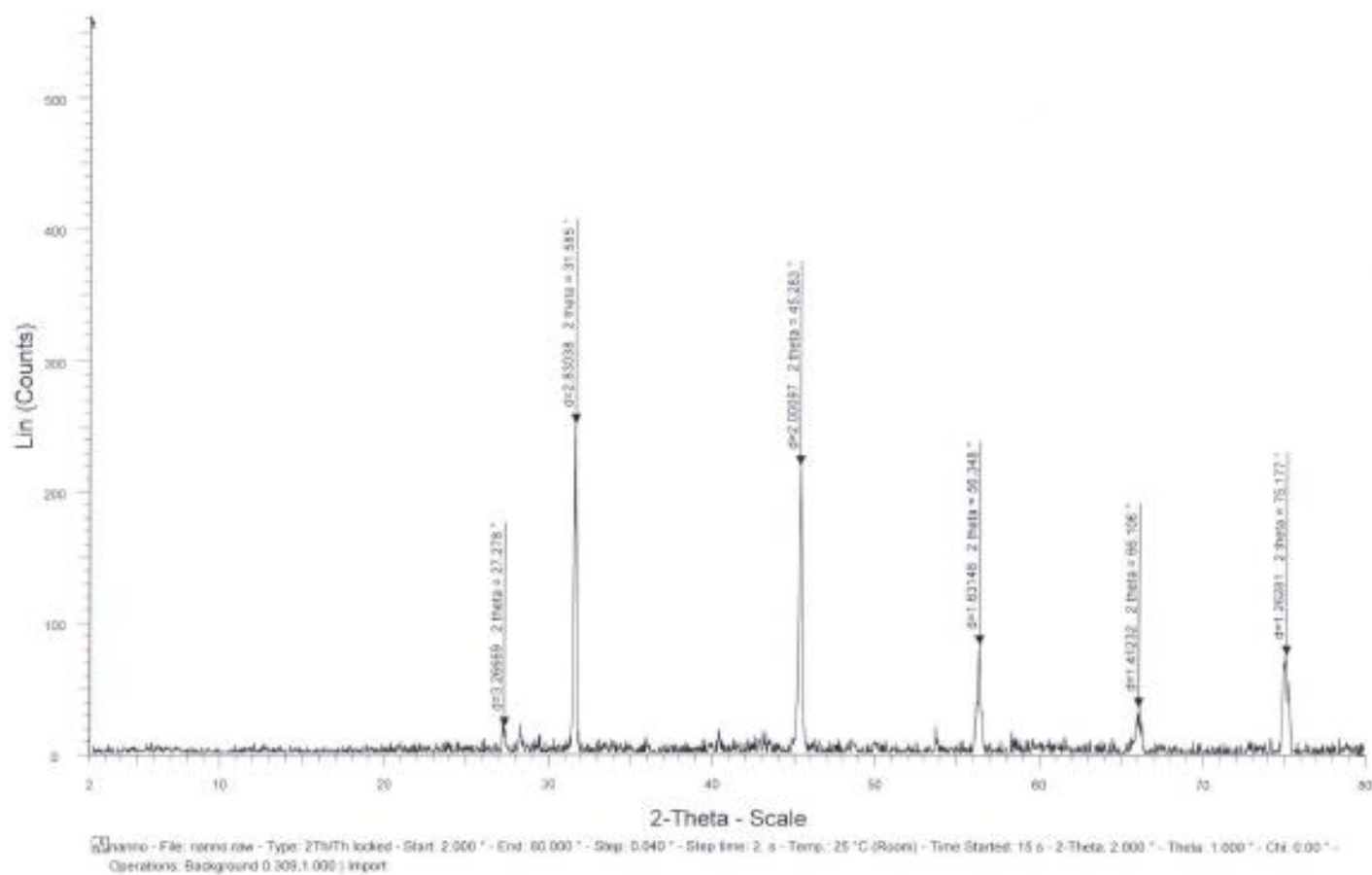


Figure 33 – XRD analysis of *Nannochloropsis*

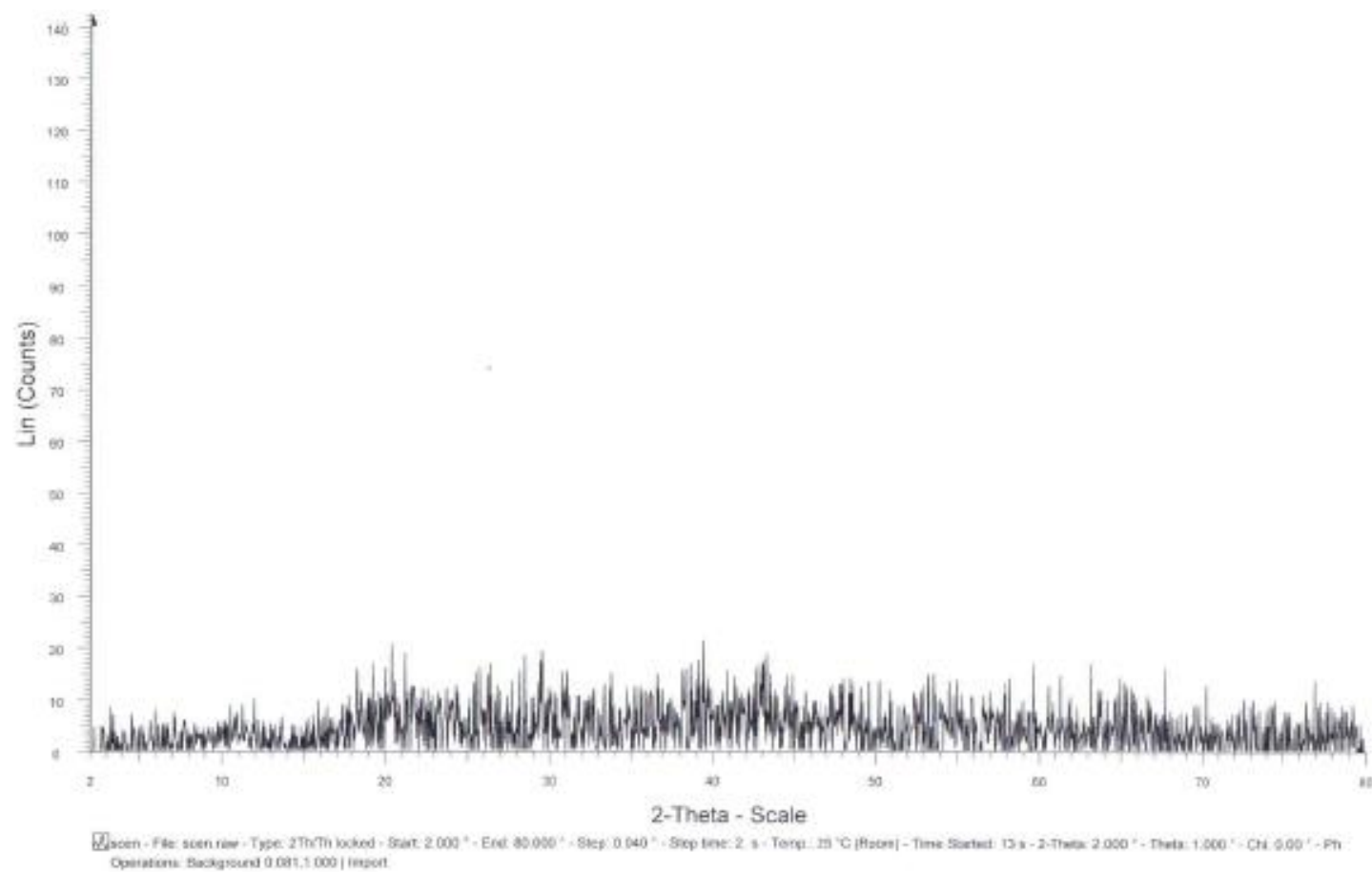


Figure 34 – XRD analysis of *Scenedesmus*

