DOCTOR OF PHILOSOPHY

Intermediate pyrolysis studies of aquatic biomass and potential applications in the BtVB-process

Katharina Kebelmann

2013

Aston University

Intermediate pyrolysis studies of aquatic biomass and potential applications in the BtVB-process

Katharina Kebelmann

Doctor of Philosophy

ASTON UNIVERSITY

November 2012

Aston University

Intermediate pyrolysis studies of aquatic biomass and potential applications in the BtVB-process

Katharina Kebelmann

Doctor of Philosophy

2012

THESIS SUMMARY

Aquatic biomass is seen as one of the major feedstocks to overcome difficulties associated with 1st generation biofuels, such as competition with food production, change of land use and further environmental issues. Although, this finding is widely accepted only little work has been carried out to investigate thermo-chemical conversion of algal specimen to produce biofuels, power and heat.

This work aims at contributing fundamental knowledge for thermo-chemical processing of aquatic biomass via intermediate pyrolysis. Therefore, it was necessary to install and commission an analytical pyrolysis apparatus which facilitates intermediate pyrolysis process conditions as well as subsequent separation and detection of pyrolysates (Py-GC/MS). In addition, a methodology was established to analyse aquatic biomass under intermediate conditions by Thermo-Gravimetric Analysis (TGA).

Several microalgae (e.g. *Chlamydomonas reinhardtii*, *Chlorella vulgaris*) and macroalgae specimen *(*e.g. *Fucus vesiculosus*) from main algal divisions and various natural habitats (fresh and saline water, temperate and polar climates) were chosen and their thermal degradation under intermediate pyrolysis conditions was studied.

In addition, it was of interest to examine the contribution of biochemical constituents of algal biomass onto the chemical compounds contained in pyrolysates. Therefore, lipid and protein fractions were extracted from microalgae biomass and analysed separately. Furthermore, investigations of residual algal materials obtained by extraction of high valuable compounds (e.g. lipids, proteins, enzymes) were included to evaluate their potential for intermediate pyrolysis processing.

On basis of these thermal degradation studies, possible applications of algal biomass and from there derived materials in the Bio-thermal Valorisation of Biomass-process (BtVB-process) are presented. It was of interest to evaluate the combination of the production of high valuable products and bioenergy generation derived by micro- and macro algal biomass.

Key words: algae, bioenergy, TGA, Py-GC/MS

Dedicated to my friend Steffen aka Old Paul

ACKNOWLEDGEMENTS

First of all, I would like to express my gratitude to my supervisor Prof. Andreas Hornung who gave me the possibility to conduct this interesting study and for his guidance throughout all the past years.

Secondly, I would like to thank Dr. Gareth Griffiths for his vitally support in the laboratory and for his scientific input.

I am very much obliged to my external supervisor Prof. Ulf Karsten and his team, for giving me the opportunity to learn the science of algae cultivation and for providing the Polar algae biomass samples.

In addition, I am thanking Dr. Dietrich Meier and Ingrid Fortmann for their help in mass-spectrometry.

My dearest thanks go to my Dad, for being my mentor in all these years, not only scientifically. Especially, I would like to thanks my Mum, for her valuable moral support during this time.

I would like to thank Manu and Yue, for crossing my life and their valuable friendship which made Birmingham such a nice home.
Furthermore, I would like to express my deepest thanks to “Die Fliegen”. Home is where doors are open any time and roots grow by friendship.

My dearest thanks go to Robert, to stand by me in these exceptional years of my life and for the “Kontrolletti”.

Last but not least, I am greatly thanking WGD GmbH for the financial and technical support.

ABBREVIATIONS

Amu	Atomic mass unit
ASTM	American Society for Testing and Materials
BtVB	Bio-thermal Valorisation of Biomass
CCAP	Culture Collection of Algae and Protozoa
CHN	Carbon hydrogen nitrogen
CHP	Combined heat and power
db	dry basis
DHA	Docosahexaenoic acid
DTG	Differential thermogravimetry
EBRI	European Bioenergy Research Institute
EI	Electronic impact ionization
EPC	Electronic pressure control
FAMEs	Fatty acid methyl esters
FID	Flame Ionization Detector
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatograph
GHG	Green house gases
HHV	Higher heating value
HTU	Hydrothermal upgrading
iD	inner diameter
IPCC	Intergovernmental Panel on Climate Change
MSD	Mass Selective Detector
MS	Mass spectrometer
MW	Molecular weight
n.d.	not detected
NIST	National Institute of Standards and Technology
NPD	Nitrogen Phosphorous Detector
NREL	National Renewable Energy Laboratory
PBR	Photo bioreactor
PUFAs	Polyunsaturated fatty acids
Py	Pyrolyser
R&D	Research and development
RSD	Relative standard derivation
SIM	Single ion monitoring
SSL	Split-/splitless inlet

TAP	Tris-acetate-phosphate
TGA	Thermogravimetric analyser
TG	Thermogravimetry
T_i	Initial pyrolysis temperature
TIC	Total ion chromatogram
TID	Thermionic detector
T_{eq}	Final pyrolysis temperature
TRT	Temperature rise time

CONTENTS

THESIS SUMMARY **2**
ACKNOWLEDGEMENTS **4**
ABBREVIATIONS **5**
CONTENTS **7**
LIST OF FIGURES **11**
LIST OF TABLES **12**

CHAPTER 1 13
1. INTRODUCTION 14
1.1. Background 14
1.2. Aquatic versus terrestrial biomass 16
1.3. Intermediate pyrolysis 17
1.4. BtVB-process 20
1.5. Introduction to Algae 23
1.5.1. Biochemistry 23
1.5.1.1. Chlorophyta 28
1.5.1.2. Rhodophyta 28
1.5.1.3. Phaeophyta 29
1.5.2. Large-scale production and applications 29
1.5.2.1. Microalgae 30
1.5.2.2. Macroalgae 34
1.5.3. Bioenergy applications 36
1.5.3.1. Bio-chemical conversion 37
1.5.3.2. Thermo-chemical conversion 38
1.5.4. Analytical thermo-chemical characterisation 41
1.5.4.1. Thermogravimetry 41
1.5.4.2. Analytical Pyrolysis 44
1.6. Conclusion 52
1.7. Algae species for intermediate pyrolysis 53
1.8. Aim and objectives of research 56
1.9. Organisation of thesis 57
CHAPTER 2 58
2. Development of analytical pyrolysis system 59
2.1. Introduction 59
2.2. Micro-furnace pyrolyser 61
2.3. Gas chromatograph 64

2.4. Detectors....64
2.4.1. Mass Selective Detector (MSD)....65
2.4.2. Flame Ionization Detector (FID)....65
2.4.3. Nitrogen Phosphorous Detector (NPD)....66
2.5. Commissioning....66
2.6. Data Interpretation....70
2.6.1. Identification of components....70
2.6.2. Quantification and deviation....72
2.7. Maintenance....75
2.8. Chapter Conclusions....77
CHAPTER 3....79
3. Materials and methods....80
3.1. Microalgae....80
3.1.1. Cultivation of *Chlamydomonas reinhardtii*....80
3.1.2. Further species....81
3.2. Macroalgae....81
3.2.1. Polar species....81
3.2.2. *Fucus vesiculosus*....81
3.3. Biomass characterisation....82
3.3.1. Sample preparation....82
3.3.2. Ultimate analysis....82
3.3.3. Higher heating value....82
3.3.4. Total lipid extraction and FAMEs analysis....83
3.3.5. Total protein extraction....83
3.3.6. Thermogravimetric analysis....84
3.4. Analytical pyrolysis....84
CHAPTER 4....88
4. Results biomass characterisation....89
4.1. Ultimate analysis, ash and calorific values....89
4.2. Total Lipid, total protein and FAMEs determination....91
4.3. Thermogravimetric analysis....94
4.3.1. Introduction....94
4.3.2. Microalgae....94
4.3.2.1. *Chlamydomonas reinhardtii* and *Chlorella vulgaris*....94
4.3.2.2. *Porphyridium purpureum*....98
4.3.3. Macroalgae....100
4.3.3.1. Polar species....100

4.3.3.2. *Fucus vesiculosus* 104
CHAPTER 5 106
5. Results analytical pyrolysis 107
5.1. Introduction 107
5.2. Microalgae 108
5.2.1. *Chlamydomonas reinhardtii* and *Chlorella vulgaris* 108
5.2.1.1. Biomass 108
5.2.1.2. Proteins 112
5.2.1.3. Lipids 115
5.2.1.4. Residues 118
5.2.1.5. Dual detector analysis 121
5.2.2. *Porphyridium purpureum* 127
5.3. Macroalgae 130
5.3.1. Arctic species 130
5.3.2. Antarctic species 136
5.3.3. *Fucus vesiculosus* 141
CHAPTER 6 144
6. Discussion biomass characterisation 145
6.1. Ultimate analysis, ash and calorific values 145
6.2. Total lipid, protein and FAMES analysis 147
6.3. Thermogravimetric analysis 149
6.3.1. Microalgae 149
6.3.1.1. *Chlamydomonas reinhardtii* and *Chlorella vulgaris* 149
6.3.1.1.1. Lipids 149
6.3.1.1.2. Proteins 150
6.3.1.1.3. Residues 150
6.3.1.1.4. Biomass 151
6.3.1.2. *Porphyridium purpureum* 152
6.3.2. Macroalgae 152
6.4. Chapter Conclusions 153
CHAPTER 7 157
7. Discussion analytical pyrolysis 158
7.1. Microalgae 158
7.1.1. *Chlamydomonas reinhardtii* and *Chlorella vulgaris* 158
7.1.1.1. Lipids 158
7.1.1.2. Proteins 159
7.1.1.3. Residues 161

7.1.1.4. Algal Biomass 161
7.1.1.5. Dual detector analysis 163
7.1.2. *Porphyridium purpureum* 163
7.2. Macroalgae 164
7.2.1. Polar species 164
7.2.2. *Fucus vesiculosus* 168
7.3. Chapter conclusions 168
CHAPTER 8 173
8. Algae applications in the BtVB-process 174
8.1. Introduction 174
8.2. Extraction of valuable products prior to pyrolysis 175
8.2.1. Lipids from microalgae 175
8.2.2. Proteins from microalgae 176
8.2.3. Coenzyme Q_{10} from microalgae 177
8.2.4. Polysaccharides from macroalgae 177
8.3. Applications of pyrolysis liquids 178
8.3.1. Phenol 179
8.3.2. Levoglucosan 179
8.3.3. Squalene 180
8.4. Biochar for fertilizers 181
8.5. Chapter conclusions 183
CHAPTER 9 185
9. Conclusions and recommendations 186
9.1. Response to main objectives 186
9.2. Recommendations for future work 187
9.2.1. Algae biomass 187
9.2.2. Analytical pyrolysis 187
9.2.3. Large-scale realisation 188
RFERENCES 189
APPENDICES 209
Appendix A – Publications 210
Appendix B – Validation report analytical pyrolysis system 211
Appendix C – TAP Medium 220
Appendix D – Pyrolysis products by Py-GC/MS at 500 °C 222

LIST OF FIGURES

Figure 1-1 BtVB-Process 22
Figure 1-2 Algal polysaccharides 27
Figure 1-3 Open pond cultivation of microalgae 32
Figure 1-4 Tubular bioreactor for microalgae cultivation 32
Figure 1-5 Bioenergy generation from algae 40
Figure 2-1 Analytical pyrolysis set-up 60
Figure 2-2 Micro-furnace pyrolyser PY2020iD 62
Figure 2-3 Chromatograms of standards 68
Figure 4-1 TG/DTG of green microalgae materials 96
Figure 4-2 TG/DTG of *Porphyridium purpureum* 99
Figure 4-3 TG/DTG Arctic species 101
Figure 4-4 TG/DTG Antarctic species 103
Figure 4-5 TG/DTG *Fucus vesiculosus* 105
Figure 5-1 Pyrograms microalgal biomass at 500 °C 110
Figure 5-2 Pyrograms of microalgae proteins at 500 °C 113
Figure 5-3 Pyrograms of microalgae lipids at 500 °C 116
Figure 5-4 Pyrograms of microalgae residues at 500 °C 119
Figure 5-5 Dual detector pyrograms of *Chlorella vulgaris* at 500 °C 122
Figure 5-6 Pyrograms of *Porphyridium purpureum* at 500 °C 128
Figure 5-7 Pyrograms of Arctic species at 500 °C 132
Figure 5-8 Pyrograms of Antarctic species at 500 °C 137
Figure 5-9 Pyrograms of *Fucus vesiculosus* at 500 °C 142

LIST OF TABLES

Table 1-1 Main classes of pyrolysis for wood 19
Table 1-2 Gross chemical composition of micro- and macroalgae 25
Table 1-3 Biochemical characteristics of main alga divisions 26
Table 1-4 Commercial microalgae production and applications 33
Table 1-5 Production of major polysaccharides of macroalgae 35
Table 1-6 Main pyrolysis temperatures of biomass by TGA 43
Table 1-7 Analytical pyrolysis units 47
Table 1-8 Capillary separation columns for Py-GC/MS 48
Table 1-9 Summary analytical studies of micro- and macroalgae 51
Table 1-10 Algae species for intermediate pyrolysis 55
Table 2-1 Specifications double-shot pyrolyser PY2020iD 63
Table 2-2 Retention times of standard compounds for MSD, NPD and FID 71
Table 2-3 Relative standard deviations of detectors 74
Table 2-4 Maintenance schedule analytical pyrolysis system 76
Table 3-1 Gas chromatograph settings 86
Table 3-2 Detector settings 87
Table 4-1 Ultimate analysis, ash content and higher heating values 90
Table 4-2 Gross chemical components microalgal biomass 92
Table 4-3 FAMEs of microalgae 93
Table 5-1 Peak areas of pyrolysis compounds - microalgal biomass 111
Table 5-2 Peak areas of pyrolysis compounds - microalgal proteins 114
Table 5-3 Peak areas of pyrolysis compounds - microalgal lipids 117
Table 5-4 Peak areas of pyrolysis compounds – microalgae residues 120
Table 5-5 Compounds identified by analytical standards 126
Table 5-6 Peak areas of pyrolysis compounds – *Porphyridium purpureum* 129
Table 5-7 Peak areas of pyrolysis compounds - Arctic species 134
Table 5-8 Peak areas of pyrolysis compounds - Antarctic algae 139
Table 5-9 Peak areas of pyrolysis compounds - *Fucus vesiculosus* 143
Table 6-1 Pyrolysis characteristics microalgae materials 155
Table 6-2 Pyrolysis characteristics macroalgae materials 156
Table 7-1 Main chemicals of microalgae pyrolysates 171
Table 7-2 Main chemicals of macroalgae pyrolysates 172

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1. Background

The demand for an energy supply independently from fossil fuel reserves has gained increased attention in the last few years. A major driver for a change to renewable resources is the strongly fluctuating and generally increasing price for crude oil. Whereas prices of crude oil were in the region of $ 25 per barrel (bbl) in November 2002, it has increased up to $ 110 bbl^{-1} till today [1].

Another pressing reason to utilize carbon neutral fuels to replace fossil fuels is the climate change due to anthropogenic green house gas (GHG) emissions. As reported by the Intergovernmental Panel on Climate Change (IPCC) global emissions of carbon dioxide (CO_2) have increased by 80 % between 1970 and 2004 [2]. With the climate change associated problems are increasing air and water temperatures (global warming), water quality problems and crop losses caused by sudden changes of weather (droughts, heavy winds, rains, floods) resulting in increased food prices [2].

Sources for renewable and sustainable energy to provide independency from fossil fuels and mitigate climate change by reducing GHG emissions include sun (e.g. photovoltaic solar cells and solar heat collectors), wind (e.g. wind turbines), water (e.g. hydropower, tidal energy), geothermal resources and biomass.

Biomass plays an important role being the only renewable resource of carbon for the production of chemicals, materials and fuels. Overall, in 2008 ca. 10 % of the global primary energy supply was supplied by biomass. However, ca. 80 % was provided by combustion of low-efficiency traditional biomass including wood, straw, and manures used for cooking, lighting and heating by the poorer populations in developing countries. Only ca. 20 % are supplied by commercial high-efficient technologies, such as heat production for district heating systems, power generation via combustion, combined heat and power plants (CHP), co-firing of biomass and fossil fuels, first-generation liquid biofuels from oil crops (biodiesel) and sugar and starch crops (ethanol) [3].

To increase the contribution of biomass derived energy to the global energy market, advanced conversion technologies and feedstock production is necessary. At present, the majority of feedstocks are terrestrial derived and provide relatively low yields with up to two harvests per year. Furthermore, their production is associated with direct competition to food production and problematic environmental changes, including land-use change, extensive water use and endangered biodiversity [4].

Aquatic biomass, i.e. algal organisms, has recently gained recurring attention to be a promising alternative to terrestrial derived feedstocks. Main advantageous include higher photosynthetic activity providing large amounts of biomass, the possibility of cultivation on non-arable land and manipulable chemical composition [5].

A press release of the European Commission from October 2012 [6] emphasizes the urgent need for alternative feedstocks by limiting the amount of food crop-based biofuels to 5 % to meet the EU's renewable energy target in the transport sector of 10 % by 2020. The aim is to reinforce the development of truly sustainable biofuels and member states of the EU will provide financial incentives for the production of 2nd generation fuels, including feedstocks like algae, straw and wastes [6].

To establish conversion routes for alternative feedstocks by more efficient technologies, intensive Research and Development (R&D) activities are carried out in the field of thermo-chemical conversion processes, including gasification and pyrolysis. Benefits of the technologies are the capability converting various types of feedstocks, typically applying the entire biomass rather than an expensive extract (i.e. lipids for biodiesel) and to produce gaseous, liquid and solid fuels at the same time [7].

At present, the main attention has been paid towards processing lignocellulosic derived feedstocks, including straw, crop residues, and short rotation crops [8], [9].

Although, the potential of aquatic biomass is widely known, only limited studies about thermo-chemical conversion are available and a further need in R&D is required to evaluate their potentials in generating sustainable bioenergy.

1.2. Aquatic versus terrestrial biomass

Various conversion technologies exist, providing biofuels, power and heat from renewable resources, such as biomass including combined heat and power plants (CHP), biodiesel, bioethanol and biogas. During the 1st generation of renewable energy from biomass, typically applied feedstocks included food crops such as rape, sugar cane and corn. Alternatives include forestry products (short rotation crops, wood chips and pellets) agricultural residues (shells, husks) and specially cultivated energy crops (miscanthus, switchgrass) [10], [11].
All these materials are terrestrially derived and with an increasing generation of bioenergy derived by biomass, further increasing conflicts are seen with food supply, water use, biodiversity and land use. Consequently, raised interest is drawn towards bio-energetic applications of aquatic derived biomaterials. Several advantages over terrestrial grown biomass are evident and are presented below [12–19].

- Photosynthetic efficiency

Algal organisms convert up to 5 % of solar energy into biomass, where as terrestrial growing plants convert up to 1.5 %. Due to a simpler cellular structure and their aqueous habitat providing sufficient amounts of water, CO_2 and other nutrients, they are more efficient in capturing CO_2, producing larger amount of biomass and exhibiting generally a faster growth. Additionally, as long as sufficient light is provided, production is not seasonal and biomass can be harvested nearly all-year-round.

- Land requirements

Compared to terrestrial energy crops, land based cultivation systems of algal organisms do not require fertile land. In addition, no freshwater irrigation is necessary as algae are capable to grow in brackish waters. Therefore, the impact on land use management, food crop production and overcoming nutrient constraints is reduced. Furthermore, cultivation in areas with low economic efficiency as well as in marginal areas, such as desserts and coastal regions is possible.

- Chemical composition and versatility

Algae organisms present a vast variety of species and due to their simple cell structures contain large amounts of easily degraded chemical constituents (i.e. lipids, starch) for bio-energy production, than terrestrial plants. This could lead to optimization of energy input for conversion processes. Furthermore, algae species occur in almost

every clime and particular species from nutrient diminished or colder habitats may offer interesting opportunities in terms of applications for bioenergy generation. Moreover, advances in genetic manipulation and cultivation techniques offer possibilities to design individualised species applicable for certain conversion processes.

- Nutrient recycling from waste streams

Production of algal biomass can apply waste water sources (industrial and municipal waste water, agricultural run-off) or effluent gas streams while utilizing it as carbon and/or nutrient (nitrogen, phosphorous) source. In combination with algae production, this provides the additional benefit of wastewater remediation or effluent gas cleaning.

- Integrated systems

A number of species are available producing high valuable products, i.e. for pharmaceutical and nutritional applications, naturally. This offers the opportunity to establish integrated systems, including the valorisation of side-products and further processing the residues for bioenergy generation in an economical feasible way. Another option may be the removal and processing of contaminated or introduced seaweeds, which are difficult to dispose or might harm the ecological balance of the environment.

1.3. Intermediate pyrolysis

Pyrolysis is a thermo-chemical degradation reaction, caused by energy provided to the feedstock. A temperature increase within the feedstock, causing formation of molecules smaller than the starting material through decomposition and elimination and the absence of oxygen, define the pyrolysis conditions. Products obtained by pyrolysis are vapours, partly condensable to pyrolysis liquids and solid residues called biochar [20], [21]. Different types of pyrolysis exists, producing other proportions of the pyrolysis products resulting from the operation conditions, including reaction temperature, hot vapour residence time and heating rate. An overview of the different pyrolysis types and their characteristic process parameters for wood are presented in Table 1-1 [8], [22], [23]. Generally, a decreasing process temperature and increasing vapour residence time reduces the amount of pyrolysis liquids and promotes the production of gas and char.

The most traditional type of pyrolysis is slow pyrolysis, producing charcoal from wood by low reaction temperatures, long vapour and solid residence times, reaching from hours to days. From there, fast pyrolysis of wood and other lignocellulosic materials was developed to produce maximum amounts of pyrolysis liquids aiming at replacing fossil derived fuels. Characteristically, feedstocks with fine particle sizes are rapidly heated up to 500 °C and very short vapour residence times are maintained. Consequently, lower amounts of gases and char are obtained by the process. A vast amount on literature, including comprehensive descriptions and reviews of the process, reactor types and potential applications of the end products exist [8], [24–27].

A novel type of pyrolysis investigated is intermediate pyrolysis, operating at reaction conditions between fast and slow pyrolysis. Reaction temperatures of 350-500 °C, hot vapour residence times of 2 to 4 s and feedstock residence times of 0.5 to 25 min are applied at moderate heating rates of 1-1000 °C s^{-1}. This affects the composition of the liquid phase as well the characteristics of the biochar. The product distribution obtained by this process is 40-60 % of pyrolysis liquids, 20-30 % non-condensable vapours and 20-35 % biochar, depending on the feedstock [28], [29].

A coaxial double screw-type pyrolysis reactor, called pyroformer is patented at Aston University [30], which thermally decomposes biomass at intermediate pyrolysis conditions. Intermediate pyrolysis offers much different product qualities as the reaction prevents formation of high molecular tars and produces dry chars suitable for applications as fertiliser or fuel for combustion. In addition, processing of ash rich material is possible, whereas process temperatures lower than ash fusion temperatures are applied [31].

An advantage of this type of pyrolysis is the possible application of feedstocks with larger particle sizes due to sufficient heating given through moderate heating rates and longer residence times. Hence, the slower reaction rates of the pyrolysis process leads to a gentler decomposition of the molecules and subsequently less tar are formed in the vapours. This leads to an easier separation of the vapours from the char. Secondly, obtained pyrolysis liquids by condensation of the vapours may contain lower concentrations of reactive components, such as alkali metals due to sequestration in the biochar. Generally, the pyrolysis oils comprise a low energy aqueous phase and a high energy oily phase, with a typical heating value of 18 MJ kg^{-1} and being less prone to polymerisations and aging leading to more stable liquids [28], [30].

Table 1-1 Main classes of pyrolysis for wood [8], [22], [23]

Mode	Conditions	Oil [%]	Gas [%]	Char [%]
Fast	- Reaction temperatures ~500 °C - Short hot vapour residence time, ~1 s - High heating rates, >1000 °C s^{-1}	75	13	12
Intermediate	- Reaction temperatures, ~350-500 °C - Intermediate hot vapour residence time, 2 to 4 s - Moderate heating rates, 1-1000 °C s^{-1}	50	25	25
Slow	- Reaction temperatures ~200-400 °C - Long hot vapour and solid residence time, hours to days - Low heating rates, 5-7 °C min^{-1}	30	35	35

1.4. BtVB-process

The “Bio-thermal Valorisation of Biomass-process” (BtVB-process) was developed by Prof. Andreas Hornung of the European Bioenergy Research Institute (EBRI) and patent-registered [23] at Aston University.

The concept is an integrated process to generate low-cost and sustainable energy by processing versatile types of biomass. A main intention is the solely application of so called 2nd generation feedstocks, while consequently abdicating materials derived from food crops. Particular ash rich feedstocks, such as algal biomass, residues from biogas plants, agriculture wastes and sewage sludge are in focus to generate heat, power, transportation fuels and alternative fertilizers, as illustrated in Figure 1-1 [23].

The technological core of the BtVB-process is the pyroformer, described in section 1.2, processing 100 kg h^{-1} biomass, with particle sizes of up to 10 mm in diameter and 10-20 mm in length.

Generally, a moisture content of about 20-40 % in the biomass is favourable for intermediate pyrolysis. Therefore, the integrated process offers heat derived by the pyrolytic process to dry feedstock, which contains higher moisture contents.

A preferred route enclosed in the BtVB-process is the direct gasification of the pyrolysis vapours. Intermediate pyrolysis is particular useful for applications with subsequent gasification of the vapours, due to capturing the inorganic parts in the biochar and conveying only low ash vapours to the interfaced gasifier to further process synthesis gas or electricity and heat.

An alternative is the condensation of the vapours to pyrolysis liquids, which are a renewable resource to produce chemicals, precursors for chemical industry and/or transportation fuels.

The preferred options for biochar are applications as a soil amendment and/or as a fertilizer to recycle nutrients from the feedstocks, particular of ash rich feedstocks and to sequester carbon simultaneously [23], [28], [29].

Overall, the BtVB-process aims at being realised on local sites in combination with horticulture, agriculture and other bioenergy generating technologies, such as bio gas plants. In addition, to the terrestrial derived residual feedstocks, algal biomass plays an important role within the BtVB-process. Firstly, algal biomass resources are seen as promising feedstocks for sustainable energy generation, due to its higher energy efficiency and lower land requirements compared to terrestrially grown biomass [14]. Secondly, its non-lignocellulosic cell structure is interesting for thermal-chemical processing due to decomposition of the biomass constituents at lower temperatures [32]. In addition, pyrolysis liquids from algal materials are characterized by better

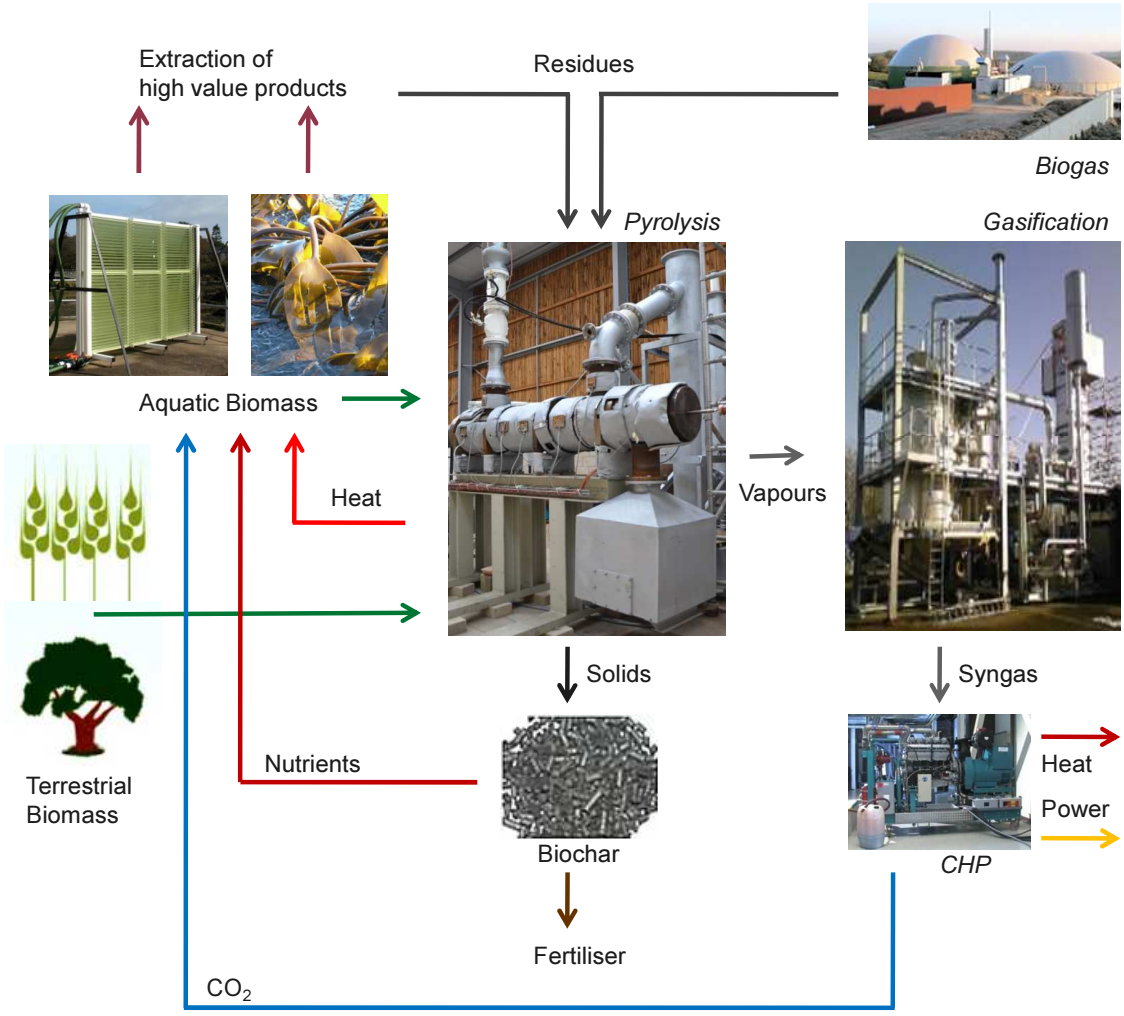
stability, lower water content, higher heating value, lower densities, and lower viscosities than those derived by terrestrial biomass, in particular wood. In addition, intermediate pyrolysis liquids of various feedstocks have comparable characteristics to biodiesel [9], [33].

Moreover, micro- and macroalgae materials are useful materials for the BtVB-process, due to their high contents of inorganic materials. The processing of these ash rich feedstock leads to higher amounts of captured nutrients in the biochar which can be recycled either into the cultivation of microalgae, while reducing their production costs or as valuable fertilizers in agriculture. In general, the application of biochar as fertilizer, offer opportunities to retain valuable amounts of phosphorous, one of the main nutrients required for plant growth where natural resources are diminishing continuously (peak phosphorous) [34].

Currently, various technologies are under investigation, to generate biofuels such as biodiesel and biogas from algal materials. However, due to high production costs of the biomass and intensive harvesting methods the production of biofuels from algal are economically not feasible, at present. Therefore, biorefinery concepts, combining bioenergy generation with the production of value added products for industrial applications are seen as opportunities [35–38].

Two options of intermediate pyrolysis of algal materials are included in the BtVB-process. The first option processes the entire biomass, such as contaminated and abundant seaweeds being difficult to dispose. The second one, processes algal derived residues after the extraction of high valuable products, such as lipids, proteins and enzymes. The opportunity to retain high valuable products for applications in chemical and pharmaceutical industries or human nutrition in combination with bioenergy generation would increase the economic feasibility of the BtVB-process drastically. For this reason the investigation of intermediate pyrolysis of various algal species, their biochemical constituents and the residues after extraction of different compounds is of high interest for the ongoing research.

Figure 1-1 BtVB-Process [23]



1.5. Introduction to Algae

The term algae refer to a large and diverse group of organisms ranging from unicellular microorganisms, i.e. *Chlorella sp.* exhibiting a cell size of 2-12 µm, called microalgae up to macroscopic sized organisms, such as *Macrocystis sp.* (Kelp) exhibiting a thallus (leaf-like structures), stems and a foot with up to 60 m in length, called macroalgae (seaweeds) [39]. Typically, microalgae species are suspended in water (planktonic), where as macroalgae species live attached to the bottom (benthic) in supralittoral- (above water level, in spray zone), intertidal- (exposed periodically in accordance to the tide) and subtidal zones (constantly submerged). Overall algae are classified into three divisions mainly based on their pigmentation into green (Chlorophyta), red (Rhodophyta) and brown (Phaeophyta) algae [39]. Organisms from all divisions occur in a broad range of habitats, such as fresh-, brackish- and marine waters and subaerial biotopes. Furthermore, micro- and macroalgae species are broadly distributed in all climate zones, while some are adapted to life in very harsh climate such as Polar Regions or in surrounding of hot springs [40].

1.5.1. Biochemistry

For commercial applications and bioenergy processing technologies, the biochemical composition of the feedstocks is of importance and the gross chemical composition of some micro- and macroalgae organisms is shown in Table 1-2. The values indicate that major algal constituents are proteins, lipids and carbohydrates. In contrast, wood and other terrestrial plants typically exhibit a cell wall build up to 60-90 % of a lignocellulosic complex consisting of cellulose, hemicellulose and lignin and only minor amounts of inorganic minerals and extractives (such as proteins, lipids, resins, pigments, waxes, starch) are present in the biomass [9], [41].

Similar to vascular plants, cell walls of algae are made of fibrils forming the skeleton and amorphous components forming the matrix in which fibrils are enclosed. However, algae do not exhibit a solidified cell wall structure, caused by the lack of the lignocellulosic complex, to maintain the flexibility of their tissues to withstand damage by turbulent water movements [42]. For classification of algal organisms the polysaccharide composition as well as the apparent pigments are used and Table 1-3 presents the classification after Bold and Wynne [39].

Primarily, the cell walls of most micro- and macroalgae species consist of polysaccharides, made of sugars and sugar acids, which are also found in land plants

including cellulose and hemicellulose. But some also contain sulphated polysaccharides in which an acidic sulphate group replaces a hydroxyl group of the sugar. In addition, algal organisms contain energy storage polysaccharides, which are unique to each division. Chemical structures of some cell wall polysaccharides and energy storage compounds are illustrated in Figure 1-2 [43].

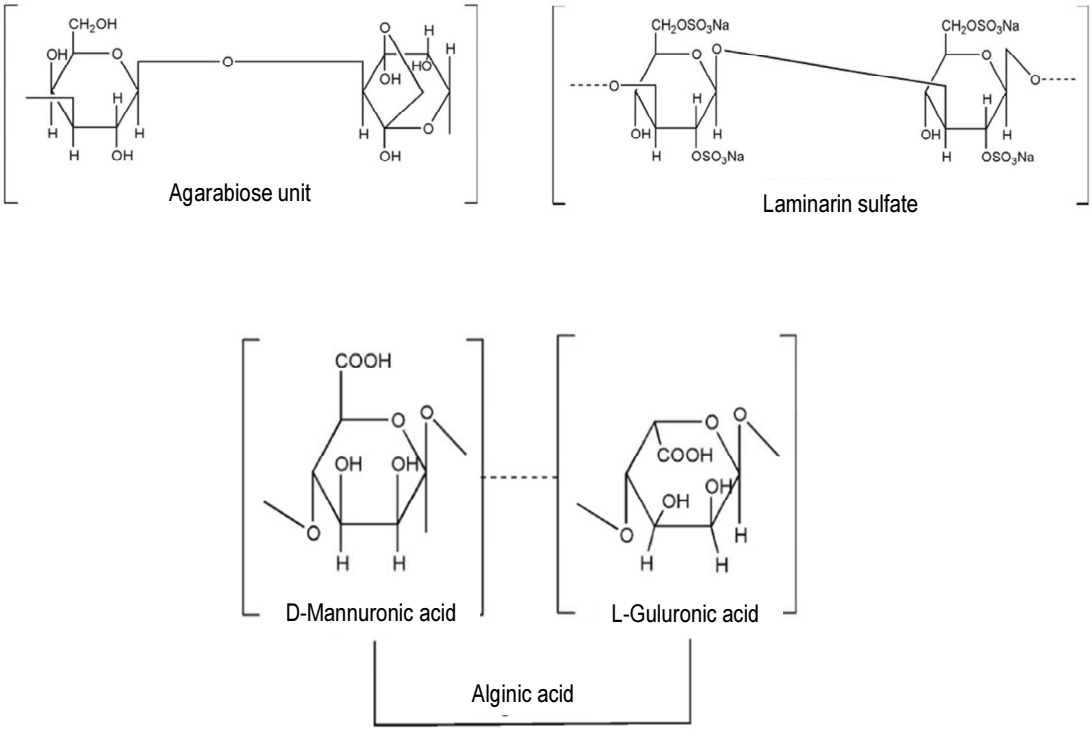
Table 1-2 Gross chemical composition of micro- and macroalgae

Type	Species	Proteins [%, db]	Carbohydrates [%, db]	Lipids [%, db]	Ref
Microalgae	*Chlamydomonas reinhardtii*	48	17	21	[44–46]
	Chlorella vulgaris	51-58	12-17	14-22	
	Porphyridium cruentum	28-39	40-57	9-14	
Macroalgae	*Ulva lactuca*	26-28	59-63	0-1	[47], [48]
	Durvillaea antarctica	10-12	57-72	1-4	

Table 1-3 Biochemical characteristics of main alga divisions [39]

Division	Cell wall	Storage product	Pigments
Chlorophyta (Green algae)	- Cellulose - Hemicelluloses (xylans, mannans) - Various heteropolysaccharides (partly sulphated) - Hydroxyproline rich glycosides - Calcified in some - Wall absent in some	- Starch (20-30 % amylose/ 70-80 % amylopectin) - Oil in some	- Chlorophyll *a* and *b* - *α*-, *β*-, and *γ*-carotenes - Xanthophylls
Rhodophyta (Red algae)	- Cellulose - Galactans - Carrageenan - Agar	- Floridean starch	- Chlorophyll *a* - Phycobilins - *α*- and *β*-carotene - Xanthophylls
Phaeophyta (Brown algae)	- Cellulose - Alginic acid - Fucoidan - Sulphated polysaccharides	- Laminarin (partly sulphated) - Mannitol	- Chlorophyll *a* and *c* - *β*- carotene - Xanthophylls

Figure 1-2 Algal polysaccharides [43]



1.5.1.1. Chlorophyta

Green algae are the major group of algae regarding abundance of species, varieties and frequency of occurrence. Benthic and planktonic species belong to this division, whereof ca. 90 % are freshwater species living in habitats ranging from oligotrophic to supersaturated waters [39], [49].

Generally, green algae contain the pigment chlorophyll *a* and *b* and manifold organisation types of the plant body exist, including unicellular, colonial and filamentous forms. Characteristic cell wall components are heteropolysaccharides containing galactose, arabinose, xylose and rhamnose being partly sulphated at the positions of some hydroxyl groups of the sugar units [50].

Comparable to terrestrial plants, Chlorophyta synthesizes xylans, containing chains of β-(1→3)-linked xylose units forming a triple helix providing a strong fibrous structure. In addition, β-(1→4)-linked mannans build a crystalline skeletal component acting as a structural polysaccharide [50]. Some green algae species are cell wall-less, some contain silica or proteins. The formation of starch as a energy product within the chloroplasts instead of the cytoplasm is unique to this division of algae [49].

1.5.1.2. Rhodophyta

Micro- and macroalgae species are members of this division, mainly living in marine seawaters at all latitudes, where as the majority of seaweeds, about 4000 species, belong to this division [49]. Characteristic is the presence of accessory photosynthetic pigments such as phycobilins, often masking the presence of chlorophyll *a*. Floridean starch, the typical energy storage product of red algal species is located outside the chloroplast being a long chain of glucose sugars α-(1→4)-linked glucans, exhibiting a similar structure to amylopectin of higher plants. Additionally, reserve products such as glycosides, floridosides, mannitol and sorbitol have been found which may account for up to 10 % of the tissue dry weight in some thalli [39], [49].

Cell walls of red macroalgae consist of an inner rigid layer made of randomly arranged cellulose forming microfibrils, and an outer amorphous mucilage layer consisting of carrageenan and agar, depending on the species. These are high molecular weight polysaccharides consisting of repeating sulphated galactose and 3,6-anhydrogalactose units accounting up to 70 % of the dry weight of the cell wall [39], [49]. In addition, carbohydrate residues such as xylose, glucose and uronic acids next to substituents

such as methyl ethers are present [51], [52]. Red microalgae species lack the cellulose structure while mainly exhibiting cells wall of sulphated polysaccharides.

1.5.1.3. Phaeophyta

Brown algae species exhibiting the greatest diversity in terms of species and occur almost exclusively in marine waters. Widely spread through the northern and southern hemisphere brown algae inhabiting rocky shores, sometimes until depths of 270 m in clear tropical waters [39]. The characteristic brown coloration evolves from the carotenoid fucoxanthin being the main pigment next to chlorophyll *a*, *b* and β-carotene [39], [49]. Similar, as Rhodophyta, the cell wall structure is composed of an inner cellulosic layer and an outer gummy layer matrix.

Amorphous matrix structures consist of alginic acid, a polymer of carbon acids, containing β-(1→4)-linked mannuronic acid, adjacent to blocks of in average 20 units of α-(1→4)-linked glucuronic acids. This polymer is localised in cell walls and intercellular spaces and accounts for up to 24 % of the dry weight of the algal cell. The proportions of the uronic acids vary depending on the species, with mannuronic acid ranging between 30-97 % [39].

Furthermore, “Fucans” (also known as fucoidin, fucoidan and sargassan), sulphated polysaccharides containing varying proportions of fucose, galactose, mannose, xylose, glucuronic acid and mannuronic acid are contained in Phaeophyta [50], [53], [54]. The general function of fucans is the reduction of desiccation effects during exposure of the organism. The energy storage compound laminarin (also known as laminaran), mainly constituted of β-(1→3)-linked glucans, with a variable degree of β-(1→6)-linkages accounts for up to 34 % of the algal cell dry weight [39], [50], [55].

1.5.2. Large-scale production and applications

Due to the knowledge of valuable products contained in micro- and macroalgae species, large-scale cultivation is carried since many decades. Today, particular high quality oils consisting of omega-3 and omega-6 fatty acids are gaining market prices of >10U$ kg^{-1} [56] and are used in food supplements.

Favourite high value compounds contained in algal materials are high quality fatty acids/oils. In addition, algal cells also contain valuable pigments such as carotenes,

proteins and starch. These compounds are used for a broad spectrum of biotechnological applications, such as food and feed production, production of chemicals for cosmetics and pharmaceuticals. The following sections present a short overview of the current state of micro- and macroalgae cultivation and biotechnological applications.

1.5.2.1. Microalgae

Mass culture of the microalgae species *Chlorella* sp. and *Arthrospira* sp. to produce high valuable products such as health food, or to extract pigments such as β-carotene or astaxanthin from *Dunaliella salina* and *Haematococcus pluvalis* is carried out for more than 40 years [57]. To produce large amounts of biomass, these extremophile[1] organisms are cultivated outdoors in open air ponds, as shown in Figure 1-3. In some cases artificial mixing via paddle wheels or rotating mixing arms for oxygen and nutrient transfers and well as moving algal cells through the illuminated zone is provided. Ponds with shallow water depths of 0.2-0.3 m and sizes of 1- 250 ha, containing up to 300.000 litres are common. In Australia *Dunaliella salina* production in open ponds containing 900.000 litres is reported. Nowadays, microalgae cultivation is a key process providing a direct source of nutrition in marine fish and shellfish aquaculture, usually performed on a much smaller scale, applying 20-40 litre containers or plastic bags and is carried out indoors in many cases [57–59].

The type of species to produce a desired product decides the factors which need to be taken into account for cultivation. Growth requirements and the biology of the species impacts on costs for land, labour, energy, water and nutrients, and hence for the overall economical outcome. Generally, outdoor cultivation in open ponds are characterised by poor light efficiency and temperature control resulting in low biomass productivity. In addition, large water losses due to evaporation are an issue. Furthermore, contaminations with other species and/or bacteria are issues, which might harm the productivity of the culture [59].

