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**BIO-OIL PRODUCTION FROM MICROALGAE BY FAST PYROLYSIS: STUDY OF  
THE EFFECTS OF BIOMASS PROPERTIES ON PRODUCT QUALITY BY USING  
WHOLE AND SPENT-MICROALGAL BIOMASS AS FEEDSTOCK**

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**DOCTORAL THESIS IN FULFILLMENT OF  
THE REQUIREMENTS FOR THE DEGREE  
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**ROBINSON EDUARDO MUÑOZ GONZÁLEZ**  
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**“Bio-oil production from microalgae by fast pyrolysis: Study of the effects of biomass properties on product quality by using whole and waste-microalgal biomass as feedstock”**

Esta tesis fue realizada bajo la supervisión del Director de tesis, Dr. Rodrigo Navia Diez perteneciente al Departamento de Ingeniería Química de la Universidad de La Frontera y la Co-Directora de tesis, Dra. Laura Azocar Ulloa del Departamento de Química Ambiental, Facultad de Ciencias, Universidad Católica de La Santísima Concepción y ha sido aprobada por los miembros de la comisión examinadora.

**ROBINSON EDUARDO MUÑOZ GONZÁLEZ**

.....

**Dr. Quiroz Andrés C.**

**DIRECTOR DEL PROGRAMA DE  
DOCTORADO EN CIENCIAS DE  
RECURSOS NATURALES**

.....

**Dra. Mónica Rubilar D.**

**DIRECTORA ACADEMICA DE  
POSTGRADO  
UNIVERSIDAD DE LA FRONTERA**

.....

**Dr. Rodrigo Navia Diez**

.....

**Dra. Laura Azócar Ulloa**

.....

**Dr. Alex Berg Gebert**

.....

**Dr. Néstor Escalona Burgos**

.....

**Dr. David Jeison Núñez**

*Dedico esta tesis a mi hija Eloísa a mi pareja Claudia y a mis hermanos Yéssica e Iván y a mi madre Alicia, quienes han compartido esta travesía y experiencia conmigo, dándome fuerzas y apoyo necesario para completar este desafío.*

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## **Summary and thesis outline**

A novel use for spent microalgal (SM) biomass following a biorefinery concept was studied. The SM is produced after lipids or protein extraction to produce high value products. The thermal conversion of SM by using fast pyrolysis has been proposed as an interesting alternative. The advantage of pyrolysis is that whole microalgal (WM) biomass can be converted into three products: bio-oil, non-condensable gases (NCG) and bio-char. Therefore, the objective of this work was to study the production of bio-oil by fast pyrolysis from SM following a biorefinery concept. To achieve the objective, we address the issue in five stages. In the first stage (Chapter I) a study of technological aspects of bio-oil production derived from microalgae biomass by fast pyrolysis was realized. Afterwards, the feasibility to produce bio-oil by fast pyrolysis from WM and SM was evaluated (in Chapter II), where the results show that lower yields of bio-oil from microalgal biomass were obtained compared with lignocellulosic biomass. In relation to bio-oil characteristics, interesting results of neutral pH were found in bio-oil from microalgal biomass.

In Chapter III the best condition of bio-oil production by fast pyrolysis from SM was studied. The study was realized analyzing the critical variables particle size, temperature and residence time by means of sequential optimization. The results show an increase in bio-oil production by increasing the reaction temperature, but simultaneously causing an increase in water content in bio-oil and in NCG. In addition, it was determined that a shorter residence time decreases the water content in the bio-oil, the product of the reduction of chain fractionation of volatiles derived from the devolatilization of microalgal biomass. Therefore, a high devolatilization of biomass allowed the generation of a high fraction of gases, which can promote a higher conversion to bio-oil under a low residence time. Finally, through statistical method "sequential optimization", theoretical conditions were obtained to produce bio-oil with high HV, being at: 480 °C of reaction temperature, 450-1,000 µm particle size range and 1 second of residence time.

In Chapter IV, we analyzed the characteristics of bio-oil produced by fast pyrolysis from the WM and SM (deproteinized microalgal biomass). The pyrolysis of WM generates bio-oil with a high heating value of 31 MJ/Kg, but with a high nitrogen compounds content. Contrarily, bio-oil produced from deproteinized microalgae had a lower number of chemical compounds than the one obtained from WM. Therefore, deproteinization promote the production of lower chemical complexity bio-oil, being able to benefit refining of bio-oil to an interesting chemical product.

The conversion yields by using the organic fraction of the WM were 30% of bio-oil, 10% of bio-char and 60% of NCG. Additionally, the conversion yields using SM were 15% of bio-oil, 3% of bio-char and 82 % of NCG. According to the obtained results, bio-oil production could be feasible to be produced on large scale associated to the microalgae biorefinery. Finally, bio-char from WM and SM was characterized by a high ash contented. Probably these ashes come from harvesting process, which could be mineralized on the bio-char during the fast pyrolysis process.

The economic feasibility of the production of rapid pyrolysis products from microalgae biomass is limited by production costs, for example, drying biomass before being pyrolyzed, this stage has a high energy expenditure. One way to reduce the total expense involved in the process is to use a biorefinery approach, in such a way as to extract and/or exploit the valuable components present in microalgae.

The present study, it demonstrated that the proteins extraction before the pyrolysis process is feasible, obtaining protein products with potential economic value and additionally to reduce the nitrogen content in the SM the one that is used like raw material for the bio-oil production for fast pyrolysis. This SM, to the being pyrolyzed a bio-oil is obtained with lower nitrogen content, in comparison to the obtained one of finished biomass.

## ABBREVIATIONS

WM	Whole Microalgae
SM	Spent Microalgae
WMB	Whole Microalgae of <i>Botryococcus braunii</i>
SMB <sub>L+P</sub>	Spent Microalgae of <i>Botryococcus braunii</i> after extraction Lipids and Proteins
SMB <sub>L</sub>	Spent Microalgae of <i>Botryococcus braunii</i> after extraction Lipids
WMN	Whole Microalgae of <i>Nannochloropsis gaditana</i>
SMB <sub>P</sub>	Spent Microalgae of <i>Botryococcus braunii</i> after extraction Proteins
SM <sub>L</sub>	Spent Microalgae after extraction Lipids
WMS	Whole Microalgae of <i>Scenedesmus almeriensis</i>
SMS <sub>P</sub>	Spent Microalgae of <i>Scenedesmus almeriensis</i> after extraction Proteins
HV	Heating Value
TGA	Thermal Gravimetical Analysis
EP	Electrostatic Precipitator
NCG	Non-Condensable Gases
FAME	Fatty Acid Methyl Ester

## TABLE OF CONTENTS

Agradecimientos / acknowledgements	i
Summary and thesis outline	ii
Abbreviations	iv
Table of contents	v
<b>CHAPTER I. General Introduction</b>	
1.1 General introduction	1
1.1.1. Introduction	1
1.1.2. Microalgae	2
1.1.3. Lipid	3
1.1.4. Carbohydrate	4
1.1.5. Protein	4
1.1.6. Biorefinery	5
1.1.7. Conversion Thermochemical	6
1.1.7.1.Pyrolysis	8
1.1.7.2.Slow Pyrolysis	9
1.1.7.3.Fast Pyrolysis	9
1.1.8. Bio-oil from microalgae	10
1.1.9. Technological aspects of bio-oil production derived from microalgae biomass by fast pyrolysis	12
1.2 Hypotheses	17
1.3 General objective	17

1.4 Specific objectives	17
1.5 References	18
<b>CHAPTER II. “Bio-oil production from microalgae following a biorefinery concept”</b>	
2.1 Introduction	31
2.2 Experimental section	32
2.2.1 Materials	32
2.2.2. Production of SM to be pyrolyzed following a biorefinery concept	33
2.2.2.1. Protein extraction	33
2.2.2.2. Lipid extraction and fatty acid methyl ester (FAME) production	33
2.2.3. Pyrolysis experiments	34
2.2.4. Characterization of feedstock and products	35
2.2.5. Characterization of FAME	36
2.2.6. Characterization of bio-oil	36
2.2.7 Characterization of NCG	36
2.3. Results and Discussion	37
2.3.1. Production of SM to be pyrolyzed following a biorefinery concept	37
2.3.1.1. Protein extraction	37
2.3.1.2. Lipid extraction and FAME production	39
2.3.2. Feedstock characterization	40
2.3.3. Bio-oil production	42
2.3.4. Analysis of bio-oil	45

2.3.5 Analysis of NCG	48
2.4. Conclusions	50
2.5. Acknowledgments	50
2.6. References	51
<b>CHAPTER III. “Study of operational conditions affecting pyrolysis conversion process of <i>Botryococcus braunii</i> spent-biomass”</b>	
3.1. Introduction	54
3.2. Methodology	56
3.2.1. Materials	56
3.2.2. Pyrolysis experiments	56
3.2.3. Characterization of feedstock and products	57
3.2.4. Characterization of bio-oil	57
3.3. Results and Discussion	58
3.3.1. Production of spent biomass to be pyrolyzed following a biorefinery concept	58
3.3.1.1. Proteins extraction	58
3.3.2. Feedstock characterization	58
3.3.3. Effect of operational conditions on pyrolysis processes of waste microalgal biomass.	60
3.3.4. Effect of the temperature on pyrolysis products yield	61
3.3.5. Effect of residence time on the reaction of pyrolysis of SMB <sub>P</sub> .	64
3.3.6. Analysis of bio-oil	68

3.4. Conclusions	74
3.5. Acknowledgments	74
3.6. References	75
<b>CHAPTER IV. “Study of the effect of microalgal biomass deproteinization on bio-oil characteristics”</b>	
4.1. Introduction	78
4.2. Methodology	79
4.2.1. Materials	79
4.2.2. Feedstock characterization	79
4.2.3. Pyrolysis experiments	80
4.2.4. Characterization of bio-oil	81
4.2. Results and Discussion	81
4.3.1 Protein extraction and feedstock characterization	82
4.3.2. Pyrolysis products yield	83
4.3.3. Effect of deproteination of the nitrogenous compounds content on bio-oil	85
4.3.4 . Characteristics of the produced bio-char and its possible applications	90
4.3.5. Characteristics of the produced NCG and its possible applications	93
4.4. Conclusions	95
4.5. Acknowledgments	96
4.6. References	97
<b>CHAPTER V. General discussion and conclusions</b>	
5.1. General discussion	106
5.2. Conclusions	109



# **CHAPTER I**

## **General introduction**

## **1.1. General introduction**

### **1.1.1. Introduction**

The industrialization has been causing an increase of dependence on developing countries with the producing fuels countries, especially of oil. World oil producers are in a limited number of countries, raising concerns associated with energy insecurity in the rest of the countries (Curtin et al., 2019). On the other hand, negative impact use of fossil fuels in gas emission of greenhouse effect, increase levels of NO<sub>x</sub>, SO<sub>x</sub> and metallic particles in environment make oil a critical issue (Bórawski et al., 2019 , Hornung, 2014).

Considering as critical issues the energy insecurity and environmental damage associated with the use of fossil fuels, the development of new alternative fuel is essential (Ubando et al., 2020 , Ganesan et al., 2020). The latter is sought through replicating fossil liquid fuels, from renewable sources such as biomass. In this context, a viable and sustainable alternative is the production of biofuels from microalga biomass, called third generation biofuels (Ganesan et al., 2020). Commercial cultivation of microalgae is 60 years old, as food or for extraction of high value nutraceuticals (Rosenberg et al., 2008 , Yan et al., 2016), reaching a worldwide biomass production of 10,000 tons per year (Nasseri et al., 2011 , Tredici et al., 2016).

The production of biodiesel (fatty acid methyl ester - FAME) from microalgae biomass has been widely studied (Ganesan et al., 2020 , Kumar and Singh, 2019). However, in these processes, the highest quantity of biomass is converted in residue because only the fatty acids are used in FAME production (Bhattacharya and Goswami, 2020). Microalgal biorefineries have recently emerged as an economically viable option for the co-production of value-added products and biofuels (Kim et al., 2019). In this sense, the thermal conversion of microalgal biomass by using pyrolysis appears as an alternative, which the whole biomass can be converted into 3 products: bio-oil, NCG and bio-

char (Pattiya, 2018 , Feroso et al., 2017). According to the aforementioned, to study the pyrolysis processes appears as a promissory subject to use the WM to produce biofuels or to be incorporated into the biorefinery concept.

### **1.1.2. Microalgae**

Algae are common aquatic organisms which can be found in marine area, freshwater, brackish water and minority in sediments and soils (Chew et al., 2017 , Savage and Hestekin, 2013). Currently, the main types of alga can be commonly grouped as macroalgae (seaweed) and microalgae based on their biological structures as well as their differences in chemical constituents (Suganya et al., 2016 , Ghayal and Pandya, 2013).

Microalgae are unicellular micro-organisms, which convert sunlight, water and carbon dioxide into algal biomass (Roselet et al., 2019). The size of microalgae, depending on the species can vary from 1 to 400  $\mu\text{m}$ . The microalgae show great utilization in product development such as pharmaceutical medicine, food ingredients, fertilizers, chemicals and biofuels due to their antimicrobial, anti-inflammatory, anti-cancer and biochemical properties (Pr  at et al., 2020 , Onen Cinar et al., 2020 , Soto-Sierra et al., 2018 , Sajjadi et al., 2018).

Therefore, its inherent properties may influence its suitability and suitability as a raw material for pyrolysis. The three main components of microalgae biomass are lipid, carbohydrate and protein (El-Sheekh et al., 2020); additionally, it contains small amounts of organic extractives and inorganic materials (Yang et al., 2019 , Chandra et al., 2019 , Suganya et al., 2016). The proportion of these components varies depending on the microalgae species (Moreno-Garcia et al., 2017). Microalgae *Botryococcus*, *Chlorella vulgaris*, *Isochrysis*, *Neochloris oleoabundans* and *Nannochloropsis* are among species contain high content in lipids (Shahi et al., 2020 , Pal et al.,

2019 , Sajjadi et al., 2018 , Gong et al., 2013). Researchers have focused on production of FAME from microalgae by utilizing the algae lipid content (Kumar and Singh, 2019 , Kim et al., 2019).

### **1.1.3 Lipid**

Microalgae lipids serve as energy reserves and structural components (membranes) of the cell (Sajjadi et al., 2018 , Wang et al., 2017). The simple fatty acid triglycerides are important energy reserves (Muñoz et al., 2020 , Kumar and Singh, 2019). Most of the microalgal oils have similar fatty acid constitutions to conventional vegetable oils (Muñoz et al., 2014). The similarity makes microalgal lipid is a promising source for fossil fuel replacement. Each of these lipids contained fatty acids in a range from C12 to C24, the common saturated straight chain fatty acids such as lauric (C12), myristic (C14), palmitic (C16) and stearic (C18) are found in microalgae (Kumar and Singh, 2019 , Kim et al., 2019 , Santos-Ballardo et al., 2015).

The composition of fatty acids present in oils from microalgae determines the quality of the produced FAME. The level of unsaturated fatty acids affects FAME properties. For example, fuels with a higher level of unsaturation of the acyl chains have a higher cloud point, which is desirable but is also much more susceptible to oxidation (Kumar and Singh, 2019 , Kim et al., 2019 , Bux, 2013). Furthermore, a high level of unsaturated fatty acids in fuel increases the danger of polymerization in the engine oil and can cause problems with oxidative stability of the fuel.

The molecular structure of lipid can be described as a long hydrocarbon chain attached with carboxyl functional group (Lee and Wilson, 2015). Typically, the types of lipid found in algal biomass differ from lignocellulosic biomass which are mostly triglycerides in nature. Lipid contents in algal biomass can be diversifying in forms of neutral lipids, phospholipids, sphingolipids, glycolipids, peptidolipids, peptidoglycolipids and triglycerides (Shahi et al., 2020). Some of these lipids can be converted and formed into oil-based products which have higher fatty

acid contents (Muñoz et al., 2015). The range of lipid content for microalgae however varies considerably from 1.10 to 51.00%, depending on the microalgal species and also relying heavily on the growth condition when more nitrogen resources are exhausted, more lipids which can be accumulated up to 90% (Francavilla et al., 2015 , Li et al., 2014 , Wang and Wang, 2012). After oil extraction, these residues algal biomass can be used for pyrolysis feedstock instead of disposing of as waste materials (Trivedi et al., 2015 , Muñoz et al., 2015).

#### **1.1.4. Carbohydrate**

Microalgal carbohydrate are usually made up of sugar monomers or polysaccharides such as glucose, cellulose and starch produced from their photosynthesis process (Sajjadi et al., 2018). Generally, microalgae possess carbohydrate contents ranging from 3.28 to 30.21% which are for extracellular protection and cell adhesion (El-Sheekh et al., 2020 , Pal et al., 2019 , Chandra et al., 2019 , Wang et al., 2017). As feasible feedstock, bioethanol and H<sub>2</sub> can be generated from glucose or starch while biologically active ingredients can be produced from polysaccharides. In addition, carbohydrates are also raw materials for synthesizing valuable chemicals such as cyclic ketones and phenols (Pal et al., 2019 , Javed et al., 2019 , Bórawski et al., 2019). Under pyrolysis process, algal carbohydrates form light organics and polycyclic aromatic hydrocarbons in the composition of pyrolytic products (Priharto et al., 2020 , Yang et al., 2014 , Liu et al., 2014).

#### **1.1.5. Protein**

Protein content is exceptionally high in microalgae (14.00 – 65.20%) (Zewdie and Ali, 2020 , Onen Cinar et al., 2020 , Bhattacharya and Goswami, 2020 , Moreno-Garcia et al., 2017), large quantities of heterocycles such as pyrroles and indoles can be produced as valuable fine chemicals for applications such as herbicides, pharmaceutical ingredients and adhesives (Bhattacharya and Goswami, 2020 , Roselet et al., 2019 , Soto-Sierra et al., 2018 , Yan et al., 2016 , Suganya et al.,

2016). The protein content in algal biomass however also contributes to the N element in the products. Under pyrolysis process, N-based species such as nitriles, nitrites and nitrogenates will either devolatilized or remain in the pyrolytic bio-oil (Soto-Sierra et al., 2018 , Wang et al., 2017). These N-based products are undesirable in pyrolytic bio-oil which may results in higher NOX emission if combusted and eventually leaving behind ash in the form of metal oxides (Imran et al., 2018 , Muñoz et al., 2015).

#### **1.1.6. Biorefinery**

In recent years, growing socioeconomic awareness and the negative impact of excessive oil consumption have reoriented research interests toward biological resources such as algae-based biomass (Soni and Karmee, 2020 , Cuevas-Castillo et al., 2020 , Bórawski et al., 2019). To meet the current challenges of bioeconomic to produce multiple high-value products at once, new integrated research and development processes are needed (Ubando et al., 2020 , Hariskos and Posten, 2014). Although several strategies have been proposed for the conversion of algae-based biomass into fuel and fine chemicals, none of them have proven to be economically viable and energy-viable. Therefore, it is necessary to look for a range of other bioproducts. In this context, the concept of a biorefinery of algae has emerged with a remarkable solution to recover multiple products from a single operating process (Ubando et al., 2020 , Soni and Karmee, 2020 , Préat et al., 2020 , Cuevas-Castillo et al., 2020 , Chandra et al., 2019 , Trivedi et al., 2015 , Hariskos and Posten, 2014).

The biorefinery concept has emerged as integrated processes for the conversion of microalgal biomass into fuel and other value-added products (Kumar and Singh, 2019 , Bastiaens et al., 2017). The multiple and complementary outputs provide a more sustainable and economical approach that focuses solely on fuel production. Biofuel production from microalgae does not have economic

viability based on current capital costs per unit of fuel production (Chandra et al., 2019 , Moreno-Garcia et al., 2017). Hence, high-value co-products must be generated to improve the economics of a microalgae biorefinery. Microalgae can be microbial factories producing various compounds other than lipids for FAME. Being composed of lipids, carbohydrates and proteins, microalgae can be ideal feedstock to commercially important value-added products (Préat et al., 2020 , Bhattacharya and Goswami, 2020 , Chew et al., 2017). Capturing the value of multiple components can be fulfilled with an integrated biorefinery, which maximize product outputs from the single biological material. A biorefinery involves a cascade of processes that can use all the raw material components while preventing loss or damage to any products (Chandra et al., 2019 , Bastiaens et al., 2017). A sustainable extraction of these compounds considering green chemistry principles is significant challenges in algae-based biorefinery. These processes are energy intensive and the maximum exploitation of microalgae biomass while using minimum energy remains the primary focus (Kumar and Singh, 2019 , Chandra et al., 2019).

#### **1.1.7. Conversion Thermochemical**

The production of liquid fuels from biomass or bio-fuels can be accomplished through several different methods. Previously, microalgal biomass has been promising feedstock for FAME production. The process involved lipid extraction and get through transesterification process to produce FAME. The microalgal residues or SM obtained after conventional transesterification are mainly composed of proteins, carbohydrates, and a fraction of unutilized lipids, which can be employed to generate liquid and gaseous fuels, and biochar solids, depending on the temperature used (Chew et al., 2017 , Bastiaens et al., 2017 , Muñoz et al., 2015). Algal biomass conversion to fuel in the form of liquid or gas is generally classified into two categories and identified as biochemical or thermochemical, involving high temperature to produce biochar, bio-oil, and NCG (Pattiya, 2018 , Bennion, 2014). Many factors influence the conversion process selection, such as

type and quantity of biomass feedstock, economic consideration, the desired form of energy and end-products (Bastiaens et al., 2017 , Acién et al., 2014).