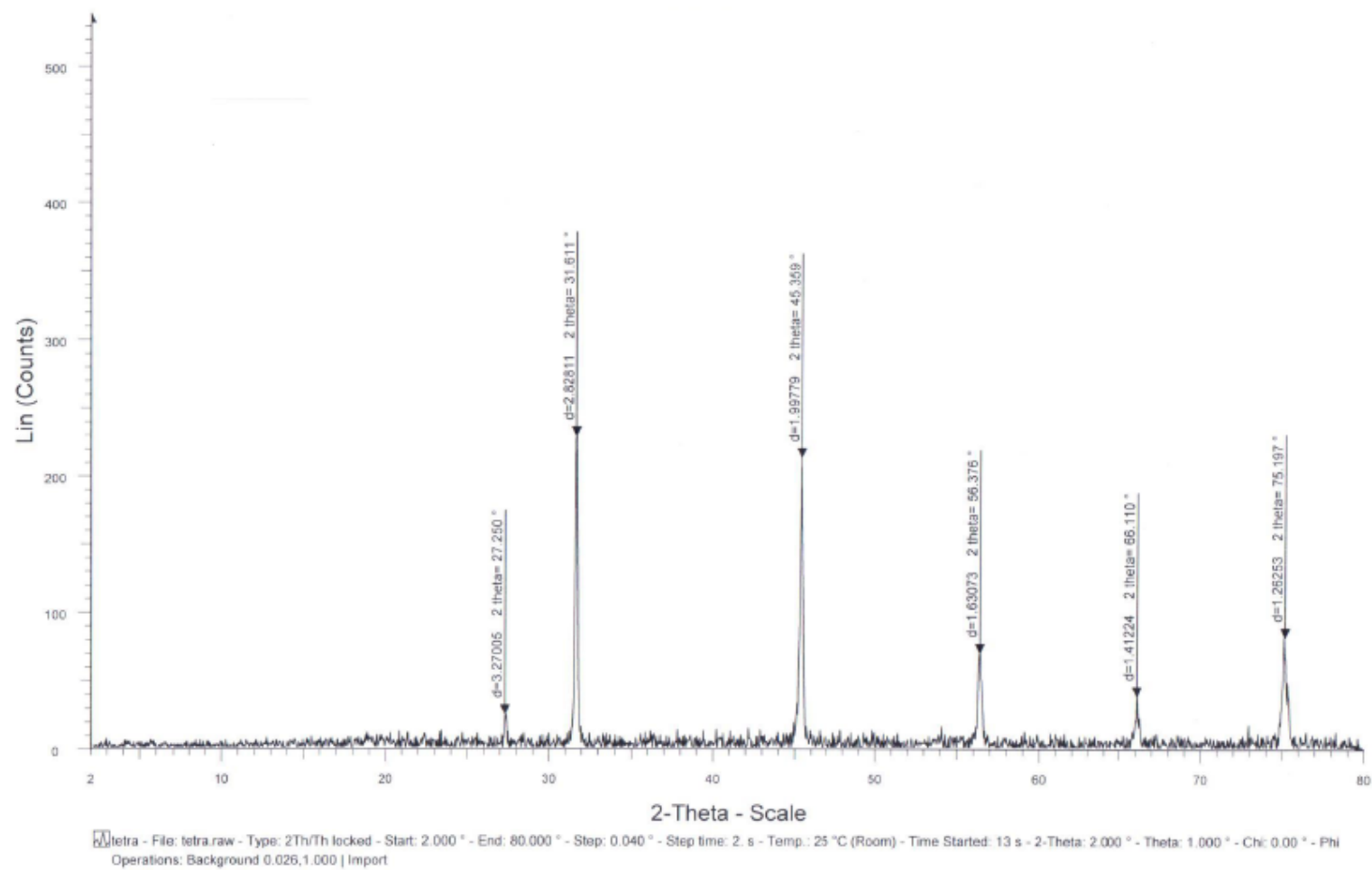


Figure 35 – XRD analysis of *Tetraselmis*



## Appendix D TGA and DrTGA of *Nannochloropsis* and *Scenedesmus* at