In recent years, the cultivation in closed systems has gained increased attentions due to the opportunity of concerted control of growth conditions, such as illumination, temperature, agitation, O_2 and CO_2 concentration and control of contamination. It is considered that the productivity, i.e. a higher biomass production is reached by cultivating in closed systems, For this, several types of photo bioreactors, including

[1] adapted to extreme habitats (i.e. highly saline), thereby eliminating competitors [57]

tubular and flat panel reactors were developed [60], as shown in Figure 1-4. Highly controllable growth conditions are of interest to influence the chemical compositions of the organisms by manipulating the growth conditions. Studies revealed that some organisms grown under suboptimal conditions produce larger amounts of substances like pigments, starch and oil. For example, excess of light stimulates the production of carotenes in the cell of some organisms. Alternatively, if the supply of nitrogen in the growth medium is limited, some algae start to accumulate oil [61].

However, because of high capital costs, difficult scale up and high energy costs due to artificial lighting when photo bioreactors are operated indoors, no commercial applications are available at present. Although, commercial microalgae culture to produce value added products is a well established industry, mainly based on outdoor cultivation. At present, more than 5000 tonnes of dry biomass are produced annually [61]. A comprehensive review of commercial production and applications of microalgae materials was presented by Spolaore et al. [62] and a summary is presented in Table 1-4. Furthermore, many research and pilot scale cultivation units are in operation worldwide, however only the commercial significant ones are presented.

Figure 1-3 Open pond cultivation of microalgae [63]

Figure 1-4 Tubular bioreactor for microalgae cultivation

Photograph by Katharina Kebelmann at Bangor University, United Kingdom

Table 1-4 Commercial microalgae production and applications [57], [62]

Species	Annual Production	Producer country	Application/ product
Arthrospira sp.	3000 t dry weight	China, India, USA, Myanmar, Japan	Human and animal nutrition Cosmetics Phycobiliproteins
Chlorella sp.	2000 t dry weight	Taiwan, Germany, Japan	Human nutrition Aquaculture Cosmetics
Dunaliella salina	1200 t dry weight	Australia, Israel, USA, China	Human nutrition Cosmetics β-carotene
Aphanizomenon flos-aquae	500 t dry weight	USA	Human nutrition
Haematococcus pluvalis	300 t dry weight	USA, India, Israel	Aquaculture Astaxanthin
Crypthecodinium cohnii	240 t DHA oil[2]	USA	DHA oil
Shizochytrium sp.	10 t DHA oil	USA	DHA oil

[2] Docosahexaenoic acid, an omega 3- fatty acid

1.5.2.2. Macroalgae

Coming from hundreds of years of traditional seaweed cultivation for food and fodder, in the 1950s large scale cultivation of seaweed biomass was developed. Overall, approximately 15.8 million tonnes of fresh macroalgae biomass were produced in 2008, from species of about 10 genera, including *Laminaria sp.*, *Monostroma sp.* and *Gracilaria sp.* Around 90 % of the production takes place in East Asia, including China, Korea, Japan and Philippines [64], [65].

The first step in seaweed cultivation is the seedstock cultivation, i.e. the production of juvenile plants. From there, adult plant cultivation starts, followed by harvesting and processing the materials into the desired commercial products. Traditionally, different ways of cultivation exist, such as land-based cultivation, indoors and outdoors conducted in tanks and ponds. Furthermore, shallow sea cultivation in intertidal or subtidal zones, either with floating or fixed supporting systems along the coastlines is common [66]. Overall, the commercial production of seaweeds for products such as human and animal feed, chemicals and pharmaceuticals is successful.

Main products are hydrocolloids, applied as gelling agents, texturisers, emulsifiers and stabilisers in human nutrition, pharmaceutics and cell culture in biology [67]. Production values of carrageenan, alginate and agar, as shown in Table 1-5 [68].

However, the application of a little number of species, limited production and cultivation area as well as no applications of genetically improved strains is criticised. Furthermore, problems in seedstock cultivation affecting the overall production and large scale seaweed cultivation is still under development [66].

Table 1-5 Production of major polysaccharides of macroalgae [68]

Product (Division)	**Production (t y $^{-1}$)**	**Algae harvested (t y $^{-1}$)**	**Value (Mio US$)**	**Species**
Carrageenan (Rhodophyta)	33.000	168.400	240	*Euchema sp.* *Kappaphycus sp.*
Alginate (Phaeophyta)	30.000	126.500	213	*Laminaria sp.* *Macrocystis sp.* *Lessonia sp.* *Ascophyllum sp.*
Agar (Rhodophyta)	7.630	55.650	137	*Gelidium sp.* *Gracilaria sp.*

1.5.3. Bioenergy applications

Additionally, to the production of high value products, many raw materials contained in algae biomass offer promising routes for sustainable and renewable bioenergy generation. Although various conversion routes for bioenergy generation are investigated and technically feasible, it is widely agreed, that the application of microalgae biomass is not economically feasible for alternative fuels, at present. Even though, prices for crude oils and from there derived fuels and products are increasing steadily, increasing prices of fertilizers, intense and ineffective harvesting technologies and low productivity when grown using natural light are still major limitations [69].

However, it is expected that more efficient and less energy consuming harvesting techniques and depletion of fossil resources enhance the commercial viability of microalgae biomass for bioenergy applications [70].

The combination of the production of value-added products and bioenergy generation in integrated systems, called biorefineries, is seen as a potential route to increase the economical feasibility and a recent review of concepts is presented by Gouveia [71].

In summary, most of the concepts utilize one or more conversion technologies to generate bioenergy and approaches to obtain high valuable co-products such as oils, polyunsaturated fatty acids (PUFAs) and pigments [71]. Some of the concepts include microalgae farming and consider the capture of CO_2 from emissions of the process or close by industrial plants [72]. However, most of the concepts utilize conversion technologies, which are dedicated to produce a specific product, i.e. biodiesel from algal derived lipids, or biogas from polysaccharides.

In contrast, intermediate pyrolysis, utilized as the main conversion technology of the BtVB-process, offers the application of a broader range of feedstocks. This gives certain flexibility, for the choice of feedstock, depending on availability. Particular, pyrolysing residues after extraction of raw material, such as lipids or proteins, is a promising option. In addition, the by-product biochar gained by intermediate pyrolysis of biomass, containing valuable inorganic materials, such as nitrogen, potassium and phosphorous can be reused as alternative fertilizer to decrease the production costs of microalgae cultivation.

The following two sections along with Figure 1-5 present an overview of currently investigated bio-chemical and thermo-chemical conversion routes to generate fuels, power and heat.

1.5.3.1. Bio-chemical conversion

The most intensively studied route is the production of biodiesel from extracted triglycerides of algal biomass via transesterification [73], [74] similar to the process normally applied for oleaginous, edible crops such as rapeseed, soybean, sunflower and palm. Favourably applied are algal species with high lipid contents, where the extracted triglycerides are converted to fatty acid methyl esters (FAMEs) and glycerol as a by-product [11]. Most of the background work was carried out at US National Renewable Energy Laboratory (NREL) between 1970s to 1990s [74]. In 1996 the main conclusion was that it is not economically viable to produce biodiesel from microalgae, due to the price being twice as high as the price of a similar quantity of petroleum diesel. Today, where the prices of fossil derived diesel have almost doubled, the feasibility of this technology is tested in pilot plants and industrial scale plants are still under development [14], [19], [69].

High starch content, polysaccharide based cell walls and the lack of lignin in micro- and macroalgal biomass makes them attractive for conversion into bioethanol via fermentation [19]. During the 1st generation of bio-fuels food crops such as sugar cane, sugar beet and corn were applied. Nowadays residual woody materials are favoured, but the lignin needs to be removed before as it is not digested by the microorganisms [11], [75]. The process includes hydrolysis of cellulose, hemicellulose and starch to monomeric sugars, which are subsequently metabolized into ethanol by yeast (i.e. *Saccharomyces cerevisiae*) [71]. After distillation and dehydration a biofuel is available, that can replace parts of fossil fuels.

An option to recover chemical bound energy from whole cells of algal biomass is biogas production via anaerobic digestion. Advantageously, this technology utilises wet biomass and the gas, consisting mainly of methane, can be directly combusted to produce heat and/or electricity or is upgraded to replace natural gas [19]. Being a well developed technology with commercial applications converting sewage sludge, animal wastes and industrial effluents, processing of algal biomass is still under development [11], [14]. Some macroalgae species have been tested successfully by anaerobic digestion whereas some microalgae species contain indigestible cell walls and a pre treatment to break them is necessary. The major limitation of anaerobic digestion is the usually incomplete conversion of the material and slow process rates compared to those of other processes.

Hydrogen is currently produced by steam reformation of fossil fuels, or large-scale electrolysis of water, but the production requires more energy than generated from the gained hydrogen [19]. Alternatively, bio-hydrogen can be produced by direct photolysis

of photosynthetically active algal organisms, by splitting water into O_2 and H_2 enzymatically. Biohydrogen offers opportunities in reducing GHG emissions in the transport sector, as it produces only water as exhaust product and no NO_x emissions when used in a fuel cell [19]. For commercial applications, storage (compressed, liquefied or stored in metal hydrides) and transportation are main drawbacks. Furthermore, no commercial fuel cells are available and more knowledge of the hydrogen production of the algal organisms is necessary [11].

1.5.3.2. Thermo-chemical conversion

Thermo-chemical routes investigated to recover energy stored in algal biomass include gasification, hydrothermal processing (thermo-liquefaction/ hydro-thermal upgrading (HTU)) and pyrolysis [11], [76]. Gasification and pyrolysis require feedstocks with a moisture content at least < 50 % and drying before processing needs to be taken into account, where as hydrothermal processing utilize biomass with higher moisture contents [77].

During gasification, biomass releases energy by partial oxidation at high temperatures in the range of 800-900 °C. Generated are combustible gases, called synthetic gas, with medium calorific values, consisting of carbon monoxide (CO), hydrogen (H_2), and methane (CH_4) which is further processed to i.e. liquid hydrocarbons and methanol [8], [11], [78]. Hydrothermal processing, sometimes called catalytic gasification, applies the entire biomass with a moisture content of 80-90 %. The process is considered as a low temperature gasification reaction (350 °C) taking place at high-pressures (20 MPa). The process is essentially steam reforming as there is no oxidizer or reagent other than water. The high moisture containing biomass, commonly combined with metal catalysts, is converted into gas consisting of CH_4 and CO_2 [79]. Laboratory scale experiments were carried out with algal biomass and a reaction mixture of medium heating value syngas, liquids and tar were obtained. However processing difficulties have been caused by biomass components and corrosive effects of water in the reactors and no commercial applications are available in the short-term [79].

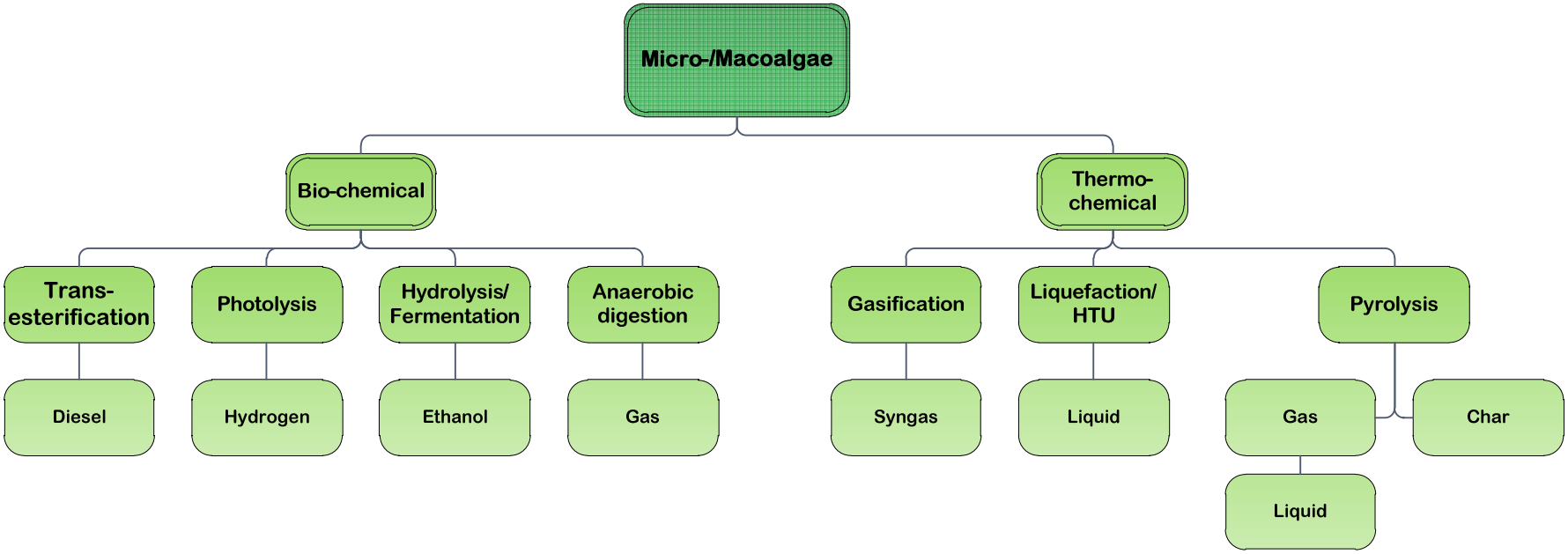
One of the most promising routes to generate bioenergy from various types of biomass is the thermo-chemical conversion via pyrolysis. Advantageously, pyrolysis produces gaseous, liquid and solid fuels in short reaction times such as minutes, efficiently [80], [81]. For further process details of pyrolysis see previous sections 1.3 and 1.4.

Overall, extensive studies have been carried out utilizing a wide variety of biomass resources, where as lignocellulosic feedstocks are the most investigated materials [11]. Furthermore, vast studies investigating fundamental pyrolytic characteristics of lignocellulosic biomass constituents are available.

Even though, the advantages of the applications of algal biomass over lignocellulosic biomass for bioenergy production are evident, only little work has been done on processing of micro- and macro algae via pyrolysis. In addition, the pyrolytic characteristics of the main chemical constitutes in algal biomass affecting the overall performance of the materials are studied rarely.

Currently, intensive investigations of intermediate pyrolysis applications of algae biomass are carried out at Aston University. For further developments of the BtVB-process, it is of interest to evaluate potential pathways for bioenergy generation from algal materials.

Figure 1-5 Bioenergy generation from algae



1.5.4. Analytical thermo-chemical characterisation

The following sections review reported studies of the thermo-chemical behaviour carried out at laboratory scale (analytical technologies), including thermogravimetry and by analytical pyrolysis of aquatic biomass. An overview of laboratory equipment applied by other researchers is enclosed.

1.5.4.1. Thermogravimetry

A major thermal analysis technique to investigate physical changes of biomass during pyrolysis is thermogravimetry analysis (TGA). The obtained data from analytical scale experiments provides useful information in terms of degradation temperatures, thermal stability, reaction mechanisms and compositional analysis. Generally, the weightloss of the material during a controlled heating as a function of temperature or time is recorded, expressed in a TGA curve. Additionally, a differential thermogravimetric (DTG) curve expresses the rate of weightloss over time or temperature during the process. Moreover, kinetic data such as activation energy and constants is obtained via TGA; and a few studies present data of algae biomass [82], [83]. However, this is not of interest in this research.

Virtually any biomass material which is applied for thermo-chemical conversion processes has been tested for thermo-chemical characteristics by TGA. Particular terrestrial biomass and its constituents (cellulose, hemicellulose and lignin) are investigated intensively [7], [84–89].

Some TGA studies of aquatic biomass are reported, studying the pyrolytic behaviour under various conditions including microalgae (i.e. *Chlorella sp., Dunaliella sp., Spirulina sp.* and *Nannochloropsis sp.)* and macroalgae species (i.e. *Fucus sp., Laminaria sp.* and *Ulva sp.*). Usually, sample sizes of 2-10 mg were pyrolysed in He or N_2 atmosphere while maintaining constant flow rates between 50 and 200 ml min^{-1}. Non-isothermal temperature programs with an initial temperature ranging from ambient temperature up to 100 °C, heating rates of 5-80 °C min^{-1} up to final temperatures between 500 and 900 °C were applied. An overview of pyrolysis temperatures obtained by various biomass materials, (aquatic, terrestrial and chemical constituents) is listed in Table 1-6. Overall the studies revealed that the temperature of the main pyrolytic activity varies with the applied heating rate [17], [32], [81], [90–97]. Furthermore,

aquatic derived feedstocks exhibit lower pyrolysis temperatures than typically derived from terrestrial biomass. This is mainly caused by the lack of high cellulose and/or lignin contents [32] and supports the potential application of aquatic materials for pyrolysis.

So far, only a few studies made first attempts to pyrolyse chemical constituents from algal biomass. Included are main carbohydrates of brown algal biomass (alginic acid, mannitol, laminarin and fucoidan) analysed with a heating rate of 25 °C min^{-1} [98]. Furthermore, early studies investigated the contribution of lipids in *Chlorella protothecoides* and *Nannochloropsis sp.* during pyrolysis [92], [99].

To expand information regarding the chemical composition of evolved products during pyrolysis of algal biomass, TGA has been coupled to a Fourier transform infrared spectroscopy (TGA/FTIR) and to a mass-spectrometer (TGA/MS) via a heated transfer line [99]. A large range of functional groups including C-H (i.e. corresponding to methylene groups) C=O-bonds (carbonylic compounds, such as aldehydes and acids) and C-O-C-bonds (ether compounds) were detected in pyrolysates of *Nannochloropsis sp.* and derived lipids. Overall, the analysis of evolved products by TGA/FTIR revealed differences between the materials, but provided only general information about the product formation during pyrolysis [99]. The study showed, that this coupled technique offers the opportunity to detect evolved gases by on-line analysis; however, the identification is limited towards functional groups only and does not provide any further details about volatile products. Another study investigated evolved products of *Nannochloropsis gaditana* by TGA coupled to a mass-spectrometer (TGA/MS). By on-line analysis, the identification of volatile products including H_2O, CH_4, CO, CO_2 and H_2 was possible [100]. Again, this technique has restrictions as only searches for certain fragment masses can be performed and no separation of the pyrolysates is provided.

Table 1-6 Main pyrolysis temperatures of biomass by TGA

Biomass material	Origin	Heating Rate [°C min^{-1}]	Main pyrolysis [°C]	Ref
Chlorella protothecoides	Microalgae (Chlorophyta)	15	330	[90]
		80	340	
Spirulina platensis		15	330	
		80	360	
Laminaria japonica	Macroalgae (Phaeophyta)	10	250	[101]
		30	266	
Alginic acid	Carbohydrate (Phaeophyta)	25	225	[98]
Mannitol		25	336	
Laminarin		25	342	
Fucoidan		25	202	
Oak	Terrestrial	5	350	[102]
		20	370	
Hemicellulose	Terrestrial Polysaccharide	10	260	[88]
Cellulose	Terrestrial Monosaccharide	10	355	
Lignin	Terrestrial Polyphenol	10	380	

1.5.4.2. Analytical Pyrolysis

Analytical pyrolysis is a well established laboratory technique to analyse solid and liquid samples which are difficult to characterise chemically in intact condition, as they may not be volatile, have low solubility in most solvents and may decompose easily during heating.

During analytical pyrolysis, the chemical degradation of the sample under highly controlled heat influence in inert atmosphere is enforced. This produces smaller molecules by decomposition and elimination reactions which can subsequently be detected by i.e. GC or GC/MS. Advantages of no required sample preparation such as extraction procedures along with the possibility of applying small sample amounts (µg-mg) makes this technique essential in fields of polymer chemistry (synthetic and natural), soil and environmental chemistry (analysis of fossil materials), microbiology (taxonomy of microorganisms), food and wood science as well as clinical and forensic science [103–105]. Comprehensive textbooks, literature reviews and studies are reporting available technologies, presenting applications, process parameters and results [20], [104–108]. The very same technology is applied to analyse the transformation of biomass into other forms of organic substances to produce fuel or raw chemical materials.

Analytical pyrolysis techniques to investigate biomass derived feedstocks include a micro-pyrolysis unit (Py) coupled to gas-chromatography (Py-GC) or gas-chromatography/ mass-spectrometry (Py-GC/MS) [109].

In contrast, to relative similar constructed thermogravimetric analyser, providing inter-laboratory comparable results, different designed pyrolysis units are available and subsequently providing different pyrolysis conditions which complicate inter-laboratory comparisons. Generally, pyrolyser are classified by their source of heating which provides the thermal energy to pyrolyse the sample and each design has specific characteristics as presented in Table 1-7 [107], [108]. Depending on the heating element, each pyrolysis unit performs a certain type of pyrolysis (flash, fast, intermediate and slow). In all cases, the temperature control of the pyrolysis unit is the main concern to produce reproducible results. Main parameters to control the temperature environment of a pyrolysis unit are the equilibrium temperature (T_{eq}), i.e. the final pyrolysis temperature and the temperature rise time (TRT), expressing the time, required to reach T_{eq}.

In a Curie-point analyser the sample is placed onto a ferromagnetic metal, which is heated by an inductive current. The T_{eq} of the wire and of the sample respectively is determined by the Curie-point (change from ferro- to para magnetism) of the applied

metal or alloy. When the temperature is reached, the heating process implied by the electrical current stops immediately. Curie-point metals are available for temperatures ranging from ca. 350-1100 °C. The heating of the sample is realized in a short TRT, commonly between 10-100 ms.

As the electric current is induced into the filament by means of a high-frequency coil, it is necessary that the coil provides enough power to permit heating of the wire until the specific Curie-Point temperature is reached. Therefore, reproducibility depends on the accuracy of the alloy, the power of the coil and the placement of the wire in the system. Advantages of the Curie-point analyser are simple sample handling, and reproducible T_{eq} when using wires of the same manufacturer. However, due to the lack of TRT control, Curie-point analysis is a tool for flash pyrolysis applications only. In addition, for comparability studies of different T_{eq}, different alloys for each experiment are required [107].

The resistively heated filament pyrolyser provides the energy by a controlled current which is passed through the filament. Similar to the Curie-point analyser, very thin films of samples are heated from ambient temperature to T_{eq} at a very short TRT. Filaments are made of materials with high electrical resistance, such as iron and platinum, but are not self-limiting in terms of $T_{eq.}$ Therefore an exact control of the filament current is essential for temperature accuracy and reproducibility [108]. In contrast to the Curie-point pyrolyser this analyser has the capability to maintain a linear heating rate, achieved by a linear increasing voltage. Disadvantages of this analyser are non-uniformly heating over the length of the filament which causes irreproducible results if the sample is not placed in the same point at each measurement [20]. Furthermore, both types of filament pyrolyser provide a rapid heating of the sample by applying very small filaments. Consequently small sample sizes are used to be compatible to the mass of the filament [20], [108].

Another common type of analytical pyrolysis units is the furnace type reactor, where a pyrolysis zone is electrically heated externally by the furnace walls. The control of the temperature is done by using thermocouples for maintaining the correct furnace temperature. Generally, furnace pyrolyser can be used for isothermal and non-isothermal experiments. In contrast to filament pyrolyser, furnaces have the capability to provide T_{eq} already before the sample is introduced. In this case, the sample is hold in a cold zone above the heated pyrolysis zone and is subsequently dropped into it. Due to no contact of the sample to the heated wall of the furnace directly, slower TRT are achieved than by filament pyrolysers. However, when the sample is dropped into the already heated furnace a direct measurement of the TRT is not possible. In

contrast to filament analyser, larger sample amount up to milligrams can be applied by furnace pyrolyser [20], [107], [108].

In terms of terrestrial and aquatic derived biomass characterisation, Py-GC/MS is the most common analysis technique as it provides an efficient separation of the pyrolysates and extra sensitivity and specificity toward identification of compounds through ion fragmentation. Additionally, Py-GC is applied in some studies mostly being equipped with a flame ionization detector (FID). Similar to Py-GC/MS this technique is able to separate isomers in complex mixtures and additionally obtains reproducible quantitative results of pyrolysis products. However, reference compounds for the identification by retention times and calibration are necessary [107].
For an efficient separation of biomass pyrolysates the most common applied capillary columns (30-60 m length, 0.25-1 µm film thickness, iD 0.25-0.32 mm) are presented in Table 1-8. Typically applied GC temperature programs cover a broad range of temperatures for efficient separation of the complex pyrolysate mixtures. Usually helium is applied as a carrier gas and an initial temperature of 20-50 °C is kept for 1-3 min, heated with 3-10 °C min^{-1} to a final temperature of 250-320 °C (depending on max. column temperature) held for 2 to 40 min [110–112]. Furthermore, in most of the studies, electron-impact mass spectrometers were applied, operating with 70 eV performing 1-2 scans s^{-1} in the range of 20-700 atomic mass unit (amu). In most cases gained mass-spectra are interpreted by applying mass-spectral libraries. Some studies employ reference compounds, which add additional complexity as they need to be analysed at the same instrument with very same parameters for reference time and mass-spectral comparison.

Table 1-7 Analytical pyrolysis units [107], [108]

Pyrolyser	Heating	T_{eq} [°C]	TRT	Heating rates	Pyrolysis mode	Sample amount	Characteristics	Model Manufacturer
Curie-point analyser	ferromagnetic wires	350-1100	ms	None	Flash	<µg	- easy sample handling - rapid heating - no temperature programs - possible catalytic effects by metal wires	GSG Pyromat GSG-Meß-und Analysengeräte Vertriebsgesellschaft mbH
Filament analyser	iron or platinum filament	1-1400	ms	0.01 °C min^{-1} to 1000 °C s^{-1}	Flash to slow	<µg	- broad temperature range - isothermal and programmed heating - complex temperature control	Pyroprobe 5200 CDS Analytical Ltd./USA
Micro furnace	heated furnace wall	40-800	s-min	1-100 °C min^{-1}	Fast to slow	mg	- T_{eq} stability of +/-1 °C - isothermal and programmed heating - no measurement of TRT	Pyrolyser Py-2020iD Frontier Laboratories/Japan

Table 1-8 Capillary separation columns for Py-GC/MS

Column name	Stationary phase	Polarity	Ref
DB 5 (BPX5)	5% Phenyl 95% Dimethylpolysiloxan	Low polar	[110]
DB 1701 (NB 1701, RTX 1701)	14% Cyanopropyl-phenyl 86% Dimethylpolysiloxan	Low- mid polar	[32], [98], [111], [113–117]
DB 1 (CP Sil 5)	100% Dimethylpolysiloxan	Non polar	[118], [119]

Analytical pyrolysis of aquatic biomass has been applied in various research fields, such as marine biology, microbiology, geochemistry and water research. Mainly flash and fast pyrolysis methodologies were applied, utilizing Curie-point analysers and filament pyrolyser coupled to GC or GC/MS. Consequently, very short TRT to reach T_{eq} were characteristic for these studies [112], [120], [121].

In general, obtained pyrograms indicated that anhydrosugars, furans and carbonyl compounds are carbohydrate (cellulose, hemicellulose) and nitrogen containing products (pyrroles, indoles and nitriles) are mostly protein derived [120], [121].

Early studies by Gelin et al. [118], [119] analysed lipids isolated from the green microalga *Botryococcus braunii* by flash pyrolysis at T_{eq}= 700 °C to reveal their role during the formation of kerogenes (crude oil precursors). Pyrolysates consisted mainly of homologues series of aliphatic hydrocarbons, several saturated and unsaturated aldehydes as well as saturated primary alcohols. Furthermore fatty acids (palmitic acid, oleic acid) phthalates and squalene were observed. However, the applied technique revealed difficulties, such as evaporation of products prior to pyrolysis, condensation of pyrolysis products inside the pyrolyser, not completed pyrolysis and transfer of pyrolysates onto the GC column [118].

Early studies investigated polysaccharide constituents of red algae including agar extracted from *Gracilaria tikvahiae* and carrageenan extracted from *Eucheuma spinosum* by Py-GC and Py-GC/MS at T_{eq}= 750°C. Major polysaccharide derived pyrolysis compounds were furans (2-methylfuran, 5-methyl-2-furaldehyde, 5-(hydroxymethyl)-2-furaldehyde) and anhydrosugars (1,4-anhydro-6-O-methyl-galactopyranose; 1,6-anhydro-*β*-D-galactopyranose) [113], [114].

At present, only little studies investigated algal biomass in terms of pyrolysis applications where as terrestrial biomass and its constituents have been investigated mainly [20], [122], [123]. Some brown macroalgae species and their polysaccharide constituents were analysed by flash pyrolysis with Py-GC/MS (CDS Pyroprobe, heating rate 20 °C ms^{-1}, T_{eq}= 500 °C) [32], [98], [115–117]. Compared to terrestrial derived pyrolysates it was revealed that macroalgae produce increased amounts of furans, nitrogen containing compounds, linear chain alcohols and less methoxyphenols. Generally, the high proportion of furans instead of phenols is seen as an advantage for the quality of bio-fuels, as furans are more easily hydro-deoxygenated [32].

The influence of inorganic portions on the chemical compounds in pyrolysates contained in macroalgae was investigated by hydrochloric acid washing of biomass of *Laminaria hyperborea* and *Fucus vesiculosus.* Up to 98 % of Na, K, Ca and Mg were removed from the biomass. However, Py-GC/MS revealed predominant changes in the

polysaccharide composition of the brown algae by pyrolysis product distributions (significant amounts of fucoidan, mannitol and laminarin have been removed) instead of changes in product formation caused by reducing the metal content [115].

Particular, Py-GC/MS studies of microalgae species in regard to bio-fuel production are rare. Recently, a study was conducted comparing *Botryococcus braunii* and *Chlamydomonas reinhardtii* at a T_{eq}= 600°C, applying a micro-furnace Double-shot pyrolyser Py-2020iD manufactured by Frontier Laboratories Ltd./Japan. Pyrolysis products such as fatty acids, their methyl esters, terpenoids, sterols, aromatics and alkanes were identified [110]. However, the temperature is too high to be applicable for intermediate pyrolysis studies.
Overall, Table 1-9 presents a summary of TGA and Py-GC/MS conducted studies along with applied micro- and macro algal species.

Table 1-9 Summary analytical studies of micro- and macroalgae

Type	Species	TGA Heating rates [°C min^{-1}] Temperature range [°C]	Py-GC/MS	Ref
Microalgae	*Chlorella protothecoides* *Spirulina platensis*	15, 40, 60, 80 ambient to 800	-	[90], [92]
	Dunaliella tertiolecta	5, 10, 20, 40 ambient to 900	-	[81]
	Chlorella vulgaris	15, 30, 40, 50 ambient to 800	-	[93]
	Nannochloropsis gaditana	5, 15, 40 40 to 900	-	[100]
	Botryococcus braunii *Chlamydomonas reinhardtii*	-	Frontier Laboratory Single-shot Pyrolyser 2020, T_{eq}= 600 °C, for 10 s	[110]
	Botryococcus braunii (lipids)	-	Curie-point pyrolyser T_{eq}= 610 °C and 779 °C, for 10 s	[118], [119]
Macroalgae	*Porphyra yezoensis* *Plocamium telfairiae* *Corallina pilulifera*	10, 30, 50 ambient to 800	-	[94]
	Laminaria digitata *Laminaria hyperborea* *Fucus vesiculosus*	25 40 to 900	CDS 5000, 20 °C ms^{-1}, T_{eq}= 500 °C	[32], [115], [117]
	Enteromorpha prolifera	10, 20, 30, 40, 50 50-700	-	[17]
	Grateloupia filicina *Ulva lactua* *Dictyopteris divaricata*	10, 20, 30, 50, 80 ambient to 700	-	[95]
	Ulva pertusa	10, 30, 50 ambient to 800	-	[96]
	Laminaria japonica *Sargassum pallidum*	10, 30, 50 ambient to 800	-	[101]

1.6. Conclusion

Bioenergy generation from aquatic derived feedstocks via intermediate pyrolysis have a large potential to substitute fuels, power and heat derived from fossil fuel resources.
Main advantages over terrestrial derived feedstocks are lesser impacts on food supply chains and the environment. In contrast to the lignocellulosic structure of terrestrial materials, algal biomass consists of proteins, carbohydrates and lipids, mainly. Particular the lack of lignin makes algal biomass an interesting resource for thermo-chemical processing, whereas a formation of less high molecular compounds and lower decomposition temperatures during pyrolysis are expected.
Two major laboratory techniques are typically applied for thermo-chemical characterisation of biomass feedstock, namely TGA and Py-GC/MS. Both analytical methods provide useful information regarding thermal decomposition and product evolution during pyrolysis processing. Whereas extensive studies about lignocellulosic materials and constituents, such as hemicellulose, cellulose and lignin are reported, only minor are found about micro- and macroalgae materials. Particular, the analysis of algal derived constituents is scarce. Beyond that, the majority of available studies are not applicable to intermediate pyrolysis process conditions, as conducted by either Curie-point or heated filament analysers (flash or fast pyrolysis).

To evaluate potential applications of algal materials in the BtVB-process a need for thermo-chemical characterisation of micro- and macroalgae as well as extracted chemical constituents conditions is seen.
Therefore, a choice of analytical equipment and/or methodologies has to be made, providing process conditions differing from reported studies while addressing intermediate pyrolysis conditions. Consequently, the author chose to install a Py-GC/MS unit which is capable of providing intermediate pyrolysis conditions to carry out fundamental research of algal biomass and from there derived materials. In addition, it is reported, that algal derived pyrolysates contain higher amounts of nitrogen containing compounds. Therefore, a secondary application of a nitrogen and phosphorous-detecting GC detector for first approaches in the interpretation of algal pyrolysis products is seen as useful.
Furthermore, a choice of algae species, being possibly valuable and applicable within the BtVB-process needs to be done.

1.7. Algae species for intermediate pyrolysis

This study is one of the first investigating intermediate pyrolysis of aquatic biomass and it is intended to study various species, originating from fresh and saline water habitats and growing in various climates. Consequently, a broad spectrum of micro- and macroalgal material for this study is chosen and presented in Table 1-10.

The green unicellular microalgal species *Chlamydomonas reinhardtii* and *Chlorella vulgaris* are chosen, as both species are well studied organisms, including their biochemistry, growth cycles and genetics. Particular, it is evident that both species lack cellulose and lignin and thereby provide a useful resource to study non-lignocellulosic materials [124–128].

Cell walls of *Chlorella vulgaris* are mainly composed of hemicellulose with rhamnose and galactose being the predominant sugars along with xylose, arabinose and mannose. Other components include glucosamine, uronic acids and proteins [127], [129]. Furthermore, *Chlorella vulgaris* is one of the most important microalgae species, grown for commercial application (human and animal nutrition), widely used in photo bioreactors and investigated for bioenergy applications [130], [131]. *Chlamydomonas reinhardtii* cell walls are composed of a multilayered structure consisting of a hydroxyproline-rich glycoprotein framework with galactose, arabinose and mannose being the predominant carbohydrate side chains [128], [132], [133]. Furthermore, the mutated strain *Chlamydomonas reinhardtii* CW15+ is included in this study due to its absent cell wall based on the incorrect formation of proteins and extracellular cross linking resulting in minimal amounts of cell walls being produced [134]. The utilization of the cell wall deficient mutant may be useful to assist in elucidation the potential role of cell wall constituents in pyrolysates.

The marine red microalgae *Porphorydium purpureum* is included in the study, because of its biotechnological applications of the Coenzyme Q_{10}, in cosmetics and in pharmaceutics as a valuable antioxidant [135], [136]. It is of interest, to study thermo-chemical characteristics of the entire biomass and of the residual biomass after Q_{10} extraction to evaluate possible routes for integrated bioenergy generation and production of high valuable products.

Fucus vesiculosus is a brown macroalgae, inhabited in the North Sea and Baltic Sea as well as in the Atlantic ocean of northern Europe. It is included in this study, as it is of interest to investigate one of the major seaweed species living along the northern Europe coastlines, to evaluate local resources. *Fucus vesiculosus* is attached with a holdfast on the surface and exhibits flattened fronds (large divided leafs, like ferns) and

is a well known source for hydrocolloids and has valuable potentials as supplements for human nutrition [67], [137].

In addition, rarely studied marine algae species derived from the Polar Regions are included in this study. Arctic derived species are *Prasiola crispa, Monostroma arcticum, Polysiphonia arctica, Devaleraea ramentacea, Odonthalia dentata, Phycodrys rubens* and *Sphacelaria plumosa*. Antarctic derived species include *Gigartina skottsbergii, Plocamium cartilagineum, Myriogramme manginii, Hymencladiopsis crustigena,* and *Kallymenia antarctica*.

All these species are highly adapted to the harsh climates and strong seasonally changing environmental factors such as light regime, temperature and nutrient availabilities [138]. The strong seasonality of the photoperiod reinforced by ice and snow cover during the winter months is characteristic for both Polar Regions and is the main factor influencing the growth of benthic macroalgae. Generally, compared to temperate and tropical regions the annual solar radiation is 30-50 % lower in Polar regions, but endemic polar macroalgal species are adapted to these low radiations, with high photosynthetic efficiency and hence a lower light requirement [138]. Furthermore, sea water temperatures in the Antarctic and Arctic region are low throughout the year and close to 0 °C. In the Antarctic, sea temperatures rise up to 5 °C and up to 8-10 °C in Arctic during summer. Additionally, in intertidal zones, variations in water level and temperature can be very large. During low tide, algae are exposed to air and dehydrate for several hours and may even freeze if air temperature falls below 0 °C [139–142].

In this study, the primarily interest is to collect information about the formation of chemical constituents and to understand the overall pyrolytic behaviour of this exceptional biomass. Due to their habitats in much colder regions than species so far applied for pyrolytic studies, it is of interest to possibly identify novel pyrolytic products, which may offer new routes for renewable chemicals.

Table 1-10 Algae species for intermediate pyrolysis

Type	Species	Division	Habitat/ Distribution	Comment	Ref
Microalgae	*Chlorella vulgaris* *Chlamydomonas reinhardtii* *Chlamydomonas reinhardtii* CW15+	Chlorophyta	- Fresh and brackish water - Global	- No cellulose and lignin - Well studied organisms - Biotechnological applications	[124–134]
	Porphorydium purpureum	Rhodophyta	- Marine water - Global	- Biotechnological application of coenzyme Q_{10}	[135], [136], [143]
Macroalgae	*Fucus vesiculosus*	Phaeophyta	- Marine water - North Sea, Baltic Sea, Atlantic Ocean	- pharmaceutical and biomedical potential - source of hydrocolloids	[67], [137]
	Prasiola crispa *Monostroma arcticum*	Chlorophyta	- marine water - Arctic	- Adapted to harsh climates - Rarely studied - Possible sources of new chemicals	[138–142]
	Polysiphonia arctica *Devaleraea ramentacea* *Odonthalia dentata* *Phycodrys rubens*	Rhodophyta			
	Sphacelaria plumosa	Phaeophyta			
	Gigartina skottsbergii *Plocamium cartilagineum* *Myriogramme manginii* *Hymencladiopsis crustigena* and *Kallymenia antarctica*	Rhodophyta	- marine water - Antarctic		

1.8. Aim and objectives of research

This study aims to contribute knowledge regarding thermo-chemical processing of aquatic biomass for sustainable bioenergy generation via intermediate pyrolysis. Therefore, several micro- and macroalgae species were chosen. It is of interest to investigate the formation of products of algal biomass and biochemical constituents during intermediate pyrolysis. Therefore, extraction and subsequent analytical pyrolysis of lipids and proteins are included in this study, as well as the characterisation of residual biomass derived after extractions. In addition, first application of the gas-chromatographic detectors flame-ionization detector (FID) and nitrogen-phosphorus-detector (NPD) for interpretation of pyrolysates derived by algal materials are investigated. Overall, this aims in generating fundamental knowledge about algal pyrolysis, as well as specific acquaintance concerning the aspect of integrated valuable product and bioenergy generation in the BtVB-process.

The four objectives of this study are the following:

- Installation of an analytical pyrolysis system, providing intermediate pyrolysis conditions, with subsequent separation and detection of pyrolysates.

- Examine the pyrolytic behaviour of algal derived biochemical constituents under intermediate pyrolysis process conditions.

- Study the thermo-chemical behaviour and chemical product formation of micro- and macroalgae biomass during intermediate pyrolysis.

- Evaluate possible applications of algal feedstocks in the BtVB-process upon basis of obtained analytical results.

1.9. Organisation of thesis

The remaining chapter of the thesis are organised as follows:

Chapter 2 describes the technical details of the analytical pyrolysis unit, developed in this study. Included are a description of the commission and a discussion of data interpretation, detectors and limitations of the instrument.

Chapter 3 encompasses the laboratory methods to cultivate algae organisms, for algae biomass characterisation and protein and lipid extraction procedures. Furthermore applied TGA and PY-GC/MS methodologies are presented.

Chapter 4 presents the obtained results of feedstock characterisation including gross chemical compositions, ultimate analysis, higher heating values and ash contents. In addition, FAMES analysis of the total lipids as well as the TGA curves for all materials are included.

Chapter 5 presents the PY-GC/MS derived pyrograms measured for all algal biomass and biochemical constituents. In addition, pyrograms obtained by dual detector analysis for *Chlorella vulgaris* materials are included.

Chapter 6 discusses the results by feedstock characterisation and the thermo-chemical behaviour of the algal materials, achieved by TGA and presents a summary of the main pyrolysis characteristics obtained by TGA.

Chapter 7 discusses the identified compounds in the pyrograms of algal biomass and biomass constituents. In addition a summary of the main pyrolytic compounds for all applied samples is included.

Chapter 8 evaluates potential routes of algal materials in the BtVB-process based on the conducted analytical studies.

Chapter 9 presents the response to the four objectives of this research and present recommendation for continuative and future work.

CHAPTER 2

DEVELOPMENT OF ANALYTICAL PYROLYSIS SYSTEM

2. Development of analytical pyrolysis system

This chapter addresses the main objective of installing an analytical pyrolysis unit for the analysis of various algal samples. The instrumental set-up includes a micro-furnace pyrolyser, online analysis of pyrolysates by gas chromatography followed by detection with various detectors. Details about the single instrument components as well as a description of commissioning and maintenance of the instrument are presented. Furthermore, a discussion of possibilities and limitations of this analytical pyrolysis instrumentation is included.

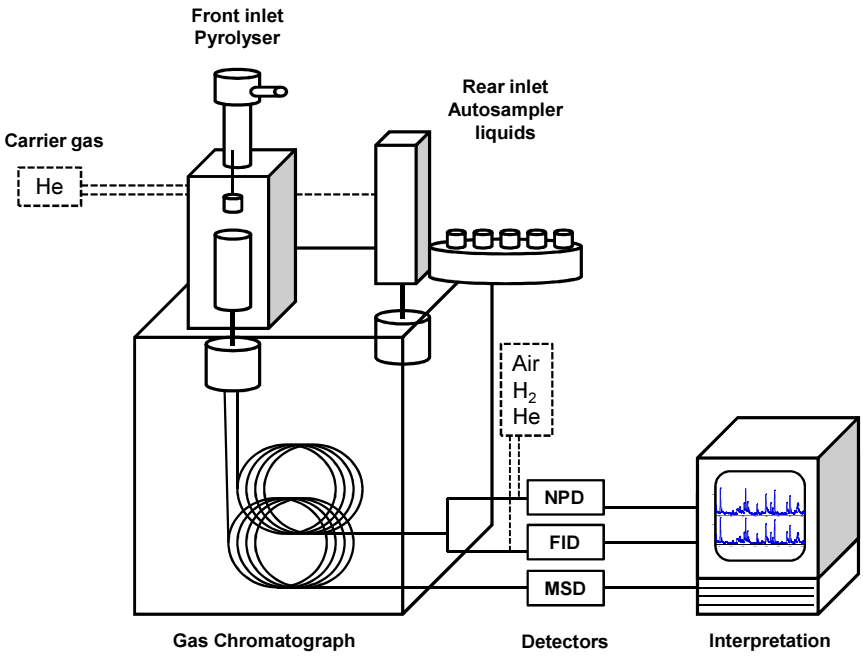
2.1. Introduction

To obtain reliable and repeatable information about chemical product formation of intermediate pyrolysis from various algal biomass samples the overall experimental design of analytical pyrolysis must consider each step of the processes, including sample preparation, pyrolysis i.e. formation of degradation compounds, separation, detection and interpretation of the results. An essential requirement for any analytical equipment is the reproducibility, by means that replicated analysis should produce the same product profile within appropriate ranges.

The set-up of the analytical pyrolysis system is illustrated in Figure 2-1. The instrument consists of a micro-furnace pyrolyser (Py) coupled to the front inlet of a Gas chromatograph (GC). At the back inlet an auto sampler allows the injection of liquid standard samples to support characterisation of the obtained pyrolysates. To multiply the information from pyrolysed algal materials and to increase the productivity of the method, the gas chromatograph set-up was chosen to be a dual column and dual detector configuration. Two identical separation columns were connected to a Mass Selective Detector (MSD), a Flame Ionization Detector (FID) or a Nitrogen Phosphorous Detector (NPD). The arrangement of the chromatographic system allowed either a simultaneous Py-GC/MSD and Py-GC/FID or a simultaneous Py-GC/MSD and Py-GC/NPD data acquisition. Furthermore, a parallel Py-GC/FID and Py-GC/NPD data acquisition is applicable.