Thermochemical conversion appears to be a promising technology to produce various biofuels compared to bio-chemical processing. The bio-oil produce can be directly implemented for power and electricity plant, or the refining bio-oils can be used as transportation fuels (Shahi et al., 2020 , Srifa et al., 2019). Furthermore, thermochemical technology can be applied to any types of biomass feedstock without the use of toxic or chemicals in the process. Besides, the conversion utilizes the WM biomass for bio-fuel production, where the biomass is thermally decomposed into bio-oil under specific conditions (Yang et al., 2019). The relative fractions of various solids, liquids and gaseous products are highly dependent on the operating conditions including temperature, pressure, heating rate and residence time (Yang et al., 2019 , Srifa et al., 2019). Pyrolysis can be carried out at moderate temperature 400 to 650 °C, in inert condition and depends on the residence time of reactions (Yang et al., 2019 , Pattiya, 2018 , Yildiz et al., 2014). Gasification runs at a higher temperature >700 °C, cracking and promoting the production of gaseous hydrocarbons and NCG (Chernova et al., 2020 , Mhilu, 2014). Bio-oil from pyrolysis need to be upgraded through deoxygenation and hydrocracking/ cracking process to produce products with same properties as gasoline, jet fuels and diesel (Srifa et al., 2019 , Saber et al., 2016). The pyrolysis has been already demonstrated at industrial scale and present lower capital costs compared to liquefaction and gasification (Bhattacharya and Goswami, 2020 , Bórawski et al., 2019).

Pyrolysis liquid final product can be transported and upgraded to fuels and valuable products such as fertilizers, resins, and other specialty chemicals that are fully compatible with existing petroleum infrastructure by downstream processing in a similar way as crude oil is processed in an oil refinery (Srifa et al., 2019 , Saber et al., 2016 , Zhang et al., 2013 , Xiu and Shahbazi, 2012).

However, the production of bio-oil from microalgae requires large quantities of biomass. The process takes place either in dry or wet conditions. Microalgae contain high water content (80-90%) (Shahid et al., 2019 , Park et al., 2019), and this is a significant drawback for the direct implementation conversion (gasification or pyrolysis) of microalgae. Therefore, like the terrestrial biomasses, water content needs to be reduced before processing the raw algae, through energy-intensive separation processes (Zewdie and Ali, 2020 , Ubando et al., 2020 , Cuevas-Castillo et al., 2020). There are a variety of methods for dewatering microalgae.

The technologies available this day carried out using lignocellulosic biomass which is different compared to the microalgae. Microalgae contained higher inorganic and ashes content, the peculiar state of aggregation depending on the harvesting methods (Zewdie and Ali, 2020 , Gong et al., 2020 , Roselet et al., 2019). The use of salty water for algae cultivation increase problem such as corrosion and solid deposition (El-Sheekh et al., 2020 , Roselet et al., 2019 , Morgan et al., 2016). Thus, a pre-treatment on the microalgae before the conversion could be an advantage on tackling the issues.

#### **1.1.7.1. Pyrolysis**

In pyrolysis, the thermal decomposition is carried out under oxygen- free or oxygen limiting conditions, resulting in the organic material being broken down into three distinct product fractions: solid (biochar and coke), liquid (bio-oil) and NCG fuels (methane and higher gaseous hydrocarbons). The main types of pyrolysis are reported in the literature: slow (low process temperatures and longer time) and fast pyrolysis (higher process temperatures and shorter time) (Ganesan et al., 2020 , Pattiya, 2018 , Ronsse, 2016).

Among those thermochemical methods, pyrolysis can produce different forms of product from algal biomass into liquid, solid and gas products at varied pyrolysis conditions. Slow and fast

pyrolysis are considered to be long-standing technologies and distinguished through several critical parametric controls such as pyrolysis temperature, pyrolysis time, heating rate, particulate size and inert gas sweeping rate (Yang et al., 2019 , Pattiya, 2018 , Feroso et al., 2017). The following section provides the review on the conventional pyrolysis and their current applications on algal biomass.

#### **1.1.7.2. Slow pyrolysis**

Slow pyrolysis is an eminent synthesis method utilized mainly to generate biochar with co-products such as bio-oil and NCG. Slow pyrolysis conditions primarily focus on moderate temperatures (250 – 550 °C), slow heating rates (< 60 °C/min) and long pyrolysis times (> 5 min) (Wang et al., 2020 , Ronsse, 2016). Slow pyrolysis covers, besides carbonization, more recent process developments such as torrefaction (Ronsse, 2016).

#### **1.1.7.3. Fast pyrolysis**

To maximize the bio-oil production, fast pyrolysis is often employed on biomass with the following pyrolysis conditions: high temperature (350 – 650 °C), fast heating rate (> 60 °C/min) and short pyrolysis time (0.5 – 10 s) (Pattiya, 2018 , Hornung, 2014). As fast pyrolysis operates with high pyrolysis temperature but short time residence, such settings can inhibit the secondary reactions of volatiles thereby preventing the cracking of the primary products, which eventually contribute to improve liquid product yields (Lee et al., 2020 , Imran et al., 2018 , Hornung, 2014). In other words, fast pyrolysis technology suppresses secondary reactions as well as polymerization of volatile intermediates (Tirapanampai et al., 2019 , Srifa et al., 2019). With fast pyrolysis processes, the algal biomass is directly converted into liquid and non-condensable gaseous products before the formation of biochar. To accommodate such processing temperature and time, the particle size of biomass used in fast pyrolysis is usually fine (< 2 mm) to ensure effective heating (Yang et al.,

2019 , Pattiya, 2018 , Ronsse, 2016). Furthermore, fast pyrolysis is capable of handling high ash content feedstock such as microalgae by concentrating those minerals or inorganics into the portion of the biochar which can be used as soil fertilizer and conditioner (Wang et al., 2020). Compared with other forms of pyrolysis, fast pyrolysis is considered to be an inexpensive way to produce pyrolytic liquid with good quality (Ubando et al., 2020 , Pinheiro Pires et al., 2019 , Saber et al., 2016).

#### **1.1.8. Bio-oil from microalgae**

From algal biomass pyrolysis, liquid products such as bio-oil, can be obtained in the form of condensate (Pattiya, 2018 , Vanderauwera and Wambeke, 2014). Bio-oil is formed with rapid quenching from the mixed volatiles at the outlet of the pyrolysis unit (Pattiya, 2018 , Saber et al., 2016 , Ronsse, 2016). Typically, the bio-oil is produced from fast pyrolysis, mostly with the appearance of dark brown, may be viscous or non-viscous and with strong, distinctive smell. Chemically, bio-oil has high complexity containing organic compounds whereby its composition is highly dependent on feedstock, pyrolysis process and synthesis operating conditions (Srifa et al., 2019 , Saber et al., 2016). To date, many researchers focus on bio-oil synthesis as it has the potential to be a viable replacement for fossil fuels. Bio-oil is made up of two different phases, namely organic phase and the aqueous phase (water soluble). The organic phase usually possesses higher energy values compared to the aqueous phase. However, pyrolytic oil suffers from high viscosity, high acidity as well as high moieties which can cause bio-oil degradation over time (Srifa et al., 2019 , Saber et al., 2016 , Ronsse, 2016 , Vanderauwera and Wambeke, 2014 , Fei et al., 2014). Due to its protein fraction, the nitrogen contents in algal bio-oil can reach up to 13%.

Compared to lignocellulosic pyrolysis oils, microalgae-derived pyrolysis oils have a higher Heating Value (HV), with values between 31 MJ/kg and 36 MJ/kg. Microalgae species with higher

carbohydrate or lipid fractions tend to give pyrolysis oils with higher HV and lower oxygen contents (Imran et al., 2018 , Rai and da Silva, 2016).

Direct utilization of pyrolysis liquids is limited due to their limited thermal stability, high oxygen content, high water content, high viscosity, and immiscibility with hydrocarbons (Echresh Zadeh et al., 2021 , Hu and Gholizadeh, 2020). Also, the high amounts of nitrogen, mainly in the form of organo-nitrogen compounds in microalgae pyrolysis oils, is not a favorable feature as it will result in NO<sub>x</sub> emissions during combustion and issues with the hydrotreating catalysts when co-processed in existing crude oil refineries (Shan Ahamed et al., 2020).

Bio-oil is a HV liquid fuel, which has a high energy density, and can be stored, transported and used as crude or refined fuel (Venderbosch and Prins, 2010 , Yang et al., 2019). Commonly, in a thermo-cracking process using lignocellulosic biomass to produce bio-oil is feasible to produce bio-oil with about 400 chemical compounds with a HV of 13 to 18 MJ/Kg (Basu, 2010). This HV is between 6 and 8 times higher than the original biomass, but lower compared with the petroleum fuels (41–43 MJ/kg) (Lu et al., 2009). Additionally, the use of bio-oil produced from lignocellulosic biomass is limited, as bio-oil is commonly viscous, corrosive, highly polar and with high oxygen content (40–50 wt. %) (Bridgwater, 2012 , Yufu et al., 2011). However, these characteristics could vary according to the operation parameters of the reaction and the kind of feedstock used for its production.

Increased demand for biofuel feedstock results in a high deficit of biomass. Besides, when microalgae are used for FAME production, high quantities of residual biomass are produced. Pan *et al.* (2011) reported that only the energy from lipids of microalgae is used in FAME production, SM biomass could reach about 60–70% of the cell dry weight. Therefore, there is a potential use of SM for bio-oil production. According to results of Miao *et al.* (2004), bio-oil obtained from WM

and SM of *C. protothecoides* had a HV of 29 (MJ/Kg) and 24.6 (MJ/Kg), respectively (Das et al., 2011). These HV were higher compared than the obtained for bio-oil of lignocellulosic biomass. Therefore, there is interest into produce bio-oil from post-harvest microalgae (WM) and SM (post-extraction of protein and/or lipids), as a complementary or alternative stage within of a microalgae biorefinery process.

#### **1.1.9. Technological aspects of bio-oil production derived from microalgae biomass by fast pyrolysis.**

The differences in chemical composition among strains of microalgae generate the main differences in conversion yield from microalgae to bio-oil by pyrolysis. Microalgae are mainly composed of three components: lipids, carbohydrates, and proteins. Grierson *et al.*, (2009) and Miao *et al.*, (2004) have used microalgal biomass of different strains, *C. vulgaris*, *D. tertiolecta* and *C. protothecoides*, achieving a bio-oil conversion yield of 41%, 24% and 18%, respectively. These results were low compared with the bio-oil production from lignocellulosic biomass (~80%) (Mark, 2006). However, quality of bio-oil obtained from *C. protothecoides* had HV of 29 (MJ/kg), which was much higher than lignocellulosic bio-oil (13-18 MJ / Kg) (Basu, 2010 , Du et al., 2011). Similarly, Du (2011) reported a low bio-oil production (28.6%), but a HV (30.70 MJ/Kg), generating a high potential as a fuel.

Du (2011) reported the chemical composition of the bio-oil obtained from *Chlorella sp* microalgae, identifying mainly hydrocarbon chain, aromatic compounds, long carbon chain compounds and several with terminations in functional groups. Unlike lignocellulosic biomass, the absence of lignin in microalgae biomass allows to obtain bio-oil with less variety of compounds, it may be of interest to study its refining to obtain products of higher purity.

Pyrolysis of carbohydrates and proteins produces a wide range of unsaturated and aromatic hydrocarbons due to secondary reactions (Meuzelaar et al., 1982). The presence of high levels of lipid and protein in the biomass generates high bio-oil and NCG, latter almost 30 (wt/wt<sub>dry basis</sub>). On the other hand, pyrolysis of lignocellulosic biomass is characterized by high yield of bio-oil conversion and low yield of NCG production (10 wt/wt<sub>dry basis</sub>). Furthermore, the presence of proteins in biomass generates bio-oil with HV similar to the HV for the fossil oil. In the presence of nitrogen compounds, oxygen replaced pyrolytic oxygenated compounds, increasing HV of bio-oil. As shown in Fig. 1.1, chemical substitution of nitrogen for oxygen has nucleophilic nature resulting from pyrolytic cracking of proteins. The rupture of peptide bonds from proteins release amine nucleophiles which react with the electrophilic organo-oxygen species derivative from carbohydrates, releasing oxygen as water. This replacement contributes significantly to increase the HV (Mullen and Boateng, 2011).

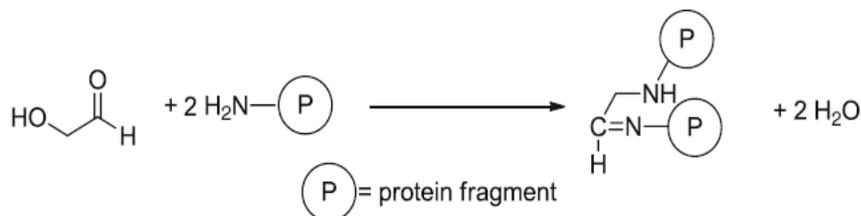


Figure 1.1: Chemical substitution of nitrogen for oxygen in oil pyrolysis (Mullen and Boateng, 2011).

Du *et al.* (2011), compared the characteristics of bio-oil produced from microalgae *Chlorella sp.* with wood bio-oils. The results show a lower oxygen content, and higher carbon, hydrogen content and HV for bio-oil from microalgae compared than bio-oil from lignocellulosic biomass. Besides, bio-oil from microalgae had a lower density than lignocellulosic bio-oil and a viscosity in the typical range of wood bio-oil.

The yields of pyrolysis for bio-oil production could be increased by modifying metabolic pathways of microalgae, from an autotrophic to heterotrophic growth. Miao and Wu (2004), have reached bio-oil yields obtained from *C. protothecoides* by fast pyrolysis of 3.4 times greater in microalgae produced under heterotrophic conditions (57.2%) compared with the produced under autotrophic (16.6%) at temperatures from 450 to 500 °C. Thus, productive differences are generated according to the chemical composition of each species. On the other hand, microalgae biomass produced under heterotrophic conditions was characterized by high lipid content up to 3.8 times more than autotrophic microalgae. High lipid content is directly related to a higher conversion in to bio-oil.

The use of SM for production of bio-oil by fast pyrolysis, generates lower production of bio-oil compared with lipid microalgal biomass. Pan et al, (2010) reported production of bio-oil from waste-microalgae biomass (post extraction of lipids) of *Nannochloropsis sp.* obtained at 400 ° C and 300 °C, achieving a low conversion of 31% and 20% of bio-oil respectively (Table 1.1). Bio-oil has a HV (24.6 MJ/kg and 6.4 MJ/kg obtained at 400°C and 300 °C respectively) significantly higher than bio-oil from lignocellulosic biomass, and slightly lower compared with bio-oil microalgal biomass derived from WM (*C. protothecoides*). This study allows us to consider this type of residual biomass as feedstock for production of bio-oil with a high HV.(Pan et al., 2010)

Table 1.1: Chemical composition of *Nannochloropsis sp.* SM and bio-oil yield by Pan (2010)

Component	SM	Bio-oil
C (wt. %)	44.1	56.13
H (wt. %)	7.09	7.63
N (wt. %)	5.51	5.34
O (wt. %)	33.40	30.09
HV (MJ/Kg)	20.7	24.4
O/C	0.75	1.63
H/C	0.16	0.40

WM *Nannochloropsis sp.* has high nitrogen content (66 - 24% by weight approx.) that can be maintained in biomass after lipid extraction process. Therefore, when whole biomass or biomass after lipid extraction is used as feedstock in fast pyrolysis reaction, bio-oil with high nitrogen content can be produced. Thus, when bio-oil is burned, NO<sub>x</sub> could be produced. Therefore, it is interesting to investigate if the characteristics of bio-oil could be improved if nitrogen components are extracted previous to fast pyrolysis. In this project the extraction of the nitrogen components (proteins) is proposed as an alternative in order to improve the quality of bio-oil. In addition, it is expected that alternative along with removing nitrogen, will allow recovering commercially valuable chemicals, such as proteins.

Therefore, the association of technology to processes of reuse of waste generated from a potential industry of microalgae could be interesting and consequently, this thesis proposes obtaining bio-oil of SM and WM with significant HV as fuel.

Production rates and bio-oil quality can also vary considerably according to the main operating parameters (reaction temperature, heating rate and residence time of gases and vapors). Basu

(2010) reported an increase in bio-oil production as the temperature increases, as well as a high heating rate and moderate end temperature (450 - 600 ° C) maximized liquid yield and minimized char yield (Demirbas, 2006). Similarly, the operation at different temperatures produces a different chemical composition in bio-oil, it could thus be possible to direct the chemical composition to a specific group of compounds. In this regard, Li *et al.* (2012) reported pyrolysis of algae biomass at different reaction temperatures, achieving a reduction of sugar in bio-oil when the temperature was increased favoring other groups of higher HV. Therefore, temperature can be considered as a method to improve bio-oil quality. The gas phase residence time is reaction time available for secondary tar reactions, hence affecting the yields of gaseous and liquid products.(Li et al., 2012)

## **1.2. Hypothesis**

### **Statements:**

The following **working hypotheses** are proposed:

- 1 The different composition of microalgal biomass compared with lignocellulosic biomass allows producing more alkaline bio-oil compared with bio-oil from lignocellulosic biomass.
- 2 A certain amount of proteins extracted from the microalgae biomass before the pyrolysis process allows to obtain a bio-oil with a higher calorific value than the lignocellulosic bio-oil.

## **1.3. General goal**

To study bio-oil production by fast pyrolysis from SM following a biorefinery concept.

## **1.4. Specific objective**

- 1 To evaluate the feasibility to produce bio-oil by fast pyrolysis from WM and SM.
- 2 To optimize the production of bio-oil by fast pyrolysis from SM.
- 3 To evaluate the characteristics of the produced bio-oil, NCG and bio-char and their possible applications.

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## **CHAPTER II**

# **“Bio-oil production from microalgae following a biorefinery concept”**

### **Chapter based in the published paper:**

Muñoz R, Navia R, Ciudad G, Tessini C, Jeison D, Mella R, Rabert C, Azócar L (2015). Preliminary biorefinery process proposal for protein and biofuels recovery from microalgae. Fuel 150. 425–433.

## 2.1. Introduction

Microalgae have been widely investigated as potential raw material for the production of biofuels and other chemicals. Specifically, microalgae characteristics present high internal lipids content and, in some cases, external hydrocarbons, generating interest to find feasible methods to extract these products (Bhattacharya and Goswami, 2020). Up to date, intensive pretreatment and/or expensive solvents have been applied to obtain both high hydrocarbons and lipids extraction yields (Alzate et al., 2012 , Magota et al., 2012).

Besides, when microalgal biomass is used for FAME or hydrocarbons production; only about 10 to 20% of WM is used and about 70% of biomass is generated as SM. Therefore, the research has been focused on finding a novel use for the SM following a biorefinery concept. In this sense, the thermal conversion of SM by using pyrolysis has been proposed as an interesting alternative. The advantage of pyrolysis is that whole biomass can be converted into bio-oil, NCG and bio-char. On the contrary, in FAME or ethanol production it is only possible to produce a biofuel using part of the feedstock (fatty acids or carbohydrates, respectively) (Pal et al., 2019).

Some research has been reported on microalgae pyrolysis processes, following a biorefinery concept. Wang *et al.* (2013) studied pyrolysis of *Chlorella vulgaris* SM, after lipid extraction. The authors reported bio-oil, bio-char and NCG yields of 53, 31 and 10 wt.% respectively, using a fluidized bed reactor at 500°C. Torri *et al.* (2011) reported the production of photo-biogenic hydrogen during the culture, FAME from lipids and finally bio-oil, bio-char and NCG by pyrolysis of *Chlamydomonas reinhardtii*. The biomass was pyrolyzed in a fixed bed reactor at 350 °C obtaining 44% biochar (w/w dry-biomass) and 28% bio-oil (w/w<sub>dry-biomass</sub>).

Although the interesting results obtained in pyrolysis from microalgal biomass, there is a very important disadvantage of using microalgal biomass as feedstock as microalgae are characterized by a high protein (nitrogen) content, which could be finally present in the

produced bio-oil. This high nitrogen content in bio-oil could generate NO<sub>x</sub> when the bio-oil is burned. In addition, when lipids are extracted in a first step, proteins are concentrated in the biomass, increasing the nitrogen content in the final bio-oil. Wang *et al.* (2013) reported that 60.3% original nitrogen content in the biomass ended up in the bio-oil and 30% ended up in the bio-char. In this sense, it could be interesting to study the alternative of carrying out a protein extraction previous to pyrolysis process to reduce the nitrogen content in the bio-oil.

In addition, it has been reported that the main drawback of bio-oil from lignocellulosic feedstock is low pH (2-3) as the acidity can produce extensive corrosion of the metal surface of internal combustion engines (Mahfud *et al.*, 2008). This low pH is caused by the presence of large amounts of organic acids such as acetic acid, formic acid and propionic acid, which are produced from the lignocellulosic biomass composed by cellulose, lignocellulose and hemicellulose. Nevertheless, microalgal biomass is mainly composed of lipids, proteins and hydrocarbons. Therefore, it seems interesting to study the characteristics of the bio-oil produced from microalgal biomass and compare these properties with conventional bio-oil obtained from lignocellulosic raw material.

According to previous statements, the aim of this work was to investigate the pyrolysis of microalgae biomass to analyze the characteristics of the produced bio-oil, and compare it with conventional lignocellulosic bio-oil. In addition, the biorefinery concept was applied in this research by comparing the results with SM pyrolyzed after both, protein and lipid extractions.

## **2.2. Experimental section**

### **2.2.1. Materials**

Freshwater microalgae *Botryococcus braunii* (WMB) and saline microalgae *Nannochloropsis gaditana* (WMN) were used as feedstock. Both microalgae were produced in the Atacama Desert (Chile) according to the conditions reported by Bazaes *et al.* (2012) and concentrated up to about 200 g/L as microalgal paste. In addition, radiate pine from plantations in the Southern

Chile was also used as feedstock to compare the results with lignocellulosic biomass. (Bazaes et al., 2012)

## **2.2.2. Production of SM to be pyrolyzed following a biorefinery concept**

### **2.2.2.1. Protein extraction**

Protein extraction from WMB was carried out adapting the methodology of Kale *et al.* (2011). The microalgal biomass was resuspended in distilled water at a water/ microalgae ratio of 16:1 (v/wt<sub>dry basis</sub>). Subsequently, NaOH 2M was used to adjust the pH of the sample at 11 and protein extraction was carried out under continuous stirring at 150 rpm during 13 minutes and room temperature. The resulting mixture was centrifuged at 4400 rpm during 15 minutes and the two phases obtained were separated. The precipitate of SM *B. braunii* after extraction proteins (SMB<sub>P</sub>) was stored and used in the next step of lipid extraction. The supernatant pH was adjusted at 3.3 (isoelectric point) with HCl 1M and centrifuged to obtain a precipitate of microalgal protein fraction. The protein fraction and remaining biomass were gravimetrically quantified.