different sonication energies

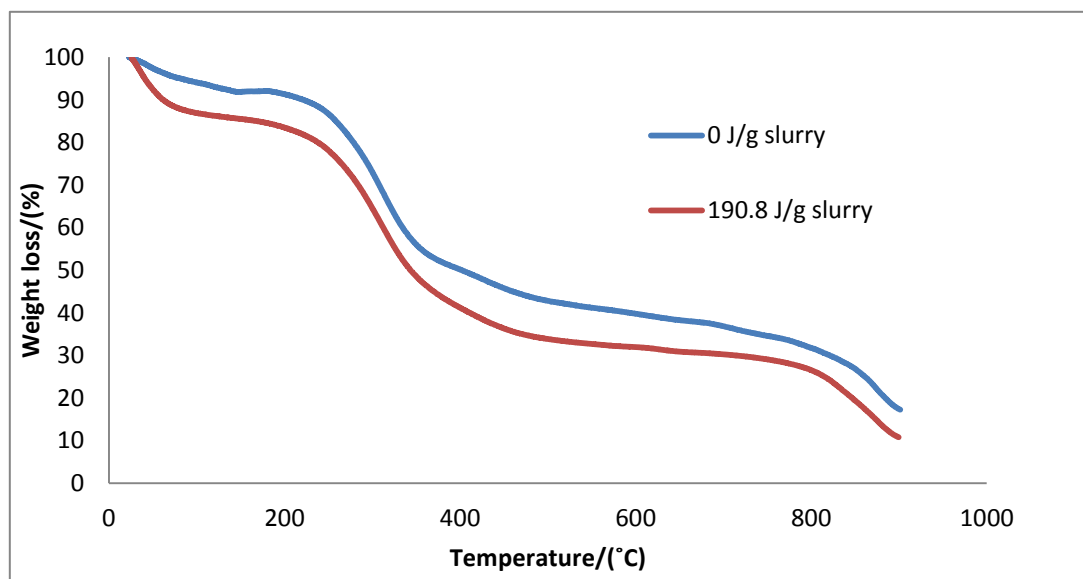


Figure 36 – TGA of *Nannochloropsis* after using different sonication energies as pretreatment