The following sections are presenting technical details regarding the instrumental set-up, applied for intermediate pyrolysis of various algal specimens and derived materials.

Figure 2-1 Analytical pyrolysis set-up



2.2. Micro-furnace pyrolyser

Based on review of literature and manufacturer information the decision was drawn towards a micro-furnace pyrolyser manufactured by Frontier Laboratories/ Japan type Double-shot pyrolyser PY2020iD. The design of the Double-Shot Pyrolyser (Py) is a vertical micro-furnace capable to analyse any liquid and solid sample. An illustration of the micro-furnace is shown in Figure 2-2. Due to its technical specifications, presented in Table 2-1, stable pyrolysis process conditions are maintained and this equipment covers a large field of analytical applications like polymer chemistry, environmental, food and soil chemistry as well as paper manufacture and wood science [144].

The sample amount applied depends on the coupled analytical system and ranges from about 0.1 mg up to 6 mg. Overall, it is necessary, to apply homogenous and small particle sized samples to ensure even heat distribution within the sample. Stainless steel cups with a capacity of 50 µl holding the sample. An inert carrier gas enters the micro-furnace at the back to maintain an oxygen free atmosphere within the system.

The sample holder allows holding the sample in standby position within the carrier gas purged chamber maintained at ambient temperature above the pyrolysis zone. In the illustration the upper sample position is indicated by the upper green spot. The green arrows indicate the cooling air flow to maintain ambient temperatures. Effectively, this prevents any unwanted decomposition before the actual thermal decomposition.

For pyrolysis the sample is inserted inside the quartz tube to the lower sample position, indicated by the other marked spot. Pyrolysates are transported by the stable gas flow through the stainless steel needle onto the GC column. To prevent major condensation of pyrolysates, the interface of the micro-furnace, is either maintained at a set temperature up to 400 °C or is stepwise increased with increasing pyrolysis temperatures.

The unit offers the possibility to either insert the sample directly into the preheated furnace providing already T_{eq} within the range of 40-800 °C or it can be introduced into the furnace at low temperatures followed by programmed heating, applying heating rates of 1-100 °C min^{-1}. Overall, the temperature stability of the unit is +/- 1 °C, being essential for pyrolytic studies, due to high temperature dependencies of primary bond fission reactions of biomass materials.

Figure 2-2 Micro-furnace pyrolyser PY2020iD [145]

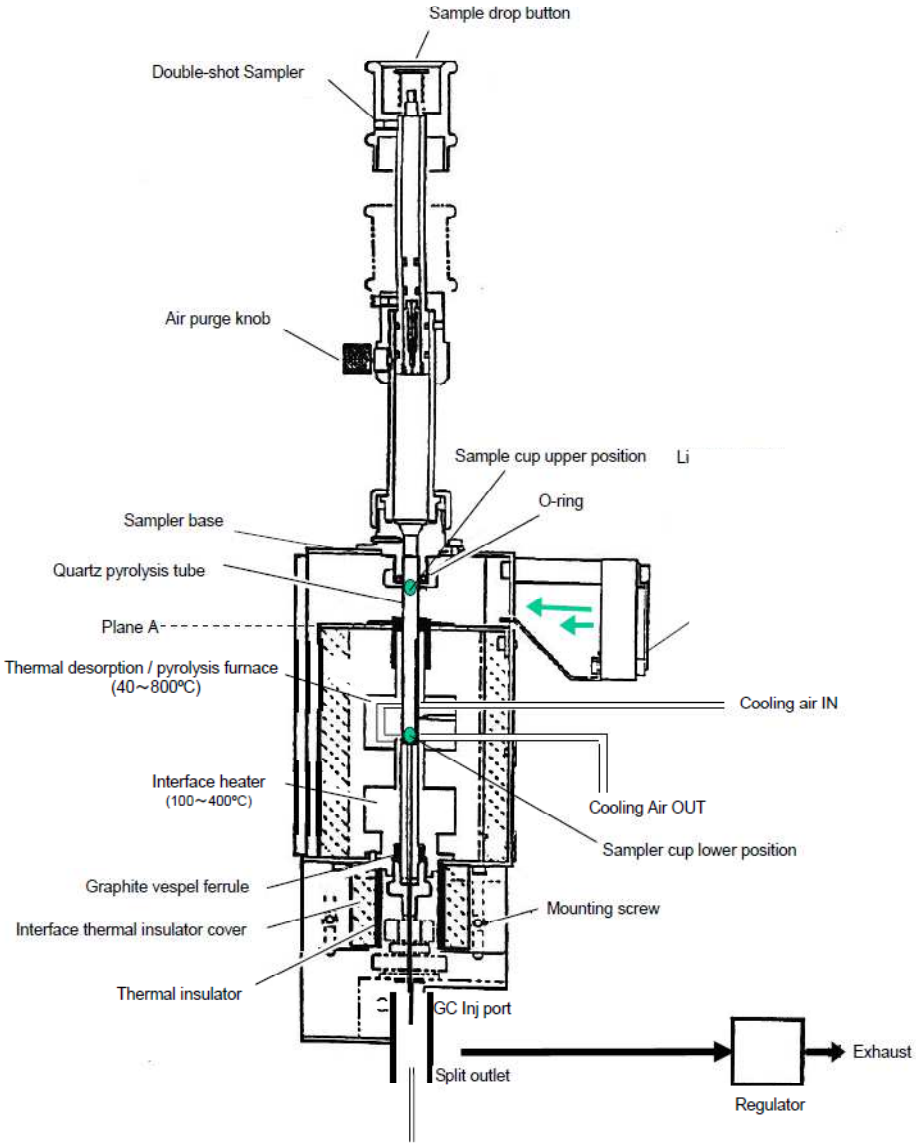


Table 2-1 Specifications double-shot pyrolyser PY2020iD [144]

Parameter	Value
Temperature Range	40-800 °C, +/- 1 °C
Over heating protection	820 °C
Heating Rate	1 - 100 °C min^{-1}
Interface Temperature	Maximum 400 °C, +/-1 °C
Sample weight	0.1 - 6 mg

2.3. Gas chromatograph

The micro-furnace is interfaced at the front split-/splitless (SSL) inlet port of a 5890 Series II Gas chromatograph (Hewlett Packard, Agilent Technologies). At the rear SSL inlet port an auto sampler 7673 for liquid samples was installed.

Two identical capillary separation columns of the type DB 1701 (Agilent Technologies), with a length of 60 m, an inner diameter of 0.25 mm and a film thickness of 0.25 µm, holding a mid to low polarity, were fitted into the injection port of the GC, which allows a wide range of SSL and detector combinations with a high sensitivity. To realise the installation of two columns in one injector the holes of the injector nut and gold plated seal were enlarged to 1 mm and an injector ferrule with 0.8 mm opening was applied to avoid breaking off the column ends and to maintain a tight fit. One of the columns was connected to the MSD and the other one either to the FID or to the NPD. As an alternative a single column and dual detector set up could have been chosen. In this case a post column split, for example using a “Y” connector, would have been installed at the end of the column to reach both detectors [146].

In general, 30 and 60 m DB 1701 columns were applied to separate lignin and polysaccharide derived pyrolysis products from wood, to study chemical additives in paper via Py-GC/MSD [147–149]. Additionally, the DB 1701 columns were applied for chemical characterisation of pyrolysis liquids derived from various biomass feedstocks via offline analysis [150–152]. The main difference between utilization of a 30 m instead a 60 m capillary column is the duration of the analysis and accuracy in compound separation. A shorter column provides a shorter analysis time whereas a longer column provides a more accurate separation as a result of the higher plate number of the column. Consequently, a 60 m column has been chosen for this study. Alternatively, a high temperature GC column might have lead to better separation of high molecular compounds, but a comparison of the obtained data of algal pyrolysis with other biomass pyrolysis studies would have been difficult.

2.4. Detectors

This study aims at setting up a manifold analytical pyrolysis unit for data analysis and interpretation of algal derived intermediate pyrolysis products. Therefore, the gas chromatograph is equipped with three different detectors, offering various detection modes. The following sections are describing the chosen detectors.

2.4.1. Mass Selective Detector (MSD)

By mass-spectrometric detection, molecules of generated pyrolysates are electronically ionized inside the MDS and undergo characteristic fragmentations to yield fragment ions which are separated based upon mass (m/z). In combination with the measurement of the abundance of each fragment ion a mass spectra is obtained, where the largest mass commonly represent the molecular ion, revealing the molecular weight of the parent compound, unless it is too unstable for detection [107]. Electron impact ionization (EI) is the most common mode of MSD operation, where molecules are ionized by passing through a high-energy beam with 70 eV generated within the ion source. Approaches of applying reduced ionization energies of 10-20 eV to minimise fragmentation and to simplify interpretation, due to a mass spectra with a more frequent molecular ion were made [107]. However, the main mass spectral libraries (NIST, Wiley) providing spectra for identification are based on EI with 70 eV and are therefore not applicable for other ionization methods.
For the analysis of algae derived pyrolysates a 5972 Series Mass Selective Detector (EI 70 eV) has been chosen. Generally, a MSD can be operated in scan mode which covers a certain range of masses or SIM mode (single ion monitoring), which detects only a few specifically selected masses. The specifications of an MSD depend on the system, but a sensitivity of a MSD in scan mode accounts for around 10 nanograms and 10 picograms in SIM mode in general. In terms of selectivity a MSD is a universal detector and its applications vary in a broad range [153].

2.4.2. Flame Ionization Detector (FID)

The FID is one of the most widely used GC detectors, due to its simplicity in use and its large range of organic compounds which can be detected in high sensitivity due to its high linear range. The main principle of the detection is ionization of eluted compounds reaching from the separation column into a hydrogen and air flame. A collector with polarizing voltage attracts the ions coming from the flame and produces a current measured in a coupled electronic amplifier, which is proportional to the amount of sample compound in the flame. The minimum detectable level, depending on the molecular structure of the compound ranges from 10-100 picograms. The FID response to all compounds containing C-H bonds, considered as organic compounds. Furthermore, the detector does not response or has little response to H_2O, CO_2, CO,

N_2, O_2, CS_2 and inert gases. Applications for the FID range from environmental, pharmaceutical, food and flavour analysis. Particular its utilization is common in the petroleum industry, i.e. the analysis of gasoline or kerosene due to its high sensitivity to hydrocarbons [154].

2.4.3. Nitrogen Phosphorous Detector (NPD)

The technical design and operation method of a NPD, also called thermo ionic detector (TID), utilizes a jet to provide a hydrogen/air flow for ionization of the compound and a heated rubidium salt coated pearl, to make the NPD selective for nitrogen and phosphorous containing organic compounds (GC 5890 and following types, Agilent Technologies).
Compared to an FID, air and hydrogen flows are lower which minimizes the ionization of hydrocarbons and increases the ionization of N or P containing compounds. The minimum detectable level for nitrogen containing compounds ranges from 0.4 to 10 picograms and for phosphorous from 0.1 to 1 picograms. Inorganic nitrogen containing compounds such as N_2 or ammonia (NH_3) are not detected. Typical applications for NPD are pharmaceutical or environmental analysis [153].

2.5. Commissioning

The correct performance of the instrument parts is accredited by the validation report provided by the supplier. This document includes performance tests of temperature stability of the GC, description of the general state of the micro-furnace (temperature stability, current stability) and presents an air and water check and a tune report of the MSD (Appendix B).
After installation in the laboratory, the performance of the GC was tested by analysing certified standard materials and obtained chromatograms are shown in Figure 2-3. For performance tests of the capillary columns, of the MSD and FID a liquid diesel standard (BAM-K010) accredited by the “German Federal Institute for Materials Research and Testing” was injected at the rear injection port. This standard consists of hydrocarbons within the range of C10-C40, whereas hydrocarbons larger than C20 were not detected with this instrumental set-up. The performance of the NPD was tested by measuring a Triazine pesticides analytical standard mix (Supelco) containing ametryn, atrazine,

prometon, prometryn, propazine and terbutryn. The applications of those standards allow the optimisation of instrument parameters including inlet pressures, column flows and temperature programs.

To establish an appropriate GC oven temperature profile to separate pyrolysates of algal materials, biomass of *Chlorella vulgaris* was applied. Optimisation of the GC parameters revealed, that programmed GC temperatures i.e. from 50-290 °C at a low heating rate (i.e. 3 °C min^{-1}) lead to evenly spaced and clearly eluted peaks. A typical pyrogram (T_{eq}= 500 °C) obtained by the MSD of *Chlorella vulgaris* biomass is also shown in Figure 2-3. Due to high temperatures at the end of the program, the sensitivity for late peaks is substantially improved. Generally, an increase to high temperatures towards the end of the measurements increases the degree of column bleed (raise of baseline) and shortens the lifetime of the separation column. However, high temperatures at the end of a ramped program prevent column contamination due to sufficient elution of high molecular compounds and are a part of the instrument maintenance.

The mass range of the MSD was set to 50-500 amu, to eliminate water and carbon dioxide contributions in the pyrograms. To prevent an overload of the analytical system and to ensure a satisfying separation of compounds, 0.1 mg of homogenous samples and a split ratio of the GC injector of 20:1 are applied in this study.

Overall, it is of importance, that for the whole study a certain temperature profile and stable instrument settings (gas flows, pressures) are maintained due to the direct impact onto retention times and peak heights of eluted compounds.

Figure 2-3 Chromatograms of standards

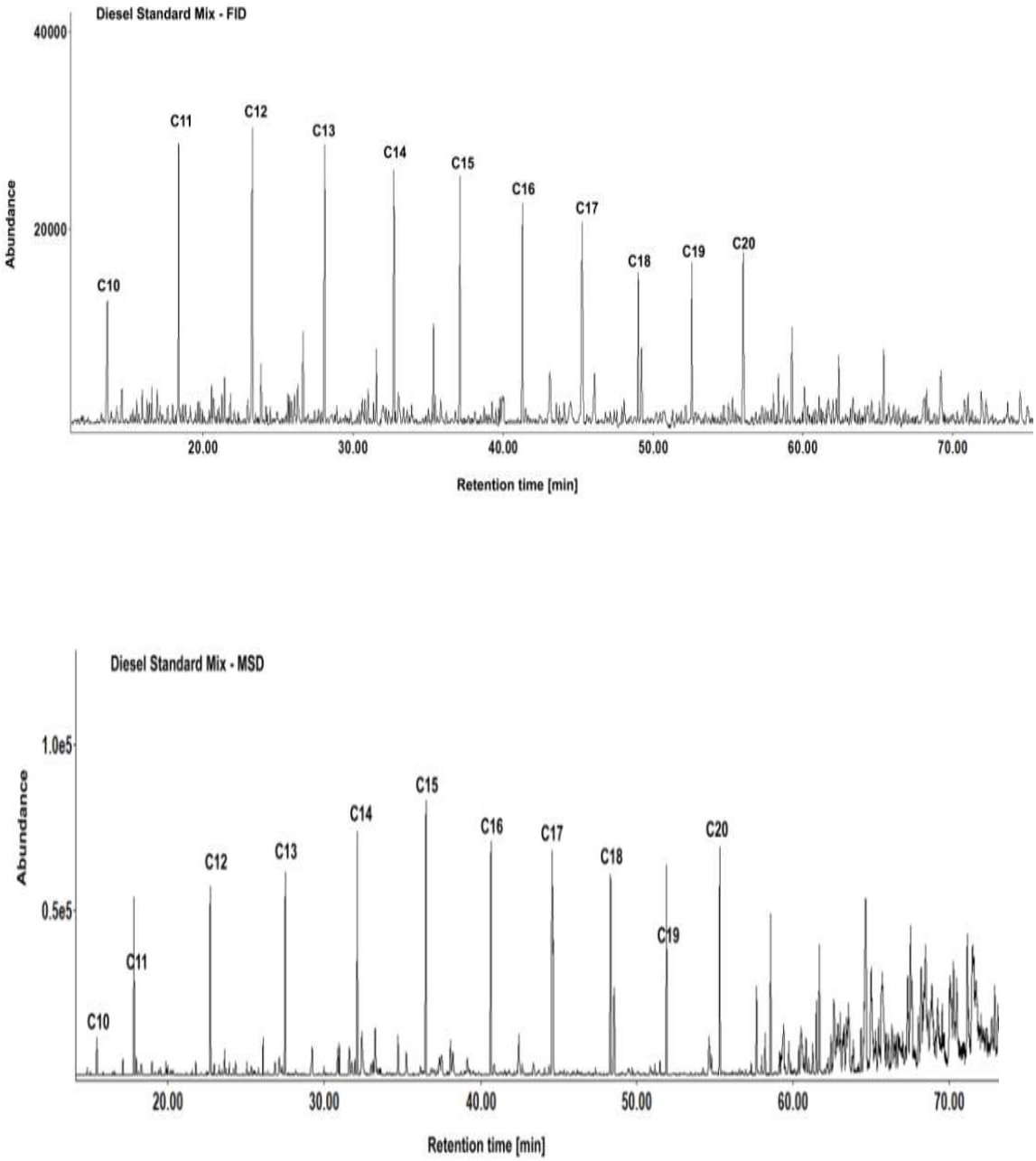
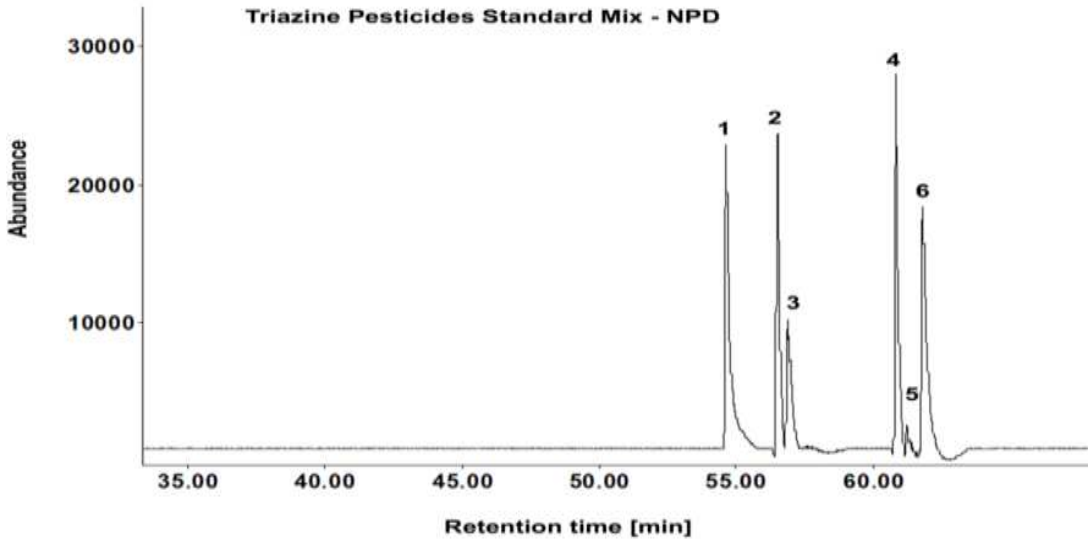
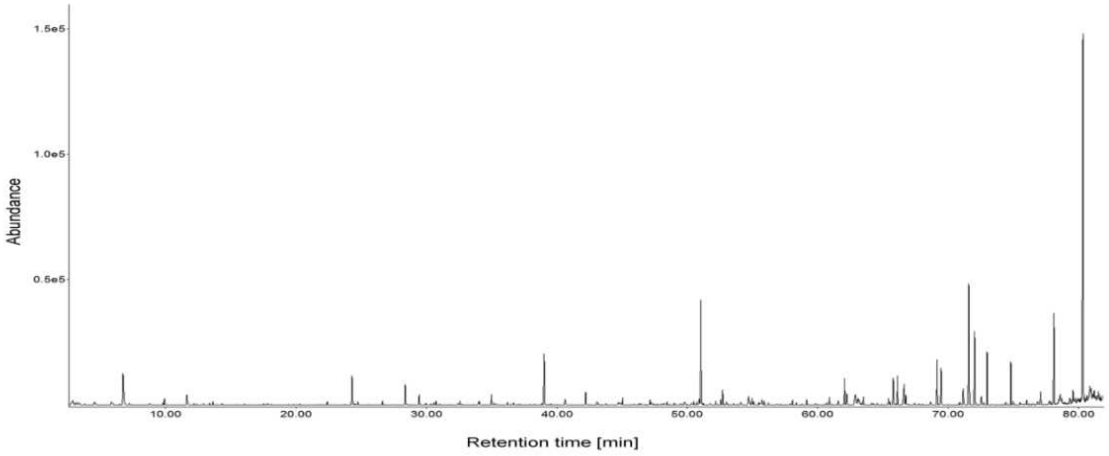


Figure 2-3 continued



1-Prometon 2-Propazin 3-Atrazine 4-Prometryne 5-Ametryne 6-Terbutryn



Pyrogram - *Chlorella vulgaris* Biomass

2.6. Data Interpretation

2.6.1. Identification of components

Pyrolysates of biomass materials are complex mixtures with up to typically 200 components. The assignment of MSD peaks was done by a mass spectral databases NIST08 (National Institute of Standards and Technology, 2002). Each compound which was identified by the library by means of a certain library match (usually ca. 80-95 %) was checked for plausibility.

Overall, the advantage of mass spectral information of a compound is the independency of the GC parameters which were applied for the measurement.

In contrast, peak assignments in chromatograms obtained by FID and NPD data requires retention time data from standard components, which have been analysed with exactly the same method as the pyrolysate.

This study included the construction of parallel MSD/NPD and parallel MSD/FID measurements and to exemplarily present data interpretation obtained from these pyrograms by retention time, some analytical standards were measured with the automatic sampler at the rear inlet port. Chosen standards for the MSD/NPD were the nitrogen containing compounds pyridine, pyrrole, 3-phenylpropionitrile, picolinamide and indole. Analytical standards applied for the MSD/FID were toluene, ethylbenzene, styrene, phenol and 4-methylphenol and retention times are presented in Table 2-2.

Related to the different outlet pressures of the installed columns and detectors, retention time shifts between the detectors are unavoidable. As the MSD operates in vacuum atmosphere and FID and NPD under ambient pressure, variations of retention times from identical compounds are evident, although two matching separation columns were installed. Generally, the retention times obtained by the MSD are slightly shorter than those obtained by the FID or NPD. The reason is a suction effect of the MSD due to its vacuum atmosphere, which leads to a higher column flow of carrier gas and separated substances and therefore to shorter retention times. Overall, a retention time shift of ca. 0.3 min along the pyrograms has been investigated for simultaneously obtained MSD/FID and MSD/NPD pyrograms.

Table 2-2 Retention times of standard compounds for MSD, NPD and FID

Compound	MSD [min]	NPD [min]	FID [min]
Pyridine	7.21	7.46	x
Pyrrole	9.99	10.34	x
3-Phenylpropionitrile	35.01	35.33	x
Picolinamide	36.22	36.52	x
Indole	38.99	39.37	x
3-Methylindole	42.19	42.55	x
Toluene	6.72	x	6.80
Ethylbenzene	9.60	x	9.80
Styrene	11.60	x	11.79
Phenol	24.28	x	24.53
4-Methylphenol	28.44	x	28.70

2.6.2. Quantification and deviation

To quantify individual chemical components in a pyrolysis mixture GC is a useful tool. In this study peak areas obtained from the integrated chromatograms are used for quantification. Typically, single components in complex mixtures, such as pyrolysates of biomass, are quantified by the straightforward area percent method, applying equation (1).

The relative quantity of the component (*i*) is calculated from the normalized peak area, by dividing its peak area (A_i) by the sum of areas of all peaks ($\sum_A$). Even though, this method is not taking the response factors of each component into account it is accepted as a useful method for inter-sample comparisons of complex mixtures particular with a high portion of unknown compounds.

To express the variation of obtained peak areas, the Relative Standard Derivation (RDS) was calculated for each detector. For this, from each detector 10 individual measured peak areas of the same sample were obtained. Ethanol was used for the MSD and FID and fenitrothion ($C_9H_{12}NO_5PS$) for the NPD.

The RSD, expressing the variation of each individual area from the average in percent, was calculated by following equations. Firstly, the average ($\bar{x}$) of the obtained peak areas was calculated by summing the individual results and dividing this sum by the number (n) of individual values, as shown in the equation (2). Secondly, the standard derivation (s) was calculated by equation (3). Subsequently, the relative standard derivation expressing the variation of the individual results from the average in percent was obtained by equation (4) and presented for each detector in Table 2-3.

$$\frac{A_i \times 100\ \%}{\sum_A} = \text{area \% component } i \qquad (1)$$

$$\overline{X} = \frac{1}{n} \times \sum_{i=0}^{n} xi \qquad (2)$$

$$s = \sqrt{\frac{\sum (x - x)^2}{n - 1}} \qquad (3)$$

$$RSD = \frac{100 \times s}{\overline{x}} \qquad (4)$$

Table 2-3 Relative standard deviations of detectors

Detector	Compound	RSD [%]
MSD	Ethanol	1.8
FID	Ethanol	1.9
NPD	Fenitrothion	2.6

2.7. Maintenance

To eliminate any background effects which might lead to manipulation or irreproducible results, the performance of the MSD, FID and NPD was tested once a week and after every change of the set-up (i.e. cleaning, detector change, replacement of helium bottle) by running the appropriate standard material.

When no abnormalities, such as retention time shifts out of the expected range, spikes or unexpected peaks, tailing or overlapping peaks and/or drastically changed pyrolysis products distribution were observed in any of these measurements, the system performance was adequate. Furthermore, regular backing of the column (keeping the column at high temperatures for 20 min) is applied to eliminate contaminations. In addition, the needle and quartz tube of the furnace was cleaned by organic solvents regularly. A further procedure includes cleaning of sample holder and crucibles after every experiment, by rinsing them with acetone and subsequently cleaned in a high temperature furnace at 900 °C for 20 min.

For the maintenance of the FID and NPD no particular procedures need to be carried out. However, to avoid any condensation in the FID the temperature need to reach >150 °C before ignition. During the operation of a NPD it is suggested to increase the temperature slowly, i.e. 100 °C every 10 min, to prevent a damage of the rubidium pearl.

Overall, by following these steps, including the maintenance schedule listed in Table 2-4 a reliable analytical pyrolysis unit producing reproducible results is available and no operating problems have been encountered.

Table 2-4 Maintenance schedule analytical pyrolysis system

Instrument part	Activity	Period
MSD	Air & water Check Control vacuum pressure	daily
	Standard spectra auto tune	weekly
MSD, NPD, FID	Standard materials	weekly
Column	Backing (290 °C)	on demand
Pyrolyser	Blank run (empty crucible) *Chlorella vulgaris*	after every 3rd experiment after every 10th experiment
	Cleaning crucibles Cleaning needle and quartz tube	after every experiment on demand

2.8. Chapter Conclusions

The installation and commission of the Py-GC allocates a powerful tool to analyse pyrolysates obtained by intermediate pyrolysis of algal materials. The instrument offers stable pyrolysis process conditions given by the micro-furnace pyrolyser, followed by online separation of the pyrolysate mixture by GC and subsequent detection with various detectors including a MSD, FID and NPD.

For this study, the MSD is the main detector to identify chemical compounds in algal derived pyrolysates by their mass spectral information. Therefore, a mass spectral database is applied. However, this database might limit the identification of chemicals due to the complex nature of pyrolysis mixtures obtained from biomass.

Furthermore, the detection of compounds with high molecular weights and being unstable at high temperatures is limited by analytical pyrolysis. As well, polar saccharides derived by the pyrolysis of lignocellulosic materials or polysaccharides as contained in algae biomass, are not detected. Overall, it is expected that up to 70 % of the pyrolysates can be detected, although many components can not be identified [155].

Another constraint of this unit is caused by the maximum temperatures of the single parts of the analytical device, such as injector, columns and detectors. The possibility remains that high molecular compounds are not detected, due to condensations within the unit. Subsequently, these condensations can lead to contamination and manipulation of following measurements. Furthermore, large sample amounts may lead to an overload of the analytical device and cause contamination of the column and MSD, which might be carried over into the next analysis.

A disadvantage of the established method is the long analysis time (ca. 90 min) for each sample. However, this is required to ensure proper separation and sufficient time for slowly eluting products and complex mixtures, which may contain more than 200 chemical components.

The dual column set-up and installation of three detectors offers various combinations for parallel analysis of the pyrolysates. Furthermore, the automatic sampler at the rear inlet port allows the injection of up to 100 liquids samples.

Both, FID and MSD detect organic compounds and obtained pyrograms exhibit a similar appearance. However, chromatograms may differ in peak intensities due to different response factors of detectors. Reasons for the additional installation of a FID are simpler operation and lower maintenance costs due to the less complex apparatus compared to a MSD. Moreover, a FID is less sensitive against contamination and

possible operation at temperatures up to 400 °C are advantages for applications in pyrolysis research.

The review of algal pyrolysis revealed that pyrolysates contain higher proportion of nitrogen containing compounds [32]. Therefore the NPD was installed in the Py-GC unit, which may assist in identifying nitrogen containing compounds. The NPD is able to detect phosphorous containing compounds, however, at present none are reported in pyrolysates derived from biomass. Consequently, this unit offers further investigations towards phosphorous containing chemicals in algal pyrolysates.

For continuative research work it is suggested to set up retention time libraries containing numerous analytical standards for further interpretation of biomass pyrolysates. Therefore, an auto sampler carrying up to 100 liquid samples was installed at the rear inlet of the GC. The identification of N- and if present P-containing chemicals in pyrolysates, may assist in developing alternative fertilizers within the BtVB process.

This study carried out the preliminary work including installation, commission and testing of the dual column set up by a set of standard materials. Furthermore, some biomass samples will be analysed, however extensive work with the FID and NPD is beyond the scope of this study.

CHAPTER 3

Materials and Methods

3. Materials and methods

This chapter presents the algal materials employed for intermediate pyrolysis studies. The cultivation method of *Chlamydomonas reinhardtii* strains is described. Furthermore, the methodologies for general characterisation of the biomass and for extraction of lipids and proteins are included. For biomass characterisation via TGA and detecting chemical products in algal pyrolysates, instrumentation and methodical details of thermogravimetric analysis and pyrolysis- gas chromatography are presented.

3.1. Microalgae

3.1.1. Cultivation of *Chlamydomonas reinhardtii*

Two strains of the green microalgal species *Chlamydomonas reinhardtii* were cultivated for this study. The strains *Chlamydomonas reinhardtii* wild type CCAP 11/32 and the cell wall mutant *Chlamydomonas reinhardtii* CCAP 11/32 CW15+ were obtained from the Culture Collection of Algae and Protozoa, Oban, Scotland/UK (CCAP). Cultivation was performed in Tris-Acetate-Phosphate media (TAP) (Appendix C) under axenic conditions [156]. For cultivation, Erlenmeyer flasks with a volume of 200 ml were incubated and kept in a New Brunswick Scientific orbital shaker with continuous rotary agitation at 120 rpm. Growth temperature was kept at 20 °C and cool white fluorescent lighting about 15 cm from the culture with an intensity of ca. 50 µmol $m^{-2}s^{-1}$ with a 12 h photoperiod was provided. The growth of the culture was accompanied by a daily cell count applying a haemocytometer and the cells were harvested at the late logarithmic growth stage, providing a cell density of ca 1-2x10^6 cells per ml. The biomass was separated from the growth media by centrifugation at 3000 g for 15 min, followed by washing with distilled water, another centrifugation step and subsequently drying at 70 °C for 24 h. Overall, the cultivation of the organisms was performed to obtain biomass only.

3.1.2. Further species

Biomass of *Chlorella vulgaris* (CCAP 211/11B) was procured as a powder from Ingrepro B.V./Netherlands, grown in a PBR in F/2 Medium.
Dried biomass and a residual biomass obtained after extraction of the Coenzyme Q_{10} of the red micro-alga *Porphyridium purpureum* (strain SAG 1380-1f) were provided by Dr.-Ing. Barbara Klein, Institute of Bioprocess Engineering, University of Erlangen-Nürnberg, Erlangen/Germany. The biomass was cultivated in a 120 litre PBR (type Airliftschlaufenreaktor "Medusa") designed at the Institute of Bioprocess Engineering in an artificial seawater medium. After cultivation, an accelerated solvent extraction (ASE®) instrumentation manufactured by Dionex [157] was applied to isolate the Coenzyme Q_{10}, by extraction with methanol for 1 minute at 100 Bar.

3.2. Macroalgae

3.2.1. Polar species

Dried biomass of algal species from Polar Regions was provided by Prof. Ulf Karsten, Institute of Biological Sciences, Applied Ecology, University of Rostock/Germany. Arctic specimens were collected in Kongsfjorden (Spitsbergen, Norway, 78°55.5'N;11°56.0'E) including *Prasiola crispa, Monostroma arcticum, Polysiphonia arctica, Devaleraea ramentacea, Odonthalia dentata, Phycodrys rubens* and *Sphacelaria plumosa.* Antarctic specimens were collected in Potter Cove (King George Island, Antarctic Peninsula, 62°14'S;58°40'W) and include *Gigartina skottsbergii, Plocamium cartilagineum, Myriogramme manginii, Hymencladiopsis crustigena* and *Kallymenia antarctica.*

3.2.2. *Fucus vesiculosus*

Dried biomass of the brown macro-algae *Fucus vesiculosus* and a residue after polysaccharide extraction was provided by Dipl.-Chem. Thomas Hahn, Faculty of Mechanical and Process Engineering, Technical University of Kaiserslautern, Kaiserslautern/Germany. The biomass was collected at the German coast of the North Sea and polysaccharides were extracted by the following procedure: After an incubation of the biomass in a mixture of formaldehyde, ethanol and water (unknown

ratio and duration) the biomass was washed by acetone. Subsequently, the extraction was performed while keeping the biomass for 3 hours in a $CaCl_2$ (1 %) solution at 70 °C.

3.3. Biomass characterisation

3.3.1. Sample preparation

Prior to analysis, the algal biomass was ground and sieved to obtain homogenous samples with a particle size of 250 µm. Subsequently the biomass was dried at 70 °C for 24 h to evaporate residual surface water. Until further use, samples were stored in clean and sealed glass containers, placed in a desiccator.

3.3.2. Ultimate analysis

Elemental analysis was performed by an external company (MEDAC Ltd., Surrey/UK) to determine the basic elemental composition of algal biomass samples. Therefore, carbon, hydrogen and nitrogen (CHN) were analysed using a Carlo Erba Flash 1112 elemental analyzer with ±0.3 % absolute accuracy. The oxygen content was calculated by difference. CHN analysis was performed in duplicates.

3.3.3. Higher heating value

The higher heating value (HHV) of the biomass was calculated using equation (5) including C, H and N in mass percentages on dry basis [158].

$$HHV = 5.22C^2 - 319C - 1647H + 38.6C \times H + 133N + 21028 \quad (5)$$

3.3.4. Total lipid extraction and FAMEs analysis

Total lipids were extracted from the biomass in glass tubes by a modified Bligh & Dyer method [159], [160]. The biomass was acidified with 0.15 mol acetic acid (1 ml) followed by the addition of chloroform/methanol (1:2, 7.5 ml), distilled water (2.25 ml) and chloroform (2.25 ml). After phase separation, the lower chloroform layer containing the lipids was removed and evaporated to dryness under nitrogen. Samples were resuspended in chloroform and stored at -20 °C under N_2 until required. FAMEs of total lipid extracts were prepared by transmethylation in 2.5 % sulphuric acid in anhydrous methanol (2 ml) [161]. To avoid contaminations all glass ware cleaned with chromic acid.

A lipid free residue was obtained, containing polymers, proteins, other extracts and ash. This was evaporated to dryness under nitrogen and kept sealed until analysis.

GC-MS analysis of the FAMEs was employed to determine the fatty acid composition of the algae on a DB 23 capillary column (length 30 m, iD 0.25 mm, film thickness 0.25 µm). The GC injector was operated in split mode (50:1) with an inlet temperature of 250 °C. The column temperature was kept at 50 °C for 1 min then increased at 25 °C min^{-1} to 175 °C, followed by a second ramp with a heating of 4 °C min^{-1} up to 235 °C held for 15 min. Assignments of main FAMEs were made by using a NIST08 MS library.

The weight of total lipid and residue is expressed relative to the known weight of the sample. All experiments were performed in triplicates and average results are presented.

3.3.5. Total protein extraction

For total protein extraction, algae were homogenised with 1 ml of 10 % trichloroacetic acid applying a potter homogenizer. Subsequently the proteinaceous residue was separated by centrifugation 4000 g for 10 min. Subsequently 5 washes with 5 ml acetone to remove main portions of the chlorophyll were performed [162]. After each wash the proteins were recovered by centrifugation. The solvent was evaporated under N_2 until dryness and the sample stored sealed in a desiccator. The weight of the total proteins is expressed relative to the weight of biomass. Total protein extraction was performed in triplicates and average results are presented.

3.3.6. Thermogravimetric analysis

Intermediate pyrolysis and ash determination was performed on the basis of the ASTM Standard E 1131-03 [163] for compositional analysis by TGA.
For each analysis, approximately 5 mg of sample were placed in a 70 µl alumina crucible. To determine the volatile matter under intermediate pyrolysis conditions, the sample was initially heated to 105 °C with a linear heating rate of 10 °C min^{-1} and held for 10 min to ensure complete removal of moisture. Subsequently, a linear heating rate of 100 °C min^{-1} was applied to reach the final pyrolysis temperature of 900 °C, followed by a dwell time of 10 min, with an overall flow rate of 50 ml min^{-1} helium.

For ash determination, a slow ashing procedure within oxidative atmosphere was performed. The sample was analysed in air atmosphere (50 ml min^{-1}) and a heating rate of 5 °C min^{-1} from 40 °C to 575 °C and a final hold time of 15 min was applied.
All TGA experiments were replicated in triplicates and average values are presented.

To eliminate background effects of the instrument, for each method a “blank run” (empty crucible) was performed and a background curve obtained. This was stored with the according method and automatically subtracted from each experiment. To avoid contamination through sample residues, the furnace was cleaned by backing, (keeping the furnace temperature at 950 °C for 30 min) after every 5^{th} measurement. Additionally, used crucibles were immersed in acetone and subsequently cleaned in a muffle furnace at 950 °C for 20 min. The temperature accuracy of the instrument according to the manufacturer is $\pm$ 0.25 °C.

3.4. Analytical pyrolysis

Intermediate pyrolysis studies and online analysis of pyrolysates of algal biomass and derived materials were conducted by the developed analytical pyrolysis system, presented in chapter 2.
Sample amounts of 0.1 mg were pyrolysed in a Double-shot pyrolyser PY2020iD micro-furnace from Frontier Laboratories/ Japan at 500 °C in helium atmosphere (20 ml min^{-1}). Once the pyrolysis zone reached the 500 °C, the sample was inserted by releasing the button of the sample holder and the crucible containing the sample was inserted by free fall into the quartz tube situated in the hot chamber. These process

conditions provide intermediate pyrolysis conditions including a TRT of the sample of 0.04 min and a retention time of 1.6 min (revealed by kinetic analysis of *Chlorella vulgaris* biomass by Neeranuch Phusunti, EBRI/Aston University, 2011).

The micro-furnace pyrolyser was interfaced to a split-/splitless (SSL) inlet port of a 5890 Series II Gas chromatograph (Hewlett Packard, Agilent Technologies). A helium flow of 20 ml min^{-1} swept the pyrolysis vapours onto two DB 1701 capillary separation columns (length 60 m, iD 0.25 mm, film thickness 0.25 µm). The interface of the micro-furnace was maintained isotherm at 350 °C. The split mode of the GC injector was 20:1 and the inlet temperature 250 °C. The column temperature was kept isothermal at 50 °C for 1 min, followed by an linearly increase of 3 °C min^{-1} to the final column temperature at 290 °C, which was held for 10 min before the column was cooled down rapidly to the initial temperature. Detection of chemical compounds of the pyrolysates was performed applying parallel data acquisition by MSD/FID or MSD/NPD.

In Table 3-1 the instrumentation setting of the GC and in Table 3-2 the configurations of the detectors are presented. All measurements were carried out in triplicates and mean values are presented.

Table 3-1 Gas chromatograph settings

Parameter	**Column I**	**Column II**	
Detector	MSD	FID	NPD
Carrier gas	Helium	Helium	Helium
Inlet control mode	Constant pressure	Constant pressure	Constant pressure
Inlet pressure [kPa/psi]	250/ 36	60/ 8.7	60/ 8.7
Injector temperature [°C]	250	250	250
Split flow [ml min^{-1}]	20	20	20
Split ratio	20:1	20:1	20:1
Initial column flow [ml min^{-1}]	2.8	2.56	2.56
Average velocity [cm/s]	44.4	38.3	38.3
Initial temperature [°C]	50	50	50
Initial time [min]	1	1	1
Heating rate [°C]	3	3	3
Final temperature [°C]	290	290	290
Final Hold time [min]	10	10	10
Full Program time [min]	91	91	91

Table 3-2 Detector settings

Detector	Parameter	Value
MSD	Column interface temperature [°C]	280
	Solvent delay [min]	2
	Mass range, scan mode [amu]	50-500
	Scans per second	1.5
	Helium flow [ml min^{-1}]	2.8
	Outlet pressure	Vacuum
	Ionization energy [eV]	70
FID	Temperature [°C]	250
	Hydrogen flow [ml min^{-1}]	40
	Air flow [ml min^{-1}]	350
	Helium (makeup flow) [ml min^{-1}]	15
	Outlet pressure	Ambient
NPD	Temperature [°C]	250
	Hydrogen flow [ml min^{-1}]	4
	Air flow [ml min^{-1}]	100
	Helium (makeup flow) [ml min^{-1}]	15
	Outlet pressure	Ambient

CHAPTER 4

Results

Biomass Characterisation

4. Results biomass characterisation

4.1. Ultimate analysis, ash and calorific values

The obtained CHNO, ash and HHV of all algae species are presented in Table 4-1. Both *Chlamydomonas reinhardtii* strains exhibited carbon contents of ca. 50-52 %, a hydrogen content of ca. 7 %, about 11 % of nitrogen and ca. 30 % of oxygen. *Chlorella vulgaris* exhibited lower amounts of carbon accounting for 43.9 %, hydrogen of 6.2 % and nitrogen of 6.7 %, and a higher oxygen content of ca. 43 %. The red alga *Porphyridium purpureum* exhibited a significant lower C and N and higher O content, of 35 %, 1.3 % and 58 %, respectively. The obtained ash values of microalgae species range between 13.6-15.9 % and the HHV between 14.9-23 MJ kg^{-1}.