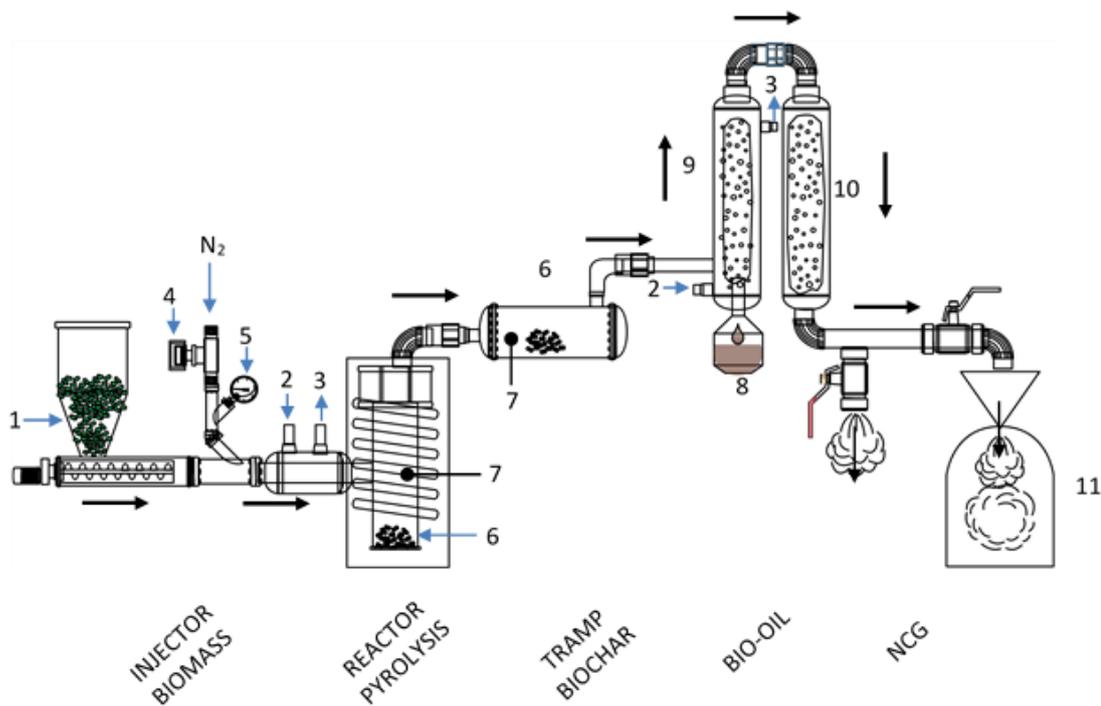
### **2.2.2.2. Lipid extraction and fatty acid methyl ester (FAME) production**

The SMB<sub>P</sub> after protein extraction was disposed in a dried tunnel for 24 hours and then, milled in a mortar. Subsequently, lipids were extracted using soxhlet extraction system with petroleum ether as solvent at a solvent/biomass ratio of 14:1 (v/wt<sub>dry basis</sub>), during 16 hours. The SM obtained after lipid extraction was dried at 40 °C during 1 hour in order to eliminate the remaining solvent in biomass. The resulting SMB<sub>L+P</sub> was gravimetrically quantified and used on pyrolysis assays. FAME production using the extracted lipids was carried out according to Miao *et al.* 2006, as follows: Catalyst to oil ratio of 100:1 (v H<sub>2</sub>SO<sub>4</sub>/wt<sub>dry basis</sub>), methanol to oil molar ratio 56:1 (mol/mol), at 30 °C during 4 hours of reaction time. FAME products were quantified by gaseous chromatography (GC).

### 2.2.3. Pyrolysis experiments

Pyrolysis of WMB, WMN (WM of *N. gaditana*), from SMB<sub>L+P</sub> and radiate pine was investigated. WMB and WMN were previously dehydrated in a tunnel. All the feedstock was also milled and sifted to a particle size of 100-500  $\mu\text{m}$ . Additionally, previous to pyrolysis experiments TGA were performed to study the thermal behavior of the microalgal biomass investigated. TGA were carried out on a NETZSCH TG 209F1 thermogravimetry analyzer. Small samples between 4 to 10 mg were loaded into an Al crucible in order to avoid heat and mass transfer limitations. The samples were heated from room temperature at a rate of 10°C/min until 150°C and were held at this temperature for 10 min to ensure the complete water removal. Then, the temperature was increased until 550 °C and held for 60 min. The TGA were performed under a nitrogen flow of 50mL/min.

Pyrolysis was performed with a lab-scale fed-batch reactor which was operated under fast pyrolysis conditions and atmospheric pressure with heating controlled by Data Acquisition Instrument (Fig. 2.1). Between 11 and 15 grams of biomass was continuously fed at a feeding rate of 12 mg/s using nitrogen as carrier gas. The residence time of the biomass in the pyrolysis reactor was approximately 2 sec. The reactions were carried out in a steel tube of 15.1 cm in length and 3.4 cm internal diameter. Electrical heating tape was used to maintain the pyrolysis reactor temperature at 500 °C. Bio-char was retained by filters inside the reactor. One additional filter was disposed in the reactor outlet to capture the residual bio-char. Two bio-oil fractions were obtained in the condensation system composed by a cooling water condenser at 5 °C (first fraction), followed by an EP couple to a final condenser at 5 °C (second fraction). The non-condensable gases were stored in a gas sampling bag for further analysis by GC. The experiments with each biomass were carried out with at least three replicates.



**Figure 2.1.** Scheme of pyrolysis reactor. 1) Biomass, 2) Cool water inlet, 3) Water outlet, 4) Flow meter, 5) Manometer, 6) Bio-char, 7) Thermocouple, 8) Bio-oil, 9) Condensation system, 10) EP, 11) Gas sampling bag.

#### 2.2.4. Characterization of feedstock and products

Composition of pyrolysis feedstock previously dehydrated and milled was determined. Moisture analysis was performed gravimetrically drying the biomass at 103°C during 3 hours until constant weight. Similarly, ash was quantified at 550° +/- 25 °C during 8 hours. Lipids were quantified through acid hydrolysis using Butt system and protein by Kjeldahl methodology. Carbohydrate content was expressed as the 100% - (moisture content + lipids + protein + ash).

### **2.2.5. Characterization of FAME**

Identification and quantification of fatty acids in FAME was carried out using a Clarus 600 chromatograph coupled to a Clarus 500T mass spectrometer from Perkin Elmer (GC-MS). An Elite-5ms capillary column with a length of 30 m, thickness of 0.1  $\mu\text{m}$  and internal diameter of 0.25 mm was used. The vials were prepared by adding 3  $\mu\text{g}$  of sample to 100  $\mu\text{L}$  methyl heptadecanoate as an internal standard (initial concentration of 1300 mg/L). The following temperature program was used: 50  $^{\circ}\text{C}$  for 1 min and then increasing temperature at a rate of 1.1  $^{\circ}\text{C}/\text{min}$  up to 187  $^{\circ}\text{C}$ . Both the injector and detector temperatures were 250  $^{\circ}\text{C}$  and He was used as the carrier gas (Azócar et al., 2010b).

### **2.2.6. Characterization of bio-oil**

Compounds in bio-oil detectable by GC were analyzed on a gas chromatograph HP 6890 Series coupled to a HP 5973 mass spectrometer. A VF-1701 column with a length of 60 m, thickness of 0.25  $\mu\text{m}$  and internal diameter of 0.25 mm was used. The samples were prepared diluting 20 mg of bio-oil in 1 mL of acetone as solvent. The following temperature program was used: 45  $^{\circ}\text{C}$  for 4 min until 60  $^{\circ}\text{C}$  increasing temperature at a rate of 1 ( $^{\circ}\text{C}/\text{min}$ ) and then increasing temperature 3  $^{\circ}\text{C}/\text{min}$  up to 280  $^{\circ}\text{C}$ , maintaining the final temperature during 20 minutes. The injection volume was of 1  $\mu\text{L}$  and a solvent delay of 5 minutes was used with He as carrier gas (Tessini et al., 2012 , Tessini et al., 2011).

### **2.2.7. Characterization of NCG.**

NCG obtained from the pyrolysis were measured using a Clarus 580 chromatograph coupled to a thermal conductivity detector from Perkin Elmer (GC-TCD). A carboxen <sup>TM</sup>-1000 column with a length of 15 m with an isothermal program at 120 $^{\circ}\text{C}$  were used. A gas mix standard was used to quantify the gases H, CH<sub>4</sub>, CO<sub>2</sub> and CO<sub>2</sub>.

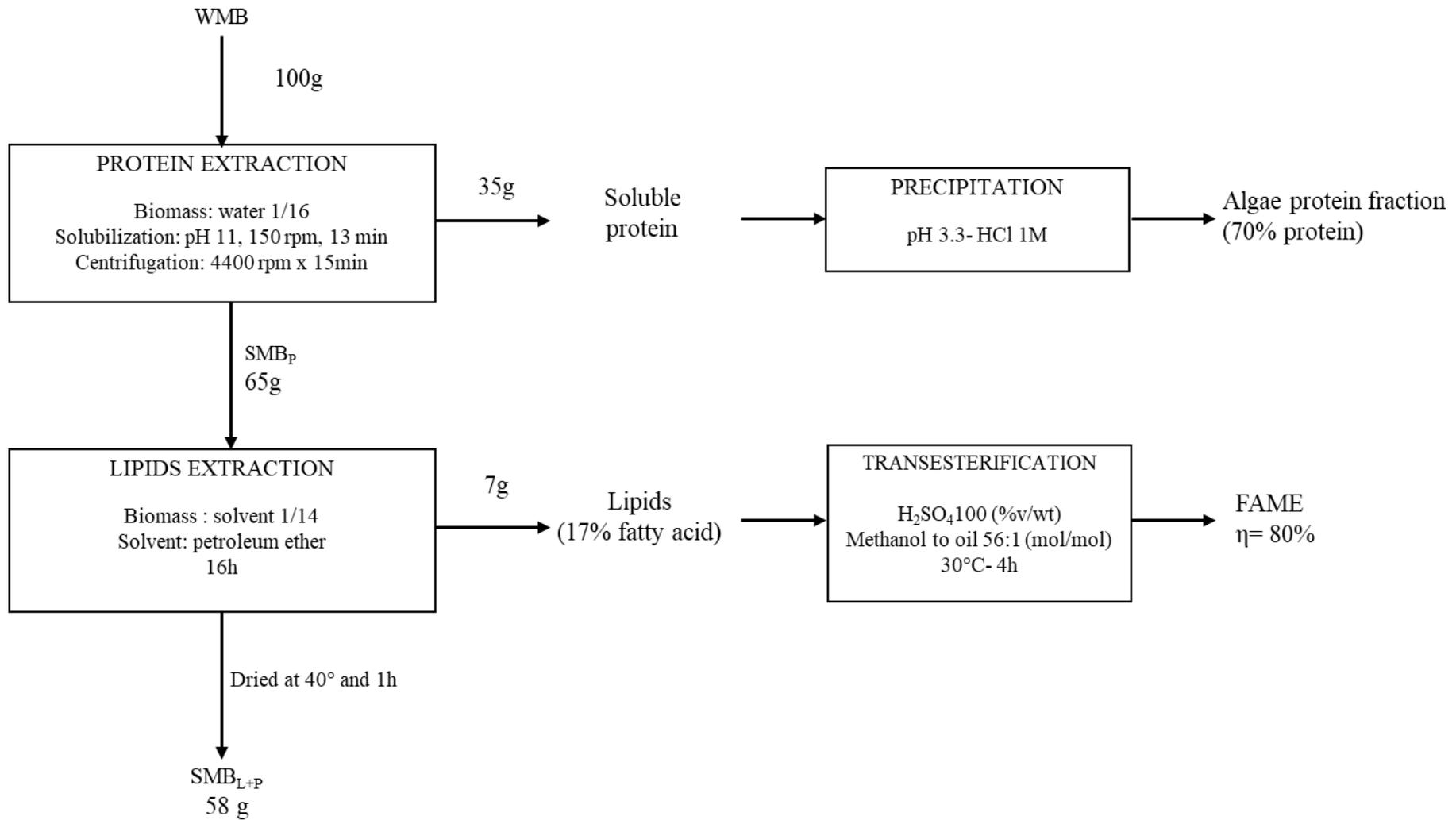
## 2.3. Results and Discussion

### 2.3.1. Production of SM to be pyrolyzed following a biorefinery concept

#### 2.3.1.1. Protein extraction

In the experiments of protein extraction an average of 35:1 (wt/wt<sub>dry basis</sub>) of biomass rich in proteins was obtained, of this 35% about 70% was protein (Fig. 2.2). The other 30% was mainly fatty acids and some lipids and carbohydrates. This loss of fatty acids is a disadvantage when and alkaline methodology is used for protein extraction, if the focus is to produce FAME with the waste biomass. The loss of fatty acids is probably produced by saponification reactions favored in alkaline conditions, which could produce soap from fatty acids.

On the other hand, considering that no previous pretreatments were performed to the biomass the reached yield is an interesting result. In this sense, Romero García *et al.* (2012) reported that without treatment a hydrolysis degree of only 15% of free-amino-acids concentrates from *Scenedesmus almeriensis* by using an enzymatic method was possible. Probably, the high yield obtained in the present research could be related to the direct use of biomass for protein extraction after harvesting by centrifugation. Instead, the use of dried or freeze-dried biomass to preserve it is commonly applied, processes that may contribute to obtain a more recalcitrant biomass. This result could be also related to those reported by Gerde *et al.* (2013), who investigated the protein extraction from defatted and non-defatted *Nannochloropsis* microalgal biomass. In this work they reported that a higher protein extraction yield was reached when non-defatted biomass was used. This could be related to the fact that during lipid extraction, some soluble proteins can be co-extracted with lipids. Therefore, if the objective is to produce several products from microalgal biomass, the order established to carry out the products extraction seems to be a very important topic (Romero García *et al.*, 2012 , Gerde *et al.*, 2013).



**Figure 2.2:** Production of  $SMB_{L+P}$ , to be pyrolyzed following a biorefinery concept. Biomass balance in dry basis.

### 2.3.1.2. Lipid extraction and FAME production

Biomass after protein extraction was used in lipid extraction by using a soxhlet extraction system. The reached yield of lipids from deproteinized biomass was 9.5:1 (wt/wt<sub>dry</sub> basis), remaining 58 g of SMB<sub>L+P</sub> for pyrolysis assays (Fig. 2.2). The lipids extracted were characterized by a very low fatty acids content of 17:1 (wt/wt dry basis). These low fatty acids content could be related to the fact that *B. braunii* is characterized by a high hydrocarbons content, which can be also extracted using petroleum ether. In addition, the low fatty acid extraction yield could be related to the fatty acid loss during protein extraction.

The lipid fractions were used as raw material to carry out a transesterification reaction under acidic conditions according to previous references. The FAME yield obtained was 80% as a function of fatty acids content. The FAME composition produced is shown in Table 1. The high content of saturated (21.5%) FAME could negatively affect the performance of FAME in engines if this is used in cold zones, being a typical value in FAME produced from waste frying oils (17%) (Azócar et al., 2010a). However, the value of methyl oleate is a very good value typical on FAME from rapeseed oil. In spite of the interesting results, a low quantity of FAME was produced: about 1.3 g of FAME from 75 g of SMB<sub>P</sub>. These results show that pyrolysis could be a very interesting alternative even to replace the FAME production to produce bio-oil, NCG and bio-char using SMB<sub>P</sub> biomass.

**Table 2.1.** FAME composition produced from SMB<sub>P</sub> biomass

Fatty acid		[%]
Methyl miristate	C14:0	0.2
Methyl palmitoleato	C16:1	3.4
Methyl palmitate	C16:0	18.6
Methyl lioledaidate	C18:3	0.0
Methyl linoleate	C18:2n6c	2.9
Methyl oleate	C18:1n9c	70.9
Methyl elaidate	C18:1n9c	0.5
Methyl estearate	C18:0	2.6
Methyl arachidonate	C20:4n6	0.3
Methyl eicosapentaenoic	C20:5n3	0.6

### 2.3.2. Feedstock characterization

Composition and physical characteristics of both WMB, WMN and SMB<sub>L+P</sub> are given in Table 2. Data for pine also are presented for a comparison with lignocellulosic biomass. Microalgal biomass is mainly composed by lipids, proteins and carbohydrates, while the main pine components are lignin, cellulose and hemicellulose. This means that it is expected to obtain bio-oil with a very different composition when using microalgal biomass compared to lignocellulosic feedstock in fast pyrolysis processes.

Microalgal biomass was characterized by a high protein content of 30% for both WMB and WMN. Lipid content was higher in WMB (17.7%) compared to WMN (12.7%). However, the lipids from WMN were composed of about 50% of fatty acids compared to the low fatty acids content in WMB (17%) (Table 2.2). In the case of SMB<sub>L+P</sub> the protein content was 20.4% and lipids content was 4.7%. This means that the methods used for protein and lipids extraction were not very effective. Indeed, it can be seen that the carbohydrates content in SMB<sub>L+P</sub> was

7.1% compared to the 15.5% in WMB. This means that a critical loss of carbohydrates happened during the extraction of lipids and/or proteins. Therefore, it seems to be interesting to optimize the methodologies to extract different components from microalgal biomass to increase a selective extraction avoiding carbohydrates loss.

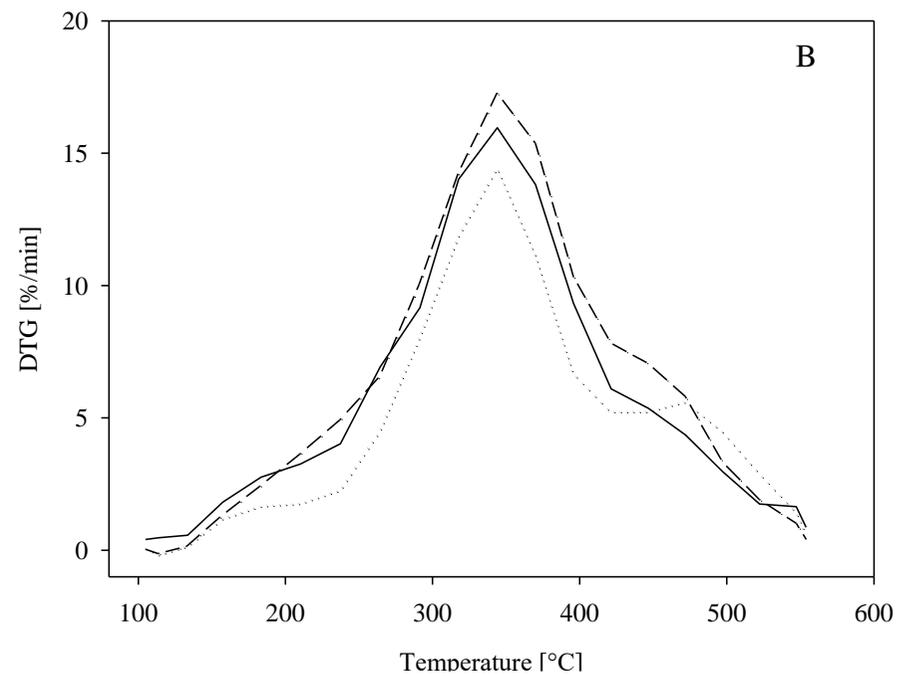
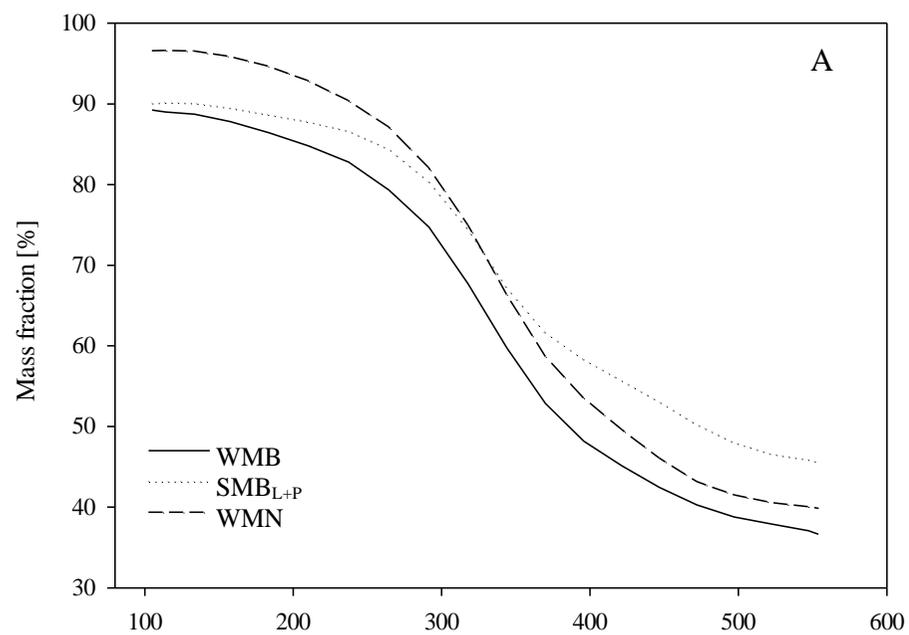
Related to moisture content in each feedstock, these are in a suitable range for pyrolysis. There for the drying tunnel used to dry the microalgal biomass could be a suitable system to be implemented before pyrolysis processes. Ash content in microalgal biomass was higher than pine biomass. According to Wang *et al.* (2013), the reason is the accumulation of inorganic salts in the cell from the culture media during the growth phase. In addition, salt from seawater could increase the ash content when saline microalgae such as *N. gaditana* are used. The ash content obtained in the present work is higher compared to the reference where were values between 3 and 16% are reported (Pan *et al.*, 2010 , Wang *et al.*, 2013). This is a very critical parameter for the pyrolysis process as a high ash content favors bio-char production and diminishes bio-oil yield and its HV. Therefore, nutrients dosage during microalgae growth phase must be revised in future research.