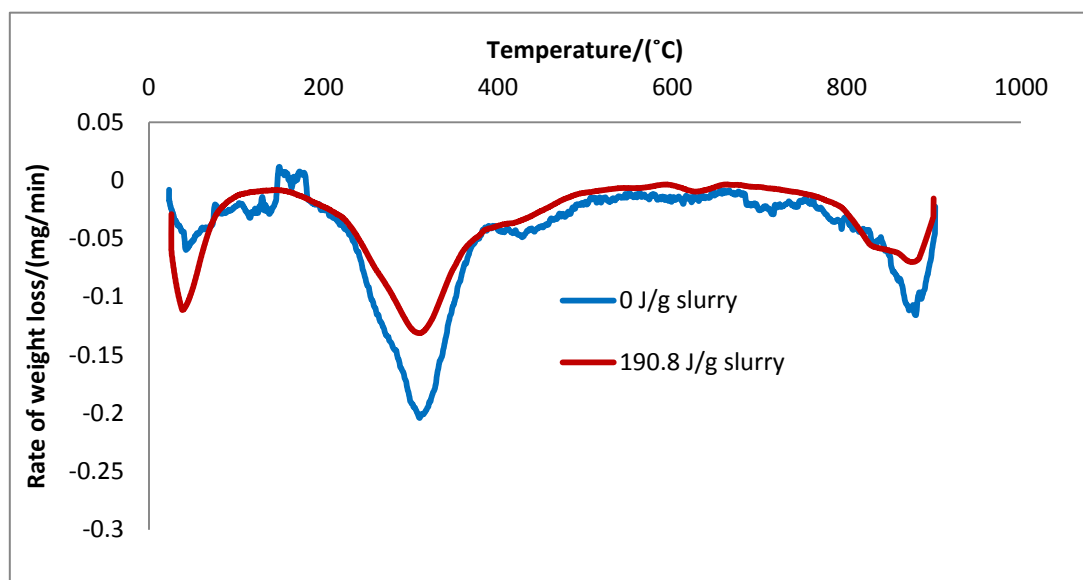


Figure 37 – DrTGA of *Nannochloropsis* after using different sonication energies as pretreatment

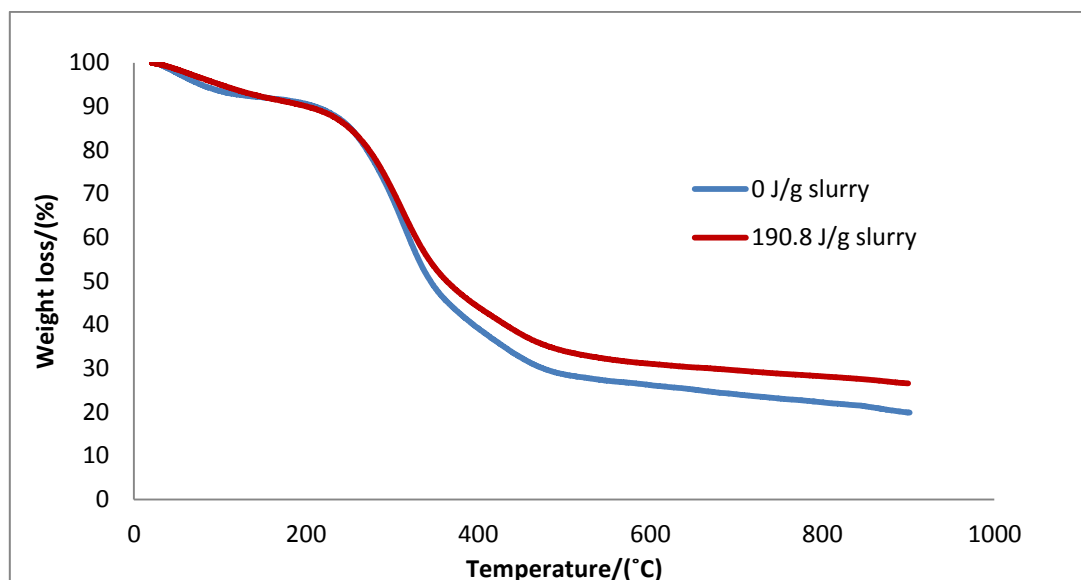


Figure 38 – TGA of *Scenedesmus* after using different sonication energies as pretreatment