Of all macroalgae species, the obtained carbon contents vary between 22.3-38.9 %, the hydrogen within 3.9-5.8 %, the nitrogen between 1.4-4 % and oxygen within 52.6-70.5 %. The highest carbon and hydrogen contents as well as the lowest oxygen content were measured within the biomass of *Prasiola crispa.* The lowest nitrogen content was obtained by *Fucus vesiculosus* and the highest by *Hymenocladiopsis crustigena.* Maximum oxygen values obtained in this study derived from biomass of *Gigartina skottsbergii*. Obtained ash values of macroalgae biomass range between 15.5-44.7 %, where as the lowest was obtained by the species *Fucus vesiculosus* and the highest by *Kallymenia antarctica.* Higher heating values vary between 13.1-16 MJ kg^{-1}within all species, where the highest was obtained by *Prasiola crispa* and the lowest by *Myriogramme manginii.*

Table 4-1 Ultimate analysis, ash content and higher heating values

Type	Species	C	H	N	O[a]	Ash	HHV [MJ kg^{-1}]
		[mass fraction %, a.r.]					
Microalgae	*Chlamydomonas reinhardtii* wild type	52.0	7.4	10.7	29.8	14.3	23.0
	Chlamydomonas reinhardtii CW15+	50.2	7.3	11.1	31.4	15.6	22.0
	Chlorella vulgaris	43.9	6.2	6.7	43.2	15.9	18.0
	Porphyridium purpureum	35.2	5.5	1.3	58.0	13.6	14.9
Macroalgae	*Prasiola crispa*	38.9	5.8	2.7	52.6	37.6	16.0
	Monostroma arcticum	34.4	5.5	1.7	58.4	36.9	14.7
	Devaleraea ramentacea	30.9	4.7	3.4	61.0	34.8	14.5
	Odonthalia dentata	35.3	5.2	3.1	56.4	34.2	15.2
	Phycodrys rubens	32.0	5.4	2.9	59.7	30.0	14.3
	Polysiphonia arctica	34.4	5.0	1.8	58.8	27.6	14.9
	Gigartina skottsbergii	23.7	3.9	1.9	70.5	40.2	13.8
	Hymenocladiopsis crustigena	28.8	4.2	4.0	63.0	44.2	14.5
	Myriogramme manginii	22.3	4.9	3.4	69.4	38.9	13.1
	Kallymenia antarctica	31.5	4.9	3.5	60.1	44.7	14.5
	Plocamium cartilagineum	30.1	4.4	3.6	61.9	36.2	14.5
	Sphacelaria plumosa	34.0	5.0	2.1	58.9	34.2	14.8
	Fucus vesiculosus	29.8	4.4	1.4	64.4	15.5	14.2

[a]= calculated by difference a.r.= as received

4.2. Total Lipid, total protein and FAMEs determination

The separation of total lipids and total proteins of *Chlamydomonas reinhardtii* wild type, *Chlamydomonas reinhardtii* CW15+ and *Chlorella* vulgaris are presented in Table 4-2. Both *Chlamydomonas reinhardtii* strains contained 45.7-47.4 % total proteins and 18.1-22.4 % total lipids. *Chlorella vulgaris* contained 54.9 % total proteins and 15.5 % total lipids. The residue of the three species after lipid extraction was obtained, which contained non-lipid fractions, such as carbohydrate, protein and other extracts, accounting for 62.3 % of *Chlamydomonas reinhardtii* wild type, for 65.6 % of *Chlamydomonas reinhardtii* CW15+ and 71.5 % for biomass of *Chlorella vulgaris.*

The FAMEs compositions as mean percentage of total fatty acids of the three microalgae are listed in Table 4-3. Both *Chlamydomonas reinhardtii* strains exhibited a fatty acid composition with α-linolenic acid (18:3, *all-cis*-9,12,15-octadecatrienoic acid) being the predominant acyl constituent accounting for 64.3-69.4 %. Furthermore, palmitic acid (16:0, hexadecanoic acid) was detected with 19.2 % in *Chlamydomonas reinhardtii* wild type and with 14.6 % in *Chlamydomonas reinhardtii* CW 15+. Other FAMEs detected within the range of 6-7 % were oleic acid (18:1, (9*Z*)-octadec-9-enoic acid) and linoleic acid (18:2, *all-cis*-9,12-octadecadienoic acid). Furthermore, stearic acid (18:0, octadecanoic acid) accounted for 2-3 % in both *Chlamydomonas reinhardtii* strains. Overall, a total of saturated FAMEs of 21.2 %, 17.7 %, 31.4 % were identified for *Chlamydomonas reinhardtii* wild type, *Chlamydomonas reinhardtii* CW15+ and *Chlorella vulgaris,* respectively. Higher amounts of total unsaturated FAMEs of 78.8 %, 82.4 %, and 68.6 % were indentified, respectively. In terms of PUFAs both *Chlamydomonas reinhardtii* strains contained ca. 71-76 % and *Chlorella vulgaris* 43.4 %.

In total lipids of *Chlorella vulgaris* various fatty acids were observed including amounts of less than 5 % myristic acid (14:0, tetradecanoic acid), palmitoleic acid (16:1, hexadec-9-enoic acid) and oleic acid. Major FAMEs are α-linolenic acid accounting for 33.4 % and palmitic acid with 27.9 %. Furthermore, 10 % of linoleic acid was detected.

Table 4-2 Gross chemical components microalgal biomass

Species	**Total Protein**	**Total Lipid**	**Residue after total lipid extraction**
	[mass fraction %, a.r.]		
***Chlamydomonas reinhardtii* wild type**	47.4	18.1	62.3
***Chlamydomonas reinhardtii* CW15+**	45.7	22.4	65.6
Chlorella vulgaris	54.9	15.5	71.5

a.r. = as received

Table 4-3 FAMEs of microalgae

FAMEs	***Chlamydomonas reinhardtii* wild type [%]**	***Chlamydomonas reinhardtii* CW15+ [%]**	***Chlorella vulgaris* [%]**
14:0	-	-	1.8
16:0	19.2	14.6	27.9
16:1	-	-	4.1
18:0	2.0	3.1	1.7
18:1	7.2	6.7	21.1
18:2	7.3	6.3	10.0
18:3	64.3	69.4	33.4
Total saturated	21.2	17.7	31.4
Total unsaturated	78.8	82.4	68.6
Total PUFAs	71.6	75.7	43.4

4.3. Thermogravimetric analysis

4.3.1. Introduction

Thermal decomposition of biomass depends on its chemical composition, process temperatures and heating rates. TGA studies with a heating rate of 100 °C min $^{-1}$ in the range of 100-900 °C were conducted to investigate pyrolysis pattern for microalgae biomass and from there derived materials under intermediate pyrolysis conditions. In this context, from microalgae biomass extracted total lipids, total proteins and residues after lipid or enzyme extractions were included to reveal valuable details about microalgal pyrolysis behaviour.

4.3.2. Microalgae

4.3.2.1. *Chlamydomonas reinhardtii* and *Chlorella vulgaris*

TG and DTG curves of total lipids, total protein, residue after lipid extraction and the biomass of the three specimens are presented in Figure 4-1.
Thermogravimetric analysis revealed a release of volatiles in three main decomposition stages, (stage I – III) throughout the pyrolytic process of all samples.

The curves of total lipid analysis obtained by *Chlamydomonas reinhardtii* revealed stage I ranging from ca. 170-250 °C releasing 6 % of the total volatiles. In stage II, from ca. 250 to 500 °C two main peaks of weight loss were detected at 300 °C and 410 °C, releasing 78 % of volatiles. Total lipids of *Chlorella vulgaris* released 3 % of volatiles in stage I and exhibited one main decomposition peak in stage II at 400 °C corresponding to the release of 82 % of volatiles. In stage III, within temperatures of 500-900 °C further 4 % of volatiles were released and a char residue of 10-14 % was formed from all three lipid samples.

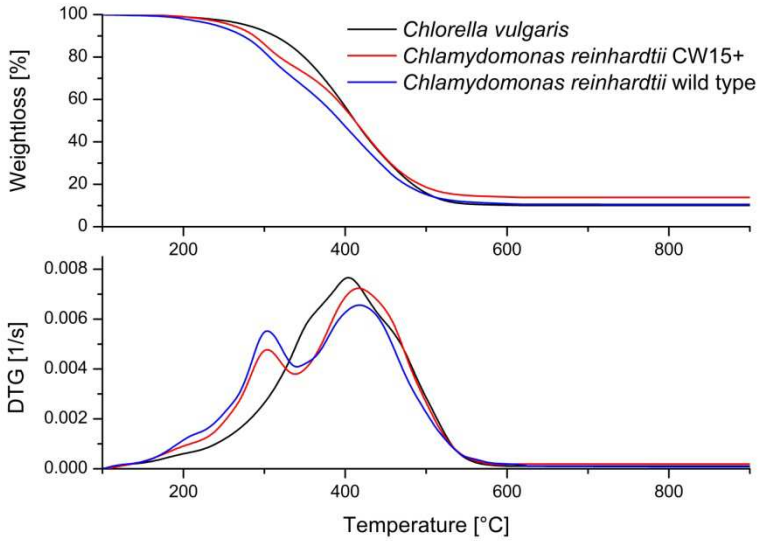
The extracted proteins of all three strains released 2 % of their volatiles at stage I. All three proteins samples exhibit their maximum weight loss during stage II (200-500 °C) peaking at 350-360 °C. Additionally, *Chlamydomonas reinhardtii* wild type shows a shoulder at 320 °C and *Chlorella vulgaris* at 490 °C. During this decomposition phase proteins from both *Chlamydomonas reinhardtii* strains release 62 % and *Chlorella vulgaris* proteins 52 % volatiles. In stage III, from 500-900 °C both *Chlamydomonas reinhardtii* strains release 5 % volatile matter and a char residue of 30 % is obtained.

Chlorella vulgaris released 10 % of volatiles in this stage, forming a slightly higher char residue of 37 %.

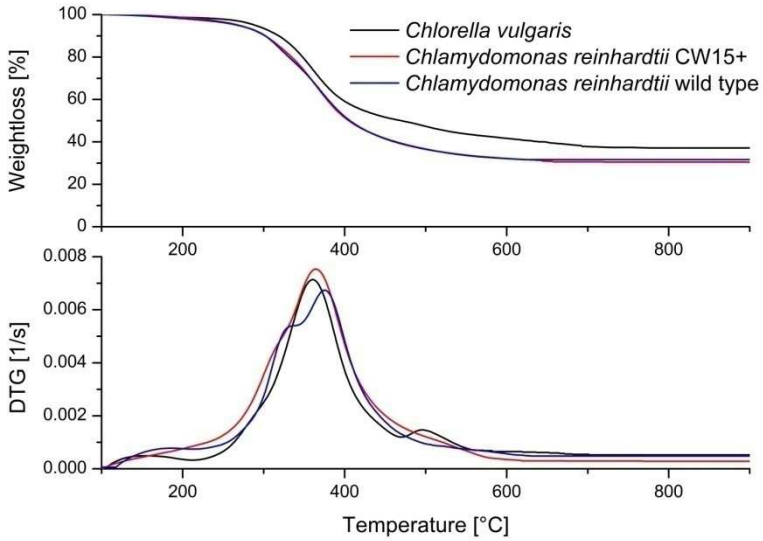
The data obtained by thermogravimetric analysis of the residue after total lipid extraction of all three specimens revealed a weightloss of 1-3 % in stage I (100-230 °C). Stage II ranged from ca. 230-500 °C and both *Chlamydomonas reinhardtii* strains released 68 % and *Chlorella vulgaris* 60 %. The maximum degradation temperatures were determined being 380 °C for *Chlorella vulgaris and* 390 °C for *Chlamydomonas reinhardtii* wild type and *Chlamydomonas reinhardtii* CW15+. In addition, the residues of *Chlamydomonas reinhardtii* wild type revealed a shoulder at 320 °C and *Chlorella vulgaris* at 500 °C. Above 500 °C another 1-3 % of volatiles were released by all residues. Obtained char values by both *Chlamydomonas reinhardtii* strains were ca. 27 % and *Chlorella vulgaris* exhibited a char fraction of ca. 37 %.

The thermal degradation of biomass samples of all three specimens revealed similar shaped TG and DTG curves. Both *Chlamydomonas reinhardtii* strains exhibited decomposition stage I within 100-270 °C, releasing 6 % of volatiles. The main pyrolytic decomposition process for all biomass samples occurred between 270-500 °C with the maximum weight loss detected at 350-360 °C. The amount of volatiles released by the two *Chlamydomonas reinhardtii* strains accounts for 60 % and for *Chlorella vulgaris* 54 %. Furthermore, the biomass of *Chlorella vulgaris* exhibited two additional decomposition steps at 490 °C and 730 °C. Within the temperatures of 500-900 °C both *Chlamydomonas reinhardtii* strains released 7 % of their volatiles and formed a char residue of 26 %, whereas *Chlorella vulgaris* released 12 % of volatiles forming a char residue of 30 %.

Figure 4-1 TG/DTG of green microalgae materials

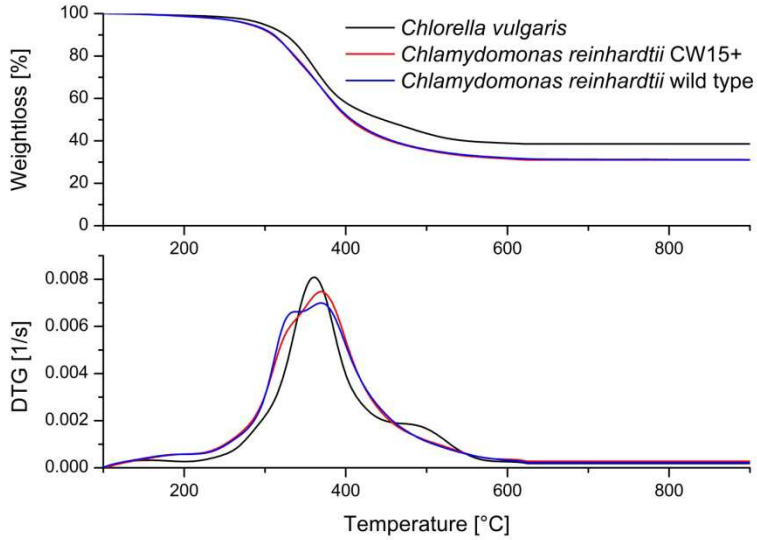


Lipids

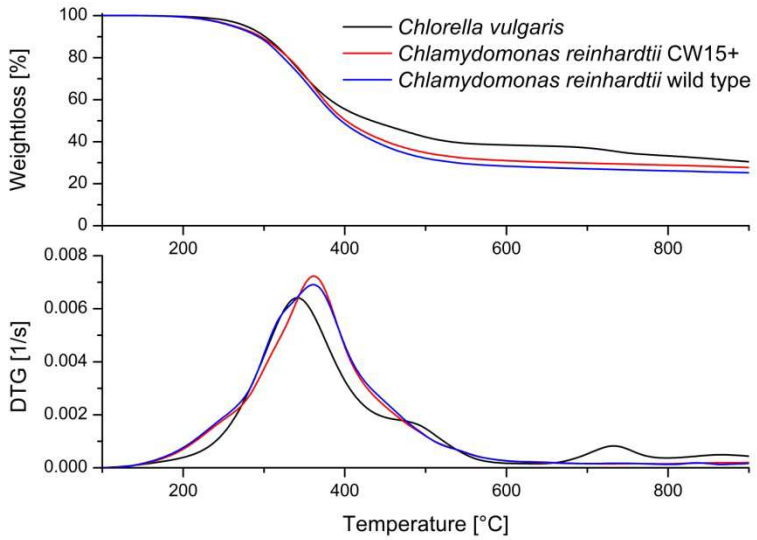


Proteins

Figure 4-1 continued



Residue



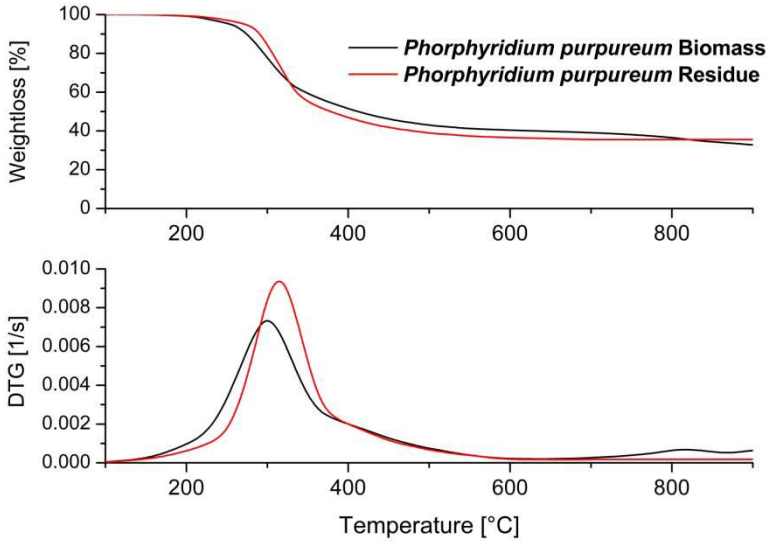
Biomass

4.3.2.2. ***Porphyridium purpureum***

Investigations of red algae biomass of *Porphyridium purpureum* and its residue after extraction of Coenzyme Q_{10} were conducted and TG and DTG curves are presented in Figure 4-2.

Three decomposition stages have been observed for the entire biomass, as well as for the residue. 2 % of the total volatiles were released from both samples within stage I, ranging from 100 to 250 °C. Within stage II the maximum degradation temperature has been determined for the biomass at 300 °C and the residue at 320 °C. The main degradation and therefore evolution of volatiles stopped with reaching 600 °C and further weightloss around 820 °C was observed for both samples. 66-69 % of volatiles were released from the biomass and the residue in stag II. At the end of the measurements, for both samples, 31-34 % char were obtained.

Figure 4-2 TG/DTG of *Porphyridium purpureum*



4.3.3. Macroalgae

4.3.3.1. Polar species

The obtained TG and DTG curves of the arctic and antarctic algae specimen are shown in Figure 4-3 and 4-4.

Similar shaped curves were obtained from the thermal analysis of all Arctic and Antarctic species, exhibiting a three staged thermal decomposition. The first stage ranging from 100 to 180 °C corresponds to a weight loss of ca. 2.5 % of volatiles. Stage two was observed at a temperature range of 180-650 °C exhibiting a weightloss of 40-60 % of the volatiles. The lowest pyrolysis temperatures of 220 °C and 240 °C were obtained from the red algal species from the Antarctic, *Myriogramme manginii*. and *Gigartina skottsbergii,* whereas the red algae *Odonthalia dentata* and brown algae *Sphacelaria plumosa* form the Arctic exhibit the highest with 310 °C and 320 °C, respectively.

Within stage three ranging from 630 to 900 °C about 2-14 % of volatiles were released for all biomass samples. The lowest amount of char residue was produced by *O. dentata*, with 33% and the highest yield of 46 % was obtained by *Myriogramme manginii*. In addition, the thermal degradation of the red algae species *Prasiola arctica, Phycodrys rubens, Hymenocladiopsis crustigena*, *Plocamium cartilagineum* and *Kallymenia antarctica* was characterised by an additional decomposition peak towards the end of stage III occurring at temperatures of 820-850 °C.

Figure 4-3 TG/DTG Arctic species

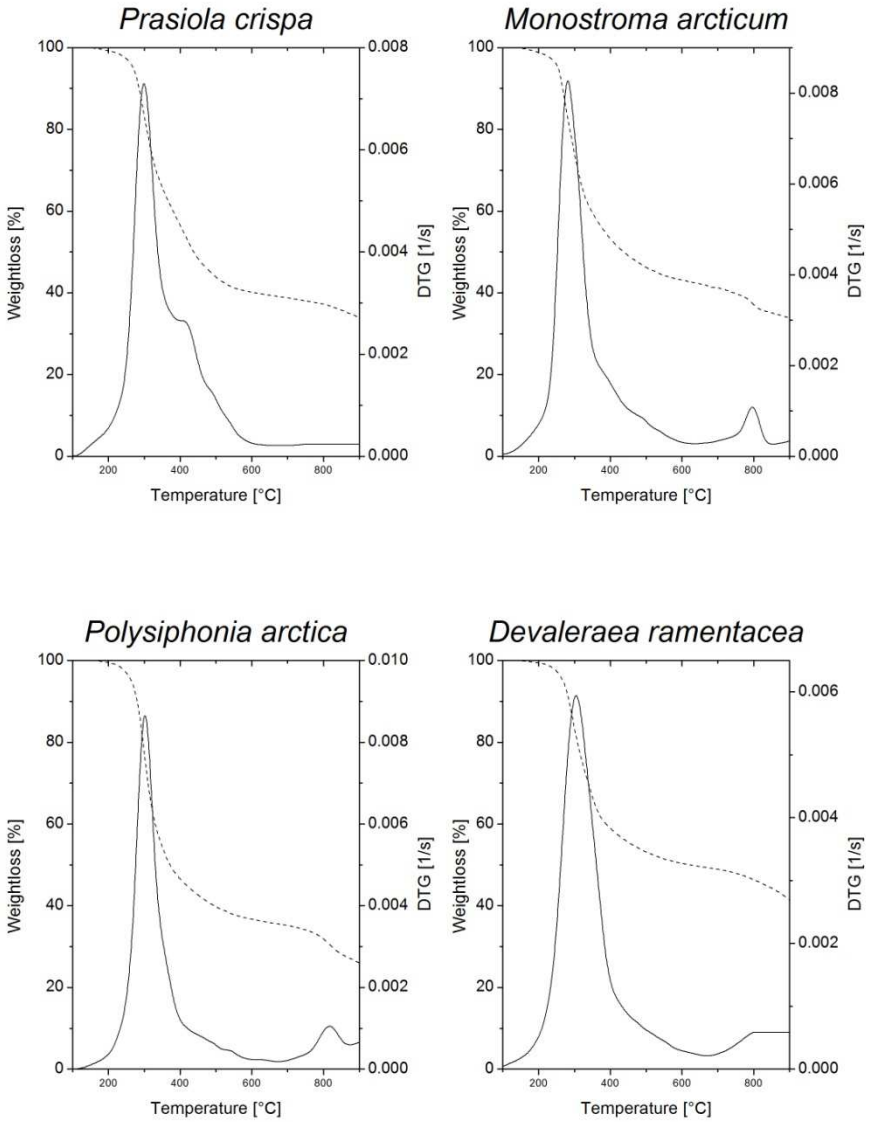


Figure 4-3 continued

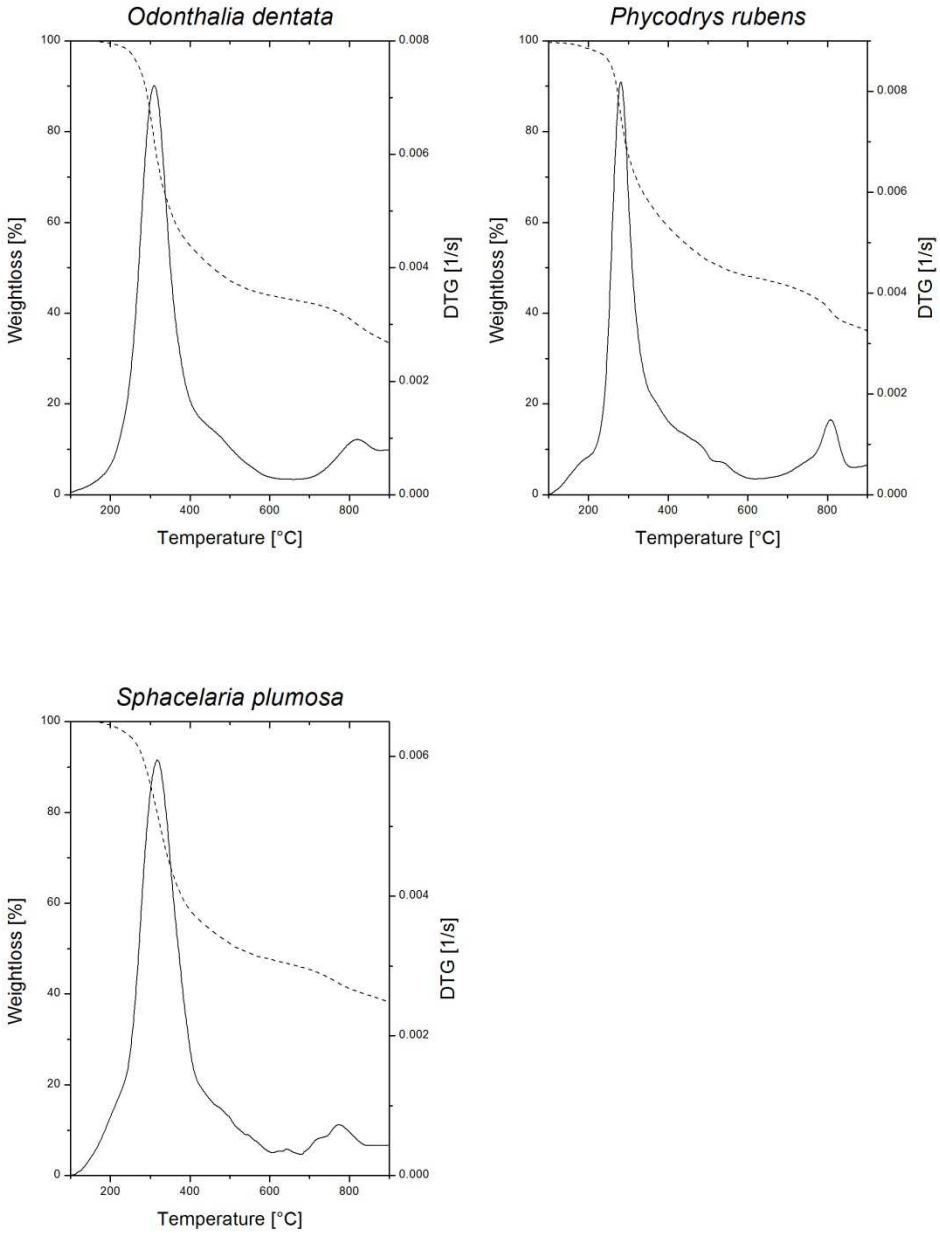
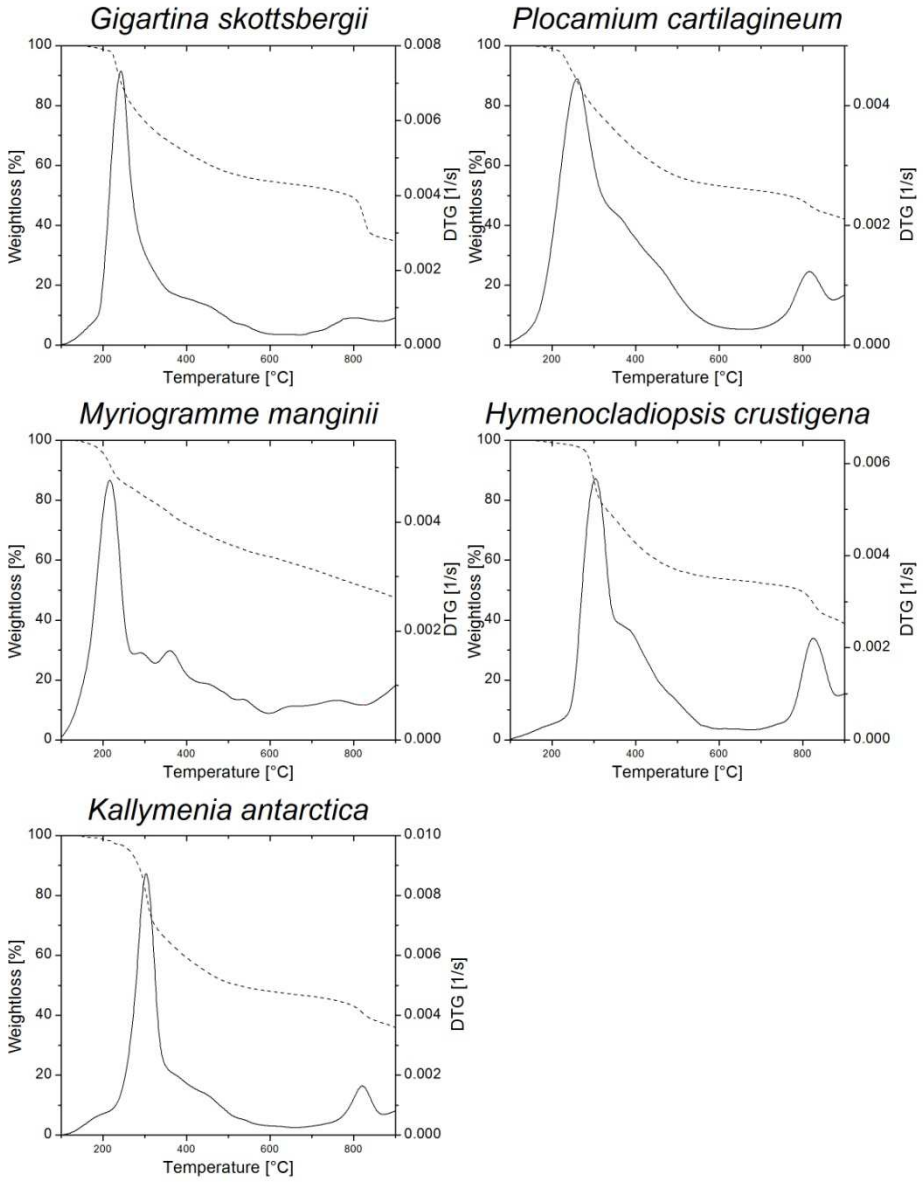


Figure 4-4 TG/DTG Antarctic species

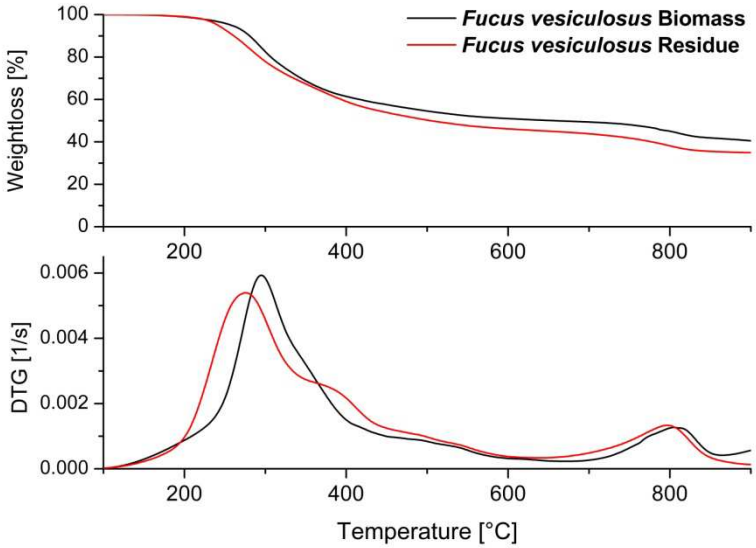


4.3.3.2. ***Fucus vesiculosus***

In addition *Fucus vesiculosus* biomass and its residue after polysaccharide extractions were investigated by TGA as shown in Figure 4-5. The main thermal decomposition takes place within stage II, ranging from 200-600 °C, with a maximum degradation temperature at 300°C for both samples. The release of volatiles obtained by the biomass accounted for 63 % and for the residue for 66 %.

Char values of 45 % and 40 % for the biomass and the residue were obtained, respectively. *Fucus vesiculosus* biomass as well as its residue exhibited an additional degradation peak at ca. 800°C.

Figure 4-5 TG/DTG *Fucus vesiculosus*



CHAPTER 5

Results

ANALYTICAL PYROLYSIS

5. Results analytical pyrolysis

5.1. Introduction

Py-GC equipped with various detectors was used to study pyrolysis products evolving from several micro- and macroalgae biomass and from there derived materials under intermediate pyrolysis conditions, at T_{eq}= 500 °C. Included are fresh and saline water microalgae species, growing from temperate to tropical regions. Furthermore, pyrolysis of extracted total lipids, total proteins and residues after lipid extractions from the microalgae *Chlamydomonas reinhardtii* wild type, its cell wall mutant CW15+ and *Chlorella vulgaris* was performed in order to obtain information about the origin of pyrolysis products. In addition, the biomass of *Porphyridium purpureum* and its residues after coenzyme Q_{10} extraction was studied. Macroalgae species from both Polar Regions as well as *Fucus vesiculosus*, endemic to the temperate climate along the North Sea coastlines of Europe are also investigated, as well as its residue after polysaccharide extraction.

Preliminary pyrograms obtained by parallel Py-MSD/FID and Py-MSD/NPD analysis from *Chlorella vulgaris* biomass and materials are a first approach of the dual detector set-up of the analytical pyrolysis unit.

At the current status of research the MSD was the main detector for identification of peaks in algal material derived pyrolysates, as it reveals valuable information by mass spectral data.

Around 50 identified chemical compounds by retention time data from standard compounds and by using a NIST08 MS library are presented in Appendix D. A semi-quantitative analysis (area % on pyrograms) was performed on pyrolysis products of all materials to reveal information about evolution of compounds by all different samples and is presented in the following sections.

5.2. Microalgae

5.2.1. *Chlamydomonas reinhardtii* and *Chlorella vulgaris*

5.2.1.1. Biomass

The pyrograms derived from biomass of *Chlamydomonas reinhardtii* wild type, its cell wall mutant CW15+ and *Chlorella vulgaris* are shown in Figure 5-1. The identified chemicals within the pyrolysates with peak number and area percentage obtained from the pyrograms are listed in Table 5-1.

In general, detected chemical compounds obtained by intermediate pyrolysis showed relative consistency between the three microalgae strains, although variations in their proportions were evident.

About 56-60 % of the total peak area of the pyrograms obtained by the biomass of both *Chlamydomonas reinhardtii* strains was identified. In terms of detected chemicals similar apparent pyrograms from both strains were obtained, and major identified components were toluene (ca. 4-5 %), 4-methylphenol (ca. 4 %) and indole (ca. 5 %). Phytol and isomers, including trans-phytol accounted for ca. 20-27 % of the total pyrogram area. Furthermore, benzoic acid alkyl ester derivatives (ca. 4-5 %) and squalene (ca. 4-5 %) was detected.

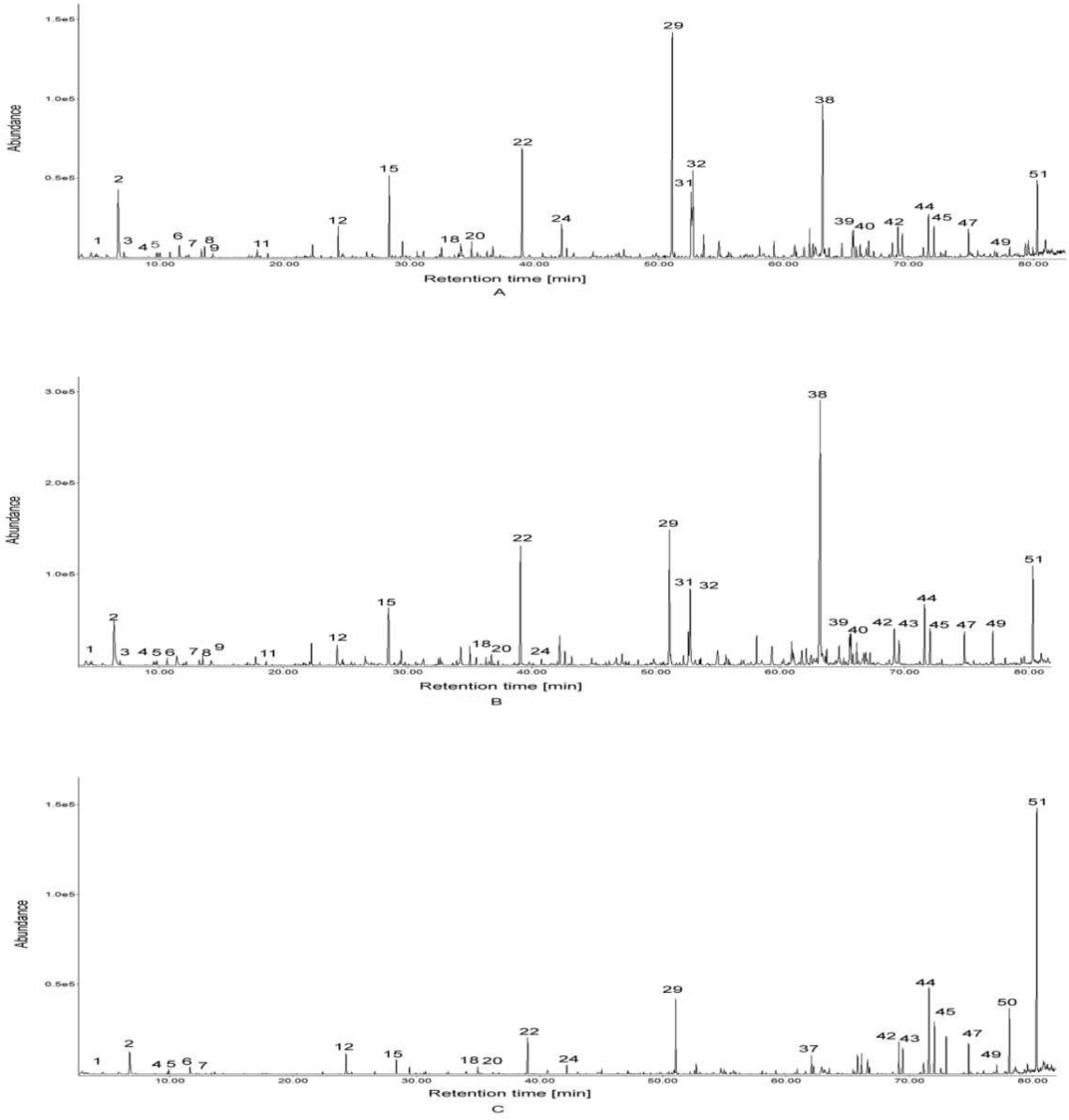
Identified compounds, with an area less than 4 % were phenol (ca. 1.4 %), 3-methylindole (ca. 1.4 %), levoglucosan (1.4-1.9 %), dipeptides (ca. 1.5 %) and 2-propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester (2-2.4 %). Furthermore, in traces (<1-1 %) detected compounds include acetic acid, pyridine, ethylbenzene, pyrrole, styrene, furfural, pyrrole, 2- or 3-methyl, 2-furaldehyde, 5-methyl, 3-phenylpropionitrile, picolinamide, hexadecanamide and benzenedicarboxylic acid, alkyl ester derivative.

Major pyrolysis compounds detected in pyrolysates of *Chlorella vulgaris* biomass were phytol (5.8 %), 2-propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester (8 %), benzoic acid alkyl ester derivatives (8.5 %). The peak area of squalene accounted for 25.5 % and was the major pyrolysis product obtained of *Chlorella vulgaris* biomass.

Minor compounds, with peak areas less than 4 % were toluene (2.9 %), phenol (1.9 %), indole (3.3 %) and hexadecanamide (2.6 %). Compounds detected in *Chlorella vulgaris* pyrolysates only were 1-hexadecene (1.3 %) and octadecanoic acid, octyl ester (5.6 %). Compared to *Chlamydomonas reinhardtii* strains no trans-phytol, dipeptides and levoglucosan was detected. Amounts detected in traces (<1-1 %) include acetic acid,

ethylbenzene, pyrrole, styrene, furfural, 3-phenylpropionnitrile, picolinamide, 3-methylindole, and benzenedicarboxylic acid, alkyl ester derivative.

Figure 5-1 Pyrograms microalgal biomass at 500 °C



A= *Chlamydomonas reinhardtii* wild type

B= *Chlamydomonas reinhardtii* CW15+

C= *Chlorella vulgaris*

Table 5-1 Peak areas of pyrolysis compounds - microalgal biomass

		Area percent [%]		
Peak No.	**Chemical Compound**	***Chlamydomonas reinhardtii* Wild type**	***Chlamydomonas reinhardtii* CW15+**	***Chlorella vulgaris***
1	Acetic Acid	<1	<1	<1
2	Toluene	5.1	4.4	2.9
3	Pyridine	<1	<1	n.d.
4	Ethylbenzene	<1	<1	<1
5	Pyrrole	<1	<1	<1
6	Styrene	<1	<1	<1
7	Furfural	<1	<1	<1
8	Pyrrole, 2 or 3-methyl	<1	<1	n.d.
9	Isomer of 8	<1	<1	n.d.
11	2-Furaldehyde, 5-methyl	<1	<1	n.d.
12	Phenol	1.4	1.3	1.9
15	4-Methylphenol	4.4	4.1	1.1
18	3-Phenylpropionitrile	<1	<1	<1
20	Picolinamide	<1	<1	<1
22	Indole	4.8	5.0	3.3
24	3-Methylindole	1.4	1.2	<1
29	Phytol	12.1	5.7	5.8
31	Levoglucosan	1.9	1.3	n.d.
32	Isomer of 29	3.3	2.6	<1
37	1-Hexadecene	n.d.	n.d.	1.3
38	Trans-phytol	8.2	16.3	n.d.
39	Dipeptide	1.4	1.5	n.d.
40	Dipeptide	1.4	1.5	n.d.
42	Benzoic acid, alkyl ester derivative	1.9	2.0	1.8
43	Hexadecanamide	<1	1.2	2.6
44	2-Propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester	2.0	2.4	8.0
45	Isomer 42	1.3	1.6	5.0
47	Isomer 42	1.4	1.5	1.7
49	Benzenedicarboxylic acid, alkyl ester derivative	<1	1.4	<1
50	Octadecanoic acid, octyl ester	n.d.	n.d.	5.6
51	Squalene	4.3	4.7	25.5
Total Identified Area %		**56.3**	**59.7**	**66.5**

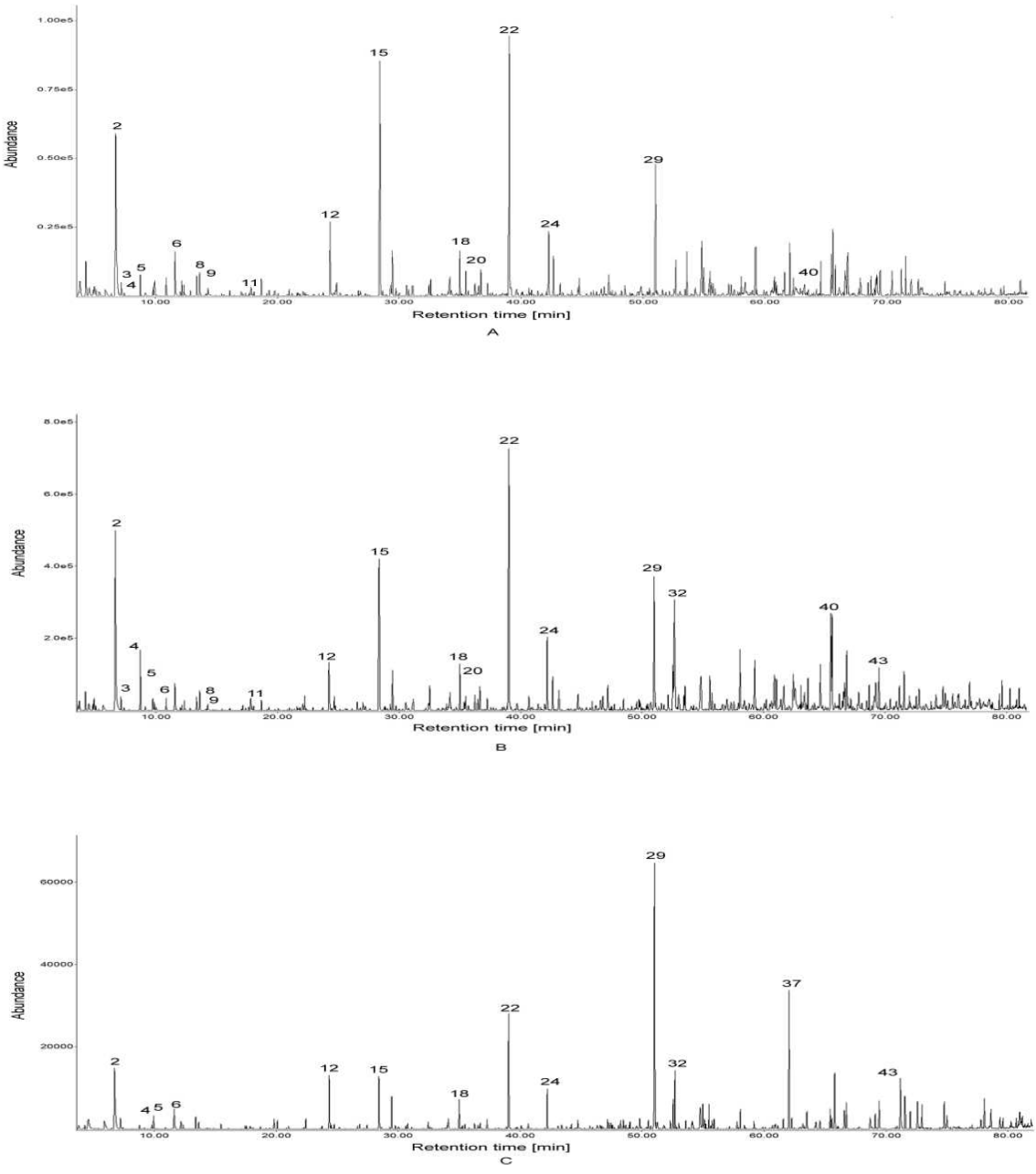
5.2.1.2. Proteins

The pyrograms of extracted total proteins from *Chlamydomonas reinhardtii* wild type, its cell wall mutant CW15+ and *Chlorella vulgaris* are shown in Figure 5-2 along with the identified chemical compounds and their area percentages in Table 5-2.
Main chemical compounds in pyrolysates of total proteins extracted from *Chlamydomonas reinhardtii* wild type and its cell wall mutant CW15+ were toluene (ca. 7-9 %), 4-methylphenol (ca. 7-8 %) and indole (ca. 8-9 %).
Products detected in minor amounts include styrene (<1-1.8 %), phenol (ca. 2 %), 3-phenylpropionitrile (ca. 1.2 %), 3-methyl-indole (ca. 2 %), phytol and isomers (ca. 4-9 %) and a dipeptide (ca. 2-3 %). Products, detected with a peak area less than 1 % were pyridine, ethylbenzene, pyrrole, pyrrole, 2– or 3- methyl and isomer, 2-furaldehyde, 5-methyl and picolinamide.