**Table 2.2:** Composition and physical characteristics of pyrolysis feedstock

<b>Feedstock</b>	<b>WMB</b>	<b>SMB<sub>L+P</sub></b>	<b>WMN</b>	<b>Pine</b>
<b>Composition (%dry basis)</b>				
Lipid	17.7	4.7	12.7	-
Protein	30.4	20.4	30.1	-
Carbohydrate	15.5	7.1	21.6	-
Moisture	8.0	10.3	4.6	6.7
Ash	28.5	36.0	31.0	6.7

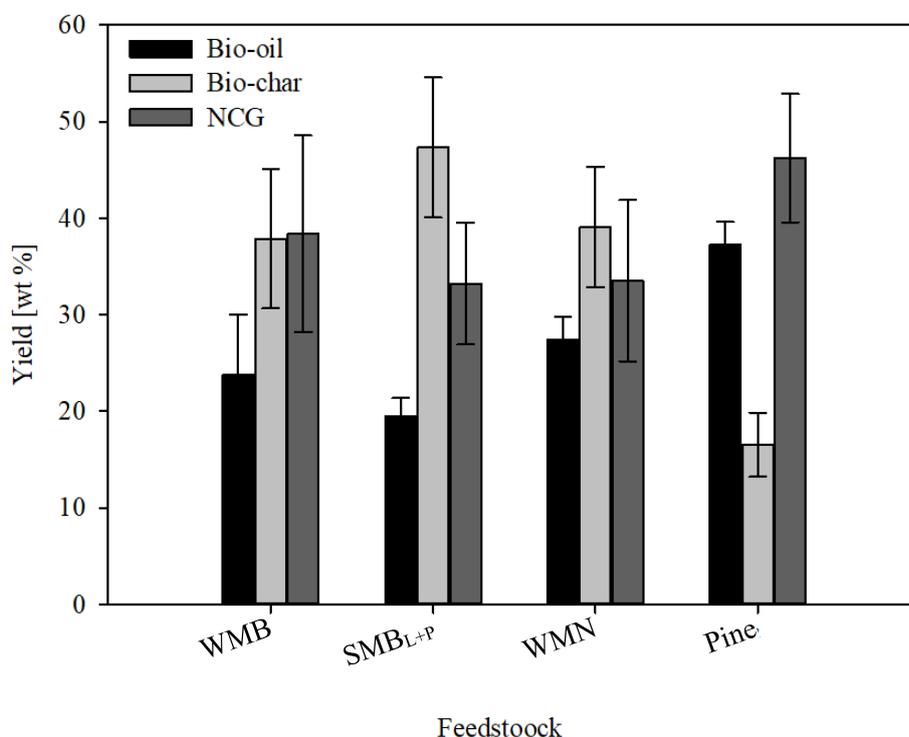
### 2.3.3. Bio-oil production

Preliminarily, thermo-gravimetric analyses of microalgal biomass were carried out to study the thermal behavior of biomass and its different components (Fig. 2.3). In Fig. 2.3A it is possible to see a similar behavior of the three types of microalgal biomass studied. However, it can be observed that in the case of SMB<sub>L+P</sub> there is still about 50% remaining biomass at the end of the analysis, suggesting a higher ash content in the pre-extracted biomass. Differential thermo-gravimetric analysis (DTG) curves show that the higher zone of decomposition was at about 250 °C – 450 °C (Fig. 2.3B), where protein and carbohydrates decomposition could happen. In addition, another zone with a moderate peak (about 400 °C to 500 °C) denoted lipids decomposition. Additionally, it has been reported that volatiles at around 350 °C could be related to decomposed hemicelluloses which could form part of the cell wall in the microalgal biomass investigated (Kebelmann et al., 2013). According to the previous, pyrolysis assays were carried out at 500 °C.



**Figure 2.3.** TGA/DTG of microalgal biomass

The product yields obtained by fast pyrolysis are shown in Fig. 2.4. The total bio-oil yields were WMB 38.3%,  $SMB_{L+P}$  33.2%, WMN 39.7% and Pine 39.9%. These yields are lower compared to previously reported data (Pan et al., 2010 , Wang et al., 2013 , Miao et al., 2004), which could be related to the necessity to optimize the operational parameters and the performance of the pyrolysis reactor. However, there is a very interesting result obtained using the WM biomass, where a bio-oil production yield similar to that obtained using pine was obtained.  $SMB_{L+P}$  bio-oil production yield was very low. This can be related to the previous lipid extraction process along with the loss of fatty acids during protein extraction, and the loss of carbohydrates during both extraction processes. Considering the low efficiency of FAME production from the extracted lipids, it seems interesting to extract only proteins previous to the pyrolysis process. In addition, the optimization of protein extraction avoiding fatty acids loss should be studied.



**Figure 2.4.** Comparison of fast pyrolysis products yield for WMB. SMB. WMN and pine sawdust.

#### 2.3.4. Analysis of bio-oil

Different amount of bio-oil was obtained in the two bio-oil fractions analyzed. The largest quantity of products was obtained in the first fraction: WMB (63.9 wt.%), SMB<sub>L+P</sub> (67.5 wt.%), WMN (63.4 wt.%) and Pine (85.1 wt.%). A lower number of compounds is observed in the first fraction when using microalgae biomass compared to lignocellulosic biomass. These results are in accordance with Wang *et al.* (Wang et al., 2013), as a large portion of bio-oil from microalgae is not condensable in a first fraction due to aerosols presence, which may be recovered in a posterior recovery step.

According to Table 2.3, a higher number of compounds in bio-oil produced from different microalgae samples were identified in fraction 2 compared to fraction 1. In addition, fewer and different compounds were identified in bio-oil from microalgae compared to bio-oil from pine biomass. This could be related to the methodologies used which have been developed to identify specific compounds present in bio-oil from lignocellulosic biomass. Additionally, no fewer protein-derived compounds are present in bio-oil from SMB<sub>L+P</sub>, as previously expected. However, no lipids-derived compounds were detected in this bio-oil. Therefore, to improve protein extraction to diminish protein-derived compounds presence in bio-oil should be a critical target of further research. In addition, lipid extraction from biomass could be avoided to increase lipid derived compounds in bio-oil to additionally increase its HV.

Clearly, differences in feedstock composition produce differences in bio-oil products from microalgal biomass compared to lignocellulosic biomass (Table 2.3). As expected, bio-oil from lignocellulosic biomass was characterized by a high cellulose, hemicelluloses and lignin derived compounds content. On the other hand, bio-oil from microalgal biomass was characterized for high protein-derived compounds content and some carbohydrate and lipid derived compounds, in accordance with related studies (Belotti et al., 2014 , Wang et al., 2013).

The results show that acetic acid, hydroxyacetaldehyde and acetol, typical products from pyrolysis of lignocellulosic biomass were not present in bio-oil from microalgae. This is in agreement with the results reported by Wang *et al.* (2013), where only 1.3% of acetic acid was found on bio-oil produced from fats pyrolysis of WM *C. vulgaris*. Due to the absence of acidic compounds in bio-oil from microalgae, the pH of bio-oils was measured (Fig. 2.5).

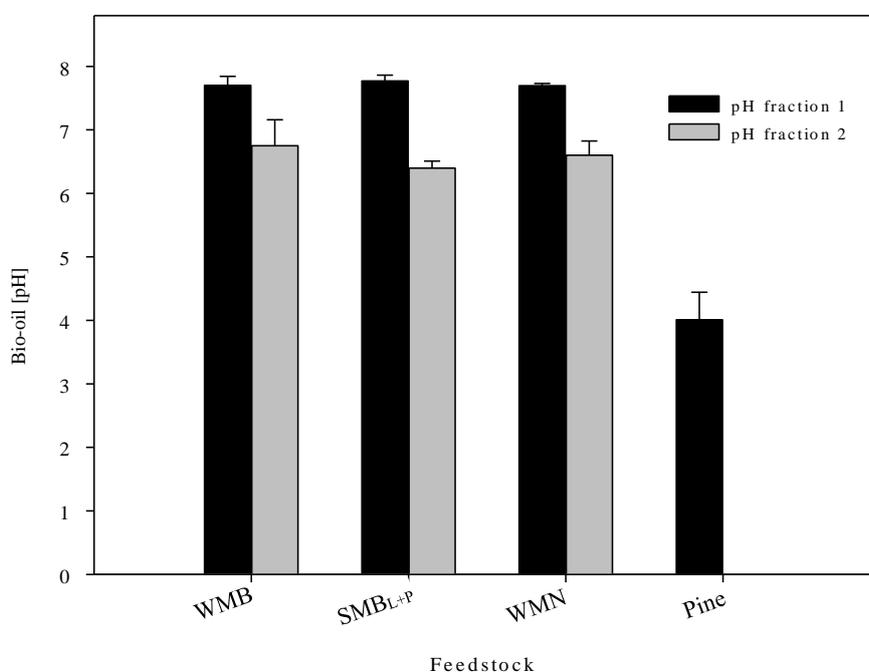
The results showed values near to neutral pH for bio-oil from microalgae. Contrarily, bio-oil from pine was characterized by a pH of 4, which is in agreement with related references (Mahfud *et al.*, 2008). The low pH is a critical characteristic of bio-oil produced from lignocellulosic feedstock as this acidity can produce extensive corrosion of the metal surface of internal combustion engines. Indeed, Mahfud *et al.* (2008) established that crude bio-oil from lignocellulosic feedstock is not suitable as a fuel for engines combustion and upgrading is required. Therefore, the neutral pH obtained in bio-oil from microalgal biomass is an interesting result. It has been already reported that nitrogen content in microalgae could favor an alkaline pH of the produced bio-oil by the presence of indole, pyridine, amides and ammonia, among other compounds (Du *et al.*, 2011).

Patwardhan *et al.* (2010) reported that the presence of organic salts and ash could act as catalysts in the reaction favoring the production of some compounds such as acetol and formic acid, among others. As previously commented, in the present study high levels of ash were found in microalgal biomass, which could produce interference in the pyrolysis reaction and therefore, in bio-oil composition (Yildiz *et al.*, 2015).

**Table 2.3.** Bio-oil composition

Compounds	Formula	Feedstock / Area [%]			
		WMB	SMB <sub>L+P</sub>	WMN	Pine
<b>Protein derived compounds</b>					
2-Pyrrolidone	C <sub>4</sub> H <sub>7</sub> NO			1.1	
2.2.6.6-Tetramethyl-4-piperidone	C <sub>9</sub> H <sub>17</sub> NO	1.9	8.0	11.0	
Phenol	C <sub>6</sub> H <sub>5</sub> OH	1.8	2.7	1.0	
4-Methyl-phenol (p-Cresol)	C <sub>7</sub> H <sub>8</sub> O	3.3	5.4	2.0	
Indole	C <sub>9</sub> H <sub>9</sub> N	4.6	6.3	2.3	
3-Methyl-1H-indole	C <sub>9</sub> H <sub>9</sub> N		5.9	1.2	
<b>Lipids-derived compounds</b>					
Tridecanoic Acid	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>	8.8			
Pentadecane	C <sub>15</sub> H <sub>32</sub>			0.7	
3-Tetradecene	C <sub>14</sub> H <sub>28</sub>			1.3	
<b>Carbohydrate/Cellulose/Hemicellulose-derived compounds</b>					
4-Hydroxy-4-methyl-2-pentanone	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	2.2	13.7	19.0	2.7
4-methyl-3-penten-2-one	C <sub>6</sub> H <sub>10</sub> O		2.0	19.2	
Di-anhydro-mannitol	C <sub>6</sub> H <sub>10</sub> O <sub>4</sub>	3.4		5.2	
Hydroxyacetaldehyde	C <sub>4</sub> H <sub>8</sub> O <sub>4</sub>				13.7
Aceticacid	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>				18.3
Acetol	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>				21.5
Methylacetate	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>				5.8

Propanal	C <sub>3</sub> H <sub>6</sub> O				9.2
2(5 <i>H</i> )-Furanone	C <sub>4</sub> H <sub>4</sub> O <sub>2</sub>				2.8
Levoglucosan	C <sub>6</sub> H <sub>10</sub> O <sub>5</sub>				5.5
<b>Lignin-derived compounds</b>					
2-methoxyphenol	C <sub>7</sub> H <sub>8</sub> O <sub>2</sub>				5.9
Creosol	C <sub>8</sub> H <sub>10</sub> O <sub>2</sub>				7.1
2-methoxy-4-(1-propenyl)-phenol (Isoeugenol)	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>				1.8

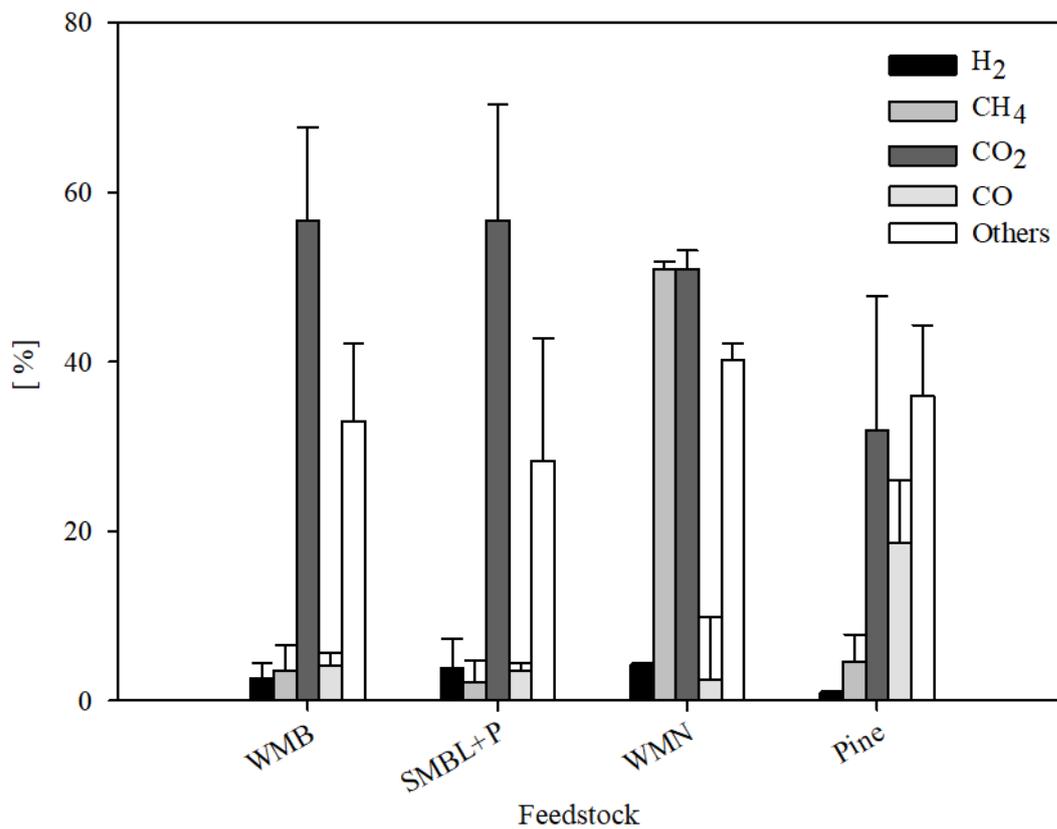


**Figure 2.5.** Comparison of pH in bio-oil produced by pyrolysis at 500 °C from WMB, SMB<sub>L+P</sub>, WMN and pine sawdust.

### 2.3.5. Analysis of NCG

NCG produced by fast pyrolysis is often overlooked and considered as a by-product of process. Normally, NCG components produced have light phase composition such as H<sub>2</sub>, CO, CO<sub>2</sub>, CH<sub>4</sub>, light hydrocarbons (ethane and propane) (Domínguez et al., 2007; Wang et al., 2009).

Quantitative results of four main components are shown in Fig. 2.6. Dominant gaseous species of the NCG produced for all microalgae raw materials was CO<sub>2</sub> varying from 55% to 68% and other light hydrocarbons such as CO, CH<sub>4</sub> and H<sub>2</sub>.



**Figure 2.6:** NCG composition

## **2.4. Conclusions**

Pyrolysis of microalgal biomass was investigated to analyze the characteristics of produced bio-oil and compare it with conventional lignocellulosic bio-oil. The results show that lower yields of bio-oil from microalgal biomass were reached compared with lignocellulosic biomass. Therefore, the process should be optimized for this new raw material. In relation to bio-oil characteristics, interesting results of neutral pH was found in bio-oil from microalgal biomass. Additionally, the biorefinery concept was applied in this research concluding that is necessary to optimize protein extraction process to avoid nitrogen derived compounds presence in bio-oil. Finally, it is proposed that lipid extraction should be avoided previous to pyrolysis because to very low yield of FAME reached.

## **2.5. Acknowledgments.**

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## **CHAPTER III**

**“Study of operational conditions affecting pyrolysis  
conversion process of *Botryococcus braunii* spent-  
biomass”**

### 3.1 Introduction

When microalgae biomass is used in FAME production, only about 2 to 20% of the WM biomass is used and about 70% of the biomass is generated as  $SM_L$ . Therefore, the research has been focused on finding a novel use for the WM, following a biorefinery concept (Vanthoor-Koopmans et al., 2013). In this sense, the thermal conversion of microalgal biomass by using pyrolysis has been proposed as an interesting alternative. The advantage of pyrolysis is that the WM can be converted in three products: bio-oil, NCG and bio-char.

Some research has been recently reported on microalgae pyrolysis processes, following a biorefinery concept. Wang *et al.* (2013) studied the pyrolysis reaction of lipid-spent microalgae *Chlorella vulgaris*. The authors reported bio-oil, bio-char and NCG yields of 53, 31 and 10 % respectively, using a fluidized bed reactor at 500 °C. However, pyrolysis from microalgal biomass has a very important disadvantage about using microalgal biomass as feedstock, because microalgae are characterized by high protein (nitrogen) content, which could be finally present in the produced bio-oil. This high nitrogen content in bio-oil could generate  $NO_x$  when the bio-oil is burned (Ren et al., 2010).

A biorefinery concept has been applied performing the extraction of soluble proteins, afterwards, lipid extraction for the FAME production and, finally, the pyrolysis of waste microalgal biomass ( $SMB_{L+P}$ ). This allowed the conclusion that lipid extraction of microalgal biomass should be avoided previous to pyrolysis because of the very low yield reached of FAME (approximately 1% of initial complete microalgal biomass) (Muñoz et al., 2015). These lipids can be exploited for the production of bio-oil with a higher HV. The extraction of soluble proteins of microalgal biomass allows the production of a HV product and the reduction of nitrogen concentration in the feedstock used for the production of bio-oil in comparison with WM.

The pyrolysis of lignocellulosic biomass has been widely investigated for obtaining bio-oil for fuels or fine chemicals. On the other hand, research of microalgal biomass pyrolysis is of recent interest and there is no information that establishes the conditions or factors that significantly affect the pyrolytic process or its effect on the characteristics of bio-oil, bio-char and NCG derived of microalgal biomass (Ali-Ahmad et al., 2020).

According to the previous statements, the aim of this work was to investigate the effects of temperature and residence time in the pyrolysis of SM and analyze the characteristics of bio-oil, bio-char and NCG produced. In addition, the biorefinery concept was applied in this study using biomass after protein extraction.

## **3.2 Methodology**

### **3.2.1. Materials**

Freshwater microalgae WMB were used as feedstock. Production of SMB<sub>P</sub> for pyrolysis was performed by protein extraction from WMB. Protein extraction from WMB was carried out adapting the methodology of Kale *et al.* (2011), according to the already described methodology in section 2.1.

### **3.2.2. Pyrolysis experiments**

By means of sequential optimization the conditions of SMB<sub>P</sub> determined greater conversion of biomass to bio-oil and established if there was a significant effect of the temperature and residence time in the conversion of SM to pyrolytic products. This statistical method sets the variation of a variable at a time, making it possible to identify a critical point of the factor in the process. Subsequently, once the critical point of the first variable was defined (temperature reaction), it was selected and maintained constant; then, the initial identification of the critical point of the second variable (residence time). This methodology allows define the critical point of each factor at the discretion of the investigator, but mainly allows identify the effect of the factors on the reaction as the space research for the development of an array of robust optimization.

All the SMB<sub>P</sub> was also milled and sifted to a particle size of 450-1,000 μm. Additionally, TGA experiments were performed previously to pyrolysis to study the thermal behavior of the microalgal biomass investigated.

Pyrolysis was performed with a lab-scale fed-batch reactor, which was operated under fast pyrolysis conditions according to the previously described methodology in section 2.3.

### **3.2.3. Characterization of feedstock and products**

Composition of pyrolysis feedstock previously dehydrated and milled was determined. Moisture analysis was performed gravimetrically drying the biomass at 103°C until constant weight. Similarly, ashes were quantified at 550° +/- 25 °C for 8 hours.

Lipids were quantified by Bligh/Dyer extraction of total lipids (Li et al., 2014), and protein by Kjeldahl methodology. Carbohydrate content was expressed as 100% - (moisture content + lipids + protein + ash).