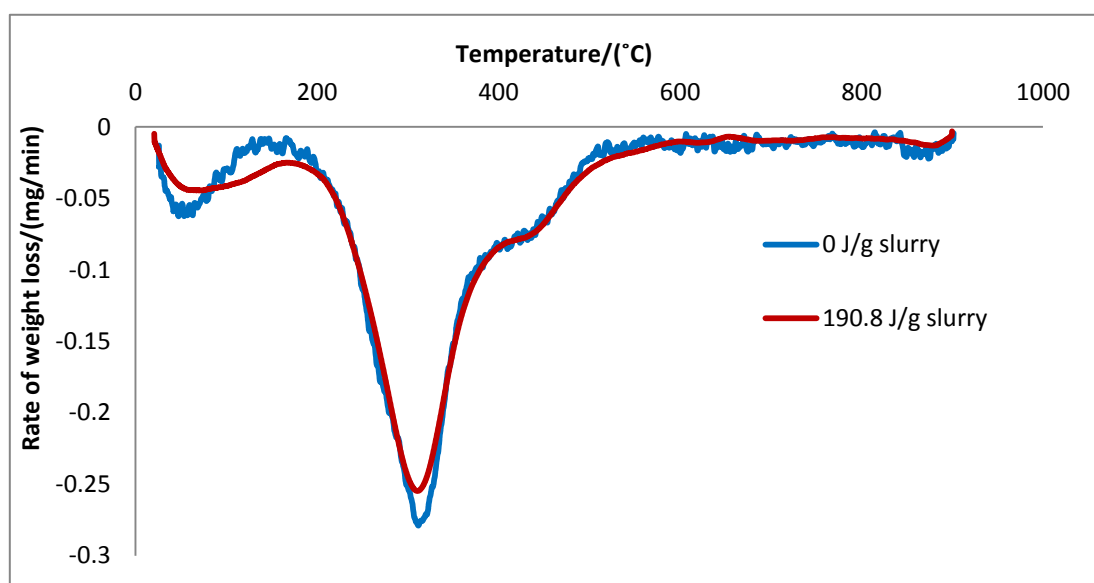


Figure 39 – DrTGA of *Scenedesmus* after using different sonication energies as pretreatment

## Appendix E FTIR of the biocrude from *Scenedesmus* and *Tetraselmis* at different sonication energies

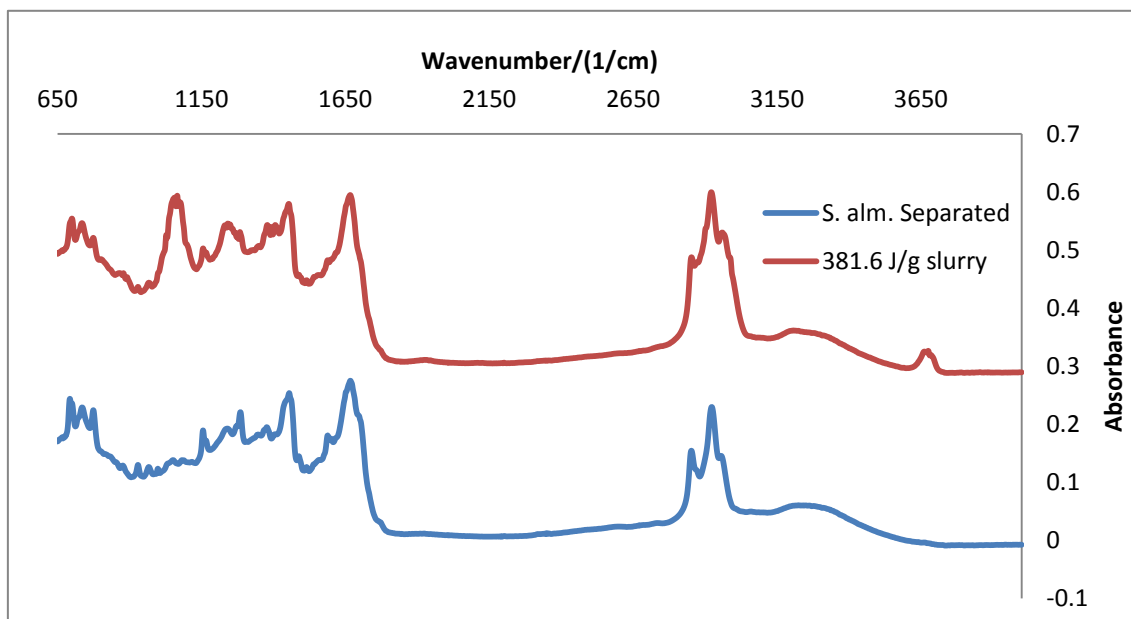


Figure 40 – FTIR of the biocrude of *Scenedesmus* using different sonication energies as pretreatment. “Separated” means that the microalgae biomass and the water have been fed separately into the reactor; therefore, the microalgae slurry has not suffered any pretreatment (0 J/g slurry).