In pyrolysates of total proteins extracted from *Chlorella vulgaris* main compounds indentified were toluene (6.2 %) and indole (6.7 %). Furthermore, compounds detected in this pyrolysate only include 1-hexadecene with 7.2 % and hexadecanamide with 1.7 %. Other compounds detected in minor amounts include styrene (1.5 %), phenol (3 %), 4-methylphenol (2.8 %), 3-phenylpropionitrile (1.4 %), 3-methylindole (1.8 %) and phytol (2.6 %). Compounds detected in traces (<1 %) were pyridine, ethylbenzene, pyrrole and the isomer of phytol.

Furthermore minor amounts of 2-propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester (1.1-3.3 %) were detected in all three protein derived pyrolysates and octadecanoic acid octyl ester (1.7 %) in *Chlorella vulgaris* only.
Overall, ca. 40 % of the total peak area could be identified for both *Chlamydomonas reinhardtii* protein derived pyrograms and ca. 35 % of the *Chlorella vulgaris* pyrogram.

Figure 5-2 Pyrograms of microalgae proteins at 500 °C



A= *Chlamydomonas reinhardtii* wild type

B= *Chlamydomonas reinhardtii* CW15+

C= *Chlorella vulgaris*

Table 5-2 Peak areas of pyrolysis compounds - microalgal proteins

		Area percent [%]		
Peak No.	**Chemical Compound**	***Chlamydomonas reinhardtii* Wild type**	***Chlamydomonas reinhardtii* CW15+**	***Chlorella vulgaris***
2	Toluene	9.3	7.4	6.2
3	Pyridine	<1	<1	<1
4	Ethylbenzene	<1	<1	<1
5	Pyrrole	<1	<1	<1
6	Styrene	1.8	<1	1.5
8	Pyrrole, 2 or 3-methyl	<1	<1	n.d.
9	Isomer of 8	<1	<1	n.d.
11	2-Furaldehyde, 5-methyl	<1	<1	n.d.
12	Phenol	2.4	2.0	3.0
15	4-Methylphenol	7.7	6.9	2.8
18	3-Phenylpropionitrile	1.3	1.2	1.4
20	Picolinamide	<1	<1	n.d.
22	Indole	9.1	8.6	6.7
24	3-Methylindole	2.0	2.0	1.8
29	Phytol	2.8	3.8	2.6
32	Isomer of 29	1.3	5.2	<1
37	1-Hexadecene	n.d.	n.d.	7.2
40	Dipeptide	2	2.6	n.d.
43	Hexadecanamide	<1	1.2	1.7
Total Indentified Area %		**39.7**	**40.9**	**34.9**

5.2.1.3. Lipids

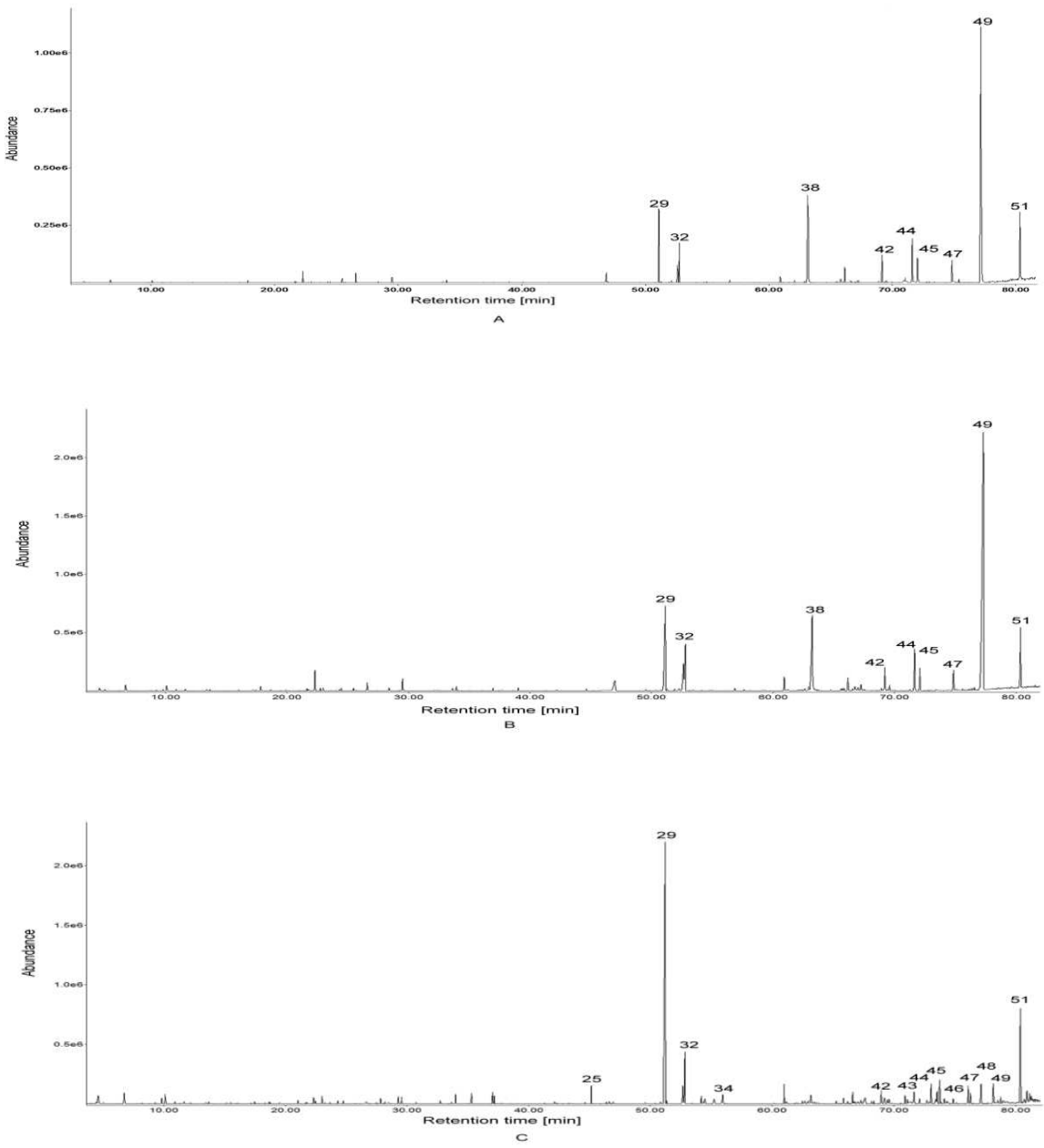
Pyrograms obtained by intermediate pyrolysis of total lipids extracted from *Chlamydomonas reinhardtii* wild type, its cell wall mutant CW15+ and *Chlorella vulgaris* are shown in Figure 5-3. Assignments of peaks and their area percentage are listed in Table 5-3.

Major pyrolysis products obtained by both *Chlamydomonas reinhardtii* strains are benzenedicarboxylic acid alkyl ester derivative accounting for 41-43.5 %. By contrast, pyrolysis of *Chlorella vulgaris* derived lipids yielded lower levels of benzenedicarboxylic acid, alkyl ester (ca. 5 %) and benzoic acid, alkyl derivates (ca. 5 %).

In addition, octadecanoic acid octyl ester (ca. 5 %), hexadencanoic acid methyl ester (ca. 3 %) and aliphatic hydrocarbons including heptadecane, 1-nonadecene and heneicosane (totalling ca. 11 %) were only detected from *Chlorella vulgaris* lipids. Squalene was detected in all three samples, accounting for ca. 5-9 % in *Chlamydomonas reinhardtii* strains and 13.8 % in *Chlorella vulgaris*.

Phytol and its isomer were detected in all three pyrolysates accounting for ca. 6-15 %. However, trans-phytol was detected in pyrolysates of *Chlamydomonas reinhardtii* strains only, accounting for 11-12 %.

Figure 5-3 Pyrograms of microalgae lipids at 500 °C



A= *Chlamydomonas reinhardtii* wild type

B= *Chlamydomonas reinhardtii* CW15+

C= *Chlorella vulgaris*

Table 5-3 Peak areas of pyrolysis compounds - microalgal lipids

		Area percent [%]		
Peak No.	**Chemical Compound**	***Chlamydomonas reinhardtii* Wild type**	***Chlamydomonas reinhardtii* CW15+**	***Chlorella vulgaris***
25	Heptadecane	n.d.	n.d.	1.9
29	Phytol	9.0	11.6	2.7
32	Isomer of 29	1.8	3.5	2.9
34	Hexadecanoic acid, methyl ester	n.d.	n.d.	2.8
38	Trans-phytol	12.3	11.2	n.d.
42	Benzoic acid, alkyl ester derivative	2.9	2.1	1.8
44	2-Propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester	5.3	3.7	3.5
45	Isomer 42	2.7	1.9	1.3
46	1-Nonadecene	n.d.	n.d.	5.5
47	Isomer 42	2.7	1.7	1.2
48	Heneicosane	n.d.	n.d.	3.6
49	Benzenedicarboxylic acid, alkyl ester derivative	41.2	43.5	5.0
50	Octadecanoic acid, octyl ester	n.d.	n.d.	5.0
51	Squalene	8.5	5.3	13.7
	Total Indentified Area %	**86.4**	**84.5**	**50.9**

5.2.1.4. Residues

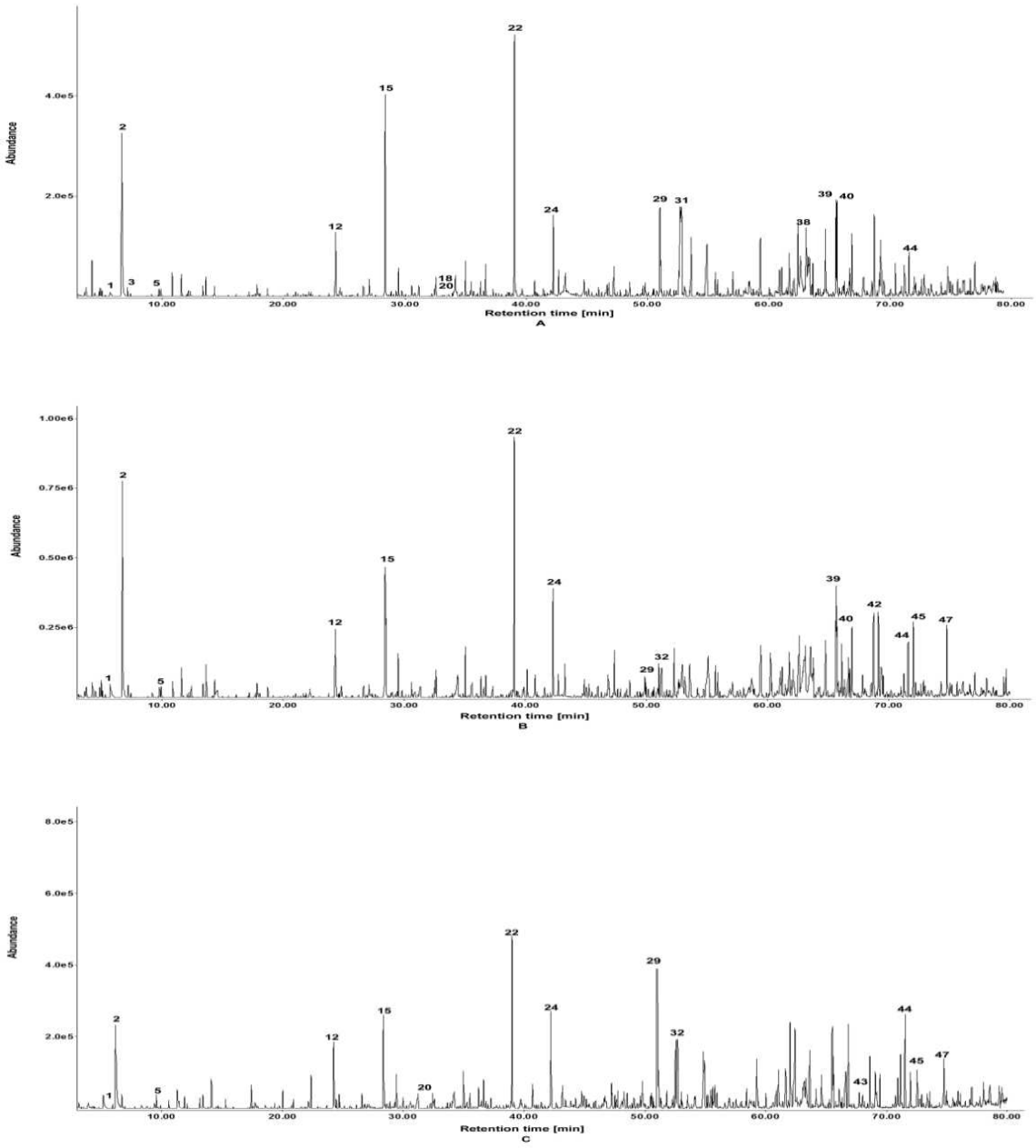
The obtained pyrograms of residual biomass derived after lipid extraction from the microalgae *Chlamydomonas reinhardtii* wild type, its cell wall mutant CW15+ and *Chlorella vulgaris* are shown in Figure 5-4 along with the assigned peaks and their area percentage in Table 5-4.

Major chemical compounds detected in the pyrolysates of both *Chlamydomonas reinhardtii* strains were toluene (ca. 6 %), 4-methylphenol (ca. 5-6.4 %) and indole (ca. 7 %). Minor amounts detected include phenol (ca. 1.8 %), 3-methylindole (ca. 1.8 %), phytol and isomers (ca. 2-4 %), dipeptides (ca. 5 %) and 2-Propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester (ca. 1-3.6 %). Identified chemical compounds with peak areas less that 1 % were acetic acid, pyridine, pyrrole, 3-phenylpropionnitrile, picolinamide. Furthermore, levoglucosan was found in the residue of the *Chlamydomonas reinhardtii* wild type derived sample with 3.3 %.

In pyrolysates of *Chlorella vulgaris* detected compounds include indole (4.2 %), toluene (3.5 %), phytol and isomers (4.9 %). Minor amounts of phenol (2.3 %), 4-methyphenol (2.6 %) 3-methylindole, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester (2.2 %) and benzoic acid, alkyl ester derivatives (ca. 1.4 %).

Overall, about 36-38 % and 23 % of the total peaks areas of both *Chlamydomonas reinhardtii strains* and *Chlorella vulgaris* were identified, respectively.

Figure 5-4 Pyrograms of microalgae residues at 500 °C



A= *Chlamydomonas reinhardtii* wild type

B= *Chlamydomonas reinhardtii* CW15+

C= *Chlorella vulgaris*

Table 5-4 Peak areas of pyrolysis compounds – microalgae residues

		Area percent [%]		
Peak No.	**Chemical Compound**	***Chlamydomonas reinhardtii* Wild type**	***Chlamydomonas reinhardtii* CW15+**	***Chlorella vulgaris***
1	Acetic Acid	<1	<1	<1
2	Toluene	5.9	6.2	3.5
3	Pyridine	<1	n.d.	n.d.
5	Pyrrole	<1	<1	<1
12	Phenol	1.7	1.8	2.3
15	4-Methylphenol	6.4	4.9	2.6
18	3-Phenylpropionitrile	<1	n.d.	n.d.
20	Picolinamide	<1	n.d.	<1
22	Indole	6.8	6.5	4.2
24	3-Methylindole	2.1	2.2	2.1
29	Phytol	2.1	1.0	3.3
31	Levoglucosan	3.3	n.d.	n.d.
32	Isomer of 29	n.d.	1.2	1.6
38	Trans-phytol	1.8	n.d.	n.d.
39	Dipeptide	2.6	3.0	n.d.
40	Dipeptide	2.5	1.8	n.d.
42	Benzoic acid, alkyl ester derivative	<1	1.6	n.d.
43	Hexadecanamide	n.d.	n.d.	<1
44	2-Propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester	1.1	1.2	2.2
45	Isomer of 42	n.d.	1.6	<1
47	Isomer of 42	n.d.	1.4	1.4
Total Identified Area %		**36.5**	**35.6**	**23.2**

5.2.1.5. Dual detector analysis

Pyrograms of *Chlorella vulgaris* biomass, extracted total proteins, extracted total lipids and the residue obtained after lipid extraction was analysed by dual detector analysis, obtained pyrograms of Py-GC-MSD/FID and Py-GC-MSD/NPD, shown in Figure 5-5.

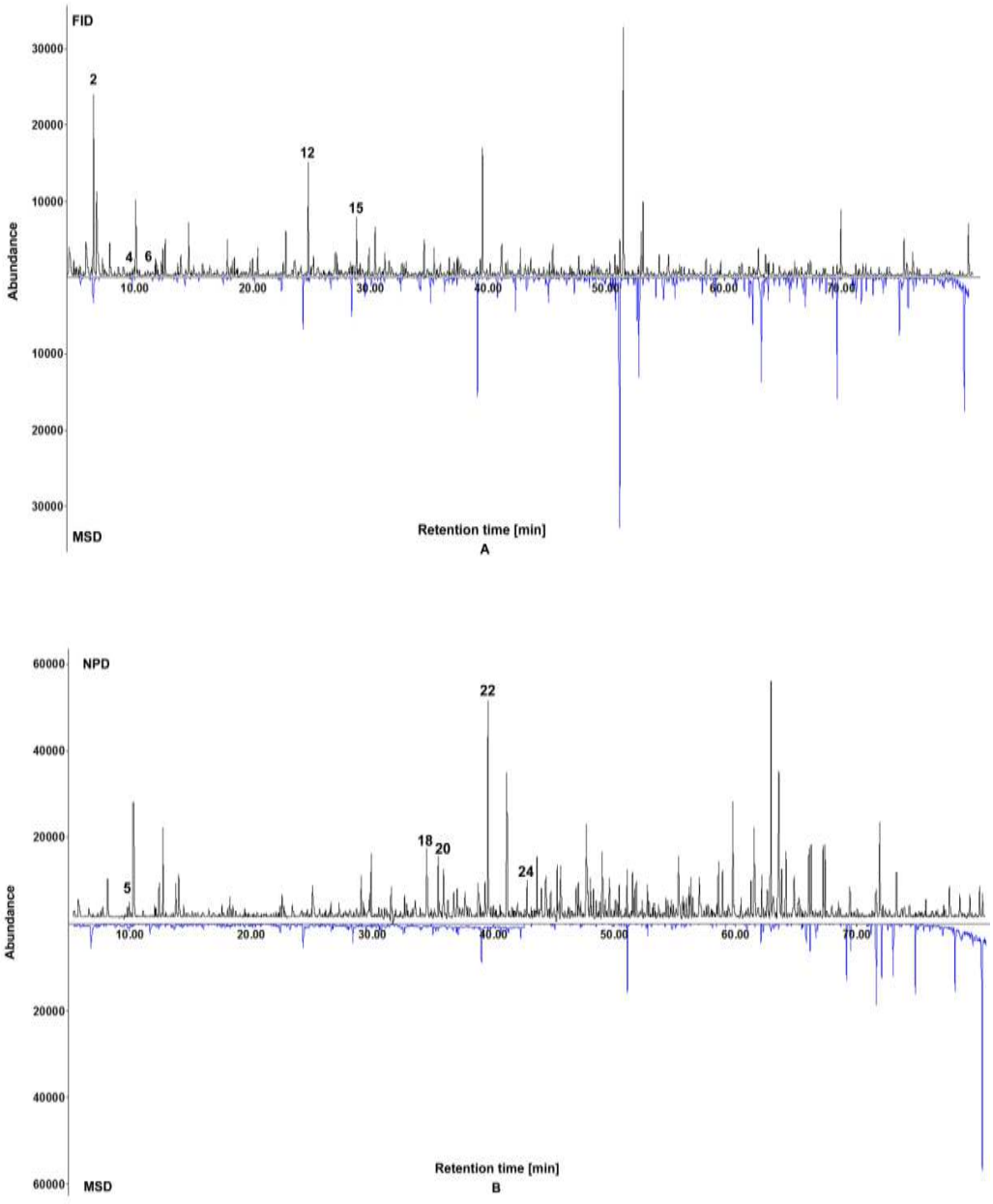
The FID produces a similar pyrogram to the MSD caused by detection of C-H containing compounds. The NPD detects only chemicals containing organic bound nitrogen and/or phosphorous. As can be seen from the NPD pyrogram, many nitrogen containing compounds are apparent in the pyrolysate of *Chlorella vulgaris* biomass.

Furthermore, a higher sensitivity of the NPD for nitrogen and/or phosphorous compounds, compared to the MSD is evident. Both, the FID and NPD require analytical standards to relate to their retention time for further interpretation of the pyrogram.

The chemical compounds, which have been identified by applying retention times of analytical standards for each detector, are presented in Table 5-5 along with their peak area. Generally, a retention time shift of around 0.3 min between the MSD and FID as well as between the MSD and NPD was observed. This is caused by the different pressure regimes within the detectors, as already outlined in the section 2.5.

Figure 5-5 Dual detector pyrograms of *Chlorella vulgaris* at 500 °C

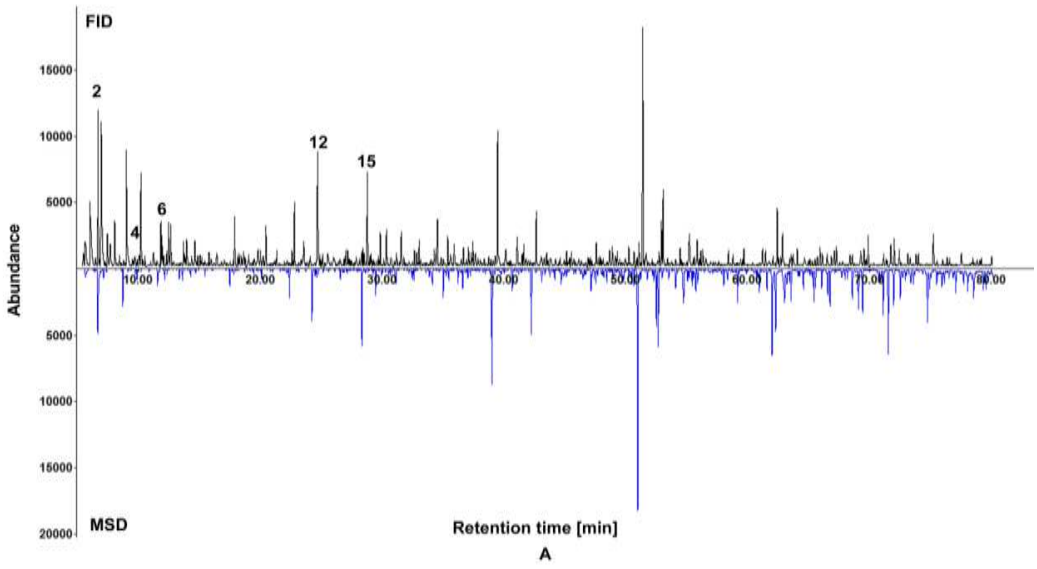
A = MSD/FID B= MSD/NPD

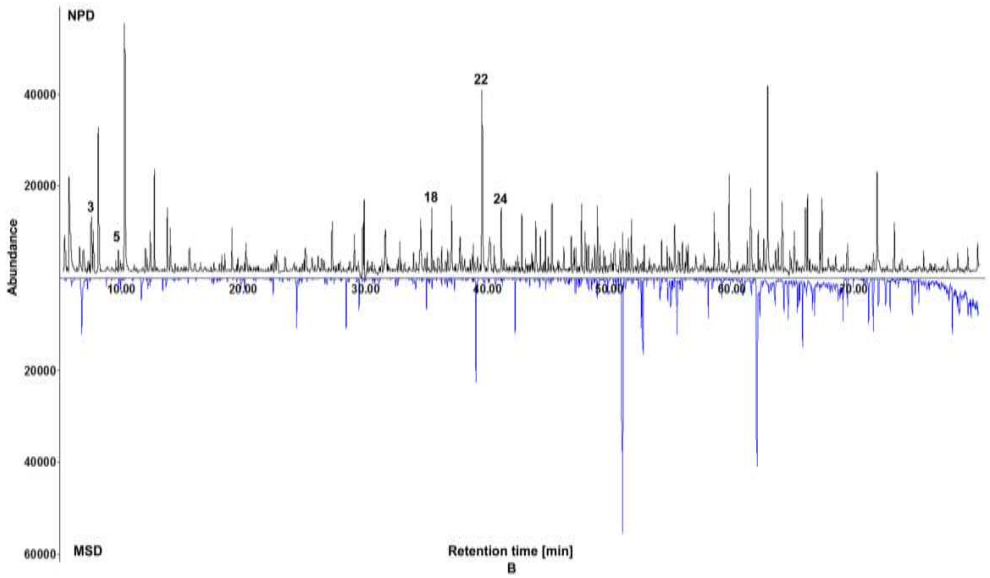


Chlorella vulgaris Biomass

Figure 5-5 continued

A = MSD/FID B= MSD/NPD

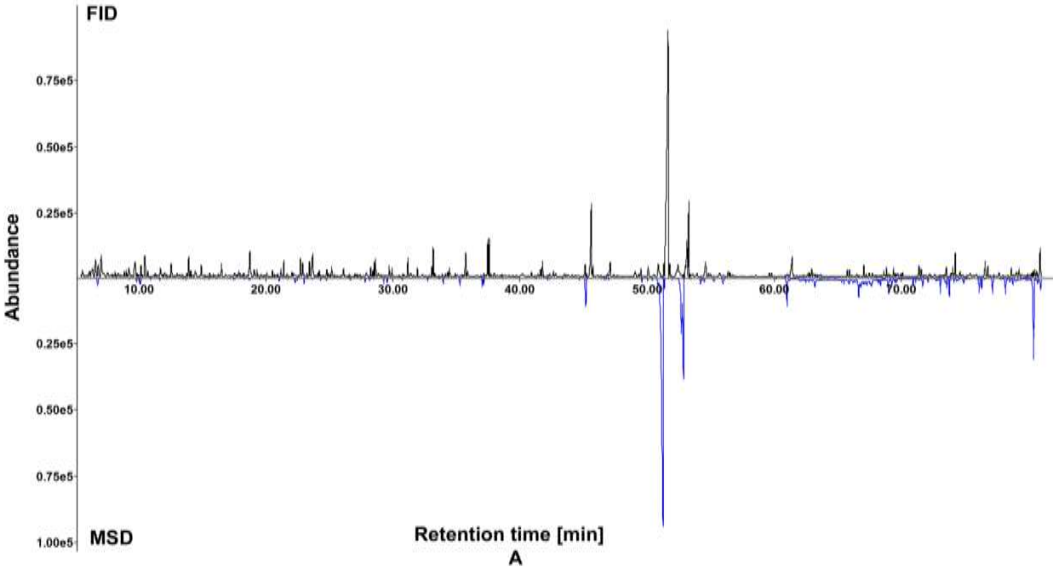


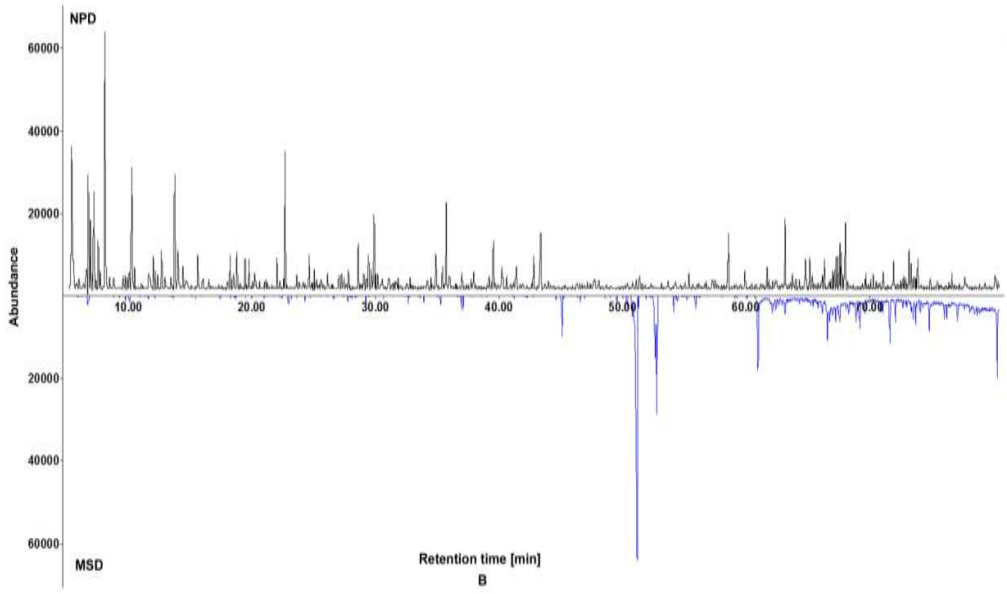


Chlorella vulgaris Proteins

Figure 5-5 continued

A = MSD/FID B= MSD/NPD

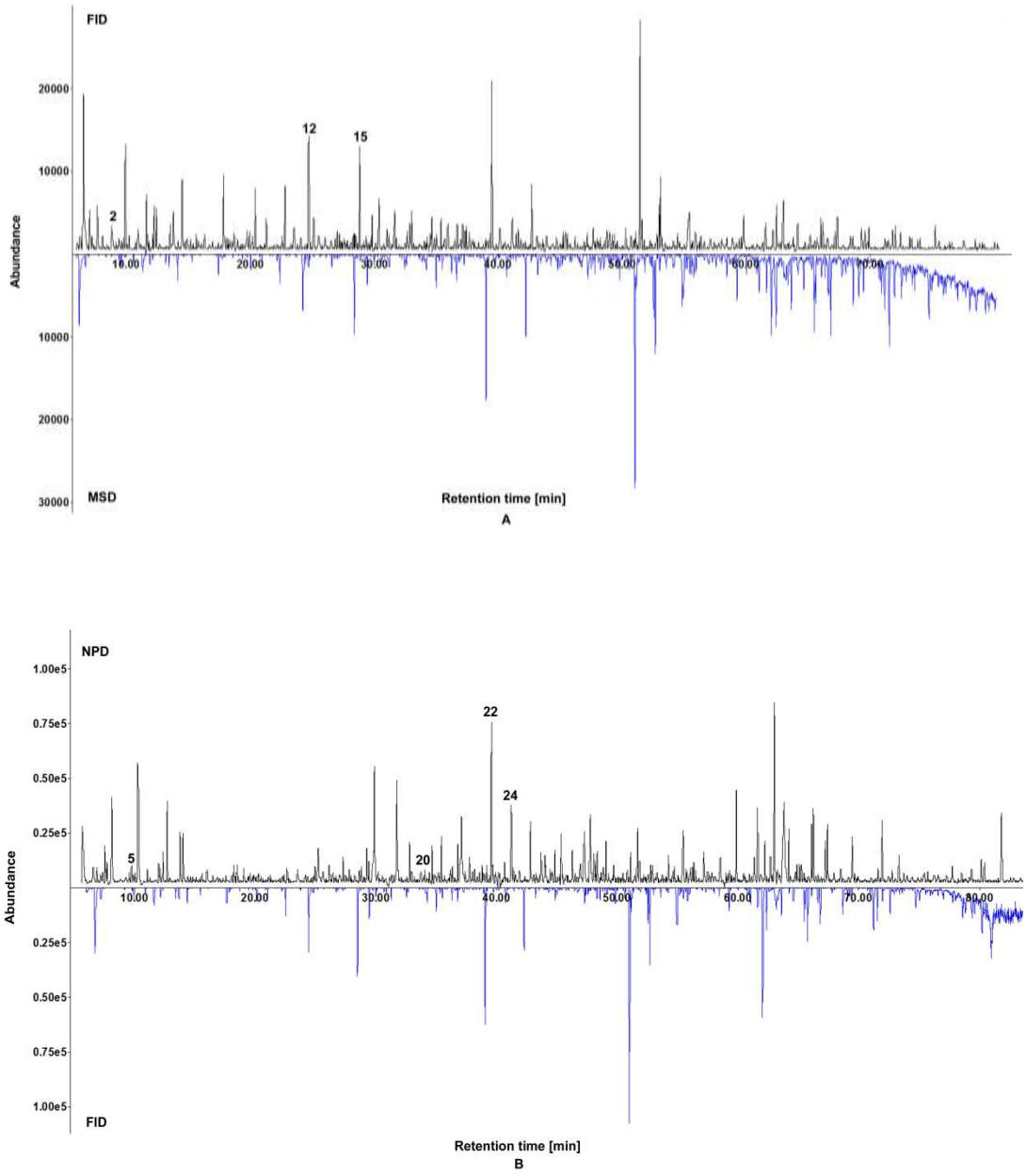




Chlorella vulgaris Lipids

Figure 5-5 continued

A = MSD/FID B= MSD/NPD



Chlorella vulgaris Residue

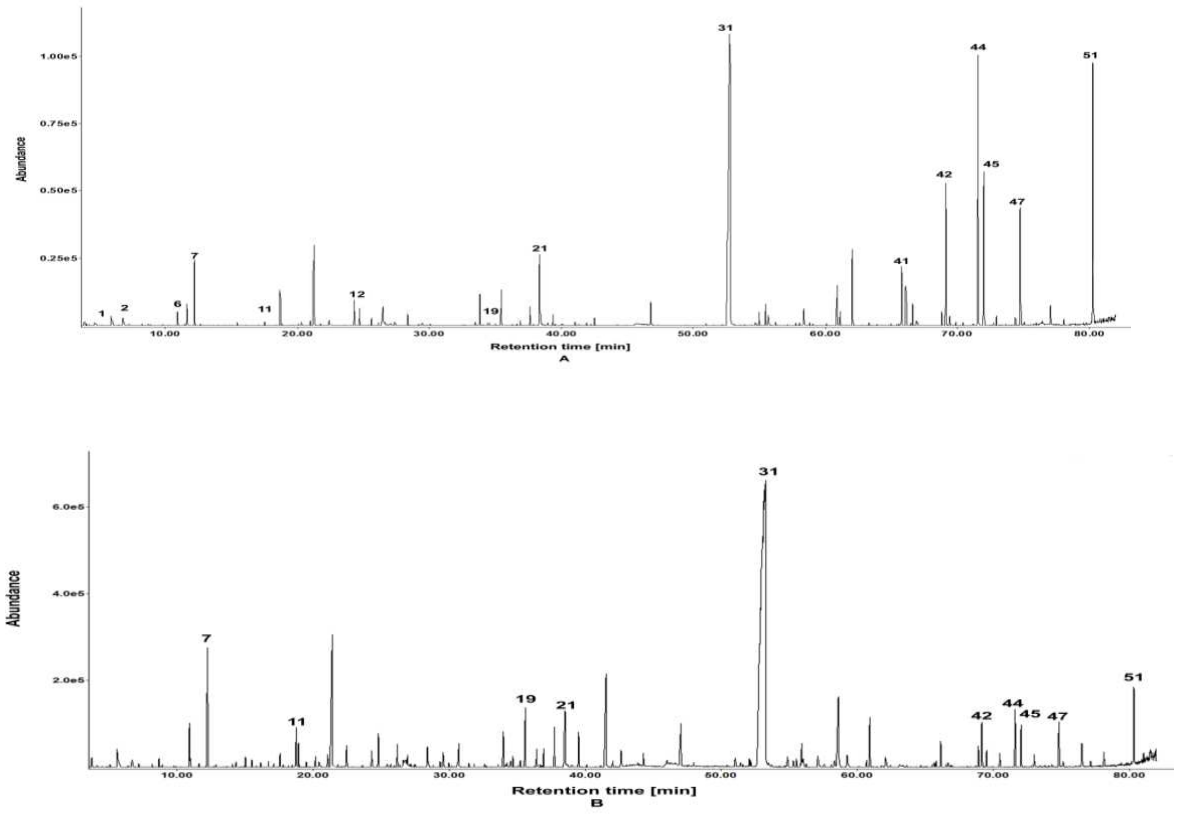
Table 5-5 Compounds identified by analytical standards

			Area percent [%]			
Detector	**Peak No.**	**Compound**	***Chlorella vulgaris* Biomass**	***Chlorella vulgaris* Proteins**	***Chlorella vulgaris* Lipids**	***Chlorella vulgaris* Residue**
FID	**2**	Toluene	3.5	7.2	n.d.	4.2
	4	Ethylbenzene	1.2	1.5	n.d.	n.d.
	6	Styrene	1.0	1.8	n.d.	n.d.
	12	Phenol	2.3	3.5	n.d.	2.7
	15	4-Methylphenol	1.6	3.7	n.d.	3.1
NPD	**3**	Pyridine	n.d.	1.3	n.d.	n.d.
	5	Pyrrole	1.5	1.6	n.d.	1.1
	18	3-Phenylpropionitrile	1.7	2.1	n.d.	n.d.
	20	Picolinamide	1.3	n.d.	n.d.	1.2
	22	Indole	3.9	7.5	n.d	4.9
	24	3-Methylindole	1.3	2.4	n.d.	2.8

5.2.2. ***Porphyridium purpureum***

The biomass and a residue after the extraction of Coenzyme Q_{10} of the red algae *Porphyridium purpureum* was used in this study and obtained pyrograms are shown in Figure 5-6. The identified peaks and their peak area percentage are listed in Table 5-6.
The most characteristic peak detected in the pyrolysate of the biomass of *Porphyridium purpureum* was levoglucosan accounting for ca. 25 %. Furthermore, benzoic acid, alkyl ester derivatives were detected with ca. 15 %, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester with 9.8 % and squalene with ca. 10.7 %. Chemical compounds detected in minor amounts were 5-(hydroxymethyl)-2-furaldehyde (3.0 %), furfural (2.1 %), triphenylmethyl chloride (2.0 %) and phenol (1.0 %). Furthermore, acetic acid, toluene, styrene, 5-methyl-2-furaldehyde and 1,4:3,6-dianhydro-α-D-glucopyranose were detected in amounts less than 1 %.
The obtained pyrogram of the residue after high pressure methanol extraction of the Coenzyme Q_{10} revealed a levoglucosan content of ca. 47 %. Other detected compounds were furfural (3.0 %), 5-(hydroxymethyl)-2-furaldehyde (2.3 %), squalene (2.2 %). In addition 1-2 % of benzoic acid, alkyl ester derivative, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester and 1,4:3,6-dianhydro-α-D-glucopyranose were detected.

Figure 5-6 Pyrograms of *Porphyridium purpureum* at 500 °C



A= *Porphyridium purpureum* biomass

B= *Porphyridium purpureum* residue

Table 5-6 Peak areas of pyrolysis compounds - *Porphyridium purpureum*

		Area percent [%]	
Peak No.	**Chemical Compound**	***Porphyridium purpureum* Biomass**	***Porphyridium purpureum* Residue**
1	Acetic Acid	<1	n.d.
2	Toluene	<1	n.d.
6	Styrene	<1	n.d.
7	Furfural	2.1	3.0
11	5-Methyl-2-furaldehyde	<1	<1.
12	Phenol	1.0	n.d.
19	1,4:3,6-Dianhydro-α-D-glucopyranose	<1	1.6
21	5-(Hydroxymethyl)-2-furaldehyde	2.3	3.0
31	Levoglucosan	25.3	46.9
41	Triphenylmethyl chloride	2.0	n.d.
42	Benzoic acid, alkyl ester derivative	5.0	1.1
44	2-Propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester	9.8	1.4
45	Isomer 42	5.2	<1
47	Isomer 42	5.0	1.4
51	Squalene	10.7	2.2
Total Identified Area %		**69.1**	**59.9**

5.3. Macroalgae

5.3.1. Arctic species

By intermediate pyrolysis generated products of arctic species including *Prasiola crispa*, *Monostroma arcticum*, *Polysiphonia arctica*, *Devaleraea ramentacea*, *Odonthalia dentata*, *Phycodrys rubens* and *Sphacelaria plumosa* were analysed.

Obtained pyrograms are presented in Figure 5-7 and identified compounds listed in Table 5-7.

The pyrograms showed a relative consistency in the types of pyrolysis products obtained although unique chemical compounds for some species were evident. The major pyrolytic chemical obtained by pyrolysis of arctic algal biomass was levoglucosan occurring within the range of 10-62 % within all obtained pyrolysates. Furthermore, 5-methyl-2-furaldehyde, furfural and 1-hexandence were found in substantial amounts of 10 % in some of the species.

Major compounds detected in pyrolysates obtained from *Prasiola crispa* include 24.2 % levoglucosan, 6.8 % D-allose, 4.9 % 5-methyl-2-furaldehyde and 4.6 % phytol. Products accounting for 1-3 % were toluene, furfural, 5-(hydroxymethyl)-2-furaldehyde, isosorbide, triphenylmethyl chloride, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester, benzoic acid, alkyl ester derivatives and octadecanoic acid, octyl ester.

Within the pyrolysates of *Monostroma arcticum* detected compounds were levoglucosan accounting for 29.3 %, 1-hexadencene with 9.4 %, 5-methyl-2-furaldehyde with 9.3 %, triphenylmethyl chloride with 8 %, benzoic acid, alkyl ester derivatives with 7.5 % and 2-Propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester with 5.3 %. Chemical compounds detected within 2-5 % were octadecanoic acid, octyl ester, 2 or 3- or 4-chlorobenzophenone, and furfural.

The major pyrolysis product obtained from *Polysiphonia arctica* was levoglucosan accounting for 61.6 %. Compounds with the amount of ca. 2 % include 5-(hydroxymethyl)-2-furaldehyde and unidentified anhydrosugar.

Main intermediate pyrolysis products of *Devalaraea ramentacea* include 10.6 % of furfural and 10.1 % of levoglucosan. Furthermore, toluene, phenol, 4-methylphenol, triphenylmethyl chloride, benzoic acid, alkyl ester derivatives, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester, octadecanoic acid, octyl ester and squalene were detected within the range of ca. 2-4 %.

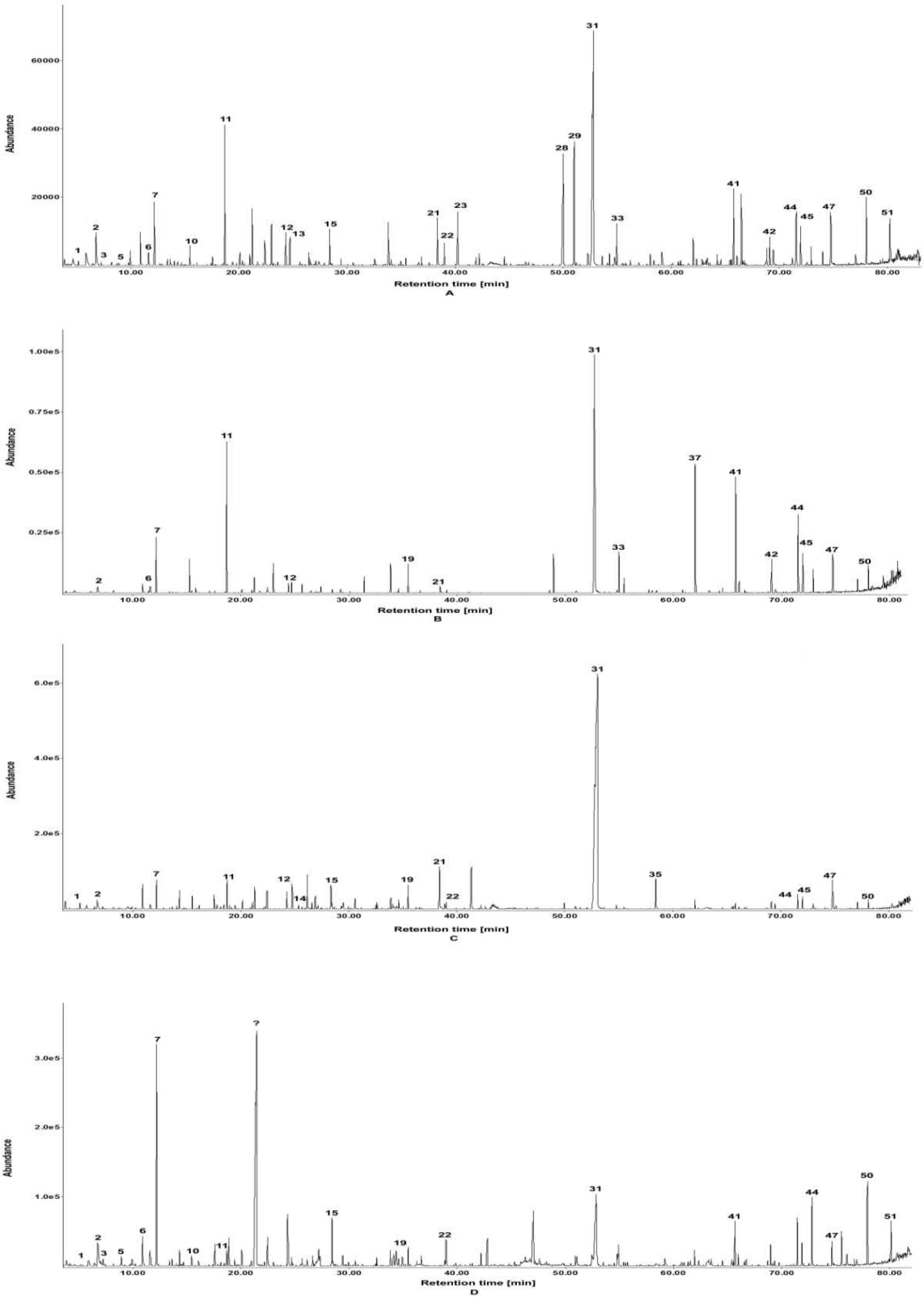
Pyrolysis of *Odonthalia dentata* biomass generated 46.2 % levoglucosan, 4.3 % squalene, 5 % benzoic acid, alkyl ester derivatives and 3.8 % 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester. In addition, toluene, furfural, 1-(2-Furanyl)-

ethanone, 5-methyl-2-furaldehyde, phenol, 3-furancarboxylic acid, methyl ester, 4-methylphenol, 1,4:3,6-dianhydro-α-D-glucopyranose and triphenylmethyl chloride accounted for 1-3 %.