### **3.2.4. Characterization of bio-oil**

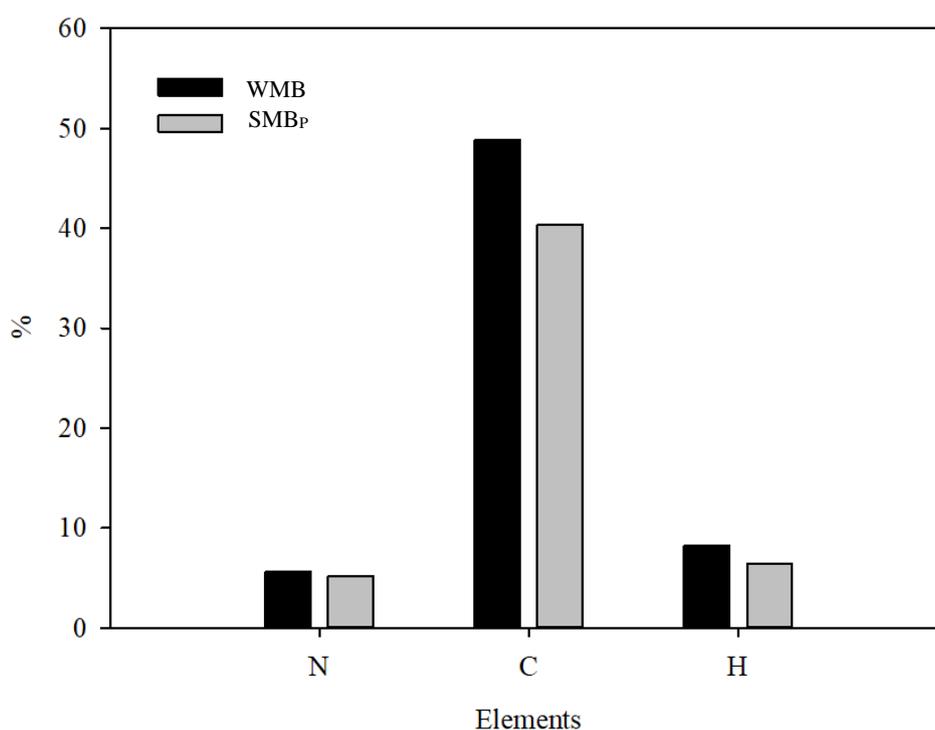
Compounds in bio-oil detectable by GC were analyzed on a gas chromatograph Clarus 600 GC/MS. A VF-1701 column 60 m long, 0.25 µm thick and internal diameter of 0.25 mm was used. Water content was measured in bio-oil with Karl Fisher, using CombiTitrant 5 aPura (Merck), titulant solution. C, H and N content in WMB, SMB<sub>P</sub>, bio-char and in bio-oil were measured using a euroEA 3000 CHNS Elemental Analyzer. Carbon Total and Organic Carbon in bio-char was measured with TOC.

### 3.3. Results and Discussion

#### 3.3.1. Production of SMB<sub>P</sub> to be pyrolyzed following a biorefinery concept

##### 3.3.1.1. Proteins extraction

Heaven *et al.* (2011), provides the empirical formula  $C_{6.0}H_{13.1}O_1N_{0.6}$  for proteins of a microalgae. Therefore, the protein extraction causes a major decrease of C and H and in less proportion N. Fig.3.1 shows a proportional reduction of C, H and N appropriate for the protein composition of the microalga. Therefore, the removal of protein helps to reduce the N total content of biomass, but it mainly allows to set a process production of proteins with a high commercial potential and without significantly altering the microalgal biomass as feedstock for the production of bio-oil (Becker, 2007 , Yen et al., 2013).(Heaven et al., 2011)



**Figure 3.1:** Elemental analysis of WMB and SMB<sub>P</sub>.

#### 3.3.2. Feedstock characterization

Composition and physical characteristics of both SMB<sub>P</sub> biomass are given in Table 3.1. The SMB<sub>P</sub> biomass is mainly composed by lipids, proteins and carbohydrates. Microalgal biomass

was characterized by a high protein content of 32%. This means that the methods used for protein extraction were not very effective in removing high nitrogen contents in biomass. It is possible that the observed high content of N does not correspond to proteins, due to an overexpression of Kjeldahl method in the determination of the protein content of the microalgal biomass. Under this hypothesis, the largest N content in the biomass is due to the micro-nutrients fixed from the cultivation of microalgae. Therefore, it is proposed for future research, make a wash or other method that makes it possible to reduce the content of inorganic N in the biomass and occupy other methods for measuring the protein content, such as modified Lowry by Herbert. In the Chapter II, the content of lipids of the  $SMB_{L+P}$  was low, in comparison to the  $SMB_P$  of the present Chapter (Table 3.1); this allows having a pyrolyzable fraction with a high energy content, which can be concentrated on the bio-oil.

**Table 3.1:** Composition of pyrolysis feedstock

	Parameters	$SMB_{L+P}$ (%)	$SMB_P$ (%)
Pyrolyzable fraction	Lipid	4.7	13.46
	Protein	20.4	32.14
	Carbohydrate	7.1	22.49
	Moisture	10.3	4.80
	Ash	36.0	27.11

In relation to moisture contents in each feedstock, these are in a suitable range for pyrolysis. Therefore, the drying tunnel used for drying the microalgal biomass could be a suitable system to be implemented before pyrolysis processes. This is a very critical parameter for the pyrolysis process with high ash content favorable for bio-char production diminishes bio-oil yield. Therefore, nutrient dosage during microalgae growth phase must be studied in future research.

### **3.3.3. Effect of operational conditions on pyrolysis processes of SMB<sub>P</sub>.**

The literature mentions the reaction temperature, residence time of the vapor and particle size as the main factors that affect operation of a fast pyrolysis process and mainly in the production of bio-oil (Zhou et al., 2013 , Liu et al., 2010 , Shen et al., 2009 , Ozlem, 2007). Due to the characteristics of fast pyrolysis, it can be operated only with particles sized between 450  $\mu\text{m}$  and 1,000  $\mu\text{m}$ . To define whether there is an effect of the particle size within the range of operation of the equipment, an experiment was carried out before fast pyrolysis at 500 °C, 1.5 s of residence time for two fractions of SMB<sub>P</sub>, the former with a distribution of size between 450  $\mu\text{m}$  to 500  $\mu\text{m}$ , the latter between 900  $\mu\text{m}$  and 1,000  $\mu\text{m}$ . As a result, it was found that there was no significant difference ( $\alpha = 0.05$ ) between the proportions of bio-oil, bio-char and NCG obtained for each particle size studied (Table 3.2). Therefore, there is considered to be despicable the effect of the factor 'size of particle' for this investigation.

**Table 3.2:** Comparison of mean of productivity bio-oil, bio-char and NCG by Student Test for particle size range between 450  $\mu\text{m}$  – 500  $\mu\text{m}$  and 900  $\mu\text{m}$  - 1,000  $\mu\text{m}$ .

Response	Mean (450 to 500 $\mu\text{m}$ )	Mean (900 to 1,000 $\mu\text{m}$ )	t-value	df	p
Bio-oil	22.82	23.28	-0.246356	2	0.828385
Bio-char	24.61	21.56	0.800660	2	0.507326
NCG	52.56	55.17	-0.606216	2	0.606012

### 3.3.4. Effect of the temperature on pyrolysis products yield

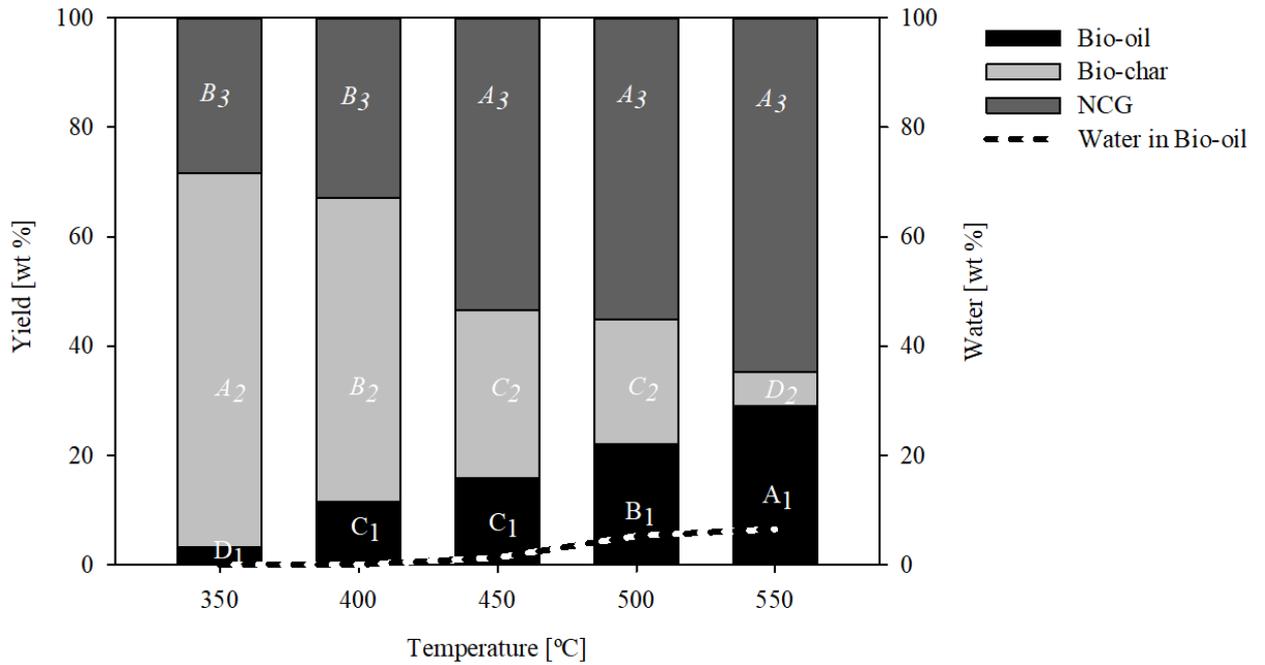
For the study of temperature effect on the pyrolysis of SMB<sub>P</sub>, a residence time of 1.5 (s) and a particle size between 450 and 1,000  $\mu\text{m}$  were established. Through the analysis of variance of the data with  $\alpha = 0.05$ , it was established that there is a significant effect of the temperature in the production of bio-oil, bio-char and NCG. Subsequently, to validate the normality of the errors, a test of homogeneity of variances was conducted ( $\alpha = 0.05$ ), stating that the differences in variances are equal, so we can establish, the significant differences between the products obtained at different temperatures by means of the Tukey test.

The product yields obtained by means of a screening of the reaction temperature of fast pyrolysis can be seen in Fig. 3.2. The total bio-oil yields were 3.5 %, 11.5 %, 15.8 %, 22.0 % and 29.0% for 350, 400, 450, 500 y 550  $^{\circ}\text{C}$ , respectively. The increase of the reaction temperature, raises the production of bio-oil, water and NCG. The generation of water will reduce the HV of bio-oil and affect its storage stability by re-polymerization (Venderbosch et al., 2010), although it improves the bio-oil flow characteristics such as viscosity (Czernik and Bridgwater, 2004).

The NCG production diminishes the bio-oil production; therefore, diminishing the NCG production can promote a major conversion of the biomass to bio-oil. In Chapter II, a differential thermo-gravimetric analysis (DTG) was performed (Fig. 3.3), curves show that the

highest zone of decomposition was at the range between 250 °C-450 °C, where proteins and carbohydrates decompose. Additionally, it has been reported that volatiles at around 350°C could be related to decomposed hemicelluloses which could form part of the cell wall in the microalgal biomass investigated (Kebelmann et al., 2013). Therefore, the generated liquid fraction during pyrolysis at temperatures  $\leq 400$  °C is derived from carbohydrates, proteins and water from the raw material. On the other hand, the high production of NCG and low bio-oil (Fig 3.2), is the product of the high residence time established for reaction, generating secondary decomposition during biomass devolatilization (Ozlem, 2007 , Zhou et al., 2013).

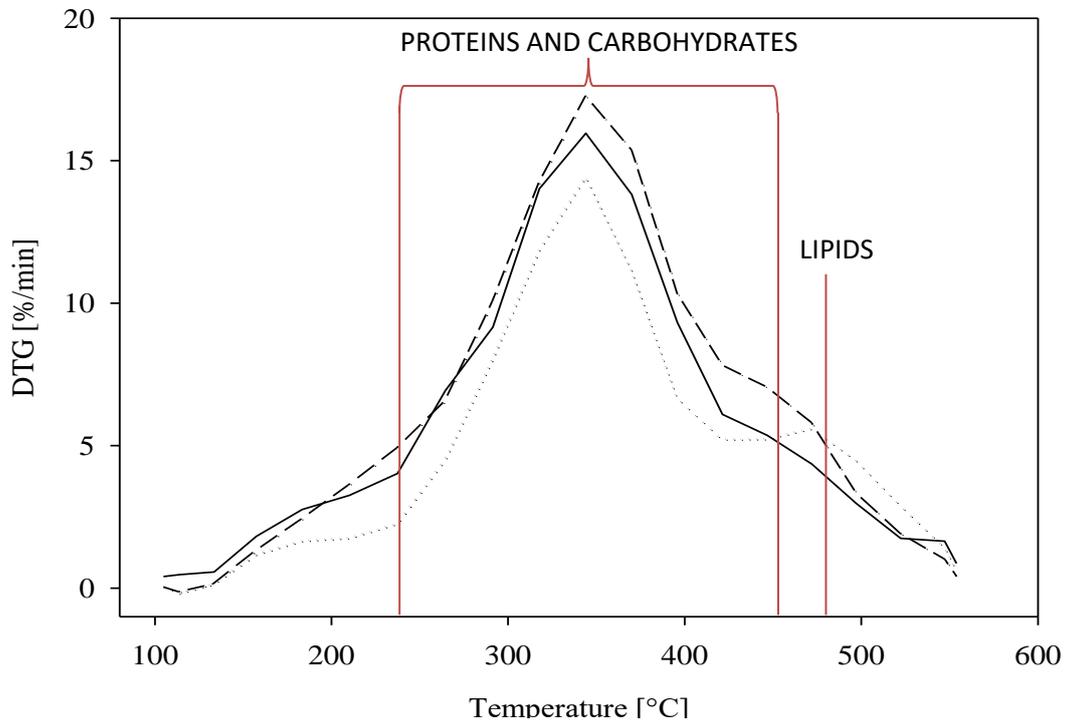
Thus, the reaction temperature defines the percentage of the biomass to be devolatilized for the formation of bio-oil and NCG. The increase in temperature causes the cracking of larger molecules, which subjected at a high residence time in the reactor, are cracked successively, raising production of NCG and water and reducing the production of bio-oil (Fig.3.2) (Demirbas, 2006 , Zhou et al., 2013). On the other hand, lower temperature causes less devolatilization of biomass, therefore, lower bio-oil production and higher biochar production.



**Figure 3.2:** Production of bio-char, bio-oil, NCG and water by pyrolysis of SMB<sub>P</sub> at different temperatures. Conditions of operation: Residence time 1.5 s, 450 μm - 1,000 μm particle size range and feeding rate of 12 mg/s. The letters on the bars symbolize average difference of Tukey test.

To define the temperature of constant work and determine the effect of time of residence, it was considered that at a temperature greater than or equal to 500 °C a quantity of water is generated (from 8% to 450 °C to 23% to 550 °C) and NCG is significantly higher. Hence, adversely affecting the quality of the bio-oil and limiting its production. Additionally, in Fig. 3.3 there is a zone with a moderate peak (about 400 to 480 °C) denoting lipid decomposition, agreeing with report by Gai *et al.*, (2013). In relation to lipid decomposition, it has been reported that it occurs from 250 to 500°C with main peaks of weight loss at 400 – 410 °C in studies carried out with *Chlamydomonas reinhardtii* and from *Chlorella vulgaris* (Kebelmann *et al* 2013). Lipids are an important source of energy of microalgal biomass. Therefore, it is a significant fraction to be pyrolyzed to obtain a bio-oil with high energy density. According to the previously mentioned, pyrolysis assays were carried out at 480 °C, assuring the decomposition of major

lipid constituents of the SMB<sub>P</sub> and diminishing the production of NCG and water (Gai et al., 2013 , Kebelmann et al., 2013).



**Figure 3.3.** DTG of microalgal biomass.

### 3.3.5. Effect of residence time on the reaction of pyrolysis of SMB<sub>P</sub>.

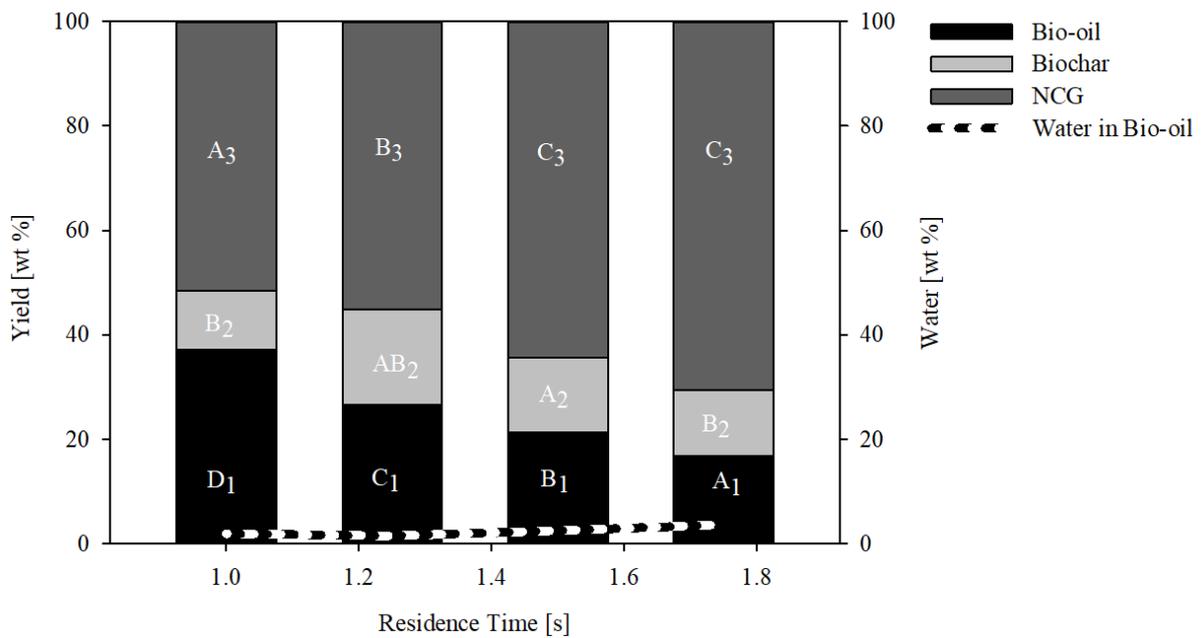
The study of residence time effect on the pyrolysis of SMB<sub>P</sub> was performed through the analysis of variance of data with an  $\alpha = 0.05$ . It was established that there is a significant effect of residence time in the production of bio-oil, bio-char and NCG. Subsequently, to validate the normality of the errors a test of homogeneity of variances was conducted ( $\alpha = 0.05$ ), stating that the differences in variances are equal, so we can establish the significant differences among the products obtained at different residence times by means of the Tukey test (Fig. 3.4).

The product yields obtained by means of a screening of residence times of fast pyrolysis can be seen in Fig.3.4. There is a significant difference in the production of bio-oil and NCG among each of the times studied, even when the difference between the residence times are 0.25 (s) approximately.

The variation of the residence time does not affect the pyrolyzable fraction, but it directly affects the production ratio of bio-oil and NCG (Amutio et al., 2012 , Demirbas, 2006 , Ozlem, 2007). With a lower residence time, higher conversion yields of bio-oil and lower of NCG were reached. On the other hand, higher residence time favors a higher production of NCG, but a lower production of bio-oil (Fig.3.4). Higher residence times prolong the primary degradation products to further exposure of secondary cracking reactions, thus converting the biomass into NCG and not into bio-oil (Zhou et al., 2013).

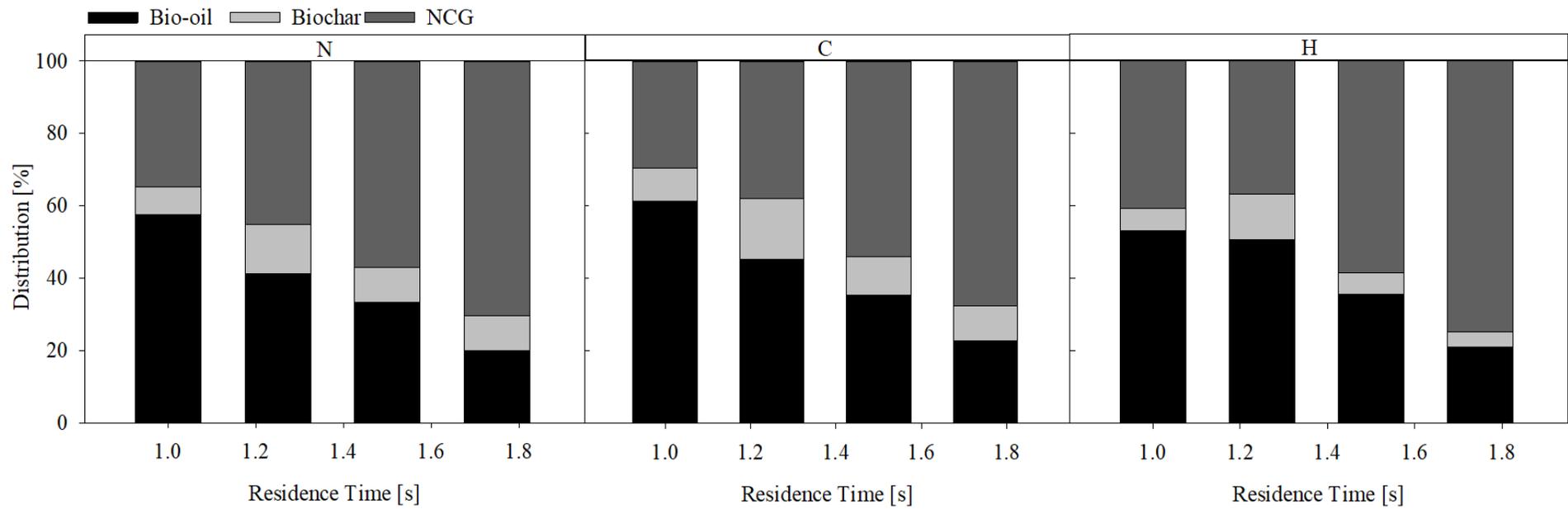
The production of bio-char does not have any significant differences with the variation of the residence time in the range of study, and is directly related to the operating temperature, which determines the fraction of the biomass to be pyrolyzed.