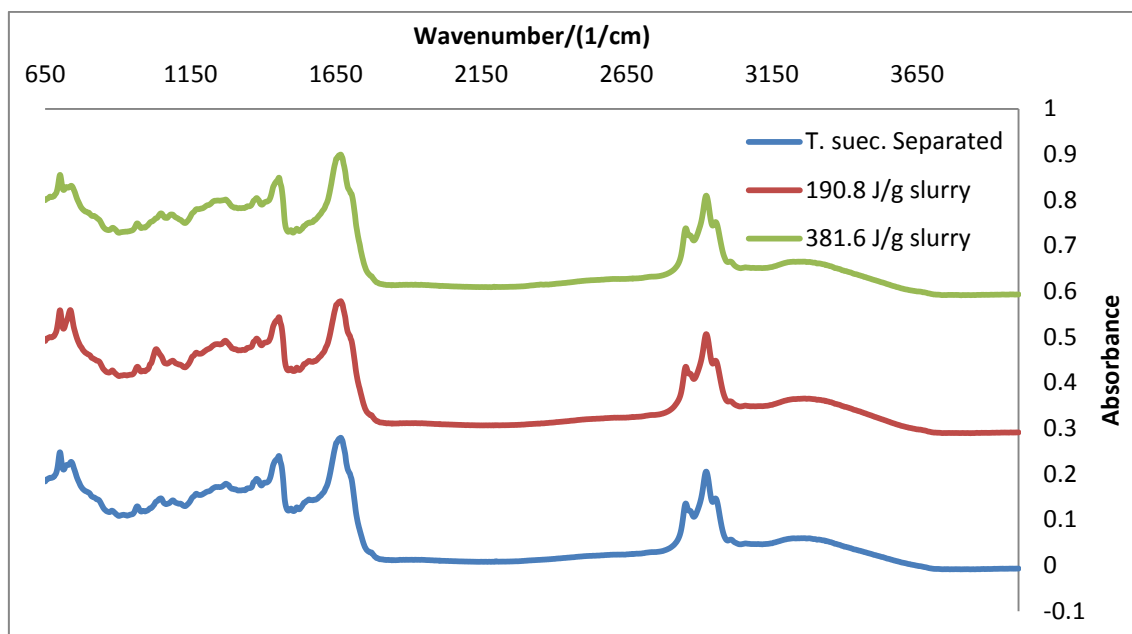


Figure 41 – FTIR of the biocrude of *Tetraselmis* using different sonication energies as pretreatment. “Separated” means that the microalgae biomass and the water have been fed separately into the reactor; therefore, the microalgae slurry has not suffered any pretreatment (0 J/g slurry).

## Appendix F GC-MS analysis of biocrude of HTL of pretreated microalgae

**Table 30 – GC-MS analysis of the biocrude from *Nannochloropsis* charged separately to the reactor**

| PK | RT      | Area<br>Pct | Library/ID                                                                                               | Qual |
|----|---------|-------------|----------------------------------------------------------------------------------------------------------|------|
| 1  | 10.1305 | 31.1922     | Phenol, 3-methyl-                                                                                        | 96   |
| 2  | 24.7961 | 2.491       | 1,3,5-Triazine-2,4(1H,3H)-dione, 6-(ethylamino)-                                                         | 43   |
| 3  | 26.8961 | 3.2574      | 3,6-Diisopropylpiperazin-2,5-dione                                                                       | 50   |
| 4  | 27.1536 | 0.8541      | Naphthalene, 1,6-dimethyl-                                                                               | 38   |
| 5  | 28.4067 | 1.0602      | 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine                                  | 80   |
| 6  | 28.5669 | 1.6575      | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-                                          | 78   |
| 7  | 28.7844 | 7.4514      | Diethyldithiophosphinic acid                                                                             | 42   |
| 8  | 29.3165 | 0.6955      | Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)]-                               | 53   |
| 9  | 29.5969 | 1.0586      | 9H-Pyrido[3,4-b]indole, 1-methyl-                                                                        | 96   |
| 10 | 29.7113 | 0.8432      | (2Z,4E)-3,7,11-Trimethyl-2,4-dodecadiene                                                                 | 81   |
| 11 | 29.9288 | 4.2846      | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-                                                    | 86   |
| 12 | 30.1119 | 2.1281      | Cyclododecyne                                                                                            | 93   |
| 13 | 30.604  | 10.0957     | n-Hexadecanoic acid                                                                                      | 98   |
| 14 | 33.4879 | 15.3151     | 9,12-Octadecadienoic acid (Z,Z)-                                                                         | 93   |
| 15 | 33.7168 | 5.5986      | 2,5-Piperazinedione, 3-benzyl-6-isopropyl-                                                               | 66   |
| 16 | 33.8999 | 2.1012      | Hexadecanamide                                                                                           | 90   |
| 17 | 34.1803 | 2.0041      | 2,5-Piperazinedione, 3-benzyl-6-isopropyl-                                                               | 43   |
| 18 | 34.535  | 0.6507      | N-Methyldodecanamide                                                                                     | 59   |
| 19 | 35.0786 | 1.4779      | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-                                            | 94   |
| 20 | 35.2503 | 2.2961      | Cyclo-(l-leucyl-l-phenylalanyl)                                                                          | 60   |
| 21 | 35.5707 | 0.8605      | Benzamide, N-4-piperidinyl-                                                                              | 46   |
|    |         |             | Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'.alpha.,10.alpha.)- |      |
| 22 | 35.874  | 0.9287      |                                                                                                          | 53   |
| 23 | 36.5949 | 1.6976      | Cyclohexene, 4-pentyl-1-(4-propylcyclohexyl)-                                                            | 91   |