The pyrolysates derived by *Phycodrys rubens* contained 13.5 % levoglucosan, 6.9 % squalene, 4.8 % of 5-methyl-2-furaldehyde and 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester, 4.2 % of furfural and 7.1 % of benzoic acid, alkyl ester derivatives. Other detected compounds were toluene (2 %), 4-mthylphenol (2.1 %) and 1,4:3,6-Dianhydro-α-D-glucopyranose (2.7 %).

Major pyrolytic chemicals identified by the pyrograms of *Sphacelaria plumosa* include levoglucosan accounting for 48.2 %, dianhydromannitol with 4.6 %, and another unidentified anhydrosugar with 3.6 %. In addition, furfural, 5-methyl-2-furaldehyde, 1,4:3,6-dianhydro-α-D-glucopyranose, and 1-hexadecene were detected within the range of ca. 1-2 %. The total identified area varies between all species within 43-78 %.

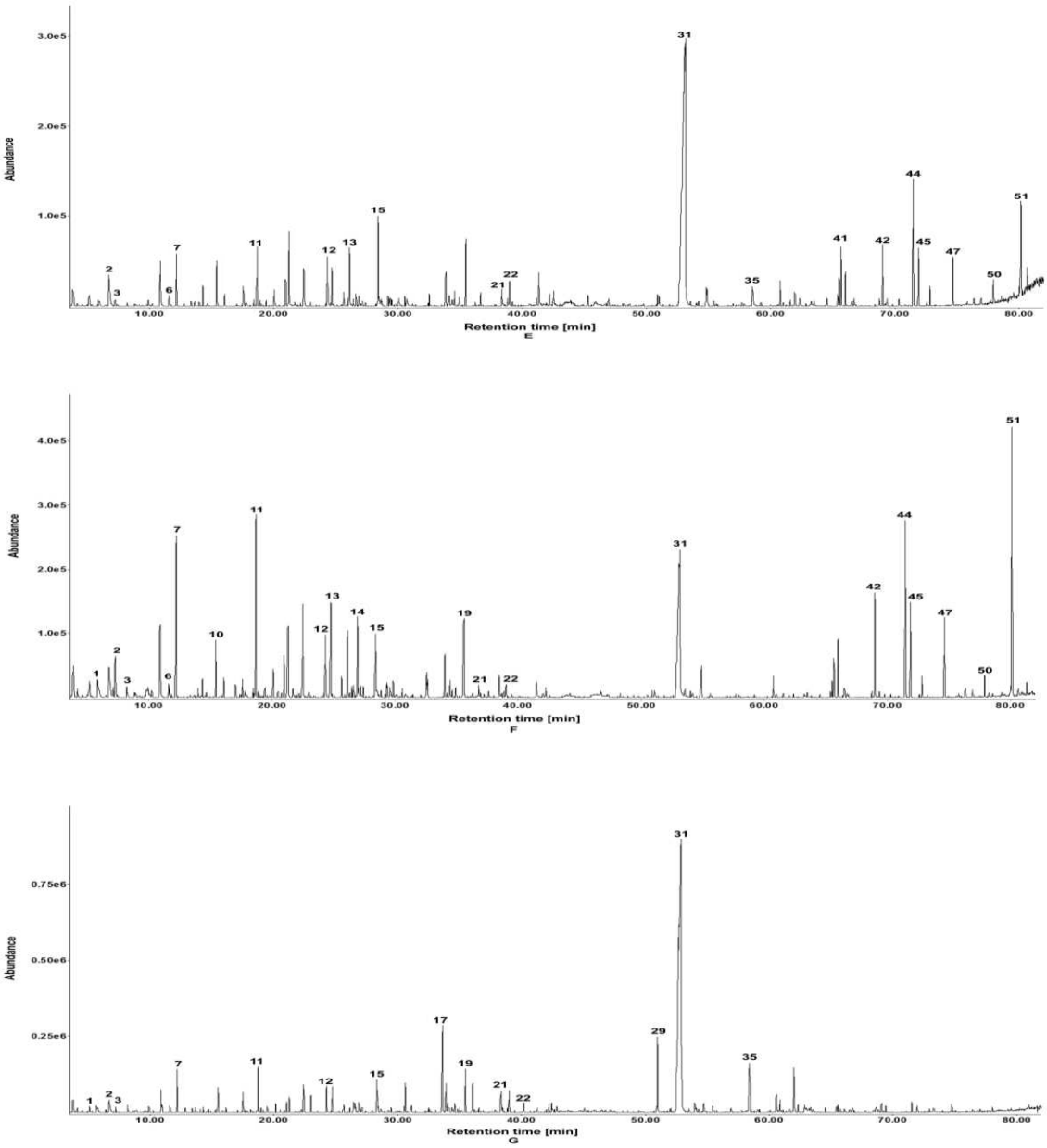
Figure 5-7 Pyrograms of Arctic species at 500 °C



A= *Prasiola crispa* B=*Monostroma arctica*

C= *Polysiphonia arctica* D= *Devaleraea ramentacea*

Figure 5-7 continued



E= *Odonthalia dentata* F= *Phycodrys rubens* G= *Sphacelaria plumosa*

Table 5-7 Peak areas of pyrolysis compounds - Arctic species

		Area percent [%]						
Peak No.	**Chemical Compound**	***Prasiola crispa***	***Monostroma arcticum***	***Polysiphonia arctica***	***Devalaraea ramentacea***	***Odonthalia dentata***	***Phycodrys rubens***	***Sphacelaria plumosa***
1	Acetic Acid	1.1	n.d.	<1	<1	<1	1.0	<1
2	Toluene	2.7	<1	<1	2.4	2.0	2.0	<1
3	Pyridine	<1	n.d.	n.d.	<1	<1	1.3	<1
5	Pyrrole	<1	n.d.	n.d.	<1	n.d.	n.d.	n.d.
6	Styrene	<1	<1	n.d.	1.1	<1	<1	n.d.
7	Furfural	2.4	3.6	1.2	10.6	1.6	4.2	1.4
10	1-(2-Furanyl)-ethanone	<1	n.d.	<1	<1	1.2	1.3	<1
11	5-Methyl-2-furaldehyde	4.9	9.3	1.3	<1	1.6	4.8	1.6
12	Phenol	1.3	<1	<1	3.2	1.7	1.8	1.1
13	3-Furancarboxylic acid, methyl ester	n.d.	n.d.	n.d.	n.d.	1.8	1.6	n.d.
14	Maltol	n.d.	n.d.	<1	n.d.	n.d.	2.4	<1
15	4-Methylphenol	1.3	n.d.	1.2	2.2	2.9	2.1	1.1
17	Dianhydromannitol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.6
19	1,4:3,6-Dianhydro-α-D-glucopyranose	n.d.	1.4	1.4	<1	1.9	2.7	1.7

Table 5-7 continued

		Area percent [%]						
Peak No.	**Chemical Compound**	***Prasiola crispa***	***Monostroma arcticum***	***Polysiphonia arctica***	***Devalaraea ramentacea***	***Odonthalia dentata***	***Phycodrys rubens***	***Sphacelaria plumosa***
21	5-(Hydroxymethyl)-2-furaldehyde	2.3	<1	2.6	n.d.	<1	<1	<1
22	Indole	<1	n.d.	<1	1.3	<1	<1	<1
23	Isosorbide	2.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
28	D-Allose	6.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
29	Phytol	4.6	n.d.	n.d.	n.d.	n.d.	n.d.	2.7
31	Levoglucosan	24.2	29.3	61.6	10.1	46.2	13.5	48.2
33	2 or 3 or 4-Chlorobenzophenone	1.6	2.6	n.d.	n.d.	n.d.	n.d.	n.d.
35	Anhydrosugar	n.d.	n.d.	2.2	n.d.	1.0	n.d.	3.6
37	1-Hexadecene	n.d.	9.4	n.d.	n.d.	n.d.	n.d.	1.9
41	Triphenylmethyl chloride	3.3	8.0	n.d.	2.1	1.8	n.d.	n.d.
42	Benzoic acid, alkyl ester derivative	1.2	2.2	n.d.	n.d.	1.8	2.6	n.d.
44	2-Propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester	2.3	5.3	<1	2.3	3.8	4.8	n.d.
45	Isomer 42	1.6	2.6	<1	1.1	1.8	2.5	n.d.
47	Isomer 42	2.3	2.7	1.8	1.3	1.6	2.0	n.d.
50	Octadecanoic acid, octyl ester	2.7	1.9	<1	3.8	<1	<1	n.d.
51	Squalene	1.7	n.d.	n.d.	2.3	4.3	6.9	n.d.
Total Identified Area %		70.5	78.3	73.3	43.8	77.0	57.5	67.9

5.3.2. Antarctic species

Py-GC/MS was used to study product evolution from the antarctic algal biomass including the species *Gigartina skottsbergii, Plocamium cartilagineum, Myriogramme manginii, Hymencladiopsis crustigena* and *Kallymenia antarctica* from intermediate pyrolysis at 500°C. Pyrograms obtained are shown in Figure 5-8 along with the peak assignments and peak areas for the identified compounds in Table 5-8.

Overall, the pyrograms showed significant differences in the types of pyrolysis products obtained from all species.

Main identified chemicals within the pyrolysate of *Gigartina skottsbergii* were furfural accounting for 13.3 %, levoglucosenone with 12.6 % and phenanthrene derivatives with 16 %. Furthermore, 4.3 % toluene, 4.7 % 5-methyl-2-furaldehyde and 3.3 % of 3-furancarboxylic acid, methyl ester and 4-methylphenol were detected.

Pyrolysates of *Plocamium cartilagineum* revealed being benzoic acid, alkyl ester derivatives (11.6 %), levoglucosenone (7.6 %), 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester (7.4 %), squalene (7.6 %) triphenylmethyl chloride (5 %) the main products. In addition, about 4-5 % of toluene, 4-methylphenol, phenanthrene derivatives and octadecanoic acid, octyl ester were found.

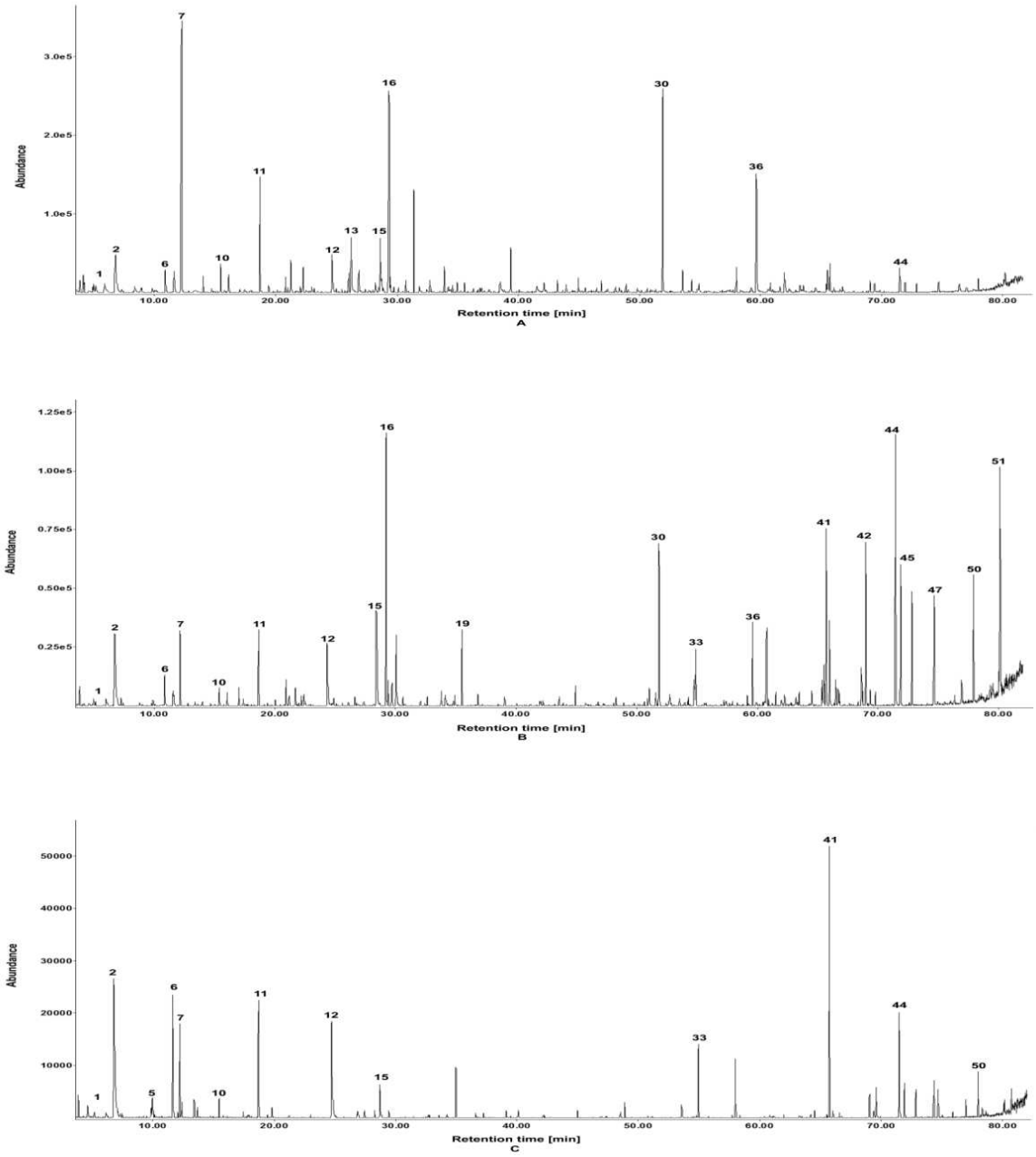
Intermediate pyrolysis of biomass of *Myriogramme manginii* generated main chemicals including toluene accounting for 18.6 %, phenol with 10 % styrene with 8.2 % and triphenylmethyl chloride with 12.5 %. Other detected compounds were furfural, 5-methyl-2-furaldehyde 2 or 3 or 4 chlorobenzophenone and 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester accounting for 4-5 %.

Hymencladiopsis crustigena derived pyrolysates contained 22.5 % levoglucosan, 12.3 % squalene, 9 % of benzoic acid, alkyl ester derivatives and an unidentified anhydrosugar accounting for 4.2 %. Further detected compounds include 1,4:3,6-dianhydro-α-D-glucopyranose, 3-methylindole, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester and octadecanoic acid, octyl ester of 2-3 %.

Main pyrolytic compounds identified within pyrolysates of *Kallymenia antarctica* were levoglucosan accounting for 23.4 %, squalene with 11.4 % and an unidentified anhydrosugar with 9.2 %. Furthermore, 1-hexadencene (3.4 %) and 4-methylphenol (2.2 %) was detected.

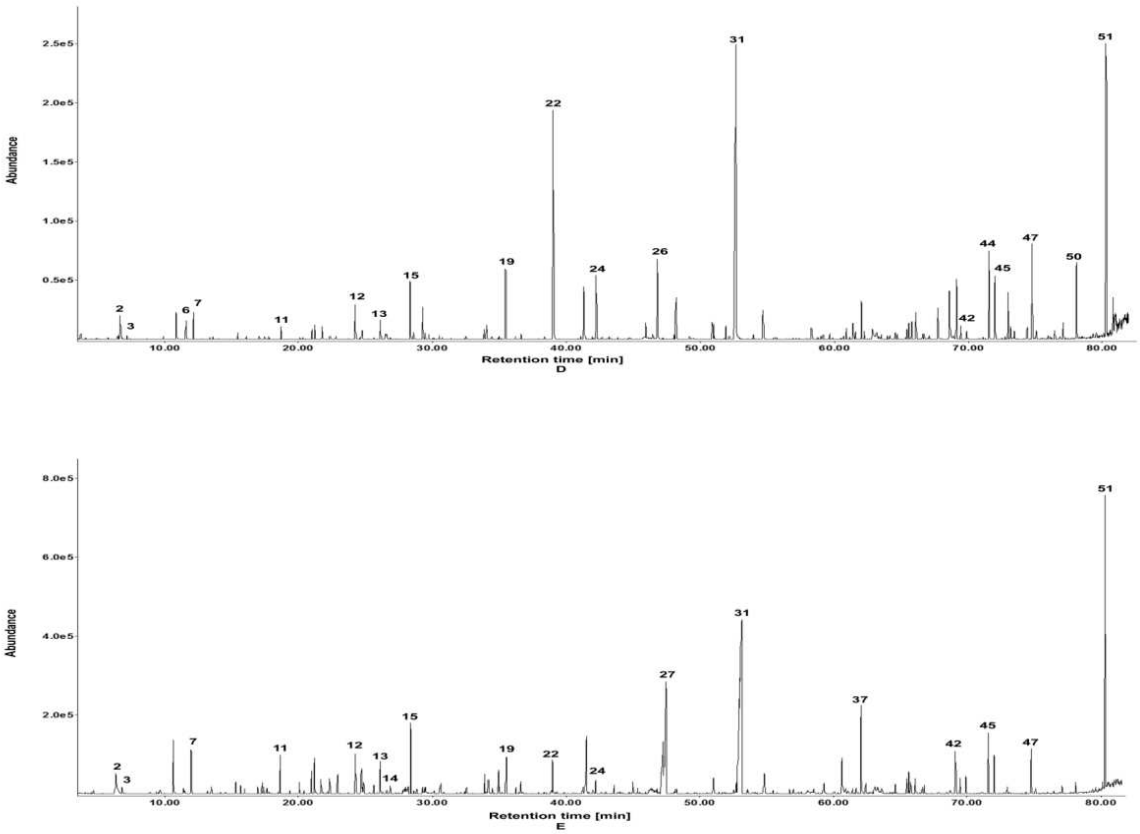
In general, 62-78 % of the total peaks areas could be indentified from pyrolysates of antarctic species.

Figure 5-8 Pyrograms of Antarctic species at 500 °C



A= *Gigartina skottsbergii* B= *Plocamium cartilagineum* C= *Myriogramme manginii*

Figure 5-8 continued



D= *Hymencladiopsis crustigena* E= *Kallymenia antarctica*

Table 5-8 Peak areas of pyrolysis compounds - Antarctic algae

		Area percent [%]				
Peak No.	**Chemical Compound**	***Gigartina skottsbergii***	***Plocamium cartilagineum***	***Myriogramme manginii***	***Hymencladiopsis crustigena***	***Kallymenia antarctica***
1	Acetic Acid	<1	<1	<1	n.d.	n.d.
2	Toluene	4.3	4.9	18.6	1.3	1.8
3	Pyridine	n.d.	n.d.	n.d.	<1	<1
5	Pyrrole	n.d.	n.d.	1.3	n.d.	n.d.
6	Styrene	1.3	<1	8.2	<1	n.d.
7	Furfural	13.3	2.0	4.8	<1	1.4
10	1-(2-Furanyl)-ethanone	1.1	<1	<1	n.d.	n.d.
11	5-Methyl-2-furaldehyde	4.7	1.9	5.1	<1	1.1
12	Phenol	2.3	2.6	10.0	1.3	1.5
13	3-Furancarboxylic acid, methyl ester	3.3	n.d.	n.d.	<1	1.1
14	Maltol	n.d.	n.d.	n.d.	n.d.	<1
15	4-Methylphenol	3.2	4.3	2.2	2.3	2.2
16	Levoglucosenone	12.6	7.6	n.d.	n.d.	n.d.
19	1,4:3,6-Dianhydro-α-D-glucopyranose	n.d.	2.3	n.d.	2.7	1.4

Table 5-8 continued

	Area percent [%]					
Peak No.	**Chemical Compound**	***Gigartina skottsbergii***	***Plocamium cartilagineum***	***Myriogramme manginii***	***Hymencladiopsis crustigena***	***Kallymenia antarctica***
22	Indole	n.d.	n.d.	n.d.	10.7	1.1
24	3-Methylindole	n.d.	n.d.	n.d.	2.5	<1
26	Anhydrosugar	n.d.	n.d.	n.d.	4.2	n.d.
27	Anhydrosugar	n.d.	n.d.	n.d.	n.d.	9.2
30	Phenanthrene derivative	10.0	4.1	n.d.	n.d.	n.d.
31	Levoglucosan	n.d.	n.d.	n.d.	22.5	23.4
33	2 or 3 or 4-Chlorobenzophenone	n.d.	1.7	4.0	n.d.	n.d.
36	Phenanthrene derivative	6.0	2.2	n.d.	n.d.	n.d.
37	1-Hexadecene	n.d.	n.d.	n.d.	n.d.	3.4
41	Triphenylmethyl chloride	n.d.	5.0	12.5	n.d.	n.d.
42	Benzoic acid, alkyl ester derivative	<1	4.3	n.d.	2.8	1.6
44	2-Propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester	1.2	7.4	5.2	3.9	n.d.
45	Isomer 42	n.d.	4.1	1.7	2.8	n.d.
47	Isomer 42	n.d.	3.2	1.7	3.8	1.7
50	Octadecanoic acid, octyl ester	n.d.	3.9	2.5	3.1	n.d.
51	Squalene	n.d.	7.6	n.d.	12.3	11.4
Total Identified Area %		63.3	69.1	77.8	76.2	62.3

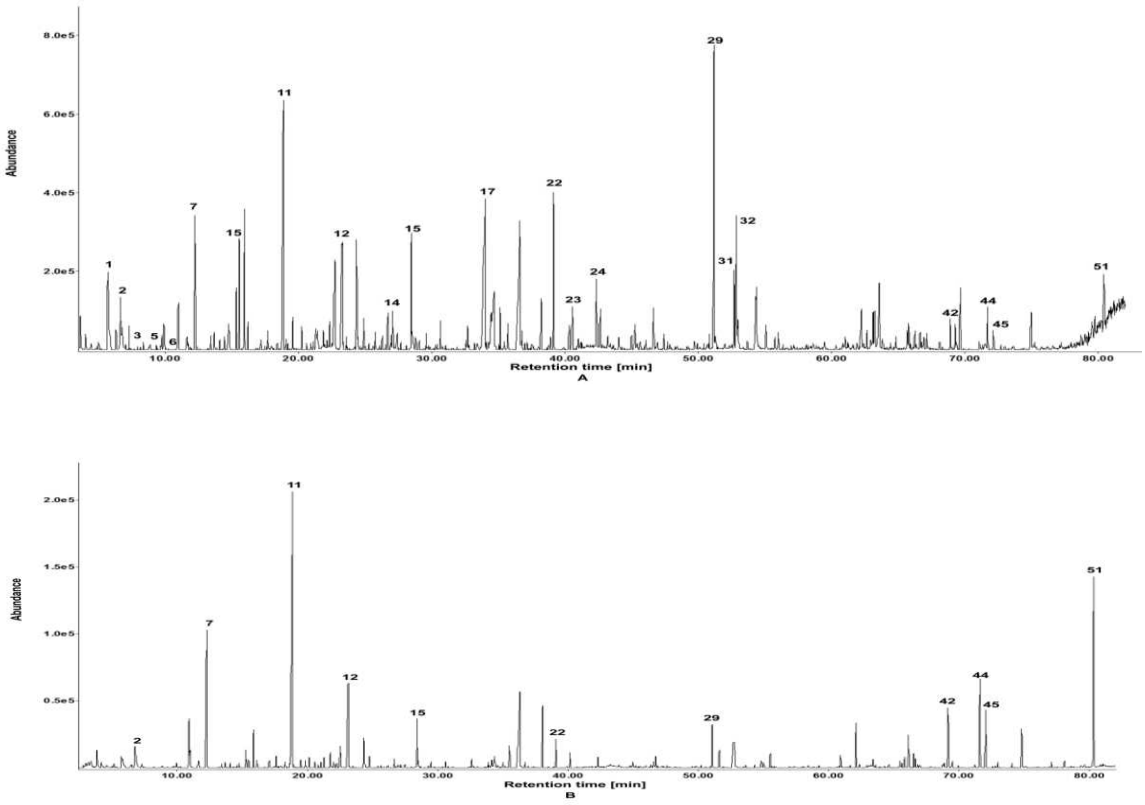
5.3.3. ***Fucus vesiculosus***

The obtained pyrograms of *Fucus vesiculosus* biomass and a residue after polysaccharide extraction are presented in Figure 5-9 and identified peaks are listed in Table 5-9.

Generally, differences in terms of distribution of the detected chemical were observed. The pyrolysate of the entire biomass consists mainly of polysaccharide derived chemicals such as 5-methyl-2-furaldehyde accounting for 5.5 %, dianhydromannitol with 7.4 %, levoglucosan with 4.1 %, isosorbide (1.2 %) and maltol (1.2 %). Further detected compounds are acetic acid (2.7 %), furfural (2.5 %), indole (2.7 %), squalene (1.5 %), and phytol with 9.2 %.

Main chemicals indentified within the pyrograms obtained by the residue are 5-methyl-2-furaldehyde with 15 %, squalene with 8 %, furfural with 6.2 % and benzoic acid alkyl ester derivative with 4.4 %. In addition, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester and squalene were found within the pyrograms of the residue.

Figure 5-9 Pyrograms of *Fucus vesiculosus* at 500 °C



A= *Fucus vesiculosus* biomass B= *Fucus vesiculosus* residue

Figure 5-9 Peak areas of pyrolysis compounds - *Fucus vesiculosus*

		Area percent [%]	
Peak No.	**Chemical compound**	***Fucus vesiculosus* Biomass**	***Fucus vesiculosus* Residue**
1	Acetic Acid	2.7	n.d.
2	Toluene	1.4	1.9
3	Pyridine	<1	n.d.
5	Pyrrole	<1	n.d.
6	Styrene	<1	n.d.
7	Furfural	2.5	6.2
10	Ethanone, 1-(2-furnaly)-	2.1	n.d.
11	2-Furaldehyde, 5-methyl	5.5	15.0
12	Phenol	2.1	1.4
14	Maltol	1.2	n.d.
15	4-Methylphenol	2.1	1.7
17	Dianhydromannitol	7.4	n.d.
22	Indole	2.7	1.0
23	Isosorbide	1.2	n.d.
24	3-Methylindole	1.1	n.d.
29	Phytol	6.2	1.5.
31	Levoglucosan	4.1	n.d.
32	Isomer of 29	3.5	n.d.
42	Benzoic acid alkyl ester derivative	<1	2.2
44	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	<1	3.4
45	Isomer 42	<1	2.2
51	Squalene	1.5	8.0
Total Identified Area %		47.3	44.5

CHAPTER 6

Discussion

Biomass Characterisation

6. Discussion biomass characterisation

This chapter discusses the main results revealed by biomass characterisation including CHNO, HHV, and ash analysis. Furthermore, the outcomes of intermediate pyrolysis performed by TGA are reviewed. At the end a summarising conclusion is presented.

6.1. Ultimate analysis, ash and calorific values

The characterisation of the microalgal biomasses revealed no significant differences between the both *Chlamydomonas reinhardtii* strains, regarding their CHNO, Ash and HHV values. In contrast, the biomass of C*hlorella vulgaris* contained lower C, N and higher O values than the two *Chlamydomonas reinhardtii* strains. In addition, the HHV of *Chlorella vulgaris* biomass was lower.

In general, higher carbon contents indicate higher amounts of lipids in algae biomass, due to their hydrocarbon structure, where up to 80 % are constituted of carbon.

In comparison, proteins contain ca. 53 % and carbohydrates ca. 40 % of carbon and higher oxygen contents than lipids [164], [165]. Consequently, higher oxygen contents indicate higher protein and/ or carbohydrate contents within the biomass.

Therefore, the high carbon and low oxygen content of both *Chlamydomonas reinhardtii* strains are related to a higher lipid and a lower carbon and/or protein content. Furthermore, the lower carbon content of *Chlorella vulgaris* biomass indicates a lower lipid and a higher protein and/or carbohydrate content.

Overall, higher carbon and lower oxygen contents lead to higher energy contents [166], expressed by the HHV. Therefore, the higher carbon and lower oxygen contents of both *Chlamydomonas reinhardtii* strains results in higher HHV of 22-23 MJ kg^{-1}, compared to *Chlorella vulgaris* biomass revealing a HHV of 18 MJ kg^{-1}.

The lowest carbon content (35.2 %) and highest oxygen content (58 %) within studied microalgal biomass was obtained by the red alga *Porphyridium purpureum.* In coherence, the biomass revealed the lowest HHV of 14.9 MJ kg^{-1} of analysed microalgae species. Consequently, when compared to the green microalgae species, lower amounts of lipids and higher amounts of proteins and/or carbohydrates can be expected in the biomass of the red algae.

In contrast to the green microalgae species, the macroalgae species exhibit significant lower carbon contents (22-39 %), and higher oxygen (52-70 %) contents. These results reveal the lower lipid and higher protein and/or carbohydrate contents, typical for

macroalgae species. In average, macroalgae biomass contains around 1-5 % lipids, 5-20 % proteins and 40-60 % carbohydrates [48], [167], [168]. As a consequence of lower lipid contents lower HHV of analysed macroalgae species were obtained in the range of 13-16 MJ kg^{-1}.

Within the analysed microalgae species, *Chlamydomonas reinhardtii* strains exhibited the highest nitrogen contents of ca. 11 %. In contrast, *Chlorella vulgaris* contained 6.7 % and *Porphyridium purpureum* 1.3 %. Within plant material nitrogen is a substantial element in amino acids, proteins, chlorophyll, enzymes and vitamins [169]. In general, due to higher amounts of proteins and pigments in algal biomass it exhibits higher nitrogen contents than in woody biomass (typically <1 %) [97]. Therefore, the detected amounts of nitrogen within the *Chlamydomonas reinhardtii* strains can indicate higher amounts of proteins and/or chlorophyll than in *Chlorella vulgaris*. The low levels of nitrogen detected in the red microalgae *Porphyridium purpureum* can indicate lower protein contents in the biomass. Amounts of nitrogen detected in macroalgal biomass ranged within 1.4-4.0 %. After all, the lower nitrogen content and higher oxygen content of red and brown algae biomass, could be related to their different pigmentation, including nitrogen-free and oxygen containing pigments such as xanthophylls [39].

Ash values determined from microalgae species range from 13.6-15.9 % whereas *Porphyridium purpureum* exhibits the lowest and *Chlorella vulgaris* the highest content. The highest ash values were determined in the biomass of macroalgae samples. Nine out of twelve species exhibited ash contents between 30-40 %. The lowest content of 15.5 % was obtained by the temperate species *Fucus vesiculosus* and the highest of 44.7 % by the antarctic species *Kallymenia antarctica.* Determined ash values from algal species, particular of macroalgae contrasts sharply with the ash content typically obtained from terrestrial biomass of ca 1-5 % [170–172].
In general, the ash content of algal biomass is related to the species, its geographical origin and seasonal and environmental variations. For macroalgae species, contents of around 40 % ash are typical. Due to their large organisms and differentiated cells building the thallus, major mineral elements are accumulated such as sodium, potassium, calcium and magnesium. Furthermore, sulphates are contained in macroalgae biomass, ranging typically from 1-5 % [167], [173].
Overall, high ash contents have an influence on the pyrolytic behaviour of biomass. In general, decomposition temperatures of polysaccharides material in the biomass lowered by high ash contents in the biomass, due to catalytic effects of the inorganic materials [174]. Furthermore, high ash contents, particular alkali metals, such as

sodium and potassium, may cause operational problems in large scale thermo-chemical conversion processes relating to corrosion and slag formation [168], [172]. In addition, the high ash contents in macroalgal biomass leads to lower HHV [166].

6.2. Total lipid, protein and FAMEs analysis

The extraction of biomass components revealed proteins being the most abundant of the three unicellular algae analysed. Between both *Chlamydomonas reinhardtii* strains no major differences in terms of amounts of total proteins were evident. In contrast, biomass of *Chlorella vulgaris* exhibited a significant higher content of proteins. These results are in coherence with obtained data from the elemental analysis, where both *Chlamydomonas reinhardtii* strains showed similar CHNO values and *Chlorella vulgaris* biomass revealed a significant lower carbon and higher oxygen content. Total lipid determination revealed that the biomass of *Chlamydomonas reinhardtii* wild type contains 18 %, its cell wall mutant ca. 22 % and *Chlorella vulgaris* ca. 16 % of total lipids.

Overall, the indication of the gross chemical composition by the values of C, H and O in the biomass was confirmed by total lipid and total protein determination, whereas both *Chlamydomonas reinhardtii* strains contain lower protein and higher lipid proportions than *Chlorella vulgaris*.

Moreover, the obtained total lipid and total protein values are in accordance with previously published data [44]. According to literature, alanine, tyrosine, and glutamine/glutamic acid are the main amino acids of *Chlorella vulgaris* [175]. Most abundant amino acids within *Chlamydomonas reinhardtii* are hydroxyproline and glutamine/glutamic acid [176].

Within the BtVB-process, it is of interest to evaluate possible extraction of value added products, such as lipids before pyrolysing the biomass. Therefore, the identification of FAMEs of the three microalgae species was of interest within this study, due to their various applications within pharmacy, nutrition and cosmetics [46].

Both *Chlamydomonas reinhardtii* strains revealed no significant differences and palmitic acid and α-linolenic acid were the two major FAMEs (>10 %) detected. Therefore, the results indicate that the genetic defect in cell wall synthesis has not perturbed lipid synthesis in this alga. In contrast, *Chlorella vulgaris* biomass presented

a broader spectrum of major FAMES, including palmitic acid, oleic acid, linoleic acid and α-linolenic acid.

Identified PUFAs include linoleic acid an omega-6 fatty acid and α-linolenic acid an omega-3 fatty acid. Linolenic acid was detected in both *Chlamydomonas reinhardtii* strains accounting for 6-7 % and in *Chlorella vulgaris* 10 % were observed. Total lipids of *Chlamydomonas reinhardtii* biomass contained 64-69 % of α-linolenic acid.

These PUFAs are essential fatty acids, by means, the human body need to ingest as it is not able to synthesise them. Main sources of α-linolenic acid are flaxseed, walnuts, and soybeans. Grains, meats and seeds are main sources of linoleic acid [177]. Effects of omega-3 fatty acids within human bodies include stimulation of hormonal activities which affects cardiovascular, immune and the central nervous system beneficially [178]. Furthermore, it has been shown, that omega-3 fatty acids reduce the risk for cardiac arrhythmias [177]. Oleic acid is a monounsaturated FAME being one of the most abundant lipids, mainly found in olive and almond oil [178] accounted for 21.1 % in *Chlorella vulgaris* total lipids. Oleic acid is used in pharmaceutical applications as a blood pressure reducing agent [179].

After lipid extraction the residual biomass was retained, accounting for ca. 62-72 % of the total biomass of all three species. This residue is of importance for this study, as it is applied to reveal the effect of lipid extraction prior intermediate pyrolysis of algal biomass. Within this residue the carbohydrate content of *Chlamydomonas reinhardtii* and *Chlorella vulgaris* accounting for 12-17 % of the dry biomass [44] as well as proteins and other extracts is contained.

In general, the proportions of biomass constituents vary dependent on species, cultivation conditions (i.e. temperature, illumination, nutrient concentration) and stage of cell growth at harvesting [180]. This study applied biomass of all three green microalgae species grown under optimum conditions, including temperature, nutrient concentration and illumination. Furthermore, each biomass was harvested at the late logarithmic growth.

6.3. Thermogravimetric analysis

6.3.1. Microalgae

6.3.1.1. *Chlamydomonas reinhardtii* and *Chlorella vulgaris*

6.3.1.1.1. Lipids

During TGA analysis, the lipid samples evolved the largest amount of highly volatile compounds of 5-7 % within temperatures of 100-200 °C. This weightloss in stage I may represent the evolution of intrinsic lipid decomposition compounds, such as aldehydes and ketones potentially formed during extraction from the cells through auto oxidation and/or enzyme catalysed routes.

The observed initial pyrolysis temperature (T_i) for lipid materials of *Chlamydomonas reinhardtii* wild type, *Chlamydomonas reinhardtii* CW 15+ and *Chlorella vulgaris* was 200 °C, respectively and is the lowest from all biomass constituents in this study. Furthermore, it has been shown, that lipids release the highest volatile matter of ca. 80 % within stage II (200-550 °C). This is related to the hydrocarbon chains of fatty acid components being the major constituents of lipids.

Deviations of thermal behaviours between the lipid extracts were observed. Different shaped TGA curves were obtained from *Chlamydomonas reinhardtii* and *Chlorella vulgaris*, whereas no significant differences were revealed between *Chlamydomonas reinhardtii* wild type and *Chlamydomonas reinhardtii* CW15+. For lipid materials from both *Chlamydomonas reinhardtii* stains two major decomposition peaks at 300 °C and 410 °C were observed. In contrast, *Chlorella vulgaris* exhibited only one main peak at 400 °C. These significant differences are consistent with the varying fatty acid compositions. Both *Chlamydomonas reinhardtii* strains contain mainly palmitic acid and α-linolenic acid. Total lipids of *Chlorella vulgaris* contained a broader spectrum of FAMEs and higher amounts of oleic acid and linoleic acid.

The amount of char obtained from all three total lipids ranged between 10-14 %. Compared to biomass, total proteins and residues these amounts of char are the lowest obtained in this study. This is in coherence with the formation of the largest amount of volatiles during stage II, due to an easier fragmentation of hydrocarbon chains into volatile components than thermal decomposition of many amino acids residues or carbohydrates. Therefore, high lipid contents in algal biomass increase the amount of volatile pyrolytic compounds and lower the amount of char.

6.3.1.1.2. Proteins

TGA analysis of the three total proteins extracts revealed T_i of 250 °C. This shows that proteins starts volatising at ca. 50 °C higher temperatures than lipids under intermediate pyrolysis conditions.

Generally, stage II lasted until 550 °C for all protein materials. *Chlamydomonas reinhardtii* wild type revealed a characteristic shoulder at 320 °C, which indicates a presence of proteins not contained in the mutant *Chlamydomonas reinhardtii* CW15+. This is consistent with the observed mutant behaviour. Certainly the motility of *Chlamydomonas reinhardtii* CW15+ appears impaired under microscopic examination and suggests that flagella proteins may be decreased in the mutant (G. Griffiths, personal observations). Additionally, components decomposed at 490 °C of *Chlorella vulgaris* derived total proteins, which were not observed in *Chlamydomonas reinhardtii* strains. This might be related to the different amino acid composition of the two green algae. TGA curves revealed that protein samples formed the largest portions of char obtained in this study, accounting for 30 % for both *Chlamydomonas reinhardtii* strains and 37 % for *Chlorella vulgaris.* Consequently, higher protein contents in algal biomass favour the formation of char during intermediate pyrolysis.

6.3.1.1.3. Residues

The analysed residues after total lipid extraction contain the total fraction of proteins and carbohydrates of the original biomass mainly. The major pyrolytic activity of these materials occurred between 230-550 °C. The residue of *Chlorella vulgaris* released 60 % of volatiles during stage II; both *Chlamydomonas reinhardtii* residues released 68 %. Furthermore, *Chlorella vulgaris* residue formed 37 % of char and *Chlamydomonas reinhardtii* derived residues ca. 27 %.

Total protein determination in biomass showed that *Chlorella vulgaris* contained about 10 % more proteins than both *Chlamydomonas reinhardtii* strains. Consequently, the residue *Chlorella vulgaris* contains higher protein content than both *Chlamydomonas reinhardtii* strains. Again, the obtained TGA curves indicate that higher protein contents in materials lower the formation of volatiles and favour the formation of char.

The carbohydrate fraction of *Chlamydomonas reinhardtii* strains residues is mainly made of galactose, arabinose and mannose originating from cell walls. The cell walls of *Chlorella vulgaris* mainly composed of hemicellulose. Both carbohydrate fractions do

not contain any cellulose and lignin. Furthermore, starch is the main storage component of the organisms, is contained in the residues [39].

Earlier studies investigated the thermal degradation of arabinose, xylose, mannose, hemicellulose and starch at heating rates between 2-20 °C min^{-1} [88], [181], [182]. Under these conditions the main pyrolytic activities for arabinose, xylose and mannose were observed within 120-310 °C. Hemicellulose decomposed within 220-260 °C and starch between 280-350 °C.

In contrast, heating rates of 100 °C min^{-1} were applied in this study to perform intermediate pyrolysis. Generally, an increase of heating rates leads to higher degradation temperatures of biomass materials. These shifts are caused by the low conductivity of heat in biomass. During heating a temperature gradient exist within the cross-section of the material. At lower heating rates, more time is given to heat the outer surface and the inner core of the biomass. At faster heating rates, shorter time is provided to distribute the heat within the biomass for appropriate evolution of the volatile matters. This causes incomplete reactions at lower temperatures, followed by higher decomposition temperatures of the same material [183].

6.3.1.1.4. Biomass

Results obtained by pyrolysis of biomass samples, revealed the main pyrolytic activity (stage II) between of 200 °C and 550 °C for all three specimens. Results obtained by the analysis of total proteins, total lipids and biomass residues show, that in stage I, between 100-200 °C low amounts of up to 5 % of highly volatile matter are released, mainly due to water evaporation and lipid derived compounds. At the beginning of stage II, at temperatures of 200 °C the lipid fraction in the biomass of the samples starts to volatise. Between 230-250 °C the proteinaceous and carbohydrate fraction starts to pyrolyse.

The maximum degradation temperature for both *Chlamydomonas reinhardtii* strains occurs at 380 °C and of *Chlorella vulgaris* at 350 °C. The main pyrolytic process lasts till ca. 550 °C were the volatilization of biomass finished. Char amounts of 30 % for *Chlorella vulgaris* and ca. 26 % for *Chlamydomonas reinhardtii* strains were observed. The slightly higher char formation of *Chlorella vulgaris* is expected to be a consequence of the higher protein content. In addition, the characteristic peak for *Chlorella vulgaris* biomass at ca. 490 °C was already observed from the protein samples and reveals consequently a breakdown of proteinaceous material.

Above 550 °C, within stage III of pyrolysis a characteristic peak at 730 °C was observed for *Chlorella vulgaris* biomass. A breakdown of material at this temperature was not related to lipid, protein or carbohydrate origin and may be related to decomposition of carbonaceous material as already reported for *Nannochloropsis* sp. above 700 °C [99], [184].

6.3.1.2. ***Porphyridium purpureum***

The results obtained by TGA analysis of the red algae *Porphyridium purpureum* show the main pyrolytic activity between 250-550 °C. Obtained maximum decomposition of the biomass was 300 °C, whereas a shift to 320 °C was observed for the residue after extraction of coenzyme Q10. However, the obtained temperatures are significantly lower, about 60 °C, than those obtained for the analysed green algae species. This indicates that the biomass constituents vary between the species.
No major differences in terms of released volatiles from biomass and residue were observed. Both samples released ca. 61-65 % volatiles and formed a char portion of 31-34 %. These amounts of volatiles and char indicate a lower lipid and a higher protein and carbohydrate content in the red algae biomass compared to green algae biomass. This is in coherence with the elemental analysis, were much lower amounts of carbon and higher amounts of oxygen were detected, indicating low lipid and high protein and/or carbohydrate contents.