Fig.3.4 confirms that the decrease in residence time diminishes molecular cracking in the reactor, hence, decreasing the production of NCG and increasing production of bio-oil. In the same way, Fig. 3.5 shows the concentrations of N, C, and H in the bio-oil.



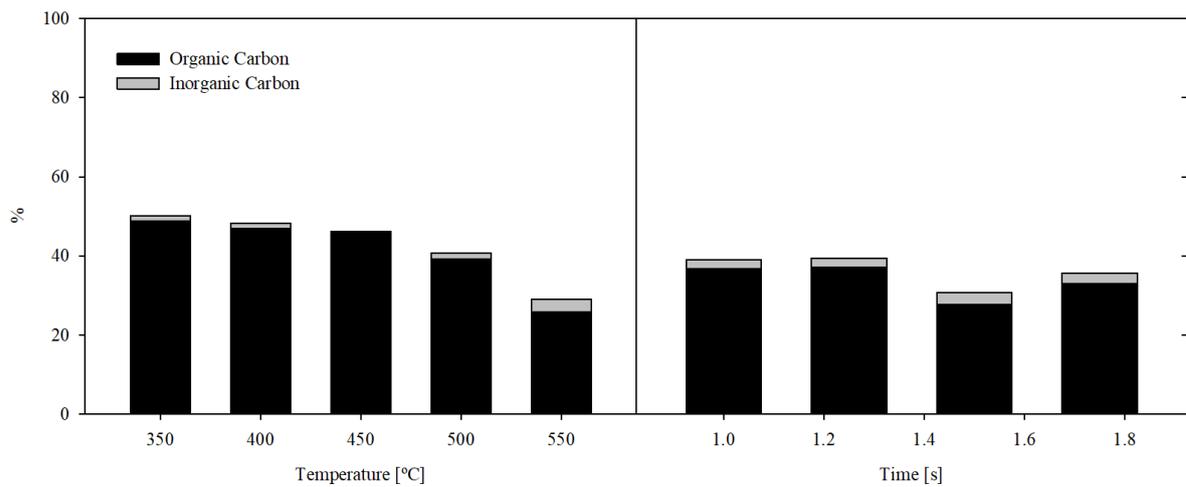
**Figure 3.4:** Production of bio-char, bio-oil, NCG and water at different residence times to pyrolysis of pyrolyzable proportion of SMB<sub>P</sub>. Conditions of operation: time residence 480 °C, 450-1,000 μm particle and size feeding rate of 12 mg/s. The letters on bars symbolize average difference of Tukey test.

On the other hand, the decline of the residence time makes N concentrate in the bio-oil. According to Tchapda and Pisupati, (2014), during the combustion of the bio-oil, the contained nitrogen is converted into nitric oxides, gaseous nitrogen y nitrous oxide. During pyrolysis of biomass with a high content of N and O enhances the direct release of NO and isocyanic acid. Therefore, the nitrogen during the pyrolysis process can generate NO, as well as during the combustion of their pyrolytic products (Ren et al., 2010). This scenario restricts the use of bio-oil of microalgae directly as fuel, so the reduction of the N content must be achieved previously. An alternative during the process of pyrolysis, is to vary the conditions of operation in order to decrease the N content and concentrate the H in the bio-oil, as shown in Fig 3.5. The variation of residence time favors the concentration of H, without increasing of the N content in the bio-oil, between 1 s to 1.25 s of residence time. The H concentration of the bio-oil sets it as a possible fuel with a high HV.



**Figure 3.5:** Balance of C, H and N in bio-oil, bio-char and NCG.

Finally, in a comparative analysis of content of organic and inorganic carbon of bio-char, produced at different temperatures and times of residences, it is noted that the content of organic and inorganic carbon is constant during the different residence times (Fig. 3.6). On the other hand, temperature affects the content of organic and inorganic carbon in bio-char, temperature is the main factor of decomposition of biomass, therefore, it defines the quality of bio-char, and the percentage of the biomass to be bio-oil and NCG (Garcia-Perez et al., 2008).



**Figure 3.6:** Organic and inorganic carbon content in biochar from SMB<sub>P</sub> pyrolysis (free ash basis)

### 3.3.6. Analysis of bio-oil

Table 3.3 shows the composition of bio-oil products from SMB<sub>P</sub> biomass. On the other hand, the bio-oil from microalgal biomass is characterized for high protein derived compound content, agreeing with Keblmann *et al.* (2013). In this sense, it can be seen that the removal of proteins does not ensure a significant reduction of nitrogenous compounds of the biomass. Therefore, it is recommended to perform an investigation which makes it possible to reduce the N content of the biomass in an effective manner. However, lipid derived compounds were detected in this bio-oil. In addition, lipid extraction from biomass should be avoided to increase

lipid derived compounds in bio-oil and, additionally, increase its HV (Šmidrkal et al., 2009 , Belotti et al., 2014 , Li et al., 2014).

The high ash content of microalgal biomass can significantly affect pyrolysis process, acting as a catalyst. The presence of metallic oxides can favor the formation of tars, reduce the production of char, promoting the formation of water, among others (Dickerson and Soria, 2013). Therefore, the reproducibility of the study is limited by the ash content present in each harvest of the crops of microalgae.

The pH obtained during the bio-oil research was measured by a pH meter, concluding that the pH of the bio-oil had an average of  $7.3 \pm 0.3$ . Therefore, the neutral pH obtained in bio-oil from microalgal biomass is a very interesting result. It has been already reported that the nitrogen content in microalgal culture could favor an alkaline pH of the produced bio-oil by the presence of pyridine, amides, and ammonia, among other compounds (Du *et al* 2011).

**Table 3.3.** Bio-oil composition

Compound Name	Temperature (°C)				Time residence (s)			
	400	450	500	550	1.75	1.5	1.25	1
	EP	C	EP	C	EP	C	EP	C
<b>Protein-derived compounds</b>								
Pyrrole								
4-amino-4-methyl-2-Pentanone				X		X	X	
Acetamide		X		X	X	X	X	X
1,2,2,3,3-Pentamethyl-aziridine			X	X		X	X	X
Phenol	X	X	X	X	X	X	X	X
2,2,6,6-tetramethyl-4-Piperidinone					X	X		X
2-methyl-Phenol				X	X			X
2-Pyrrolidinone		X	X	X	X	X	X	X
4-methyl-Phenol		X	X	X	X	X	X	X
Hexadecane				X		X		
3-methyl-Phenol						X		
Benzyl nitrile			X		X			
3-Methylbutyramide								X
Pentanamide		X	X	X		X	X	

4-methyl-Pentanamide				X	X							X
4-ethyl-Phenol												
Benzenepropanenitrile	X		X	X	X		X	X	X	X	X	X
5H-1-Pyridine												X
Indole	X		X	X	X	X	X	X	X	X	X	X
Pyrrole-2-carboxamide				X								
4-Methyl-1H-indole				X								
7-Methyl-1H-indole							X					
Phenylpropanamide				X								
(2E)-3,7,11,15-Tetramethyl-2-hexadecene	X	X	X	X	X	X	X		X	X		X
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	X	X	X	X	X	X	X	X	X	X	X	X
Pentadecanenitrile				X		X	X					
Hexadecanenitrile					X			X	X	X		X
hexahydro-Pyrrolo[1,2-a]pyrazine-1,4-dione							X		X			
Phytol	X	X	X	X	X	X	X	X	X	X	X	X
hexahydro-3-(2-methylpropyl)-Pyrrolo[1,2-a]pyrazine-1,4-dione				X	X	X		X				

2,5-Pyrrolidinedione	X		X	X	X		X	X	X	X
Hexadecanamide	X	X	X			X	X	X	X	X
(Z)-9-Octadecenamide	X	X	X						X	X
Oleamide	X	X	X	X		X	X	X	X	X
3-Methylbutyramide			X	X	X		X	X	X	X
Propanamide					X	X	X	X	X	X
2-Methyl-1-undecanol						X				
N,N-bis(1-methylethyl)-2-Propen-1-amine		X				X	X	X	X	X
<b>Lipids-derived compounds</b>										
Glycerin	X	X	X	X	X		X	X	X	X
Cholesterol			X	X	X		X		X	X
Pentadecane						X				
Hexadecane							X			
2-Heptadecanone							X			
<b>Carbohydrate/Hemicellulose-derived compounds</b>										
Acetic acid	X	X	X		X		X	X	X	X
Acetic formic anhydride				X	X		X		X	X
4-methyl-4-Penten-2-one	X		X							

4-methyl-3-Penten-2-one	X	X	X		X		X		X		X			
4-hydroxy-4-methyl-2-Pentanone	X	X	X	X	X		X	X	X	X	X		X	X
2-Furanmethanol					X		X							

C: Condenser

### **3.4. Conclusions**

The effect of temperature and residence time of a pyrolysis process of microalgal biomass was investigated to analyze the yield and characteristics of the produced bio-oil, bio-char and NCG. The results showed that lower yields of bio-oil from microalgal biomass were obtained at low temperatures, but an increase in the production of bio-oil is produced as temperature increases. This is limited by the residence time that establishes or adjusts the balance between the NCG and bio-oil. With a short residence time bio-oil production was increased, whereas a decreased NCG production was detected. Contrarily, a high residence time generates high production of NCG and less bio-oil production. In relation to bio-oil characteristics, interesting results of neutral pH were found in bio-oil from microalgal biomass. Additionally, the biorefinery concept was applied in this research, concluding that the extraction of proteins from *B. braunii*, allows to achieve a HV product and generate a SMB<sub>P</sub> available for bio-oil production. Further research to optimize the protein extraction process avoiding nitrogen derived compounds presence in bio-oil could be realized.

### **3.5. Acknowledgments**

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## **CHAPTER IV**

### **“Study of the effect of microalgal biomass deproteination on bio-oil and bio-char characteristics”**

**Chapter based in the paper submitted to Journal of Analytical and Applied Pyrolysis:**

Muñoz R, Azócar L, Hidalgo P, Navia R. (2020). Fast pyrolysis bio-oil characteristics of deproteinized microalgal biomass.

#### 4.1. Introduction

The microalgae, as photosynthetic organisms, can efficiently convert solar energy into biomass, estimating its macromolecular composition in 6–71% proteins, 5–60% carbohydrates, 7–75% lipids and 5-17% ashes (Onen Cinar et al., 2020 , El-Sheekh et al., 2020 , Tibbetts et al., 2015 , Chisti, 2007). Lipid fraction has been a crucial factor in considering the use of microalgal biomass for the production of biofuels, such as FAME. Though Muñoz *et al* 2015, reported that lipid extraction of microalgal biomass should be avoided previous to pyrolysis because of the very low yield reached of FAME (approximately 1 % of initial complete microalgal biomass). This strategy of conversion of algae into fuel has proven be economically and energetically unviable (Chandra et al., 2019). On the other hand, these lipids can be exploited for production of bio-oil with a higher calorific value.

The use of microalgae as a source of valuable products, such as proteins, is receiving increasing interest (Soto-Sierra et al., 2018 , Chandra et al., 2019). Protein extraction generates a residue, consisting a depleted microalga biomass, which can be revalued through fast pyrolysis, to produce three bio-oil, bio-char and NCG products. Though high protein content in microalgae generate to a high N content in the bio-oil, resulting in undesirable NO<sub>x</sub> emissions during combustion.

Extraction of soluble proteins of microalgal biomass allows the production of a high value product and reduction of nitrogen concentration in feedstock used for production of bio-oil in comparison with WM. The concept of algae biorefinery has emerged with a remarkable solution for recovering multiple products from a single operating process (Ubando et al., 2020 , Pr at et al., 2020).

Pyrolysis combines a cost-effective thermochemical conversion process to produce fuels and chemicals from biomass. On the one hand, bio-oil can be exploited for the production of higher

value products, including biofuels and chemicals. On the other hand, the two co-products, biochar and NCG, have potential applications in energy production (Wang et al., 2020 , Yu et al., 2018 , Ronsse, 2016 , Chernova et al., 2020).

According to the previous statements, the aim of this work was to study the effect of deproteination of the biomass on bio-oil characteristics.

## **4.2. Methods**

### **4.2.1 Materials**

Freshwater microalgae *Scenedesmus almeriensis* (WMS) were used as feedstock. The microalgae were produced in Las Palmerillas Experimental Station of Fundación Cajamar, Cajamar, (Almería - Spain) and donated by University of Almería.

Production of SMS<sub>P</sub> for pyrolysis was performed by protein extraction from WMS. For the protein extraction, first of all the microalgae, then it was lyophilized and after that it was ground using a ball mill. Then, an enzymatic hydrolysis into a 200 L reactor, with microalgae at 14% (w/w) in reactor, 4% (w/w) Alcalase 2.5 L, pH 8 was performed within a time of 2 hours. For keeping the pH at 8, NaOH was used. Then, the desired protein solubilization and a precipitate (SMS<sub>P</sub>) were obtained, by centrifugal process. The WMS and SMS<sub>P</sub> were freeze-dried and used as feedstock in the pyrolysis process.

### **4.2.2 Feedstock characterization**

Composition of previously dehydrated and milled pyrolysis feedstock was determined. Moisture analysis was performed gravimetrically drying the biomass at 103°C for 3 hours until constant weight. Similarly, ashes were quantified at 550° +/- 25°C for 8 hours.

Lipids were quantified by Bligh/Dyer extraction of total lipids (Li et al., 2014), and protein by OPA (Nielsen et al., 2001). Carbohydrates content was estimated from the mass balance according to Eq. (1):

$$\text{Carbohydrates} = 100\% - (\text{moisture content} + \text{lipids} + \text{protein} + \text{ash}) \quad (1)$$

#### 4.2.3 Pyrolysis experiments

A higher rate of thermal decomposition of microalgal biomass occurs between 400-410 °C, although lipids break between 250 to 500 °C (Muñoz et al., 2015), favoring the production of vapors that increase HV of bio-oil (Wang et al., 2017). On the other hand, one of products of biomass pyrolysis is water, derived mainly from secondary fractionation of molecules by temperature and residence time of vapors produced in the reactor (Demirbas and Balat, 2007). Therefore, pyrolysis experiments at 480°C have been established to achieve higher bio-oil productivity, with high lipid fractionation calorific power and reduced water production. Likewise, water performance decreases when particle size decreases (Shen et al., 2009). The residence time and particle size have been defined according to the minimum operating ranges of the pyrolyzer, leaving residence time of 1.0 s and particle size within the range of 450-1,000 µm.

The first pyrolysis was performed with a lab-scale fed-batch reactor, which was operated under fast pyrolysis conditions and atmospheric pressure, and heating was controlled. About 40 grams of biomass were continuously fed as according to the already described methodology in section 2.1.

The second fast pyrolysis experiments were carried out, using a micro-pyrolysis unit (FrontierLab Multi-shot pyrolyzer EGA/PY-3030D). Approximately 300 µg of untreated and treated biomass sample, with size particle smaller than 100 µm, was placed in a sample cup (composed of deactivated stainless steel) and covered with some quartz wool to prevent the

biomass from exiting the cup while falling through the reactor tube. The loaded cup fell freely into the preheated furnace by gravity during a period of 15-20 ms by which the sample was instantly heated at the desired evaluated temperatures (450, 500 and 550 °C). The micro-pyrolysis unit consists of a sampler, a quartz pyrolysis tube that can be furnace heated at the desired temperature, a heated interface and deactivated needle that is directly inserted into the GC injector. A Mettler Toledo microbalance with a sensitivity of 0.001 mg was used to weigh the sample cups before pyrolysis. Each sample was run in duplicate. The pyrolysis vapors were analyzed with a gas chromatography coupled with a mass spectrometric detection (Thermo Fisher Scientific Trace GC Ultra and Thermo ISQ MS) and integration was performed with Xcalibur data processing software. The GC/MS method was developed for the analysis of pyrolysis condensables from the thermal conversion of biomass via split injection (split ratio 1:100, injector temperature 300 °C) on a Restek capillary column (Rtx-1707, 60 m L x 0.25 mm I.D. x 0.25 µm df) with a stationary phase consisting of a crossbond 14% cyanopropylphenyl and 86% dimethyl polysiloxane and a constant helium carrier gas flow of 1ml/min. The GC oven temperature program started with a 3 min hold at 40 °C followed by heating to 280 °C at 5 °C min<sup>-1</sup>. The final temperature was held constant for 1 min.

#### **4.2.4 Characterization of bio-oil**

The content of C, H, N and S were measured using a euroEA 3000 CHNS Elemental Analyzer.

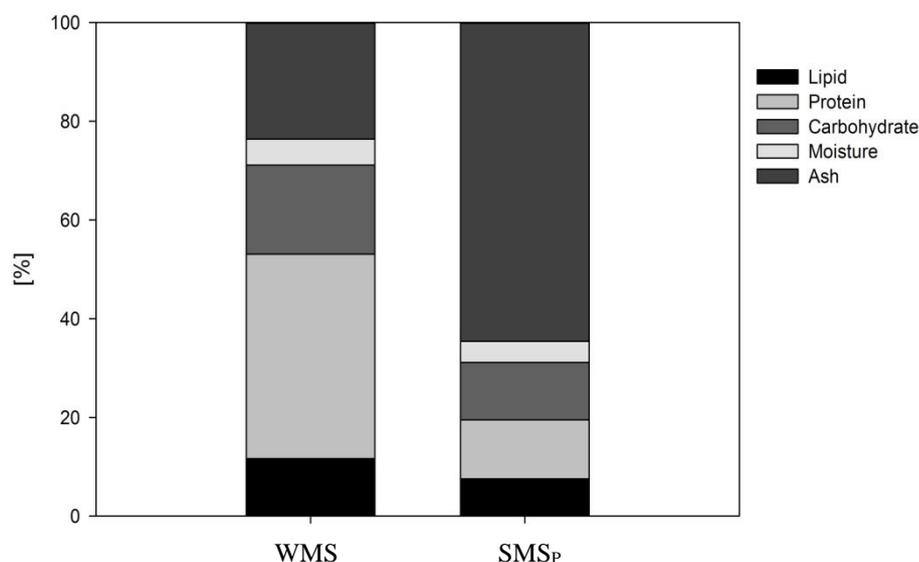
Oxygen content was calculated by difference according to Pan *et al.* (2010) (Eq. 2):

$$O_{[\%]} = 100 - C - H - N - \text{Ash} \quad (2)$$

### 4.3. Results and discussion

#### 4.3.1 Protein extraction and Feedstock characterization

Composition and physical characteristics of the microalgae biomass used are given in Fig. 4.1. The WMS was mainly composed by lipids, proteins and carbohydrates. The protein extraction was done by enzymatic hydrolysis, reaching an extraction yield of 71% and generating remaining biomass or SMS<sub>P</sub> with a high ash content (64%). This results from the process of proteins, extracting secondarily lipids and carbohydrates were removed. Additionally, the addition of sodium hydroxide as a pH regulator agent during enzymatic hydrolysis would increase the Na content on the precipitate (SMS<sub>P</sub>). The high ash content and the possible catalytic effect on the generated steam, promoting NCG formation (Dickerson and Soria, 2013 , Lee et al., 2020 , Yang et al., 2006).

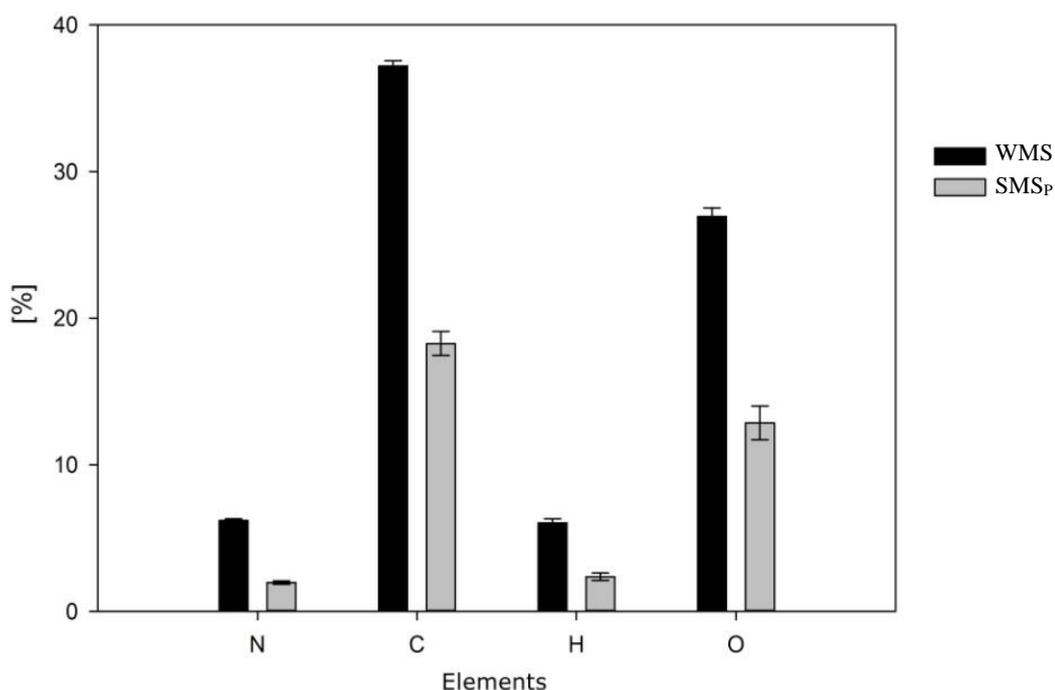


**Figure 4.1.** Composition of WMS and SMS<sub>P</sub>.

This means that the methods used for protein extraction were effective in removing high nitrogen contents in biomass (Fig 4.2), but it generated 173% more ashes than WMS because of its low specificity in protein extraction and NaOH residues deposited during the protein extraction process. The content of lipids of the SMS<sub>P</sub> was low, 35 % less in comparison with

WMS biomass (Fig. 4.1); but the energy content of the lipids can be concentrated in the bio-oil, increasing the HV.