**Table 31 – GC-MS analysis of the biocrude from *Tetraselmis* using 0 J/g slurry as sonication pretreatment**

| PK | RT      | Area Pct | Library/ID                                                              | Qual |
|----|---------|----------|-------------------------------------------------------------------------|------|
| 1  | 4.0424  | 0.3921   | 4,4-Dimethyl-2-oxazoline                                                | 64   |
| 2  | 5.965   | 0.47     | Pyrazine, 2,5-dimethyl-                                                 | 80   |
| 3  | 10.2279 | 84.2687  | p-Cresol                                                                | 97   |
| 4  | 18.399  | 0.7883   | p-Anisic acid, 4-cyanophenyl ester                                      | 78   |
| 5  | 21.1799 | 0.7011   | Phenol, 2,4-bis(1,1-dimethylethyl)-                                     | 96   |
| 6  | 26.7303 | 0.8464   | 1-[N-Methylpiperazine]ethanol                                           | 42   |
| 7  | 28.155  | 0.4553   | Benzene, 1-methoxy-2-(3-methylphenoxy)-                                 | 46   |
| 8  | 28.3839 | 0.6058   | 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine | 72   |
| 9  | 28.5728 | 0.7692   | 1,4-Benzenediamine, N-(4-methoxyphenyl)-                                | 86   |
| 10 | 28.7044 | 1.8988   | 2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone                              | 43   |
| 11 | 29.3166 | 0.5811   | 2,4-Diamino-6-benzyl-5-methylthieno[2,3-d]pyrimidine                    | 38   |
| 12 | 29.5398 | 0.8521   | 1,1'-Biphenyl, 3,3'-dimethoxy-                                          | 93   |
| 13 | 29.7057 | 0.3598   | (7E,9E)-Dodecadien-1-ol                                                 | 74   |
| 14 | 30.5697 | 1.8984   | n-Hexadecanoic acid                                                     | 98   |
| 15 | 31.2163 | 0.5192   | 1H-Indene-1,3(2H)-dione, 2-(2-methylbutylidene)-                        | 64   |
| 16 | 33.4708 | 0.7609   | cis,cis,cis-7,10,13-Hexadecatrienal                                     | 91   |
| 17 | 33.6082 | 1.796    | 2,5-Piperazinedione, 3-benzyl-6-isopropyl-                              | 78   |
| 18 | 33.8657 | 0.399    | Dodecanamide                                                            | 95   |
| 19 | 35.0387 | 0.3853   | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-           | 94   |
| 20 | 48.3481 | 1.2526   | 5-Cholestene-3-ol, 24-methyl-                                           | 84   |

**Table 32 – GC-MS analysis of the biocrude from *Tetraselmis* using 143.1 J/g slurry as sonication pretreatment**

| PK | RT      | Area<br>Pct | Library/ID                                                              | Qual |
|----|---------|-------------|-------------------------------------------------------------------------|------|
| 1  | 10.0964 | 17.3128     | Phenol, 3-methyl-                                                       | 96   |
| 2  | 26.8047 | 3.4569      | Hydantoin, 1-butyl-                                                     | 49   |
| 3  | 28.3726 | 2.5461      | 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine | 42   |
| 4  | 28.5156 | 2.1458      | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-         | 58   |
| 5  | 28.7159 | 9.5028      | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-         | 72   |
| 6  | 29.2995 | 1.2058      | Cyclohexanol, 2-methyl-5-(1-methylethyl)-                               | 43   |
| 7  | 29.5456 | 1.7332      | 9H-Pyrido[3,4-b]indole, 1-methyl-                                       | 96   |
| 8  | 29.7058 | 1.867       | (2Z,4E)-3,7,11-Trimethyl-2,4-dodecadiene                                | 83   |
| 9  | 30.587  | 15.0408     | n-Hexadecanoic acid                                                     | 98   |
| 10 | 33.4823 | 8.4548      | cis,cis,cis-7,10,13-Hexadecatrienal                                     | 93   |
| 11 | 33.6655 | 14.7538     | 6-Octadecenoic acid, (Z)-                                               | 90   |
| 12 | 33.8772 | 3.7023      | 9-Octadecenamide, (Z)-                                                  | 86   |
| 13 | 34.1518 | 2.7733      | 2,5-Piperazinedione, 3-benzyl-6-isopropyl-                              | 93   |
| 14 | 34.518  | 1.1265      | N-Methyldodecanamide                                                    | 76   |
| 15 | 35.0502 | 2.1953      | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-           | 83   |
| 16 | 35.2219 | 3.4136      | Cyclo-(l-leucyl-l-phenylalanyl)                                         | 64   |
| 17 | 35.5365 | 1.1812      | Cyclo-(l-leucyl-l-phenylalanyl)                                         | 55   |
| 18 | 35.8513 | 1.3947      | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-           | 50   |
| 19 | 48.3482 | 6.1932      | 5-Cholestene-3-ol, 24-methyl-                                           | 94   |