6.3.2. Macroalgae

Thermogravimetric analysis of macroalgae biomass and from there derived materials revealed similar shaped TG and DTG curves, than obtained for microalgae biomass while exhibiting the three stages of decomposition.
Initial pyrolysis temperatures obtained for the majority of macroalgal material revealed being 200-230 °C. Lower T_i of 150 and 180 °C were obtained by the antarctic species *Myriogramme manginii* and *Plocamium cartilagineum,* respectively. Generally, significantly lower maximum decomposition temperatures of macroalgae biomass than for microalgae biomass are evident. Whereas the T_m for macroalgae species varies within 220-320 °C, values for microalgae between 350-380 °C were obtained. In addition, the high ash contents of the materials may lower the maximum degradation

temperature [185]. Overall, macroalgae biomass released significant lower amounts of volatiles and consequently formed higher amounts of char than microalgae species. The highest amounts of volatiles observed were 58 % of *Prasiola crispa*. Lowest amounts of 41 % were released by *Myriogramme manginii*. Lower formation of volatiles are a consequence of the general lower content of lipid material in macroalgae biomass, usually ranging between 1-5 % [48], [167], [168]. Therefore higher contents of proteins and carbohydrates are apparent, which increases the amounts of char products. In addition, the high ash contents of up to 40 % typically contained in macroalgae biomass leads to higher char portions.

In addition, almost all macroalgae samples included in this study revealed a further decomposition at a high temperature range of 780-820 °C. Ross et al [98], studied the pyrolysis behaviour of macroalgae polymers and revealed that the thermal decomposition of laminarin and fucoidan carried on up to high temperatures above 600 °C and fucoidan above 700 °C. Furthermore, both carbohydrates were characterised by second degradation peaks, for laminarin at above 500 °C and for fucoidan above 700 °C [98]. Additionally, the high contents of ash might catalyse further decomposition of carbonaceous materials at high temperature.

Overall, the TG and DTG curves of macroalgae materials reveal that during pyrolysis several overlapping steps are involved, indicated by shoulders, observed for most of the samples.

The TG and DTG curved of *Fucus vesiculosus* and its residue after carbohydrate extraction revealed no major differences between the samples. However, a decrease of the main decomposition temperature from 300 to 280 °C was observed, as well a lower formation of char by the residue. This observation may indicate that higher portions of carbohydrates may favour the formation of char.

6.4. Chapter Conclusions

The ultimate analysis of all algal biomass reveals preliminary information about the gross chemical composition of the samples. In general, higher carbon contents such as 50 % in *Chlamydomonas reinhardtii* biomass indicate higher lipid contents. Higher oxygen contents such as 40 % in *Chlorella vulgaris* biomass indicate higher protein and/or carbohydrate contents. Overall, higher C and lower O contents lead to higher HHV of algal biomass. Furthermore, whereas HHV of microalgae materials range between 15- 23 MJ kg^{-1}, values for macroalgae are lower accounting for 13-16 MJ kg^{-1}.

Compared to terrestrial biomass, substantially higher amounts of nitrogen were detected in biomass samples, being an important element in proteins, pigments, enzymes and vitamins. Subsequently, higher amounts of nitrogen containing chemicals in pyrolysates of algal materials are typically detected.
Analysis of FAMEs revealed that both *Chlamydomonas reinhardtii* strains contained substantial amounts the PUFA α-linoleic acid, offering the option for the production of a high valuable product within the BtVB-process.
Furthermore, the gross chemical analysis of the green microalgae strains revealed high protein contents up to ca. 55 % in *Chlorella vulgaris* biomass, being valuable ingredients in health food for humans.

Obtained TG and DTG curves at a heating rate of 100 °C min^{-1} revealed three stages of thermal decomposition, namely dehydration (stage I), devolatilization (stage II) and char formation (stage III) [90]. Generally, stage I ranges from ca. 100 to 200 °C releasing up to 2-5 % of volatiles, mainly contributed to water evaporation (dehydration) and release of highly volatile compounds. Observations revealed that the main pyrolytic process occurs in stage II, at temperatures of ca. 200-550 °C. Above 550 °C the pyrolysis process slows down and stage III starts. During this, only minor releases of volatiles of up to 5 % were observed, mainly due to continuous decomposition of the solid carbonaceous residue forming a char, containing fixed carbon and ash.
In general, this study revealed, that algal lipids produce that largest amounts of volatiles and the smallest amounts of biochar. Moreover, extracted proteins form lower amounts of volatiles and subsequently higher amounts of char.
The major pyrolytic characteristics obtained by TGA for all microalgae and macroalgae materials are presented in Table 6-1 and 6-2, respectively.

Table 6-1 Pyrolysis characteristics microalgae materials

Microalgae species/ material	Temperatures Stage (II) [°C]				Temperature shoulder [°C]	Volatiles Stage (II) [%]	Char residue at 900 °C [%]
	T_i	T_m	T_{m2}	T_e			
***Chlamydomonas reinhardtii* Wild type**							
- **Biomass**	270	350	n.d.	500	n.d.	60	25
- **Total Lipids**	250	300	410	500	n.d.	78	14
- **Total Proteins**	200	350	n.d.	500	320	62	30
- **Residue**	230	380	n.d.	500	320	68	26
***Chlamydomonas reinhardtii* CW 15+**							
- **Biomass**	270	350	n.d.	500	n.d.	60	27
- **Total Lipids**	250	300	410	500	n.d.	78	11
- **Total Proteins**	200	350	n.d.	500	n.d.	62	30
- **Residue**	230	390	n.d.	500	n.d.	68	27
Chlorella vulgaris							
- **Biomass**	270	360	n.d.	500	490, 730	54	30
- **Total Lipids**	250	400	n.d.	500	n.d.	82	10
- **Total Proteins**	200	350	n.d.	500	490	52	37
- **Residue**	230	400	n.d.	500	490	60	37
Porphyridium purpureum							
- **Biomass**	250	300	n.d.	600	820	69	31
- **Residue**	250	320	n.d.	600	820	66	34

T_i= initial temperature, T_m = maximum temperature, T_{m2} = 2nd maximum temperature in stage II, T_e = end temperature

Table 6-2 Pyrolysis characteristics macroalgae materials

Macroalgae species/ material	Temperatures Stage (II) [°C]				Temperature shoulder [°C]	Volatiles Stage (II) [%]	Char residue at 900 °C [%]
	T_i	T_m	T_{m2}	T_e			
Prasiola crispa	230	300	n.d.	600	430	60	38
Monostroma arcticum	220	280	n.d.	630	800	57	35
Devaleraea ramentacea	200	300	n.d.	650	800	50	46
Odonthalia dentata	200	310	n.d.	650	820	57	33
Phycodrys rubens	215	280	n.d.	610	810	52	36
Polysiphonia arctica	215	300	n.d.	620	810	52	39
Gigartina skottsbergii	190	240	n.d.	630	450, 800	46	45
Hymencladiopsis crustigena	230	300	n.d.	630	390, 830	46	40
Myriogramme manginii	180	220	n.d.	590	300, 360, 450, 550, 750	41	46
Kallymenia antarctica	220	300	n.d.	630	380, 450, 800	53	35
Plocamium cartilagineum	180	260	n.d.	630	820	47	42
Sphacelaria plumosa	240	320	n.d.	600	780	52	40
Fucus vesiculosus							
- **Biomass**	200	300	n.d.	600	800	63	45
- **Residue**	200	300	n.d.	600	380, 800	66	40

T_i= initial temperature, T_m = maximum temperature, T_{m2} = 2nd maximum temperature in stage II, T_e = end temperature

CHAPTER 7

Discussion

Analytical Pyrolysis

7. Discussion analytical pyrolysis

7.1. Microalgae

7.1.1. *Chlamydomonas reinhardtii* and *Chlorella vulgaris*

7.1.1.1. Lipids

Intermediate pyrolysis of total lipid extracts derived by microalgal biomass revealed interesting outcomes towards the production of pyrolysis products. The majority of chemical compounds derived from analytical pyrolysis fell into main categories, such as carboxylic acids, esters and hydrocarbons. In terms of pyrolytic reactions it is expected, that fatty acids contained in the lipid samples generate esters by reacting with a hydroxyl group containing compound, such as an alcohol or phenol. Obtained pyrograms from total lipids showed to be less complex than those derived from total biomass, protein extracts and residues after lipid extraction, while exhibiting only up to 15 major components.

The chemical compounds detected in pyrolysates of both *Chlamydomonas reinhardtii* strains were almost identical and significant differences were revealed by comparison with pyrolysates of *Chlorella vulgaris*. These results reflect the close similarity of the fatty acid profiles of both *Chlamydomonas reinhardtii* strains and differences obtained by *Chlorella vulgaris*.

The major difference in the obtained pyrolysates was benzenedicarboxylic acid alkyl ester, accounting for 41-43 % and therefore being the dominant peak (peak 49) in pyrolysis products of *Chlamydomonas reinhardtii* specimens. In contrast, thereof only 5 % were detected in the pyrolysates of *Chlorella vulgaris* total lipid derived pyrolysates.

Three derivatives of benzoic acid alkyl esters (peaks 44, 45 and 47) were detected reaching 6-9 % for both *Chlamydomonas reinhardtii* strains and ca. 4 % in *Chlorella vulgaris* material. In addition, 2-propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester was found in all three pyrolysates accounting between ca. 3-5 %.

Also observed in all total lipid derived pyrograms was squalene (2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexane), one of the most common triterpene lipids. In this study it was the longest chain lipid product ($C_{30}H_{50}$) detected in all lipid samples accounting for ca. 5-9 % in *Chlamydomonas reinhardtii* derived lipid pyrolysates and ca. 14 % in *Chlorella vulgaris* lipids.

Squalene is an important intermediate in the biosynthesis of polycyclic triterpenes and cholesterol [186] and cannot be reproduced synthetically while being natural oils its only source. Squalene is a major product in shark oil [187] and is found in various plant tissues and plant oils [188], [189].

Furthermore, the green colonial microalgae *Botryococcus braunii* race B, investigated as a hydrocarbon rich source for biofuel applications produces squalene as a precursor for triterpenoid hydrocarbons [190].

The detection in pyrograms suggests that it could be formed as an artefact during the pyrolytic process possibly from other longer chain terpenes, such as carotenoids. This is supported by the work of Gelin et al [119], who isolated lipids from the green microalga *Botryococcus braunii* and determined their thermal behaviour via Curie point analysis. They report the detection of squalene as an erroneous decomposition product resulting from incomplete pyrolysis. Squalene has also been detected in pyrolysis oil of *Lemnar minor* (aquatic biomass, known as duckweed) [191] suggesting the widespread occurrence of this product as an unpyrolysed lipid based artefact.

In the pyrolysates of *Chlorella vulgaris* lipids, several compounds were detected which were not found on *Chlamydomonas reinhardtii* lipids pyrolysates. It has been shown that aliphatic hydrocarbons including heptadecane (1.9 %), 1-nonadecene (5.5 %) and heneicosane (3.6 %) were detected in intermediate pyrolysis derived compounds, obtained by *Chlorella vulgaris* materials, only. These may be derived from the myristic acid (14:0) and palmitic acid (16:1) which were observed in FAMES of *Chlorella vulgaris*, only. Furthermore, hexadecanoic acid methyl ester (2.8 %) and octadecanoic acid, octyl ester (5.0 %) were detected in those pyrolysates only.

During the total lipid extractions, chlorophyll partitioned from the biomass into the lipid phase. Consequently, phytol and isomers (peaks 29 and 32) were found in the pyrolysates of total lipids from all three specimens. However, trans-phytol (peak 38) was detected in pyrolysates of both *Chlamydomonas reinhardtii* strains only.

Overall, it was possible to identify ca 85 % of the total peak area of both *Chlamydomonas reinhardtii* strains and only 50 % of the area of Chlorella vulgaris lipid derived pyrogram area.

7.1.1.2. Proteins

In this study, various amino acid derived chemical compounds were detected in the pyrolysates of total protein extracts of microalgae. The main reaction pathways of thermal degradation of single amino acids are scission of the polymer chain and cleavage of the side chain (R-group) and/ or splitting of CO_2 or H_2O [20]. Similar reactions were observed for proteins and peptides leading to chemical compounds which can be traced back to single amino acids [105], [192].

Therefore, pyrograms of proteinaceous materials give evidence about amino acids contained in the original sample. The included conclusions of the amino acid appearances in the total proteins on basis of detected chemicals in pyrograms are based on earlier work of Monkhouse et al. [105] and Tsuge et al. [192].

Overall, similar compounds were observed for all three specimens, and interestingly no major differences in their distribution were evident.

The most abundant chemicals detected in all three pyrolysates were toluene, indoles and phenols. Toluene and indole were accounting for ca. 9 % in pyrolysates of *Chlamydomonas reinhardtii* wild type, 7-8 % in its cell wall mutant and for 6-7 % in *Chlorella vulgaris*. The content of toluene, being a typical pyrolysis product of the amino acid phenylalanine, and indole, a tryptophan derived compound, revealed decreasing contents of both amino acids in algal materials in the following order: *Chlamydomonas reinhardtii* wild type > *Chlamydomonas reinhardtii* CW15+ > *Chlorella vulgaris.* In addition, minor amounts of styrene (<1-2 %) and traces of ethylbenzene (<1 %), both phenylalanine derived compounds, were detected in all three pyrolysates. Another from tryptophan originated pyrolysis compound was 3-methylindole, accounting for ca. 2 % in all three pyrolysates.

Considerably large amounts of 7-8 % of 4-methylphenol were detected in both *Chlamydomonas reinhardtii* strains and 2.8 % in *Chlorella vulgaris* total protein pyrolysates. In addition, ca. 2-3 % of phenol was observed in all three total protein derived pyrolysates. Both identified chemicals indicate the amino acid tyrosine being another major amino acid constituent of the algal proteins. Furthermore, pyrrole, derived by hydroxyproline and glutamine, was detected in traces (<1 %).

Overall, the obtained pyrolysates of intermediate pyrolysis of total proteins extracted from three green microalgae species indicate a majority of aromatic chemicals, derived by amino acids containing ring structures, such as phenylalanine, tyrosine, and tryptophan.

The aliphatic hydrocarbon 1-hexadecene was detected in pyrolysates of total proteins derived from *Chlorella vulgaris* only. While accounting for 7.2 % it is one of the major chemical compounds in *Chlorella vulgaris* derived total proteins. It may have its origin from acylated proteins or acyl residues of fatty acids bound to the hydrophobic domains of the proteins. Another aliphatic nitrogen containing hydrocarbon identified, was hexadecanamide, detected in low amounts in all three pyrolysates.

Further compounds detected in pyrolysates of both *Chlamydomonas reinhardtii* proteins only were a dipeptide (2-3 %) and picolinamide, 2-furaldehyde, 5-methyl, pyrrole, 2 or 3-methyl in traces (<1 %). A tentatively assigned dipeptide is considered

being a protein derived compounds, due to its peptide structure, possibly deriving from a larger peptide molecule and due to its appearance in protein containing samples only.
In addition, a total of nitrogen containing compounds of ca. 16 % for both *Chlamydomonas reinhardtii* strains and ca. 12 % for *Chlorella vulgaris* were obtained.

7.1.1.3. Residues

This biomass residues derived by extraction of the lipid fraction from microalgae biomass is of high interest for intermediate pyrolysis applications. Due to the extraction of high valuable products such as lipids, the analysed residues of all three specimens contained proteins and carbohydrate portions of the biomass mainly. Therefore, the spectrum of pyrolysis products identified by lipids, such as carboxylic acids, esters and aliphatic hydrocarbons are not included in the pyrolysates.

The pyrograms obtained by analytical pyrolysis of the residues revealed containing protein derived compounds mainly, resulting from proteins being the main fraction of the microalgae biomasses. In coherence with the analysis of total proteins, mainly toluene, 4-methylphenol, indole and 3-methylindole were detected throughout pyrolysates of all three residues. In addition, two dipeptides were detected in the residue derived by *Chlamydomonas reinhardtii* strains only.
Furthermore, levoglucosan was detected in pyrolysates of *Chlamydomonas reinhardtii* wild type, accounting for 3.3 %, being derived by the starch content of the biomass.
Overall, about 35 % of the total peak area was identified for both *Chlamydomonas reinhardtii* strains and ca. 23 % of Chlorella vulgaris.
In comparison to the other obtained pyrograms in this study, the lowest portion of the total peak area was identified. This may indicate that the Py-GC/MS unit and mass spectral database limits the detection and identification of chemicals derived by carbohydrates and proteins.

7.1.1.4. Algal biomass

Obtained pyrograms of biomass from all three green microalgae contained pyrolysis compounds originating from contained protein, lipid and carbohydrate fractions mainly.

As revealed by analytical pyrolysis of the total protein extracts, protein derived chemicals include toluene, 4-methylpehnol, phenol, indole and 3-methylindole, where the contents vary slightly between the values obtained for both *Chlamydomonas reinhardtii* strains and *Chlorella vulgaris*. In general, lower values were observed in *Chlorella vulgaris* derived pyrograms. Furthermore, hexadecanamide, identified being protein derived was observed in pyrograms of *Chlorella vulgaris* biomass (2.6 %), mainly. Generally, higher portions of protein derived chemicals were detected in pyrolysates of both *Chlamydomonas reinhardtii* strains biomass (ca. 15 %) than in *Chlorella vulgaris* biomass (ca. 10 %).

In contrast, the gross chemical analysis revealed higher protein contents in *Chlorella vulgaris* biomass. Therefore, it is suggested, that other proteinaceous derived compounds were either not detected or could not be identified.

Identified compounds derived by the lipid fractions of biomass include carboxylic acids, esters as revealed by total lipid extracts pyrolysis. Pyrograms of microalgae biomass showed all three specimen containing similar amounts of benzoic acid alkyl ester derivatives (4-6 %), benzenedicarboxylic acid, alkyl ester (ca. <1-1 %). In contrast, 2-propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester was found in higher amounts in *Chlorella vulgaris* pyrograms (8 %) than in *Chlamydomonas reinhardtii* (ca. 2 %) ones. In addition, *Chlorella vulgaris* exhibited squalene being the major pyrolysis chemical compound accounting for 25.5 %, whereas both *Chlamydomonas reinhardtii* strains contained only 4-5 %. Furthermore, as already shown in pyrograms of the total lipid extracts, octadecanoic acid octyl ester is a chemical produced by *Chlorella vulgaris* lipid fractions only, accounting for 5.6 % in pyrograms of biomass.

Overall, the proportion of lipid derived pyrolysis products of *Chlorella vulgaris* biomass (ca. 45 %) is higher than obtained for both *Chlamydomonas reinhardtii* strains (ca. 20 %).

Polysaccharide-derived decomposition products are detected at concentrations of ca. 1-2 % of levoglucosan in *Chlamydomonas reinhardtii* biomass, only. Furthermore, furfural and 2-furaldehyde, 5-methyl are polysaccharide decomposition products of furan ring based structures [181] and these products are detected in traces only.

Again, phytol ((2*E*,7*R*,11*R*)-3,7,11,15- tetramethyl-2-hexadecen-1-ol and various isomeric forms were detected in pyrograms of biomass as already observed in other samples. Its wide spread occurrence suggests the tenacious binding of chlorophyll to both lipid and proteins fractions by means of hydrophobic interaction.

7.1.1.5. Dual detector analysis

An exemplary dual detector analysis by of Py-GC-MSD/FID and Py-GC-MSD/NPD was performed of *Chlorella vulgaris* biomass, extracted total proteins, extracted total lipids and the residue obtained after lipid extraction. Overall, due to their specification, the FID and MSD obtained pyrograms exhibit a similar appearance. In contrast the NPD detects organic bound nitrogen and/or phosphorous containing chemicals

When pyrograms of the NPD and MSD are compared the NPD exhibited a higher sensitivity. Generally, many nitrogen (and/or phosphorous) containing compounds are apparent in the pyrolysates of *Chlorella vulgaris* biomass. A set of analytical standard was applied to show the practice of a retention time library for the FID and NPD. The obtained pyrograms reveal that these detectors will provide useful information regarding the interpretation of biomass pyrolysates. In particular, when higher operation temperatures for the detection are applied, it might be possible to detect more chemicals in the pyrolysis mixtures.

7.1.2. *Porphyridium purpureum*

In this study the biomass of the red microalgae *Porphyridium purpureum* and a residue obtained after the extraction of the Coenzyme Q_{10} were included. These materials are of particular interest in terms of studying the effect of extracting high valuable products onto the pyrolysate composition.

Due to the analytical pyrolysis of the biomass constituents of the green algae species, conclusion towards the overall biomass composition can be drawn. Consequently, the pyrograms of the red algae biomass revealed lower protein contents and higher portions of carbohydrates and lipids in the sample.

The lower protein contents are revealed by minor amounts (<1-1 %) of proteinaceous derived chemical compounds, such as toluene, styrene and phenol. In coherence, almost no amounts of these compounds were observed in the pyrograms of the residual material. This indicates, that the extraction procedure for the Coenzyme Q_{10} minimises the already low amounts of pyrolysis products derived from proteins.

In addition, the lower protein content is indicated by the low nitrogen content detected in the biomass (1.3 %).

Lipid derived products found in pyrolysates of red algae biomass include ca. 15 % of benzoic acid alkyl ester derivative (peaks 42, 45 and 47), and ca. 10 % of 2-propenoic

acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester and squalene. The data obtained by analytical pyrolysis of the residue revealed that the methanol extraction applied for the separation of Q_{10} significantly lowered the lipid content of the sample. This is evident through the indentified values of only 1-2 % for each of the mentioned lipid derived compounds.

In contrast, data showed that the extraction procedure favours the production of levoglucosan, markedly. Whereas already high amounts of 25 % were detected in pyrolysates of the biomass, almost as twice as much (47 %) was detected in pyrolysates of the residual material. Generally, high levoglucosan levels are derived by the carbohydrate structure of red algae biomass, containing various polysaccharides in their cell walls (cellulose, carrageenan and agar) as well as containing floridean starch as an energy storage product [39], [181]. An increase of levoglucosan to such high values in pyrolysates of the residue might be related to a decrease of catalytic active materials through the Q_{10} extraction procedure (demineralisation effect). It was reported that mineral impurities in biomass, favour the formation of low molecular weight compounds at the cost of levoglucosan formation [16].

7.2. Macroalgae

7.2.1. Polar species

Pyrograms of arctic and antarctic derived macroalgal species revealed broad distributions of pyrolysis products originating from structural and storage polysaccharides and from lipids and proteins. On the basis of the prior identification of lipids, proteins and carbohydrate derived chemicals from microalgae biomass, results obtained by macroalgae biomass are applied to estimate whether a biomass contains mainly carbohydrates, lipids or proteins.

Overall, the pyrolysates of the macroalgae species reveal significant differences in the pyrolysate compositions, and therefore in the chemical composition of the biomass.

Interestingly, the identified chemical compounds in pyrograms of arctic species may reveal that these species contain low amounts of proteins in their biomass. For all arctic species, a sum of maximum 10 % of all protein derived chemicals was detected. In contrast, values up to 40 % were obtained by the antarctic species. The major proteinaceous derived compounds found in arctic species were toluene, phenol, and 4-methylphenol; in traces detected were pyrrole, styrene and indole. In addition, 1-hexadecene was detected in pyrolysates of *Monostroma arcticum*, accounting for 9.4

%. This was also detected in total proteins of the microalgae *Chlorella vulgaris* (7.2 %) where its origin was suggest being acylated proteins or acyl residues of fatty acids bound to the hydrophobic domains of the proteins. Overall, obtained results indicate that arctic macroalgae species contain low amounts of proteins dominated by the amino acids phenylalanine, tyrosine and tryptophan.
In contrast, higher values of proteins may be present in macroalgae biomass derived from the antarctic region. However, the same protein derived chemicals were detected as in pyrolysates from arctic species, indicating similar amino acid constitution. The species *Myriogramme manginii* exhibited the highest value of proteinaceous derived chemicals, including 18.6 % toluene, 8.2 % styrene and 10 % phenol.

Lipid derived long chain alkyl ester acids were detected in all macroalgal pyrograms, including benzoic acid alkyl ester derivatives, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester and octadecanoic acid, octyl ester. Many of these esters are likely to originate from lipids through chain scission and molecular rearrangement.
Generally, the triterpene squalene reached the highest values of lipid derived compounds, accounting for 7-12 % in the arctic species *Phycodrys rubens* and the antarctic species *Plocamium cartilagineum, Kallymenia antarctica and Hymencladiopsis crustigena*. In this study, relatively high levels of squalene have been observed in the microalgae *Chlamydomonas reinhardtii* and *Chlorella vulgaris* suggesting that it generated as lipid based artefact during pyrolysis [191].
Highest levels of 5-7 % of 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester was detected in *Plocamium cartilagineum* (antarctic) and *Monostroma arcticum* (arctic). All other lipid derived compounds were detected in lower amounts in several pyrolysates of macroalgae species. The lowest amounts (ca. 2 %) of lipid derived chemicals were detected in the arctic species *Sphacelaria plumosa*, *Polysiphonia arctica* and antarctic species *Gigartina skottsbergii*.
The diterpene phytol, the ester-linked side chain of chlorophylls, was detected in pyrolysates of the green algae *Prasiola crispa* and in pyrolysates of the brown algae *Sphacelaria plumosa* but was not detected in any of the red algal species, due to their different pigmentation.

The pyrograms obtained by intermediate pyrolysis of macroalgae species indicate that in the majority of analysed species carbohydrates are the main chemical constituents in the biomass.

Overall, levels of 10-70 % of polysaccharide derived pyrolysis products were detected in arctic and antarctic derived materials. In contrast, only ca. 2 % of polysaccharide derived chemicals were detected in *Chlamydomonas reinhardtii* biomass.

One of the main polysaccharide derived products is levoglucosan originating from pyrolysis of cellulose and starch, hexoses (e.g. D-mannose) and sugar alcohols (e.g. D-arabinitol) due to de-polymerisation mechanisms [181], [194]. Amounts of 10-62 % of the total pyrogram area were detected in all species, excluding the antarctic species *Gigartina skottsbergii, Plocamium cartilagineum* and *Myriogramme manginii*, which did not contain any. The highest levels of 40-60 % were detected in the arctic species *Odonthalia dentata, Sphacelaria plumosa* and *Polysiphonia arctica*. Amounts of 10-30 % were detected in all other species.

These high levels of levoglucosan for macroalgae species are a consequence of their polysaccharide cell wall structure and their starch contents in the cell for energy storage purposes [39]. Overall, this study revealed, that intermediate pyrolysis of red macroalgae species offer a potential route to produce large amounts of levoglucosan.

Typically, levoglucosan yields of up to 50 % contained in pyrolysis liquids of pure cellulose [195] and yields of 18-33 % in pyrolysis oils derived from waste newsprint and cotton [196] were reported. At present, almost all available studies about levoglucosan production through pyrolysis were carried out under fast pyrolysis conditions and two major routes of cellulose degradation are accepted. One route describes the fragmentation via ring scission producing lower molecular weight compounds such as hydroxyacetaldehyde, 5-hydroxymethyl-furfural and furfural. The other one favours the production of the high molecular weight compound levoglucosan due to de-polymerisation [197]. Generally, levoglucosan production is inhibited by higher process temperatures, longer residence times and higher contents of inorganic compounds (i.e. alkali metals) in the feedstock, which favours the production of the low molecular products vice versa [195], [198], [199].

Furthermore, it is documented the fast pyrolysis of raw lignocellulosic biomass produces lower yields due to the presence of inorganic compounds in the biomass and demineralization and/or pre-treatment such as acid impregnation is required to obtain high values [24], [200]. Therefore, the high ash contents in the macroalgae materials between 15-44 %, would anticipate the formation of low molecule weight compounds such as hydroxyacetaldehyde via ring scission reactions instead of levoglucosan [197]. However, the high values of levoglucosan obtained in pyrolysates revealed, that intermediate pyrolysis process conditions may favour the depolymerisation process of the cellulose and starch contained in the biomass leading to high levels of levoglucosan formation.

Interestingly, the pyrolysates of the antarctic species *Gigartina skottsbergii* free of any levoglucosan, still accounts for ca. 30 % polysaccharide derived compounds in its pyrolysate. In this case, major chemicals are furfural (13.3 %), levoglucosenone (12.6 %) and 5-methyl-2-furaldehyde (4.7 %). Both, furfural and levoglucosenone are cellulose derived pyrolytic compounds, where levoglucosenone is a dehydration product of levoglucosan [194]. Overall, furfural is detected in all pyrolysates accounting for 1-10 % and levoglucosan was found in pyrolysate of *Plocamium cartilagineum* (7.6 %).The chemical 5-methyl-2-furaldehyde is derived from galactanic, hexosic and pentosic structures and was indentified in all other pyrolysates ranging within 1-9 % [114], [181], [201].

Further sugar derived chemicals, detected in lower amounts and widely distributed thorough the majority of the analysed species 1-(2-furanyl)-ethanone (1%), 5-(hydroxymethyl)-2-furaldehyde (1-3 %) and 1,4:3,6-dianhydro-α-D-glucopyranose (<1-3 %) [181]. In addition, the carbohydrate derived methyl ester of 3-furancarboxylic acid was detected in pyrograms of *Odonthalia dentata*, *Phycodrys rubens* and *Gigartina skottsbergii* [202].

Pyrolysis products unique to the green alga *Prasiola crispa* were isosorbide (2.3 %) derived from sugar alcohols [109] and D-allose (6.8 %), previously detected in pyrolysates of cellulose [203]. Dianhydromannitol was detected in the brown alga *Sphacelaria plumosa* only, accounting for 4.6 %, consistent with earlier studies of brown algae materials [32]. In addition, maltol was detected as the only pyranoic compound in the red algal species *Phycodrys rubens,* accounting for 2.4 %, originated from starch [201].

Polycyclic aromatic hydrocarbons (PAHs) were identified in two Antarctic species, *Gigartina skottsbergii* and *Plocamium cartilagineum,* generated from both proteinaceous and carbohydrate material [39]. Chlorinated aromatics were also detected in a number of species, such as a derivative 2- chlorobenzophenone and triphenylmethyl chloride.

7.2.2. *Fucus vesiculosus*

The pyrolysate of the biomass consists mainly of polysaccharide derived chemicals such as 5-methyl-2-furaldehyde, maltol, dianhydromannitol, isosorbide and levoglucosan.

The results show, that the portions of the polysaccharide derived chemical have decreased within the residue and most of the compounds were not detected anymore. This is seen as a consequence of the polysaccharide extraction procedure. Surprisingly the content of 5-methyl-2-furaldehyde did increase significantly even thought polysaccharides were extracted. Generally, 5-methyl-2-furaldehyde originates from cellulose, galactanic, hexosic and pentosic structures and was detected across almost all macroalgae samples applied in this study. However, a portion of ca. 15 % was not detected so far in any of the samples. Furthermore, with a decrease of polysaccharide derived compounds, an increase of lipid derived compounds, such as benzoic acid, alkyl ester derivatives and squalene was observed.

A general comparison between the pyrolysates obtained by macroalgae species form Polar Regions and of *Fucus vesiculosus* do not show any obvious differences. Generally, the pyrolysates contain comparable chemicals; however their distribution is different for each specimen. Overall, pyrolysates of *Fucus vesiculosus* in general contain low amounts of protein and lipid derived chemicals and higher amounts of carbohydrate derived chemicals.

7.3. Chapter conclusions

The obtained results by Py-GC/MSD of several micro- and macroalgae species revealed interesting differences regarding chemical product formation during intermediate pyrolysis. This study showed that the main chemicals indentified by microalgae were derived mainly by lipids and proteins. As lipid derived products several benzoic acid, alkyl ester derivatives, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexylester, octadecanoic acid, octyl ester and squalene could be identified. Main products derived by proteins were toluene, 4-methylphenol, indole and 3-methylindole. In green and brown algal species phytol could be identified as a decomposition product of the pigment chlorophyll. This was not detected in any of the red algae species regarding their different pigmentation.

Results obtained by *Porphyridium purpureum* revealed levoglucosan being the main pyrolytic compound, which is unique for the microalgae species included in this study. In contrast, all other microalgae species exhibited lipid and protein derived pyrolytic products as their main compounds.

Pyrograms obtained from macroalgae species revealed that most of the Arctic and Antarctic species contained polysaccharide compounds, such as levoglucosan, 5-methyl-2-furaldehyde, 5-(hydroxymethyl)-2-furaldehyde. Further polysaccharide compounds were detected, being apparent in only a few species applied in this study, such as D-Allose in *Prasiola crispa*, Dianhydromannitol in *Sphacelaria plumosa*. Furthermore, minor amounts of isosorbide were detected in the green algae *Prasiola crispa* and the brown algae *Fucus vesiculosus*. In addition, phenanthrene derivatives were detected in only two antarctic macroalgae species, such as *Gigartina skottsbergii* and *Plocamium cartilagineum*. Minor amounts of chloride containing compounds were detected in two arctic species, i.e. *Prasiola crispa* and *Monostroma arctica* as well as in the antarctic species *Plocamium cartilagineum* and *Myriogramme manginii*.

Generally, some significant differences concerning product evolution during intermediate pyrolysis of micro- and macro algae were revealed.

However, this study showed that pyrolysis of biomass from algal species, originated from polar and temperate climatic regions, freshwater and marine water derived, collected in their natural habitats, grown in photo bioreactors and/or in the laboratory showed relatively comparable pyrograms regarding the chemical products and rather revealed differences in their distributions.

Many of the products identified in the pyrolysates of polar macroalgae are also common to those detected in terrestrial biomass. Compounds such as levoglucosan, levoglucosenone, acetic acid and 5-(hydroxymethyl)- 2-furaldehyde, toluene, styrene, phenol, maltol, furfural and phenanthrene reported here in relatively high abundance are mainly derived from cellulose and hemicellulose materials and are also found in terrestrial biomass pyrograms [192], [201], [204].

Overall, up to 80 % of the total peak area of some pyrolysates could be identified. However, this study reveals, that the detection of lipid derived products is more achievable than those derived from carbohydrates and proteins. As a consequence, no conclusions can be drawn from the pyrograms towards the proportion of lipids, proteins and carbohydrates in the biomass.

The dual detector analysis of *Chlorella vulgaris* biomass and materials revealed that this set-up could lead to valuable results towards the identification of chemicals by retention times in future studies.

Table 7-1 presents the main identified compounds accounting for 4 % or more in the analysed microalgae biomass materials. Furthermore, the compounds of pyrolysates of total lipids, total proteins and residues after lipid extraction are presented. In addition, Table 7-2 presents the obtained results from macroalgae pyrolysates.

Table 7-1 Main chemicals of microalgae pyrolysates

Species	**Major pyrolysis compounds (>4 % of total peak area)**
Chlamydomonas reinhardtii **wild type and CW 15+**	
- Biomass	toluene, 4-methylphenol, indole, phytol, trans-phytol, benzoic acid, alkyl ester derivatives, squalene
- Total Proteins	toluene, 4-methylphenol, indole
- Total Lipids	phytol, trans-phytol, 2-Propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexylester, benzenedicarboxylic acid, alkyl ester derivative, squalene
- Residue	toluene, 4-methylphenol, indole
Chlorella vulgaris	
- Biomass	phytol, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexylester, octadecanoic acid, octyl ester, squalene
- Total Proteins	toluene, indole, 1-hexadecene
- Total Lipids	1-nonadecene, benzenedicarboxylic acid, alkyl ester derivative, octadecanoic acid, octyl ester, squalene
- Residue	indole
Porphyridium purpureum	
- Biomass	levoglucosan, benzoic acid, alkyl ester derivatives, 2-Propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexylester, squalene
- Residue	levoglucosan

Table 7-2 Main chemicals of macroalgae pyrolysates

Region	Species	Main chemical compounds (>4 % of total peak area)
Arctic	*Prasiola crispa*	5-methyl-2-furaldehyde, D-Allose, phytol, levoglucosan, benzoic acid, alkyl ester derivatives
	Monostroma arcticum	5-methyl-2-furaldehyde, levoglucosan, 1-hexadecene, triphenylmethyl chloride, benzoic acid, alkyl ester derivatives, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester
	Polysiphonia arctica	levoglucosan
	Devalaraea ramentacea	furfural, levoglucosan
	Odonthalia dentata	levoglucosan, benzoic acid, alkyl ester derivatives, squalene
	Phycodrys rubens	furfural, 5-methyl-2-furaldehyde, levoglucosan, benzoic acid, alkyl ester drivatives, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester, squalene,
	Sphacelaria plumosa	dianhydromannitol, levoglucosan
Antarctic	*Gigartina skottsbergii*	toluene, furfural, 5-methyl-2-furaldehyde, levoglucosenone, phenanthrene derivatives
	Plocamium cartilagineum	toluene, 4-methylphenol, levoglucosenone, phenanthrene derivatives, trimethylphenyl chloride, benzoic acid, alkyl ester derivatives, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester, squalene
	Myriogramme manginii	toluene, styrene, furfural, 5-methyl-2-furaldehyde, phenol, 2 or 3 or 4-chlorobenzophenone, triphenylmethyl chloride, 2-propenoic acid, 3-(4-methoxyphenyl)-,2-ethylhexyl ester,
	Hymencladiopsis crustigena	indole, unidentified anhydrosugar, levoglucosan, benzoic acid, alkyl ester derivatives, squalene
	Kallymenia antarctica	unidentified anhydrosugar, levoglucosan, squalene
North Sea	*Fucus vesiculosus* Biomass	5-methyl-2-furaldehyde, dianhydromannitol, phytol, levoglucosan
	Fucus vesiculosus Residue	furfural, 5-methyl-2-furaldehyde, squalene

CHAPTER 8

Algae application in the BtVB-process

8. Algae applications in the BtVB-process

8.1. Introduction

The availability of sustainable and renewable biomass resources plays an important role for the energy supply and production of chemicals in future. Due to various advantages over terrestrial derived feedstocks, such as higher photosynthetic efficiency, less impact on arable land, environment and food chains [13], [14], aquatic feedstocks are seen as a compelling route to generate biofuels, power and heat via intermediate pyrolysis.

As already outlined in Chapter 1, commercial applications of microalgae being economically feasible are limited to relatively low-volume/high-value markets for speciality food or feed ingredients whether as whole cell preparations (e.g. *Arthrospira sp.*, *Chlorella sp.*) or extracts such as β-carotene and astaxanthin, at present [62].

In case of macroalgae biomass, the situation is comparable. The majority of commercial seaweed cultivation, mainly situated in Asia, produces goods for traditional markets and human nutrition additives (hydrocolloids). In Europe, seaweed exploitation is currently restricted to France and Norway, sustainably harvesting natural stocks by mechanised systems, which is a major challenge [205].

By knowing the current limitations of cheap algal biomass availability, the BtVB-process integrates: (1) the cultivation of microalgae biomass, (2) the production of commodity materials designed for a range of chemicals products such as pharmaceuticals and cosmetics (lipids, proteins, carbohydrates and pigments), (3) bioenergy generation and (4) the production of value-added (precursor) chemicals and bio-based materials. In addition, the recycling of process heat, CO_2 and nutrients is a step forward towards increasing efficiency and overall feasibility [23].

This study investigated fundamental analytical data, including thermo-chemical behaviour and product formation during intermediate pyrolysis of several micro- and macroalgae specimen. Based on the main results, outlooks towards the application of algae feedstocks within the BtVB-process are presented. In general, two major objectives are considered for algal biomass utilization within the BtVB-process. One of the most promising routes is the extraction of valuable products before biomass processing via intermediate pyrolysis. This will lead to an increase of the gross income and consequently improve the overall economical feasibility. Subsequently the residual materials will be further processed via intermediate pyrolysis followed by gasification to generate syngas, power and heat. Alternatively, the pyrolysis vapours are condensed

to gain pyrolysis liquids which are applied as transport fuels or as a source for chemicals. Another major aspect is the application of the biochar as a fertilizer, preferably for microalgae culture, to reduce biomass production costs [28].
Overall, the below presented approaches are based on analytical pyrolysis studies of micro- and macroalgae materials presented in this thesis. However, proof of concepts, including lifecycle assessments and economical feasibility studies, are beyond the scope of this study.

8.2. Extraction of valuable products prior to pyrolysis

8.2.1. Lipids from microalgae

One pathway within the BtVB-process considers the prior extraction of high valuable lipids from the biomass and subsequently pyrolysing the residual biomass.
As revealed by this study, *Chlamydomonas reinhardtii* and *Chlorella vulgaris* derived lipids contain portions of PUFAs accounting up to 75 % and 43 %, respectively, including linoleic acid (18:2) and α-linolenic acid (18:3). These omega-3 (18:3) and omega-6 (18:2) fatty acids are value added products due to the known health effects on the human body including a general stimulation of hormonal activity, affecting the cardiovascular, immune and the central nervous system beneficially. Furthermore, it has been shown, that omega-3 fatty acids reduce the risk for cardiac arrhythmias [177–179].
After solvent extraction of the lipid portion from *Chlamydomonas reinhardtii* and *Chlorella vulgaris* a residue accounting for ca. 60-70 % of the entire biomass was obtained. Intermediate pyrolysis of the residue after lipid extraction revealed pyrolysates mainly containing protein derived compounds such as toluene, 4-methylphenol and indole. In contrast, typical lipid derived chemicals such as benzenedicarboxylic acid alkyl ester, squalene and 2-propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexylester were not detected in pyrolysates of the residues.
As a conclusion, intermediate pyrolysis of a lipid free residual biomass provides a pyrolysis liquid containing mainly aromatic hydrocarbons, which might be a source for chemicals and /or subsequent gasification to produce syngas.

In addition, obtained results showed that the pyrolysis of lipids produce pyrolysis liquids mainly containing hydrocarbons, carboxylic acids, esters and ketones while exhibiting

comparable compositions than diesel fuel. Furthermore, possible applications of these liquids include the production of lubricants, solvents or lacquers [206].
Overall, this study revealed that algal biomass containing high portions of lipids, exhibit higher HHV, due to higher carbon content in the biomass. Moreover, TGA revealed that the hydrocarbon structure of the lipids leads to the formation of high portions of volatiles, being advantageous to produce higher amounts of pyrolysis vapours for subsequent processing.

8.2.2. Proteins from microalgae

Algal derived proteins are seen as valuable products for many applications including pharmacy, cosmetics, human nutrition and animal feed [62]. It is well known that the nutritional value of algal derived proteins is comparable with that of conventional food proteins such as derived by eggs and soybeans. Furthermore, the pharmacological values of algae proteins, including antioxidative and immune-stimulant activities potentially preventing health issues and disorders like cancer, cardiovascular diseases and diabetes mellitus are of high interest for human nutrition [44]. The fact that proteins need to be resolved form the algal cell to be accessible for digestive enzymes from the human body, makes the combination of producing proteins prior to pyrolysis reasonable.
Analytical pyrolysis studies revealed that the proteins contained in microalgae biomass lead to the formation of aromatic compounds with low molecular weights, typically including toluene, indoles and phenols. As a result of protein extraction, the pyrolysates of residual biomass lack the main proportions of these aromatic hydrocarbons.
Furthermore, the study revealed that algal materials with high contents of proteins contain higher oxygen contents and consequently exhibit lower HHV. Moreover, TGA revealed the proteins cause higher char and consequently lower formation of volatiles during processing via intermediate pyrolysis. In contrast, as nitrogen is a main element of proteins, biochar obtained by biomass containing proteins may exhibit higher nitrogen contents.

8.2.3. Coenzyme Q_{10} from microalgae

Currently, the global market for the Coenzyme Q_{10} is expected to exceed US $133 Mio by 2015, due to its emerging role in pharmacy and cosmetics, including heart health, cognitive health and anti-ageing products. In addition, large scale clinical tests are carried out to evaluate the potential to delay Parkinson diseases and to treat chronic heart failures [207–209]. To increase the economical feasibility of the BtVB-process, organisms containing high valuable products are of interest, and due to the high market value of Q_{10} *Porphyridium purpureum* is considered being one of the most interesting biomass investigated in this study.

Conducted intermediate pyrolysis studies of the pure biomass and a residual biomass after Q_{10} extraction revealed valuable results for applications in the BtVB-process. The obtained pyrograms indicated that the solvent extraction had a side effect of demineralisation and possibly increased the levoglucosan content in the pyrolysate of the residue by almost 100 %, compared to the pure biomass. Overall, levoglucosan is a pyrolysis product of interest due to its various applications, further outlined in section 8.3.2. Moreover, TGA analysis revealed that the extraction of Q_{10} had no effect on the formation of volatiles and chars, however, a slight shift (20 °C) towards higher degradation temperatures was observed.