In relation to moisture contents in each feedstock, these are in a suitable range for pyrolysis.

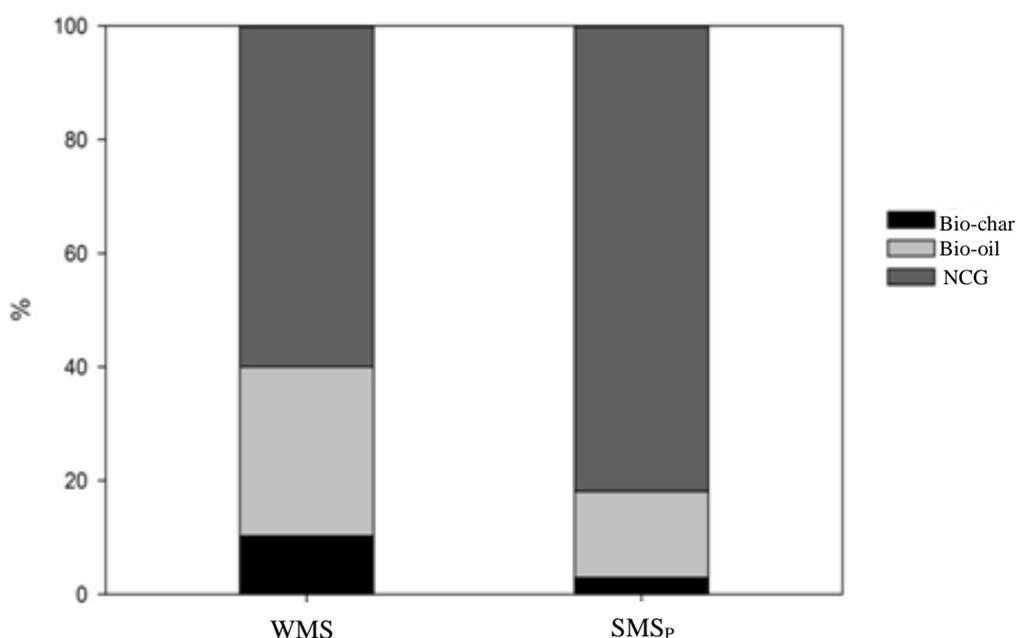


**Figure 4.2.** Elemental analysis WMS and SMS<sub>P</sub>.

#### 4.3.2 Pyrolysis products yield

Pyrolysis of WMS and SMS<sub>P</sub> produced 30% and 14% bio-oil, 10% and 3% biochar, 60% and 83% NCG respectively (Fig. 4.3). The high protein and carbohydrate content of the initial biomass facilitates the formation of NCG at 480°C, because the thermal decomposition of this fraction is between 250°C and 450°C (Kebelmann et al., 2013, Raveendran et al., 1996), being lower than the temperature exposed during pyrolysis. On the other hand, Yildiz et al., 2015, details the effect that a high ash content on biomass can change pyrolysis conditions, mainly by a possible catalytic effect of ash, being able to change the distribution and composition of products, increasing NCG production. To overcome the disadvantages of biomass ash in rapid pyrolysis, the biomass raw material must be leached (ash removal) before being introduced into

the process and biochar (containing a large majority of biomass ash) must be physically removed prior to the entry of the new raw material into the reactor.



**Figure 4.3.** Production of bio-oil, bio-char and NCG by pyrolysis of WMS and SMS<sub>p</sub>. Conditions of operation: Temperature 480°C, residence time 1.0 s, 450-1,000 µm particle size range and feeding rate of 12 mg/s.

Bio-oil obtained from WMS has 31 MJ/Kg (Table 4.1), very similar to the reported levels for bio-oil obtained from other algae varieties (Cadavillo and Capareda, 2013, Du et al., 2011, Miao et al., 2004, Miao and Wu, 2004). Although the NCG represents 60% of the initial pyrolyzed mass and HV of 12 MJ/Kg, it suggests that optimal pyrolysis conditions are at residence time or lower temperature defined by sequential method (Chapter III, section 3.3.5), therefore, Productivity and HV could be increased toward bio-oil as obtained by Miao et al. 2004, reporting 58% bio-oil Chlorella prototype productivity with a high HV (41 MJ/Kg).

Characteristics of bio-oil derived from microalgal biomass, such as high HV and its pH~7, make its direct use in boiler attractive. Unlike the lignocellulosic bio-oil, which has a lower HV (13-18 MJ / Kg) (Basu, 2010) and acid pH, wearing or directly damaging the boiler metal.

The productivity of SMS<sub>P</sub> bio-oil was lower than WMS, making it difficult to take direct liquid sample to be characterized and determine its HV. This leaves a fundamental point pending for defining its potential as biofuel.

Table 4.1: HV of pyrolysis products from *S. almeriensis*

Parameters	WMS (MJ/Kg)	SMS <sub>P</sub> (MJ/Kg)
Bio-oil	30.89	N.A.
Bio-char	5.82	3.02
NCG	11.68	N.A.
Feedstock	16.86	7.16

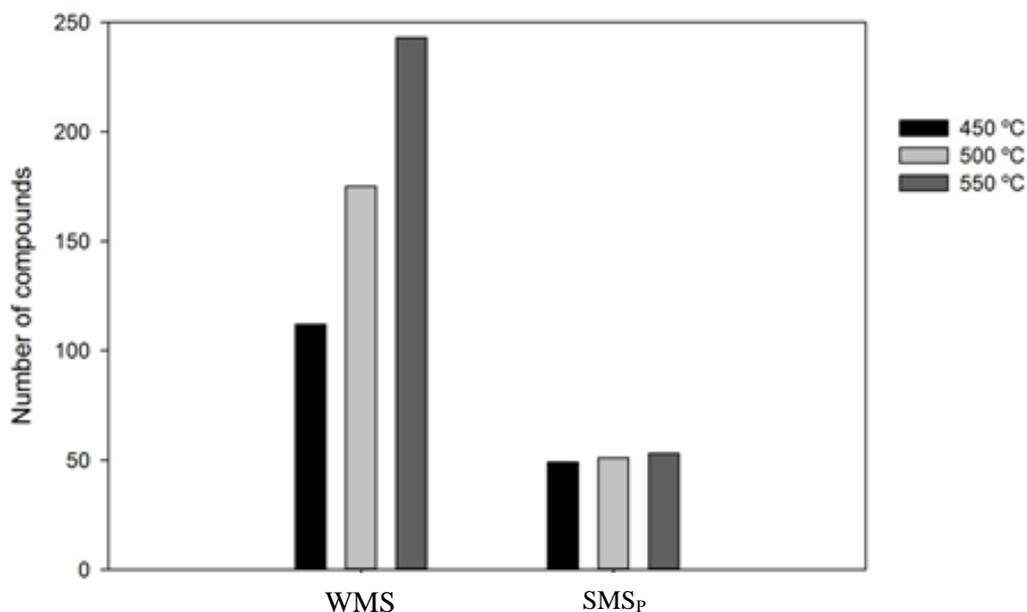
N.A.: No available.

Fast pyrolysis of WMB has produced 30% bio-oil, with approximately 60% of the nitrogen from the initial biomass (Chapter III, section 3.3.5, Fig 3.5). Based on exposed results that allow establishing bio-oil possible uses as fuel, it is necessary to determine the effect on the protein extraction as a process that allows the generation of a low nitrogen content bio-oil. According to Tchapda and Pisupati (Tchapda and Pisupati, 2014), during combustion of bio-oil, the contained nitrogen is converted into nitric oxides, gaseous nitrogen and nitrous oxide. Therefore, nitrogen can generate NO during the pyrolysis process, as well as during the combustion of its pyrolytic products (Ren et al., 2010). This scenario restricts the use of bio-oil of microalgae directly as fuel, so the reduction of the N content must be achieved previously.

#### **4.3.3 Effect of deproteination of the nitrogenous compounds content on bio-oil.**

This section evaluates the effect of biomass deproteinization on bio-oil composition, specifically vapors and gases obtained by fast pyrolysis of WMS and SMS<sub>P</sub> at 450, 500 and 550°C. The reduction of complex compounds (proteins) in biomass allows the obtaining of fewer compounds in the vapors and gases produced by rapid pyrolysis (Fig.4.4). Similarly,

production of NO<sub>x</sub> from combustion of pyrolytic products such as bio-oil and NCG would be reduced (Ren et al., 2010). Finally, the reduction of compounds in a bio-oil would allow a subsequent application of a simpler refinery process of liquid fraction (Shan Ahamed et al., 2020, Srifa et al., 2019), for the extraction of products with higher commercial value (Bórawski et al., 2019).

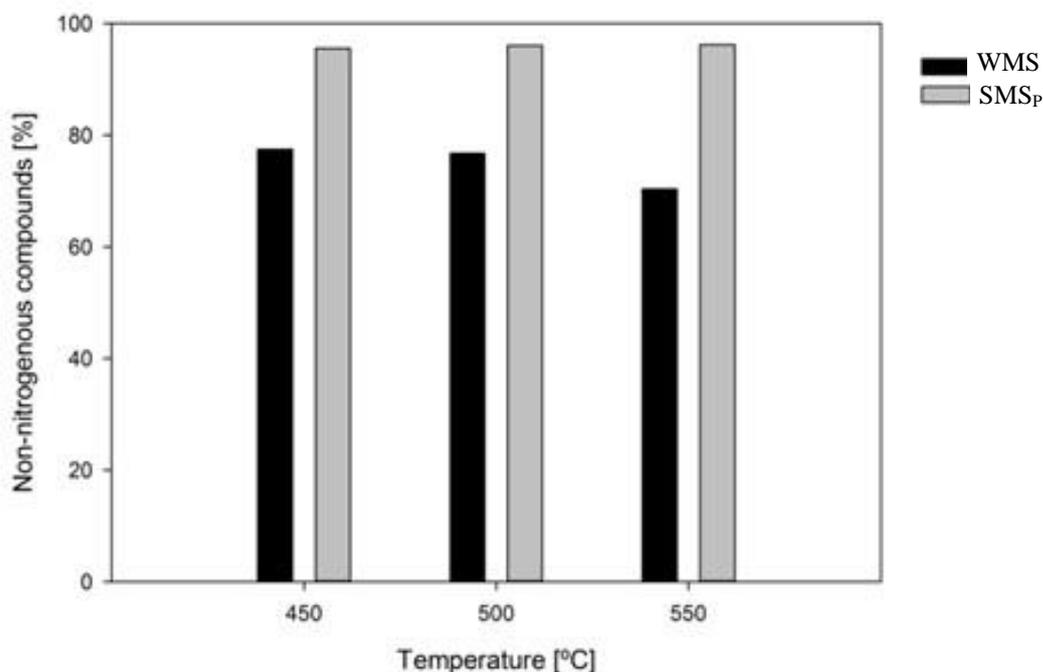


**Figure 4.4.** Compound number identified by GC/MS on the vapor and gases pyrolytics.

Fig. 4.5 shows a decrease of 80, 83 and 87% in the formation of micropyrolysis-induced nitrogenous compounds at 450 °C, 500 °C and 550 °C respectively. The higher the temperature, the higher the number of molecules fractionation, therefore the number of compounds increases, especially not nitrogenous compounds.

However, there is no effect on variability in the number of nitrogenous compounds produced from SMS<sub>P</sub> pyrolysis. However, as the temperature increases, the number of compounds produced increases (Fig. 4.4 and Fig. 4.5), due to the formation of smaller, non-condensable molecules, affecting the production of bio-oil and favoring the NCG (Chapter III, Section 3.3.4, Fig. 3.2).

Therefore, the extraction of proteins (amino acids) from microalgae biomass succeeded in reducing the main sources of nitrogen in biomass and therefore would limit the generation of NO<sub>x</sub> from combustion of bio-oil derived from microalgal biomass previously deproteinized.

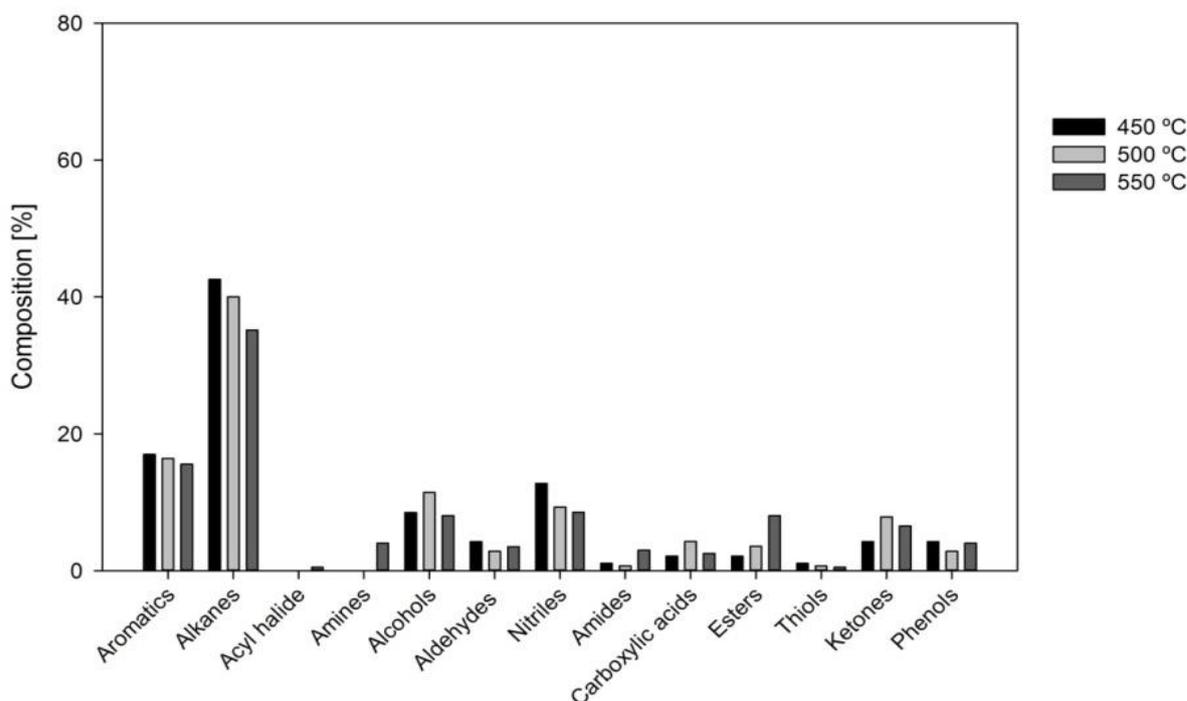


**Figure 4.5:** Non nitrogenous compound on the condensable and gases identified by GC/MS on the vapor and gas pyrolytics.

WMS pyrolysis compared to SMS<sub>p</sub>, produces a greater number of functional groups (Fig. 4.6) and compounds (Fig. 4.7), being able to reach 250 compounds derived from WMS and approximately 50 compounds derived from SMS (Fig. 4.4). In contrast, condensable fraction of rapid pyrolysis of lignocellulosic biomass, can contain more than 400 different compounds (Basu, 2010, Bridgwater, 2004), making it difficult and possible to apply refinery to this bio-oil. Therefore, techniques such as pyrolytic catalysis have been developed, which allows the performance of certain products to be improved and facilitate a less complex refinery process.

Nevertheless, any productive process involving adding multiple techniques for a single product makes it unfeasible. Although, compared to the lignocellulosic bio-oil, WMS bio-oil is less complex and much less the derivative of SMS<sub>p</sub>. Even the latter can be compared to a bio-oil of

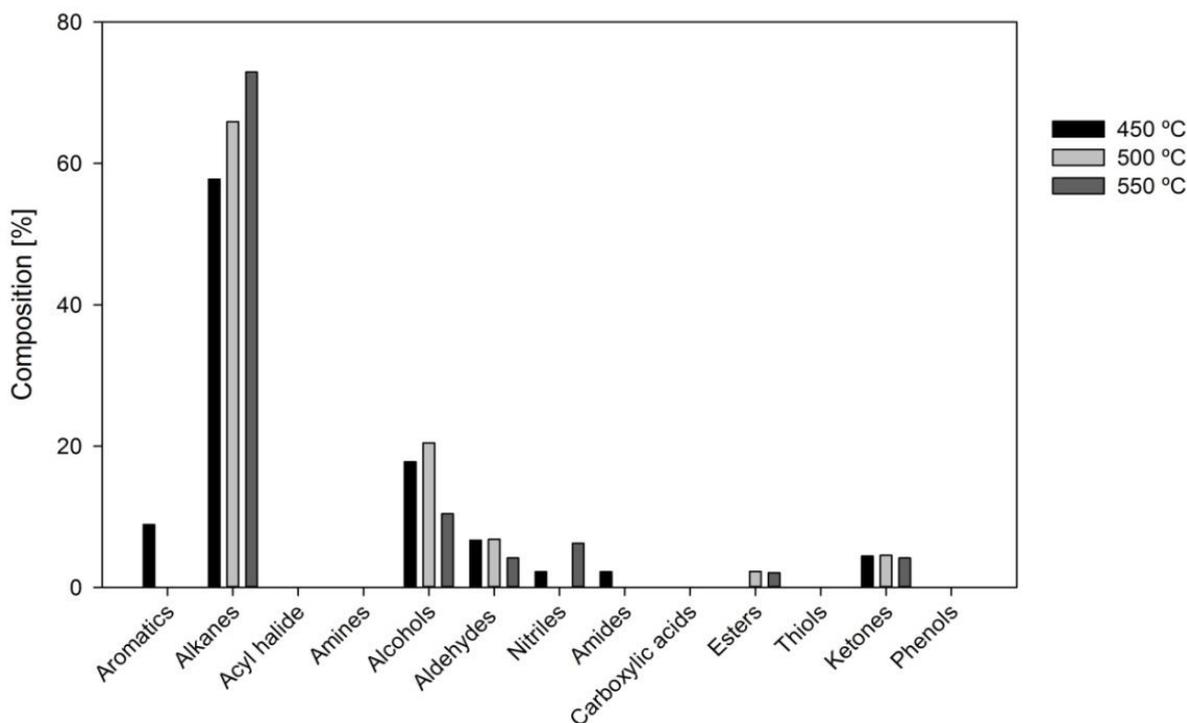
catalytic pyrolysis, as it has a higher alkane content than its WMS version, and this is the result of previous extraction of protein products.



**Figure 4.6:** Composition of vapors and gases generated by micropyrolysis on WMS. The constituents were grouped according to their chemical functional groups (GC/MS detectable only).

The main constituents of the pyrolyzable fraction of SMS<sub>P</sub> are carbohydrates and lipids, the latter is clearly observed in typical pyrolytic products in the form of alkane, from 58% to 72% of the vapors and gases produced, depending on the temperature of the reaction (Fig. 4.7). Alkanes are essential components for the chemical industry worldwide, will grow in the future and their supply is now dependent on oil and natural gas production. To close the growing gap between demand and supply, alternative technologies are being developed to produce alkenes from renewable resources. There are research such as the production of alkenes from intermediates derived from biomass such as furan, synthesis gas, methanol, ethanol and butanol, but the one with an attractive approach is rapid catalytic pyrolysis, since it would allow the production of alkenes directly from biomass, although the results of catalytic pyrolysis are

encouraging, there is a need to further improve this process to generate alkenes with higher yields. It is for this reason that the use of SMS<sub>P</sub> can be a valuable source of biomass for the production of alkanes without requiring a complex catalytic pyrolysis.



**Figure 4.7:** Composition of vapors and gases generated by micropyrolysis on SMS<sub>P</sub>. The constituents were grouped according to their chemical functional groups (GC/MS detectable only).

The effect extraction proteins of microalgal biomass on pyrolytic products is shown in Fig. 4.6 and 4.7, where WMS derivatives have a greater number of protein derivatives, such as amine, amid and nitril groups (Mullen and Boateng, 2011, Schnitzer et al., 2007), compared to SMS<sub>P</sub> pyrolytic compounds.

These results therefore allow two advantages of the deproteinization of microalgae biomass to be visualized, the first: Protein extraction from microalgae allows high commercial protein products and the second: deproteinization can be considered as a bio-oil refinery process, as its

characteristics are of low complexity, low nitrogen content and high alkanes content, ideal as a fuel or source of products of interest.

#### **4.3.4 . Characteristics of the produced bio-char and its possible applications.**

Biochar is produced mainly through thermochemical conversion processes, irreversibly changing the physical state and chemical composition of biomass into biochar. The solid product produced for slow pyrolysis is rich in carbon, but the content of carbon and yield to produce biochar they can change completely due to differences in heating speed and reaction temperature. Generally, biochar performance decreases with faster heating speed. For this work, biochar is a co-product of the rapid pyrolysis process, where its characteristics are not the best to be purchased with a slow pyrolysis bio-char and less with a lignocellulosic biomass.

Microalga biochar has high concentrations of trace elements, which in particular correspond to nutrients such as N, P, K, Ca and Mg (Zewdie and Ali, 2020 , Chew et al., 2017), suggests that the biochar produced from the pyrolysis of algae has good prospects as fertilizer (Wang et al., 2020). But the presence of elements such as Al, Ni, Zn and Sr (Table 4.2), raises opposite results, where the benefits of applying biochar as a soil amendment are limited to particular conditions such as types of biochar, application rates, type and conditions of soil and crop species. Systematic research is therefore needed to clarify the relationships between the properties of the microalga fast pyrolysis biochar and its possible application as an amendment to for agricultural and forestry systems.