**Table 33 – GC-MS analysis of the biocrude from *Tetraselmis* using 190.8 J/g slurry as sonication pretreatment**

| PK | RT      | Area<br>Pct | Library/ID                                                                                               | Qual |
|----|---------|-------------|----------------------------------------------------------------------------------------------------------|------|
| 1  | 10.102  | 3.2843      | p-Cresol                                                                                                 | 96   |
| 2  | 26.8046 | 3.6316      | 3-Nonanone, 2-methyl-                                                                                    | 38   |
| 3  | 28.3668 | 2.4526      | 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine                                  | 78   |
| 4  | 28.4926 | 3.7077      | 3-Methyldec-3-ene                                                                                        | 43   |
| 5  | 28.7101 | 10.9963     | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-                                          | 64   |
| 6  | 29.2994 | 2.0295      | (2Z,4E)-3,7,11-Trimethyl-2,4-dodecadiene                                                                 | 59   |
| 7  | 29.5512 | 2.807       | 9H-Pyrido[3,4-b]indole, 1-methyl-                                                                        | 98   |
| 8  | 29.7057 | 3.1213      | (2Z,4E)-3,7,11-Trimethyl-2,4-dodecadiene                                                                 | 76   |
| 9  | 29.8831 | 1.6594      | Pentadecanoic acid, 14-methyl-, methyl ester                                                             | 93   |
| 10 | 30.5926 | 16.8394     | n-Hexadecanoic acid                                                                                      | 99   |
| 11 | 33.4765 | 8.0764      | Methyl 8,11,14-heptadecatrienoate                                                                        | 93   |
| 12 | 33.6653 | 16.0023     | cis-Vaccenic acid                                                                                        | 95   |
| 13 | 33.8828 | 4.0305      | 9-Octadecenamide, (Z)-                                                                                   | 86   |
| 14 | 34.1574 | 3.0675      | 2,5-Piperazinedione, 3-benzyl-6-isopropyl-                                                               | 96   |
| 15 | 34.5237 | 1.9906      | N-Methyldodecanamide                                                                                     | 64   |
| 16 | 35.0558 | 2.4922      | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-                                            | 94   |
| 17 | 35.2446 | 3.487       | 1H-Cyclopenta[a]pentalen-7-ol, decahydro-3,3,4,7a-tetramethyl-, acetate                                  | 91   |
|    |         |             | Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'.alpha.,10.alpha.)- |      |
| 18 | 35.8569 | 1.3854      |                                                                                                          | 52   |
| 19 | 39.1757 | 1.4217      | Adipic acid, nonyl 2,3,6-trichlorophenyl ester                                                           | 49   |
| 20 | 48.3538 | 7.5173      | 5-Cholestene-3-ol, 24-methyl-                                                                            | 94   |

**Table 34 – GC-MS analysis of the biocrude from *Tetraselmis* using 381.6 J/g slurry as sonication pretreatment**

| PK | RT      | Area<br>Pct | Library/ID                                                      | Qual |
|----|---------|-------------|-----------------------------------------------------------------|------|
| 1  | 18.2329 | 1.0156      | Cyclopentane, 1,1,3-trimethyl-3-(2-methyl-2-propenyl)-          | 43   |
| 2  | 21.1912 | 5.1932      | Phenol, 2,4-bis(1,1-dimethylethyl)-                             | 97   |
| 3  | 23.4514 | 0.9601      | 2-Tetradecene, (E)-                                             | 97   |
| 4  | 26.7988 | 3.4766      | 3,6-Diisopropylpiperazin-2,5-dione                              | 50   |
| 5  | 27.6113 | 2.4429      | 5-Octadecene, (E)-                                              | 96   |
| 6  | 28.3724 | 2.6312      | 5-Isopropylidene-3,3-dimethyl-dihydrofuran-2-one                | 43   |
| 7  | 28.5097 | 1.591       | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- | 91   |
| 8  | 28.7099 | 7.1366      | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- | 59   |
| 9  | 29.5568 | 1.8232      | 9H-Pyrido[3,4-b]indole, 1-methyl-                               | 98   |
| 10 | 29.7056 | 1.845       | m-Menth-6-ene, (R)-(+)-                                         | 59   |
| 11 | 30.5868 | 13.5105     | n-Hexadecanoic acid                                             | 98   |
| 12 | 31.3936 | 1.7587      | 1-Heptadecene                                                   | 98   |
| 13 | 33.4764 | 8.2923      | Methyl 8,11,14-heptadecatrienoate                               | 97   |
| 14 | 33.6709 | 14.059      | 9,12-Octadecadienoic acid (Z,Z)-                                | 93   |
| 15 | 33.8827 | 3.3531      | Dodecanamide                                                    | 91   |
| 16 | 34.1516 | 3.4344      | 2,5-Piperazinedione, 3-benzyl-6-isopropyl-                      | 98   |
| 17 | 34.5235 | 1.6493      | N-Methyldodecanamide                                            | 53   |
| 18 | 34.8611 | 1.202       | Cyclopentadecane                                                | 95   |
| 19 | 35.05   | 1.7844      | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-   | 94   |
| 20 | 35.2331 | 2.5112      | Cyclo-(l-leucyl-l-phenylalanyl)                                 | 86   |
| 21 | 39.1698 | 1.0378      | 3-Thiazoline-5-methanamine, 2-iodomethyl-                       | 53   |
| 22 | 44.6115 | 1.1002      | L-Tryptophan                                                    | 76   |
| 23 | 48.3537 | 7.2134      | 5-Cholestene-3-ol, 24-methyl-                                   | 95   |
| 24 | 52.8512 | 10.9783     | Tris(tert-butyl)dimethylsilyloxyarsane                          | 38   |