Microalgae bio-char has been studied as soil recovery on desert areas, where they contribute with nutrients, carbon and retaining water. Likewise, it has been reported as immobilizer of pollutants present on the land, microorganisms, and increase the microbiological activity on the soil (Suganya et al., 2016 , Ippolito et al., 2016 , Bui et al., 2016 , Bordoloi et al., 2016). Biochar is an exceptional bio-resource for agronomy as soil amendment as concentrated coal, activated coal and especially for the elements that it has, apart from its physical characteristics such as

superficial area, porosity, distribution of pore, total pore and sorption, according to recent publications (Tsai et al., 2017 , Kumar et al., 2017 , Barbera et al., 2017 , Lu et al., 2016 , Laghari et al., 2016), but the biochar of rapid microalga pyrolysis contains low carbon content, corresponding to 3% of the total carbon content of initial biomass (Fig. 3.5 Section 3.3.5), low porosity and mineralized ash, they restrict the possibility of obtaining relevant results in soil recovery or acting as a pollutant immobilizer.

Biochar of biomass WM and SM registered an HV of 5.82 and 3.02 MJ/Kg respectively; significantly lower compared to biochar HV produced from lignocellulosic biomass by slow pyrolysis (~ 26 MJ / Kg). The potential use of microalgal biomass as a fuel is limited, due to its high ash content, which could increase the generation of particulate matter with environmentally harmful elements such as V, Cd, Cu and Pb (Liaw *et al* 2016). In addition, it may generate blockage in combustion systems by agglomeration and ash mineralization (Nanda *et al.*, 2016). However, the high ash content and its composition may give the biochar some qualities to be used as a catalyst in fast pyrolysis (Raheem et al., 2017 , Vichaphund et al., 2016 , Yildiz et al., 2015).

The presence of elements such as Ni, Zn and Fe, are reported as excellent catalysts on fast pyrolysis for the production of bio-oil with low levels of nitrogenous and oxygenate compounds (Raheem et al., 2017 , Gao et al., 2017 , Sun et al., 2016 , Liu et al., 2016).

Table 4.2: Elements concentration on WMS, SMS<sub>P</sub>, and biochar of WMS and SMS<sub>P</sub>.

Sample	Ag	Al	As	B	Ba	Be	Bi	Cd	Co	Cr	Cu	Fe
	[µg.g <sup>-1</sup> ]											
WMS (1)	<0.1	9.0	0.8	11.1	102.2	<0.1	<0.1	<0.1	<0.1	<0.2	30.8	1907.8
SMS <sub>P</sub> (2)	<0.1	78.0	0.6	4.4	185.8	<0.1	<0.1	<0.1	<0.1	5.2	21.4	2797.2
Bio-char 1	<0.1	60.1	0.9	17.1	184.2	<0.1	<0.1	<0.1	0.6	37.5	285.0	3951.0
Bio-char 2	<0.1	100.4	0.4	5.8	218.5	<0.1	<0.1	<0.1	0.1	9.5	27.1	32.19.3

Sample	Li	Mn	Mo	Ni	Pb	Se	Sr	Ti	U	V	Zn
	[µg.g <sup>-1</sup> ]										
WMS (1)	<0.5	503.1	1.29	<0.1	0.5	0.6	1037.7	<0.1	1.2	1.2	59.7
SMS <sub>P</sub> (2)	0.35	730.2	0.6	3.8	1.1	0.3	1728.6	<0.1	1.5	1.5	103.0
Bio-char 1	0.37	932.7	4.4	28.1	7.4	1.3	1850.0	<0.1	2.4	2.4	221.6
Bio-char 2	<0.5	849.7	1.2	3.9	1.2	0.3	2007.5	<0.1	1.7	1.7	128.0

The use of algae biochar as amendment for eroded soils recovery and for fertile soil upgrading, has been reported by Jung *et al.*, (2016). Biochar allows phosphates sorption, being able to be used as single manure, before the appliance of phosphates on the soil. This characteristic is connected to the conversion temperature from biomass into char, while temperature reaches or overcomes 400° C, it loses its sorption capacity because of the pore blocking during pyrolysis (Jung *et al.*, 2016).

Bio-char production, through slow pyrolysis for being used as amendment, can create environmental drawbacks because of the COV's release, even when the presence of these has not been associated to adverse effects on the ground nor on the seeds germination (Ghidotti *et al.*, 2017). Microalgae bio-char produced by fast pyrolysis does not have COVs, so it doesn't release COV's to the environment. It also acts as an excellent soil aerator, fixing polluting compounds, etc.

As a summary, biochar is insoluble mineralized residual coal, which for its physical and chemical characteristics previously mentioned, is ideal for being used as amendment for soil and catalyst, despite its production as amendment could be depleted because of economic feasibility.

#### **4.3.5 . Characteristics of the produced NCGs and its possible applications.**

Quantitative results of four main components (H<sub>2</sub>, CO, CO<sub>2</sub> and CH<sub>4</sub>) are shown in Fig. 2.6. Dominant gaseous species of NCG produced for all microalgae raw materials was CO<sub>2</sub> varying from 55% to 68% and other light hydrocarbons such as CO, CH<sub>4</sub> and H<sub>2</sub>. (Maguyon-Detras *et al.*, 2020 , Moreira *et al.*, 2017).

The conditions evaluated for higher HV in microalga bio-oil were at 480 °C and 1 second residence time, but under these conditions the WMS and SMS<sub>P</sub> biomass was converted between 60 and 80% in NCG. Nevertheless, NCG possesses about one third HV of the bio-oil and twice

the biochar (Table 4.1), being used as a "poor gas" of combustion to generate heat for pyrolysis (Banks and Bridgwater, 2016) and/or to perform the previous drying of the raw material to be pyrolyzed.

Another use of NCG is like gas carrier of vapors and gases generated during pyrolysis at home of the reactor. According to Shen et al., 2017, pyrolysis of biomass in the presence of CO<sub>2</sub> can achieve higher thermal cracking of harmful organic compounds, thus suppressing the formation of benzene derivatives (e.g. volatile organic compounds) and polycyclic aromatic hydrocarbons and can also increase CO production during biomass gasification.

The NCG produced could be used for fluidization in fast pyrolysis reactors (Ringer et al., 2006); causing generated gases and vapors to dilute with fluidizing gas and increase the hydrogen/carbon ratio within the pyrolysis reactor, producing higher hydrocarbon content in bio-oil (Yang et al., 2018 , Yan et al., 2020 , Banks and Bridgwater, 2016). Similarly, it would help to reduce or eliminate the use typically used gases such as N<sub>2</sub>, He and Ar in laboratory reactors. Pala et al., 2020, emphasizes that its use may obviate the need for catalytic pyrolysis or soft hydrotreatment processes, although this posture requires further validation.

The main gas present in NCG is CO<sub>2</sub> (Chapter II, Section 2.3.5, Fig. 2.6), its concentration is an indicator of fast pyrolysis performance, for example: High concentration of CO<sub>2</sub> in NCG indicates a high number of secondary reactions caused by high temperature rates in a pyrolysis reactor (Moreira et al., 2017) and therefore lower reaction temperatures should be considered, favoring higher bio-oil production.

Similarly, residence time is a critical parameter for defining biomass productivity to bio-oil and NCG, where the decrease in residence time from 1.5 to 1 s meant doubling the productivity of bio-oil (Chapter III, Section 3.3.5, Fig 3.4) and a reduction in CO<sub>2</sub> in NCG (Lee et al., 2020 , Safdari et al., 2019).

Decreasing residence time is the most recommended option to scale this process to the industrial level, achieving high bio-oil productivity in time, but requires the development of technology that allows to operate at high gas flows and small sizes of raw material, avoiding system obstruction or contamination of bio-oil by solids. Although, it is necessary to be considered that the recommendations are subject to the type of raw material, type of reactor and configuration of process (Sarafraz et al., 2019).

#### 4.4. Conclusions

The effect of protein extraction on microalgal biomass was investigated, analyzing the characteristics of WMS, SMS<sub>p</sub> and bio-oil produced by rapid pyrolysis. The enzymatic hydrolysis methodology for protein extraction achieved a 71% protein removal. Proteins are the main nitrogen-containing compounds in the biomass; their extraction allowed a 68% reduction of N in the biomass. Due to this, the process of deproteinization by enzymatic hydrolysis allows the extraction of a high level of proteins that could have a high economic value and also the reduction of nitrogen from the biomass for production of bio-oil.

The pyrolysis of WMS produces a bio-oil of high calorific value (31 MJ/Kg), but with a high nitrogen content. However, the bio-oil produced from SMS<sub>p</sub> has a lower nitrogen content and also a lower number of chemical compounds than that obtained from WMS, with alkane production predominating. Due to this, the lack of protection encourages the production of bio-oil of less chemical complexity, and the refinement of bio-oil to an interesting chemical product can benefit from it.

WMS and SMS<sub>p</sub> biomass biochar have a high ash content, derived from the harvesting process, which was mineralized in the biochar during the rapid pyrolysis process. The characteristics of biochar from microalgae biomass (with a high level of ash) give it qualities of a catalyst that could be used in the pyrolytic reaction itself, to reduce the presence of nitrogenous and oxygenated compounds in the bio-oil. The only economically viable way, is to associate the production to the microalgae biorefinery, as shown in this study, with the previous extraction of proteins, which could have a high commercial value and the pyrolysis as a sustainable process of elimination and revaluation of waste.

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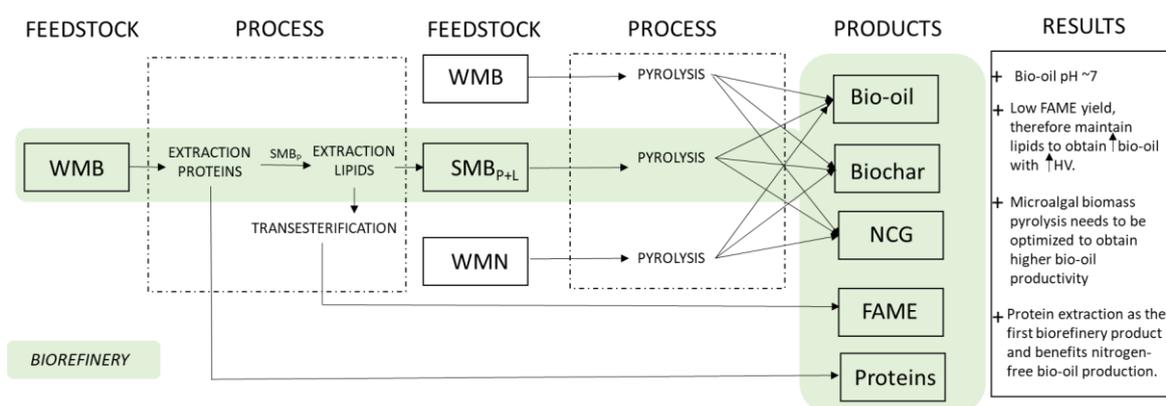
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## **CHAPTER V**

### **GENERAL DISCUSSION AND CONCLUSIONS**

## 5.1. General Discussion

This thesis reports on the production of bio-oil from microalgal biomass, associated with the concept of biorefinery. To address this problem, first the feasibility of producing bio-oil by rapid pyrolysis within the microalgae biorefinery was preliminarily evaluated, as shown in Fig. 5.1. The protein fraction was extracted from WM, thus obtaining the first product. Later, the lipid fraction was extracted to produce the second product (FAME), leaving finally a mass rich in carbohydrates and ash, which was subjected to rapid pyrolysis to produce bio-oil and by-products (bio-char and NCG), thus occupying 100% of the raw material.

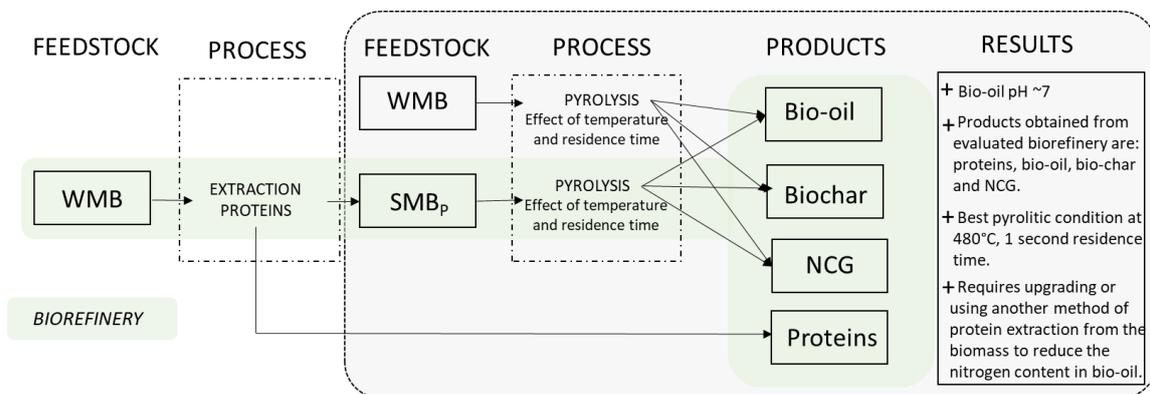


**Figure 5.1:** Diagram of the main works and results obtained in chapter II.

The most relevant results of this stage (Chapter II) are: a) The lipidic fraction of the microalgae has a low amount of methylable fatty acids, obtaining a poor production of FAME, therefore, it is established not to perform the extraction of lipids, favoring the obtaining of a greater production of bio-oil with more HV. b) The extraction of proteins allows to obtain the first product of microalgae biorefinery and favours the production of bio-oil with lower nitrogen content. c) The bio-oil obtained from microalgae biomass has a pH 7, confirming the first hypothesis of this thesis. This pH makes it interesting as a fuel, because it would be less corrosive and more stable than lignocellulosic bio-oil which has a pH 2.

Therefore, it is preliminarily confirmed the feasibility of carrying out a biorefinery of the microalgae, firstly performing the extraction of protein fraction and then the rapid pyrolysis of the depleted biomass (SMp).

In chapter II, an assessment was made regarding the conditions that would best favour the production of bio-oil within the microalgae biorefinery, considering the results obtained previously. For this, it was carried out the deproteinization of the biomass WMB, generating SMBp, which was later taken to fast pyrolysis, evaluating the effect of temperature and residence time of reaction and thus determine the best condition to achieve greater production of bio-oil with high HV (Fig. 5.2).

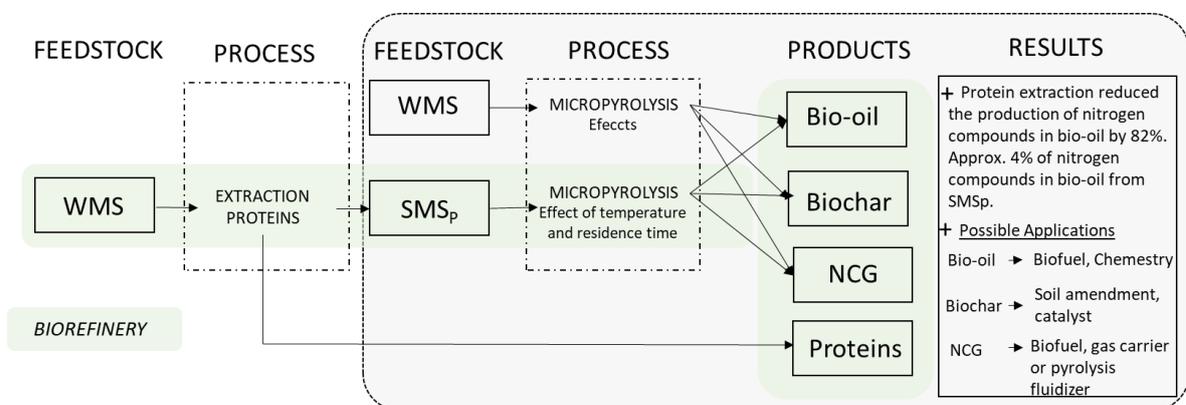


**Figure 5.2:** Diagram of the main works and results obtained in chapter III.

The main results obtained from this stage (Chapter III) have been: a) The best condition for rapid pyrolysis of microalgal biomass is at 480°C and 1 second of residence time, favoring the highest bio-oil productivity and higher HV by incorporating lipids as part of the raw material to be pyrolyzed. b) The evaluated range of residence time and the results obtained, allow inferring that the optimal point can be out of this one, that is to say to less than 1 second, still it is suggested to make an optimization of the process by means of a more robust statistical analysis, for example, MSR. c) It was observed high production of biochar, composed mainly of ashes derived from biomass, from culture medium and sodium from the process of soluble protein extraction. This suggests the implementation of washing process of biomass before

being pyrolyzed or intervene the process of production of biomass so that this does not drag high contents of salts. d) The bio-oil of WM and SM at different conditions evaluated, the pH was always close to neutral. e) The protein extraction allowed obtaining the first product of the process and also reduced the nitrogen content in the bio-oil, but it is recommended to improve the process of protein extraction to obtain a better result in the production of protein product and bio-oil without nitrogen.

Finally, chapter IV presents a final evaluation of the rapid pyrolysis products under the conditions defined in the previous chapter and the effect of deproteinization on them (Fig. 5.3). For this purpose, rapid pyrolysis of WMS and SMS at 480°C and 1 second residence time was performed. High production of NCG was observed (up to 80%), with HV of 11.7 MJ/Kg, composed mainly of CO<sub>2</sub> (up to 68% of NCG), indicating a high number of secondary reactions, which can be controlled by reducing the residence time of generated vapors. Even so, the NCG could be used for fluidization of the pyrolysis reactor or as a gas carrier and even as a lean fuel and generate heat for drying the raw material. On the other hand, SMS<sub>p</sub> biochar is mainly composed of ashes, due to the reduction of the pyrolysis fraction of the biomass and the incorporation of sodium, product of protein extraction. Its possible use is in agriculture, but due to its high ash content it is suggested to evaluate catalytic characteristics for pyrolysis or other processes.

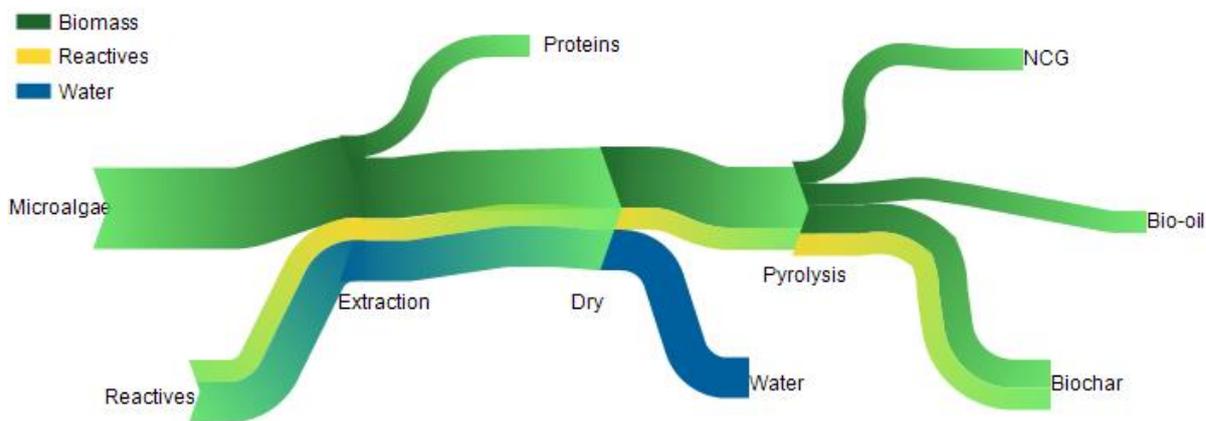


**Figure 5.3:** Diagram of the main works and results obtained in chapter IV.

The extraction of proteins at WMS, managed to reduce the nitrogen content in approximately 68% of the raw material. Similarly, nitrogenous compounds were reduced by up to 87% from vapors and gases obtained by micro-pyrolysis, obtaining up to 98% of non-nitrogenous compounds, compared to 76% of WMS-derived vapors and gases. The vapors and gases produced from SMSp have a low number of compounds (50 approximately) v/s the WM derivatives (250 approximately), which would favor the application of bio-oil improvement or the extraction of compounds of interest. This point is relevant, because the lignocellulosic bio-oil has a high number of compounds, reported over 450, making it difficult and expensive to refine it, or resort to techniques such as catalytic pyrolysis.

The bio-oil obtained from WMS has an HV of 30.89 MJ/Kg, but it has nitrogenous compounds, which limit its use as a bio-fuel. On the other hand, the bio-oil from SMSp would have a low nitrogen content and be composed mainly of alkanes, derived from pyrolysis of the lipid fraction of raw material. Due to the low sample volume of bio-oil produced from SMSp, its HV could not be quantified, but based on data obtained by micro-pyrolysis, it can be inferred that its calorific value is higher than lignocellulosic bio-oil and close to WMS bio-oil.

Finally, this work has allowed defining a biorefinery process of microalgae, achieving the use of 100% of biomass and obtaining four products, proteins, bio-oil, biochar and NCG (Fig. 5.4). The deproteinization of biomass has favored the production of bio-oil with high potential to be used as fuel for its high calorific value and low nitrogen content.



**Figure 5.4:** Biomass microalgae biorefinery process proposed

## 5.2. Conclusions

Taking into account the main results, it can be concluded that:

The conversion of microalgae biomass to bio-oil is low, being mainly devolatilized to NCG, although the bio-oil obtained by rapid pyrolysis has up to 3 times the value of the calorific value of the lignocellulosic biomass derivative and a pH around neutral. These last characteristics still make it attractive as a fuel.

The extraction of proteins allows the reduction of nitrogen in a bio-oil with potential use as fuel, on the other hand, the biomass convertible into bio-oil is considerably reduced.

In general, the potential of rapid pyrolysis products on microalgae biomass is limited by production costs, where the biomass must be previously dried (high energy expenditure). The only economically viable way is to associate the production to the microalgae biorefinery, as seen in this study, with the previous extraction of proteins, which could have a high commercial value and pyrolysis as a sustainable process of waste disposal, revalued by pyrolysis.

# **ANNEX**

## Papers ISI

Muñoz R, Navia R, Ciudad G, Tessini C, Jeison D, Mella R, Rabert C, Azócar L.(2015). Preliminary biorefinery process proposal for protein and biofuels recovery from microalgae. *Fuel [J]*, 150: 425-433.

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