8.2.4. Polysaccharides from macroalgae

Commercial macroalgae production includes the production of ca. 30.000 t hydrocolloid polysaccharides per year. Polysaccharides such as carrageenan, alginate and agar being applied as thickening agents in human nutrition, pharmaceuticals and cell culture in biology and are extracted from red and brown macroalgae species [39], [68].

This study investigated the pyrolytic characteristics of the brown algae *Fucus vesiculosus* before and after extraction of value added polysaccharides, including alginate, laminarin and fucoidan.

The main outcome was a decrease of polysaccharide derived chemicals in the pyrolysates, including levoglucosan, dianhydromannitol and isosorbide. Interestingly, an increase from the lipid derived product squalene was evident vice versa.

On the basis of these results, the application of residual biomass obtained by prior extraction of polysaccharides is seen as a promising route within the BtVB-process. On the one hand valuable polysaccharide based products can be produced, while still

useful pyrolysates are recovered, offering applications in chemistry processes or for subsequent gasification.

8.3. Applications of pyrolysis liquids

The pyrolysates obtained from analytical pyrolysis of various algal materials contained more than 200 compounds of different molecular sizes, mostly being degradation products of the three key biomass building blocks, being proteins, lipids and carbohydrates. Major groups of compounds detected in this study include aromatic and aliphatic hydrocarbons, anhydrosugars, acids and terpenes.

Overall, algae derived pyrolysis liquids can find applications as substitutes for fuel oils or diesel, applied in boilers, furnaces, engines and turbines used for electricity generation [9].

In addition to process biofuels, the BtVB-process considers a direct coupling of the pyroformer outlet to the gasifier. With this, the pyroformer acts an ash filter, while capturing the valuable inorganic compounds in the biochar and the low ash containing vapours are directly applied to produce synthetic gas, power and heat. Via this pathway no pyrolysis liquids, i.e. condensates are produced.

However, biomass is the only renewable resource of carbon which is available for the production of chemicals typically derived by fossil resources.

As a consequence, investigations of separation techniques to retain chemicals, and precursor materials form pyrolysis liquids are a key technologies for biorefinery concepts and consequently for R&D.

Generally, fractional distillation and isolation techniques of selected key chemicals derived by pyrolysis liquids are difficult due to their thermal and chemical instability and the content of different chemical compounds. Due to the high content of oxygenated compounds, the liquids have a polar and hydrophilic nature and are almost immiscible with liquid hydrocarbons [9], [210].

After all, no commercially available methods for fractionation of pyrolysis liquids are available, at present. However, due to increasing demands on carbon based chemicals and the decrease of fossil resources, investigations towards recovery of chemicals from pyrolysis liquids are ongoing.

Mostly investigated chemicals also found in algal derived pyrolysis liquids are phenol (protein derived) and levoglucosan (carbohydrate derived) and their potentials are

outlined in the following sections. Furthermore, squalene was identified as a lipid derived pyrolysis products and possible applications are presented, too.

8.3.1. Phenol

Nowadays, phenol is a petroleum derived bulk chemical used for various applications, such as a precursor for various plastics (polycarbonates, nylon), pesticides, insecticides and binding agents in the manufacture of plywood [211]. As determined in this study, the phenolic fraction in algal intermediate pyrolysates is mainly made of phenol and 4-methylphenol. Whereas no methods about extraction of phenolic compounds from algal derived pyrolysis are reported, some are studied to obtain phenol from lignocellulosic derived fast pyrolysis liquids. Typically, these liquids contain a heavy oil fraction which is made of lignin derived phenolic fractions (mainly composed of phenols, cresols, guaiacols, syringols, catechols) which find application without as an extender and a partly replacement of phenol-formaldehyde resins in plywood industry [9], [155].

Due to their limited solubility in water, further methods include treatments with different solvents, including alkali solutions (sodium hydroxide) and organic polar solvents. In addition, procedures applying supercritical CO_2 fluid extraction were investigated. Overall, although recoveries of up to 50 % of the phenol fraction were reported, no industrial application of a proposed process is available at present [155], [210], [212–215].

8.3.2. Levoglucosan

In contrast to other chemicals produced from fossil resource and found in biomass derived pyrolysates, the anhydrosugar levoglucosan is produced by pyrolysis of carbohydrates, solely.

Being the major pyrolytic product of cellulose and starch it was found in intermediate pyrolysates of various macroalgal species, accounting for up to 20-60 % of the pyrolysates of arctic (*Monostroma arcticum, Sphacelaria plumosa, Odonthalia dentata, Polysiphonia arctica*) and antarctic species (*Hymencladiopsis crustigena, Kallymenia antarctica*). In addition, up to 47 % were found in the residue of the red microalgae *Porphyridium purpureum* after solvent extraction of Coenzyme Q_{10}. Overall this study

revealed that most of the red algae species produce large amounts of levoglucosan, even higher amounts than typically retained from lignocellulosic biomass.

The product levoglucosan is of interest, as its commercial production is limited and the prices go up to US$10.000/kg for purified levoglucosan, at present [216]. Broad ranges for its utilization are studied and a high potential for becoming an important renewable chemical is seen. Various applications are tested, such its fermentation products, cosmetically and pharmaceutical potentials and applications for manufacturing of biodegradable polymers [24], [217]. Studies revealed, that naturally occurring microorganisms such as bacteria, yeasts and fungi can utilize levoglucosan as a carbon substrate via the enzyme levoglucosan kinase and subsequently convert it to ethanol and organic acids (lactic acid, citric acid) [218]. For a faster fermentation process, the anhydrosugar must be hydrolysed to glucose before, usually applying sulphuric acid. Overall, ethanol yields obtained by fermentation of levoglucosan were comparable to those from glucose [218], [219]. An interesting study carried out by Luyen et al [220] investigated the application of levoglucosan as an cell-growth enhancing substance to increase microalgae growth and thus the feasibility of microalgae production. It was found that the cell growth of various cultured microalgae species was increased by approximately 50 % when levoglucosan was added to the culture medium [220]. Another promising route to utilize levoglucosan is the production of biodegradable and renewable alkyl glycoside surfactants for soaps and cosmetics. These high valuable products are produced by condensation reactions of the sugars with vegetable oil derived alcohols or acids [217]. Furthermore, levoglucosan has been utilized as a precursor for pharmaceutical important agent such as antibiotics [221], [222].
Technically, due to its polarity and solubility, levoglucosan is contained in the aqueous phase of the pyrolysis oils and several methods applying various solvents are patented for the extraction and purification of levoglucosan [219], [223–225]. However, a low-cost recovery and purification procedures is not available, at present [24], [200].

8.3.3. Squalene

Another interesting outcome of this analytical study is the high level of the lipid derived artefact squalene which was detected in various algal materials, accounting for 25 % in the pyrolysates of *Chlorella vulgaris* biomass. So far, such high levels are not reported

in any other biomass derived pyrolysates. It is possible, that the production of this high molecular weight and long chain hydrocarbon ($C_{30}H_{50}$, MW 410) is a result of the moderate heating rates and gentle decomposition of the biomass molecules utilized during intermediate pyrolysis [23].

In cosmetic and pharmacy industry, squalene is an important ingredient due to its photo protective characteristics and its potential to decrease cancer. In addition, it is applied for the production of fine chemicals, such as lubricants for computer disks and magnetic tape [226].

Squalene cannot be reproduced synthetically and natural oils such as shark and plant oils are its only source as it is part of the biosynthesis of triterpenes and cholesterol [186–189]. In addition, the green microalgae *Botryococcus braunii* race B produces squalene as a precursor for triterpenoid hydrocarbons and is investigated as a hydrocarbon rich source for biofuel applications [190].

Due to the main delivery of squalene from fish liver oil and the increasing concern about marine animal protection, it is of interest to identify alternative sources. Current investigations include supercritical CO_2 extraction from olive oil deodorization distillate (by product of olive oil refining) and amaranth oil [227], [228]. Hardly any sources are published about squalene contained in algal derived pyrolysates and from this point it seems reasonable to investigate algae biomass and algal pyrolysates as a possible new source for the production of squalene.

8.4. Biochar for fertilisers

As outlined in section 1.3, biochar accounting for ca. 25 % of the amount of applied feedstock is obtained by intermediate pyrolysis of biomass.

At present, much attention towards biochar application in environmental management is paid, and main objectives include (1) soil improvement, (2) waste management and (3) climate change mitigations [229].

The addition of biochar to the soil is associated with improvement of the overall soil productivity. Due to its highly porous structure it possibly increases water retention capacity and surface areas of soils. In addition, while containing the inorganic portions of the pyrolysed biomass, the addition to soils is associated with an fertilising effect [229], [230].

By pyrolysing waste materials, such as sewage sludge, biogas plant and agricultural residues, not only energy is recovered, but volume and weight of the waste material is

reduced, dependently on the feedstock, down to the amount of biochar obtained. In addition, if pathogens are contained in the waste or residual material, pyrolysis offers a route to remove them, due to process temperatures above 350 °C and easier disposal is possible [229].
Pyrolysis of photosynthetic active feedstocks, i.e. plant and algae biomass, and subsequent adding of the biochar to soils, reduces the atmospheric CO_2 due to carbon sequestration soils [229–231].

In addition, within the BtVB-process the approach is included to retain water soluble nutrient fractions from the biochar to recycle them into the microalgae culture, to reduce their overall production costs. Therefore, an early study of the author investigated the water solubility of the main algal required nutrients including N, P and K from algal biochar [232]. A biochar, obtained by intermediate pyrolysis of *Chlorella vulgaris* biomass, was subjected to hot water extraction by applying a Soxhlet apparatus. It has been found, that after two hours of extraction about 100 % of the contained potassium, sulphur and ca. 90 % of the chlorine was obtained. Furthermore, about 5 % of the sodium and minor amounts of phosphorous and nitrogen were recovered [232]. In addition, it has been revealed that the phosphorous was easier released from biochar with smaller particle sizes.
Based on these results, promising applications for the water soluble potassium fraction are seen for microalgae cultures. Furthermore, the nitrogen and phosphorous still remained within the biochar after water extraction could be applied a slow releasing fertiliser in agriculture. In terms of supposed future phosphorous shortages [34], the opportunity to recover this important nutrient via intermediate pyrolysis of biomass will gain more attraction.

An unconventional but valuable resource, to valuable produce biochar is macroalgae biomass which is harmful for the environment. Overall, rapid growth rates and assimilation of nutrients such as nitrogen and phosphorous form the oceans are characteristic for macroalgae organisms [167], [233]. The ability of heavy metal assimilation is used as bioremediation of waste waters of aquaculture systems and offer a robust solution for treating eutrophic (N and P- rich) waters [234].
In contrary, green or red tides, i.e. excessive growth in eutrophic environments and abnormal biomass proliferations of some species (i.e. *Ulva sp., Cladophora sp, Gracilaria sp.*) create serious problems along coastlines and shallow lagoons [17], [235–237]. Examples are recurring algae blooms of *Ulva prolifera* in the Yellow Sea, off China, with estimated areas up to 30.000 km^2 [236], [237]. Environmentally, the water

quality of oceans and lakes is endangered, affecting fish and other organisms due to abnormal biomass proliferation. In many cases, large amounts of biomass need to be removed causing difficulties especially when the biomass is contaminated, i.e. with heavy metals [238], [239]. In those cases, where no applications are available and disposal is complicated, pyrolysis of the materials may offer a route to generate bioenergy form this problematic feedstock, while reducing the amount of disposable materials down to the amounts of biochar after pyrolysis (ca. 25 % of the feedstock).
In addition, with appropriate separation technologies the valuable nutrients may be recovered from the biochar and are available for further applications.

8.5. Chapter conclusions

Due to a large diversity and complexity of aquatic biomass, exploitation of its full potential for pyrolytic applications is still required. At present, most of the reported studies investigating pyrolytic characteristics of aquatic biomass under fast pyrolysis conditions.
This conducted analytical study presents one of the first activities in applying aquatic biomass for intermediate pyrolysis and from there derived possible applications within the BtVB-process were presented.
Potential routes include the extraction of lipids or proteins from microalgae and polysaccharides from macroalgae biomass. In particular, the compelling potential of the recovery of the high valuable product Coenzyme Q_{10} prior to intermediate pyrolysis of the red algae *Porphyridium purpureum* offers potential applications for being integrated in the BtVB-process.
In addition, a major outcome of this study showed that pyrolysis of various algal specimen, originated from polar and temperate climatic regions, freshwater and marine water derived, collected in their natural habitats, grown photo bioreactors and/or in the laboratory showed relatively comparable pyrograms regarding the chemical products and rather revealed differences in their distributions.
It has been shown, that red micro- and macroalgae species produce large amounts of levoglucosan, mainly derived by their polysaccharide structure, consisting of cellulose, galactans, carrageenan, agar and starch.
Overall it has been shown, that algal materials are a source of highly versatile feedstocks offering numerous applications. Therefore, most effective and most valuable routes need to be identified for every algal material, individually.

Major aspects are the gross chemical composition of an algal biomass, the possibility to retain a high valuable product prior pyrolysis and the composition of the pyrolysates obtained by the residual biomass.

In addition, this study revealed that the application of macroalgae biomass is a useful route for bioenergy generation and the production of renewable chemical resources. Furthermore, large potentials are seen for the development of alternative fertilizers, by capturing valuable nutrients from the oceans in the biochar via intermediate pyrolysis.

CHAPTER 9

Conclusions and Recommendations

9. Conclusions and recommendations

9.1. Response to main objectives

Objective 1:
Installation of an analytical pyrolysis system, providing intermediate pyrolysis conditions, with subsequent separation and detection of pyrolysates.

An analytical pyrolysis unit consisting of a micro-furnace pyrolyser, a gas-chromatograph with a dual column set-up has been installed. For detection and identification of pyrolysates, three detectors, a MSD, FID and NPD were installed, facilitating dual detector analysis. Overall, a reliable instrument providing intermediate pyrolysis process condition has been commissioned, delivering reproducible results for any feedstock testing.

Objective 2:
Examine the pyrolytic behaviour of algal derived biochemical constituents under intermediate pyrolysis process conditions.

Total lipids and total proteins were extracted from *Chlamydomonas reinhardtii* and *Chlorella vulgaris* biomass and subsequently analysed under intermediate pyrolysis conditions. Overall, thermo-chemical characteristics were studied and lipid and protein derived pyrolysis products were identified.

Objective 3:
Study the thermo-chemical behaviour and chemical product formation of micro- and macroalgae biomass during intermediate pyrolysis.

Via TGA and Py-GC/MS analysis intermediate pyrolysis studies of various micro- and macroalgae biomass was studied. Obtained results revealed characteristically behaviour of micro- and macroalgae biomass during intermediate pyrolysis. In addition, chemical compounds contained in biomass pyrolysates were identified.

Objective 4:
Evaluate possible applications of algal feedstocks in the BtVB-process upon basis of obtained analytical results.

On the basis of the gained analytical results various applications for algae feedstocks within the BtVB-process have been demonstrated. Included are applications for micro- and macroalgae biomass, as well as residual materials obtained by extraction of high valuable products prior to pyrolysis.

9.2. Recommendations for future work

9.2.1. Algae biomass

It is advisable to carry on with a screening of algal biomass to further investigate the formation of chemicals formed under intermediate pyrolysis conditions.
The obtained results by analysing the biomass of *Porphyridium purpureum* and the residue obtained after the extraction of Coenzyme Q_{10} revealed that the recovery of a high valuable product offers interesting possibilities to increase the overall feasibility of the BtVB-process.
It is advisable to find collaboration partners from research and industry, who are working on manipulating chemical compositions of biomass and extraction of valuable products.

9.2.2. Analytical pyrolysis

This study provided an analytical pyrolysis unit facilitating intermediate pyrolysis conditions. For further in-depth identification of chemical compounds in pyrolysates of algal biomass and any other feedstock it is suggested to set-up retention time libraries for the FID and NPD. For the convenient handling of the standard materials an auto sampler carrying up to 100 samples was installed.
With this, it is suggested to carry out calibrations of recurring pyrolysis products to obtain quantification results, taking the response factors of the detectors into account.
In addition, it is suggested to change to a high temperature separation column to and to analyse pyrolysates with GC temperatures up to 400 °C.

This study revealed by TGA that maximum degradation temperatures of algae biomass and biochemical constituents are below 500 °C. Therefore, it is suggested to investigate chemical product formation at lower pyrolysis temperatures than applied in

this study. Moreover, an investigation of staged pyrolysis to produce a certain spectrum of chemicals in the pyrolysates would be useful.

9.2.3. Large-scale realisation

It is suggested to carry out large-scale tests applying micro- and macroalgae biomass with the 100kg h^{-1} pyroformer to obtain pyrolysis liquids and biochar for further characterisation. It is of importance to record mass and energy balances to evaluate the overall efficiency of intermediate pyrolysis. If possible, large scale testing with the pyroformer coupled to a gasifier would be useful.
In addition, lifecycle assessments and economical feasibility studies including various feedstocks are necessary to rate the overall prospect of the BtVB-process.

RFERENCES

[1] "Crude Oil prices." [Online]. Available: http://www.indexmundi.com/commodities/?commodity=crude-oil-brent&months=120. [Accessed: 21-Nov-2012].

[2] M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden, and C. E. Hanson, "Climate Change 2007: Impacts, Adaptation and Vulnerability," Cambridge, UK, 2007.

[3] O. Edenhofer, R. Pichs Madruga, Y. Sokona, K. Seyboth, P. Matschoss, S. Kadner, T. Zwickel, P. Eickemeier, G. Hansen, S. Schlömer, and C. von Stechow, "Renewable Energy Sources and Climate Change Mitigation Special Report of the Intergovernmental Panel on Climate Change," Cambridge, UK, 2012.

[4] S. Raghu, J. L. Spencer, A. S. Davis, and R. N. Wiedenmann, "Ecological considerations in the sustainable development of terrestrial biofuel crops," *Current Opinion in Environmental Sustainability*, vol. 3, no. 12, pp. 15–23.

[5] A. Singh, S. I. Olsen, and P. S. Nigam, "A viable technology to generate third-generation biofuel," *Journal of Chemical Technology and Biotechnology*, vol. 86, no. 11, pp. 1349–1353.

[6] "Press Release European Commission IP/12/1112: New Commission proposal to minimise the climate impacts of biofuel production," Brussels, Belgium.

[7] F. Shafizadeh, "Introduction to pyrolysis of biomass," *Journal of Analytical and Applied Pyrolysis*, vol. 3, no. 4, pp. 283–305, 1982.

[8] A. V Bridgwater, D. Meier, and D. Radlein, "An overview of fast pyrolysis of biomass," *Organic Geochemistry*, vol. 30, pp. 1479–1493, 1999.

[9] D. Mohan, C. U. Pittman, and P. H. Steele, "Pyrolysis of Wood/Biomass for Bio-oil: A Critical Review," *Energy & Fuels*, vol. 20, no. 3, pp. 848–889, 2006.

[10] R. Slade, R. Saunders, R. Gross, and A. Bauen, "Energy from biomass: the size of the global resource, Imperial College Centre for Energy Policy and Technology and UK Energy Research Centre," London, 2011.

[11] R. Luque, L. Herrero-Davila, J. M. Campelo, J. H. Clark, J. M. Hidalgo, D. Luna, J. M. Marinas, and A. A. Romero, "Biofuels: a technological perspective," *Energy & Environmental Science*, vol. 1, no. 5, pp. 542–564, 2008.

[12] P. Schenk, S. Thomas-Hall, E. Stephens, U. Marx, J. Mussgnug, C. Posten, O. Kruse, and B. Hankamer, "Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production," *BioEnergy Research*, vol. 1, no. 1, pp. 20–43, 2008.

[13] J. N. Rosenberg, G. A. Oyler, L. Wilkinson, and M. J. Betenbaugh, “A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution,” *Current Opinion in Biotechnology*, vol. 19, no. 5, pp. 430–436, 2008.

[14] C. Posten and G. Schaub, “Microalgae and terrestrial biomass as source for fuels: A process view,” *Journal of Biotechnology*, vol. 142, no. 1, pp. 64–69, 2009.

[15] C. Torri, C. Samorì, A. Adamiano, D. Fabbri, C. Faraloni, and G. Torzillo, “Preliminary investigation on the production of fuels and bio-char from Chlamydomonas reinhardtii biomass residue after bio-hydrogen production,” *Bioresource Technology*, vol. 102, no. 18, pp. 8707–8713, 2011.

[16] X. L. Miao and Q. Y. Wu, “High yield bio-oil production from fast pyrolysis by metabolic controlling of Chlorella protothecoides,” *Journal of Biotechnology*, vol. 110, no. 1, pp. 85–93, 2004.

[17] H. Zhao, H. Yan, M. Liu, C. Zhang, and S. Qin, “Pyrolytic characteristics and kinetics of the marine green tide macroalgae, *Enteromorpha prolifera*. *Chinese Journal of Oceanology and Limnology*, vol. 29, no. 5, pp. 996–1001.

[18] A. Singh, P. S. Nigam, and J. D. Murphy, “Renewable fuels from algae: An answer to debatable land based fuels,” *Bioresource Technology*, vol. 102, no. 1, pp. 10–16.

[19] “Algae -Based Biofuels: A Review of Challenges and Opportunities for Developing Countries,” Rome.

[20] S. C. Moldoveanu, *Analytical pyrolysis of natural organic polymers*. Amsterdam: Elsevier Science B.V., 1998, p. 496.

[21] A. Demirbas and G. Arin, “An overview of Biomass pyrolysis,” *Energy Sources*, vol. 24, no. 5, pp. 471–482, 2002.

[22] H. B. Goyal, D. Seal, and R. C. Saxena, “Bio-fuels from thermochemical conversion of renewable resources: A review,” *Renewable and Sustainable Energy Reviews*, vol. 12, no. 2, pp. 504–517, 2008.

[23] A. Hornung and A. Apfelbacher, “Biomass Processing,” U.S. Patent PCT/GB2009/0012052009.

[24] S. Czernik and A. V Bridgwater, “Overview of Applications of Biomass Fast Pyrolysis Oil,” *Energy & Fuels*, vol. 18, no. 2, pp. 590–598, 2004.

[25] A. V Bridgwater, *Fast pyrolysis of biomass : a handbook*. Newbury: CPL Press, 1999, p. 188 p.

[26] A. V Bridgwater, *Fast Pyrolysis of Biomass: A Handbook*, no. Bd. 2. Cpl Press, 2008.

[27] Z. Öztürk and J. F. Merklin, “Fast pyrolysis of cellulose with reactive methane gas in a single-pulse shock tube,” *Journal of Applied Polymer Science*, vol. 52, no. 6, pp. 747–753, 1994.

[28] A. Hornung, A. Apfelbacher, and S. Sagi, “Intermediate pyrolysis: A sustainable biomass-to-energy concept - Biothermal valorisation of Biomass (BtVB) Process,” *Journal of Scientific & Industrial Research*, vol. 70, pp. 664–667, 2011.

[29] A. Hornung, A. Apfelbacher, and S. Sagi, “INTEGRATIVE, CO2 NEGATIVE, HIGH EFFICIENT POWER GENERATION (ICONE POWER) FROM ASH RICH BIOMASS COUPLED WITH PRODUCTION OF ALGAE BASED BIO OILS AS WELL AS BLACK EARTH AVOIDING AFFLUENTS AT HAINHAUS/ODENWALD USING THE BTVB PROCESS,” in *Proceedigns of the 17th European Biomass Conference & Exhibition , June 2009, (Hamburg/Germany)*.

[30] A. Hornung and A. Apfelbacher, “Thermal Treatment of Biomass,” U.S. Patent WO2009/138757 A22009.

[31] M. K. Misra, K. W. Ragland, and A. J. Baker, “Wood ash composition as a function of furnace temperature,” *Biomass and Bioenergy*, vol. 4, no. 2, pp. 103–116, 1993.

[32] A. B. Ross, J. M. Jones, M. L. Kubacki, and T. Bridgeman, “Classification of macroalgae as fuel and its thermochemical behaviour,” *Bioresource Technology*, vol. 99, no. 14, pp. 6494–6504, 2008.

[33] Y. Yang, J. G. Brammer, M. Ouadi, J. Samanya, A. Hornung, H. M. Xu, and Y. Li, “Characterisation of waste derived intermediate pyrolysis oils for use as diesel engine fuels,” *Fuel*, no. in press.

[34] D. P. Van Vuuren, A. F. Bouwman, and A. H. W. Beusen, “Phosphorus demand for the 1970-2100 period: A scenario analysis of resource depletion,” *Global Environmental Change*, vol. 20, no. 3, pp. 428–439.

[35] M. Vanthoor-Koopmans, R. H. Wijffels, M. J. Barbosa, and M. H. M. Eppink, “Biorefinery of microalgae for food and fuel,” *Bioresource Technology*, no. in press.

[36] J. H. Mussgnug, V. Klassen, A. Schlüter, and O. Kruse, “Microalgae as substrates for fermentative biogas production in a combined biorefinery concept,” *Journal of Biotechnology*, vol. 150, no. 1, pp. 51–56, 2010.

[37] L. G. Alba, C. Torri, C. Samori, J. van der Spek, D. Fabbri, S. R. A. Kersten, and D. W. F. Brilman, “Hydrothermal Treatment (HIT) of Microalgae: Evaluation of the Process As Conversion Method in an Algae Biorefinery Concept,” *Energy & Fuels*, vol. 26, no. 1, pp. 642–657.

[38] J. N. Rosenberg, A. Mathias, K. Korth, M. J. Betenbaugh, and G. A. Oyler, “Microalgal biomass production and carbon dioxide sequestration from an integrated ethanol biorefinery in Iowa: A technical appraisal and economic feasibility evaluation,” *Biomass & Bioenergy*, vol. 35, no. 9, pp. 3865–3876.

[39] H. C. Bold and M. J. Wynne, *Introduction to the Algae: structure and reproduction*. New Jersey: Prentice-Hall Inc., 1978, p. 706.

[40] J. M. G. L. W. W. Linda E. Graham, *Algae*, 2nd ed. San Fransisco: Pearson Education Inc., Pearson Benjamin Cummings, 2009.

[41] G. W. Huber, S. Iborra, and A. Corma, “Synthesis of Transportation Fuels from Biomass:Chemistry, Catalysts, and Engineering,” *Chemical Reviews*, vol. 106, no. 9, pp. 4044–4098, 2006.

[42] W. Tanner and F. A. Loewus, Eds., *Encyclopedia of Plant Physiology New Series Volume 13B - Plant Carbohydrates II Extracellular Carbohydrates*. Berlin: Springer-Verlag Berlin, 1981, p. 769.

[43] D. S. Bhakuni and D. S. Rawat, *Bioactive Marine Natural Products*. New Delhi: Co-published by Springer New York with Anamaya Publishers, New Delhi, India, 2005, p. 382.

[44] E. W. Becker, *Microalgae, Biotechnology and Microbiology*. Cambridge University Press, 1994.

[45] Ö. Tokuşoglu and M. K. üUnal, “Biomass Nutrient Profiles of Three Microalgae: Spirulina platensis, Chlorella vulgaris, and Isochrisis galbana,” *Journal of Food Science*, vol. 68, no. 4, pp. 1144–1148, 2003.

[46] S. D. Varfolomeev and L. A. Wasserman, “Microalgae as Source of Biofuel, Food, Fodder, and Medicines,” *Applied Biochemistry and Microbiology*, vol. 47, no. 9, pp. 789–807.

[47] J. Ortiz, N. Romero, P. Robert, J. Araya, J. Lopez-HernÃ¡ndez, C. Bozzo, E. Navarrete, A. Osorio, and A. Rios, “Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds Ulva lactuca and Durvillaea antarctica,” *Food Chemistry*, vol. 99, no. 1, pp. 98–104, 2006.

[48] L. E. Cruz-Suarez, M. Tapia-Salazar, M. G. Nieto-LÓPez, C. Guajardo-Barbosa, and D. Ricque-Marie, “Comparison of Ulva clathrata and the kelps Macrocystis pyrifera and Ascophyllum nodosum as ingredients in shrimp feeds,” *Aquaculture Nutrition*, vol. 15, no. 4, pp. 421–430, 2009.

[49] R. E. Lee, *Phycology*, 4th ed. New York: Cambridge University Press, 2008.

[50] E. Percival, “The polysaccharides of green, red and brown seaweeds: Their basic structure, biosynthesis and function,” *British Phycological Journal*, vol. 14, no. 2, pp. 103–117, 1979.

[51] G. A. De Ruiter and B. Rudolph, “Carrageenan biotechnology,” *Trends in Food Science & Technology*, vol. 8, no. 12, pp. 389–395, 1997.

[52] V. L. Campo, D. Fã¡. Kawano, Dã. B. da Silva Jr, and I. Carvalho, “Carrageenans: Biological properties, chemical modifications and structural analysis: A review,” *Carbohydrate Polymers*, vol. 77, no. 2, pp. 167–180, 2009.

[53] Y. Ovodov, V. Khomenko, and T. Guseva, “Polysaccharides of brown algae,” *Chemistry of Natural Compounds*, vol. 6, no. 3, pp. 285–288, 1970.

[54] T. A. Davis, B. Volesky, and A. Mucci, “A review of the biochemistry of heavy metal biosorption by brown algae,” *Water Research*, vol. 37, no. 18, pp. 4311–4330, 2003.

[55] K. Ito and K. Hori, “Seaweed: Chemical composition and potential food uses,” *Food Reviews International*, vol. 5, no. 1, pp. 101–144, 1989.

[56] A. S. Carlsson, V. J. Beilen, R. Möller, and D. Clayton, “MICRO- AND MACRO-ALGAE: UTILITY FOR INDUSTRIAL APPLICATIONS - Outputs from the EPOBIO project September 2007,” 2007.

[57] M. A. Borowitzka, “Commercial production of microalgae: ponds, tanks, tubes and fermenters,” 1998, pp. 313–321.

[58] A. Demirbas, “Use of algae as biofuel sources,” *Energy Conversion and Management*, vol. 51, no. 12, pp. 2738–2749, 2010.

[59] T. M. Mata, A. A. Martins, and N. S. Caetano, “Microalgae for biodiesel production and other applications: A review,” *Renewable & Sustainable Energy Reviews*, vol. 14, no. 1, pp. 217–232.

[60] F. Lehr and C. Posten, “Closed photo-bioreactors as tools for biofuel production,” *Current Opinion in Biotechnology*, vol. 20, no. 3, pp. 280–285, 2009.

[61] H. Wolkers, M. Barbarosa, D. Kleinegires, R. Bosma, and R. H. Wijffels, “Microalgae: the green gold of the future? - Large-scale sustainable cultivation of microalgae for the production of bulk commodities,” 2011.

[62] P. Spolaore, C. Joannis-Cassan, E. Duran, and A. Isambert, “Commercial applications of microalgae,” *Journal of Bioscience and Bioengineering*, vol. 101, no. 2, pp. 87–96, 2006.

[63] “Open Pond cultivation of Microalgae.” [Online]. Available: http://www.guardian.co.uk/environment/2008/oct/23/biofuels-energy. [Accessed: 15-Mar-2013].

[64] “Fisheries and Aquaculture Department (FAO) PART 1 - WORLD REVIEW OF FISHERIES AND AQUACULTURE,” 2010.

[65] K. Lüning and S. Pang, “Mass cultivation of seaweeds: current aspects and approaches,” *Journal of Applied Phycology*, vol. 15, no. 2, pp. 115–119, 2003.

[66] F. Xiu-geng, B. Ying, and L. Shan, “Seaweed cultivation: Traditional way and its reformation,” *Chinese Journal of Oceanology and Limnology*, vol. 17, no. 3, pp. 193–199, 1999.

[67] M. Shanmugam and K. H. Mody, “Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents,” *Current Science*, vol. 79, no. 12, pp. 1672–1682, 2000.

[68] D. J. McHugh, “A guide to the seaweed industry - FAO FISHERIES TECHNICAL PAPER 441,” Rome, 2003.

[69] H. C. Greenwell, L. M. L. Laurens, R. J. Shields, R. W. Lovitt, and K. J. Flynn, “Placing microalgae on the biofuels priority list: a review of the technological challenges,” *Journal of the Royal Society Interface*, vol. 7, no. 46, pp. 703–726, 2010.

[70] C.-Y. Chen, K.-L. Yeh, R. Aisyah, D.-J. Lee, and J.-S. Chang, “Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review,” *Bioresource Technology*, vol. 102, no. 1, pp. 71–81.

[71] L. Gouveia, “Microalgae as a Feedstock for Biofuels,” *Springer Briefs in Microbiology*, 2011.

[72] Y. Li, M. Horsman, N. Wu, C. Q. Lan, N. Dubois-Calero, and E. Canadian Soc Chem, “Biofuels from microalgae,” 2007, pp. 815–820.

[73] Q. Hu, C. Zhang, and M. Sommerfeld, “Biodiesel from algae: Lessons learned over the past 60 years and future perspectives,” *Journal of Phycology*, vol. 42, p. 37, 2006.

[74] J. Sheehan, T. Dunahay, J. Benemann, and P. Roessler, “NREL/TP-580-24190 - A Look Back at the U.S. Department of Energy’s Aquatic Species Program: Biodiesel from Algae.”

[75] A. Almodares and M. R. Hadi, “Production of bioethanol from sweet sorghum: A review,” *African Journal of Agricultural Research*, vol. 4, no. 9, pp. 772–780, 2009.

[76] L. Brennan and P. Owende, “Biofuels from microalgae--A review of technologies for production, processing, and extractions of biofuels and co-products,” *Renewable and Sustainable Energy Reviews*, vol. 14, no. 2, pp. 557–577, 2010.

[77] S. Amin, “Review on biofuel oil and gas production processes from microalgae,” *Energy Conversion and Management*, vol. 50, no. 7, pp. 1834–1840, 2009.

[78] M. Peter, “Energy production from biomass (part 2): conversion technologies,” *Bioresource Technology*, vol. 83, no. 1, pp. 47–54, 2002.

[79] D. C. Elliott, G. G. Neuenschwander, T. R. Hart, R. S. Butner, A. H. Zacher, M. H. Engelhard, J. S. Young, and D. E. McCready, “Chemical Processing in High-

Pressure Aqueous Environments. 7. Process Development for Catalytic Gasification of Wet Biomass Feedstocks," *Industrial & Engineering Chemistry Research*, vol. 43, no. 9, pp. 1999–2004, 2004.

[80] A. Demirbas, "Oily products from mosses and algae via pyrolysis," *Energy Sources Part a-Recovery Utilization and Environmental Effects*, vol. 28, no. 10, pp. 933–940, 2006.

[81] Z. Shuping, W. Yulong, Y. Mingde, L. Chun, and T. Junmao, "Pyrolysis characteristics and kinetics of the marine microalgae Dunaliella tertiolecta using thermogravimetric analyzer," *Bioresour Technol*, vol. 101, no. 1, pp. 359–365, 2010.

[82] D. Li, L. Chen, X. Zhang, N. Ye, and F. Xing, "Pyrolytic characteristics and kinetic studies of three kinds of red algae," *Biomass and Bioenergy*, vol. 35, no. 5, pp. 1765–1772.

[83] K. Kirtania and S. Bhattacharya, "Application of the distributed activation energy model to the kinetic study of pyrolysis of the fresh water algae Chlorococcum humicola," *Bioresource Technology*, vol. 107, no. 0, pp. 476–481.

[84] C. Vasile and M. C. Popescu, "THERMAL BEHAVIOUR/TREATMENT OF SOME VEGETABLE RESIDUES. II. SPANISH BROOM (SPARTIUM JUNCEUM) FIBERS," *Cellulose Chemistry and Technology*, vol. 42, no. 7–8, pp. 335–344, 2008.

[85] C. Vasile, M. Brebu, and M. C. Popescu, "THERMAL BEHAVIOUR/TREATMENT OF AGRICULTURAL BIOMASS I. THERMAL BEHAVIOUR OF OILSEED RAPE (BRASSICA NAPUS L.)," *Cellulose Chemistry and Technology*, vol. 42, no. 4–6, pp. 159–169, 2008.

[86] C. Vasile, C.-M. Popescu, M.-C. Popescu, M. Brebu, and S. Willfor, "THERMAL BEHAVIOUR/TREATMENT OF SOME VEGETABLE RESIDUES. IV. THERMAL DECOMPOSITION OF EUCALYPTUS WOOD," *Cellulose Chemistry and Technology*, vol. 45, no. 1–2, pp. 29–42.

[87] D. J. Nowakowski and J. M. Jones, "Uncatalysed and potassium-catalysed pyrolysis of the cell-wall constituents of biomass and their model compounds," *Journal of Analytical and Applied Pyrolysis*, vol. 83, no. 1, pp. 12–25, 2008.

[88] H. Yang, R. Yan, H. Chen, C. Zheng, D. H. Lee, and D. T. Liang, "In-Depth Investigation of Biomass Pyrolysis Based on Three Major Components: Hemicellulose, Cellulose and Lignin," *Energy & Fuels*, vol. 20, no. 1, pp. 388–393, 2005.

[89] M. Balat, “Mechanisms of thermochemical biomass conversion processes. Part 1: Reactions of pyrolysis,” *Energy Sources Part a-Recovery Utilization and Environmental Effects*, vol. 30, no. 7, pp. 620–635, 2008.

[90] W. Peng, Q. Wu, P. Tu, and N. Zhao, “Pyrolytic characteristics of microalgae as renewable energy source determined by thermogravimetric analysis,” *Bioresource Technology*, vol. 80, no. 1, pp. 1–7, 2001.

[91] W. M. Peng, Q. Y. Wu, and P. G. Tu, “Effects of temperature and holding time on production of renewable fuels from pyrolysis of *Chlorella protothecoides*,” *Journal of Applied Phycology*, vol. 12, no. 2, pp. 147–152, 2000.

[92] W. M. Peng, Q. Y. Wu, and P. G. Tu, “Pyrolytic characteristics of heterotrophic *Chlorella protothecoides* for renewable bio-fuel production,” *Journal of Applied Phycology*, vol. 13, no. 1, pp. 5–12, 2001.

[93] V. Bhola, R. Desikan, S. K. Santosh, K. Subburamu, E. Sanniyasi, and F. Bux, “Effects of parameters affecting biomass yield and thermal behaviour of *Chlorella vulgaris*,” *Journal of Bioscience and Bioengineering*, vol. 111, no. 3, pp. 377–382, 2011.

[94] D. Li, L. Chen, X. Zhang, N. Ye, and F. Xing, “Pyrolytic characteristics and kinetic studies of three kinds of red algae,” *Biomass and Bioenergy*, vol. 35, no. 5, pp. 1765–1772, 2011.

[95] J. Wang, G. Wang, M. Zhang, M. Chen, D. Li, F. Min, M. Chen, S. Zhang, Z. Ren, and Y. Yan, “A comparative study of thermolysis characteristics and kinetics of seaweeds and fir wood,” *Process Biochemistry*, vol. 41, no. 8, pp. 1883–1886, 2006.

[96] N. Ye, D. Li, L. Chen, X. Zhang, and D. Xu, “Comparative Studies of the Pyrolytic and Kinetic Characteristics of Maize Straw and the Seaweed *Ulva pertusa*,” *PLoS ONE*, vol. 5, no. 9, p. e12641.

[97] B. Maddi, S. Viamajala, and S. Varanasi, “Comparative study of pyrolysis of algal biomass from natural lake blooms with lignocellulosic biomass,” *Bioresource Technology*, vol. 102, no. 23, pp. 11018–11026, 2011.

[98] K. Anastasakis, A. B. Ross, and J. M. Jones, “Pyrolysis behaviour of the main carbohydrates of brown macro-algae,” *Fuel*, vol. 90, no. 2, pp. 598–607, 2011.

[99] A. Marcilla, A. Gomez-Siurana, C. Gomis, E. Chapuli, M. C. Catala, and F. J. Valdes, “Characterization of microalgal species through TGA/FTIR analysis: Application to *Nannochloropsis sp*,” *Thermochimica Acta*, vol. 484, no. 1–2, pp. 41–47, 2009.

[100] L. Sanchez-Silva, D. Lopez-Gonzalez, J. Villaseaor, P. Sanchez, and J. L. Valverde, “Thermogravimetric-mass spectrometric analysis of lignocellulosic and

marine biomass pyrolysis," *Bioresource Technology*, vol. 109, no. 0, pp. 163–172.

[101] D. M. Li, L. M. Chen, X. J. Yi, X. W. Zhang, and N. H. Ye, "Pyrolytic characteristics and kinetics of two brown algae and sodium alginate," *Bioresource Technology*, vol. 101, no. 18, pp. 7131–7136, 2010.

[102] Y.-H. Park, J. Kim, S.-S. Kim, and Y.-K. Park, "Pyrolysis characteristics and kinetics of oak trees using thermogravimetric analyzer and micro-tubing reactor," *Bioresource Technology*, vol. 100, no. 1, pp. 400–405, 2009.

[103] S. Tsuge, "ANALYTICAL PYROLYSIS - PAST, PRESENT AND FUTURE," *Journal of Analytical and Applied Pyrolysis*, vol. 32, pp. 1–6, 1995.

[104] C. S. Gutteridge, "6 Characterization of Microorganisms by Pyrolysis Mass Spectrometry," in *Methods in Microbiology*, vol. Volume 19, R. R. Colwell and R. Grigorova, Eds. Academic Press, 1988, pp. 227–272.

[105] P. M. Monkhouse and H. R. Schulten, "Pyrolysis mass spectrometry of recent and fossil biomaterials. Compendium and atlas. : by H.L.C. Meuzelaar, J. Haverkamp and F.D. Hileman. Elsevier, Amsterdam, Oxford, New York, 1982, XIII - 293 pp. price Dfl. 145.00. ISBN 0-444-42099-1," *Journal of Analytical and Applied Pyrolysis*, vol. 4, no. 3, pp. 257–259, 1982.

[106] S. C. Moldoveanu, *Analytical pyrolysis of synthetic organic polymers*, no. v 25. Amsterdam ; London: Elsevier, 2005.

[107] W. J. Irwin, *Analytical pyrolysis : a comprehensive guide*, no. v 22. New York, N.Y.: M. Dekker, 1982, p. 578.

[108] T. P. Wampler, *Applied pyrolysis handbook*. New York: M. Dekker, 1995, p. 361.

[109] Șerban C Moldoveanu, *Pyrolysis of Organic Molecules: Applications to Health and Environmental Issues*, vol. TECHNIQUES. Elsevier B.V., 2010, pp. 259–263.

[110] D. Barupal, T. Kind, S. Kothari, D. Lee, and O. Fiehn, "Hydrocarbon phenotyping of algal species using pyrolysis-gas chromatography mass spectrometry," *BMC Biotechnology*, vol. 10, no. 1, p. 40, 2010.

[111] T. Lehtonen, J. Peuravuori, and K. Pihlaja, "Characterisation of lake-aquatic humic matter isolated with two different sorbing solid techniques: tetramethylammonium hydroxide treatment and pyrolysis-gas chromatography/mass spectrometry," *Analytica Chimica Acta*, vol. 424, no. 1, pp. 91–103, 2000.

[112] D. L. Widrig, K. A. Gray, and K. S. McAuliffe, "Removal of algal-derived organic material by preozonation and coagulation: Monitoring changes in organic quality by pyrolysis-GC-MS," *Water Research*, vol. 30, no. 11, pp. 2621–2632, 1996.

[113] R. J. Helleur, E. R. Hayes, J. S. Craigie, and J. L. McLachlan, “Characterization of polysaccharides of red algae by pyrolysis-capillary gas chromatography,” *Journal of Analytical and Applied Pyrolysis*, vol. 8, pp. 349–357, 1985.

[114] R. J. Helleur, E. R. Hayes, W. D. Jamieson, and J. S. Craigie, “Analysis of polysaccharide pyrolysate of red algae by capillary gas chromatography-mass spectrometry,” *Journal of Analytical and Applied Pyrolysis*, vol. 8, no. 0, pp. 333–347, 1985.

[115] A. B. Ross, K. Anastasakis, M. Kubacki, J. M. Jones, S. Spanish Minist, S. C. S. R. U. L. P. Innovat, and C. de Gran, “Investigation of the pyrolysis behaviour of brown algae before and after pre-treatment using PY-GC/MS and TGA,” 2008, pp. 3–10.

[116] A. B. Ross, C. Hall, K. Anastasakis, A. Westwood, J. M. Jones, and R. J. Crewe, “Influence of cation on the pyrolysis and oxidation of alginates,” *Journal of Analytical and Applied Pyrolysis*, vol. 91, no. 2, pp. 344–351, 2011.

[117] J. M. M. Adams, A. B. Ross, K. Anastasakis, E. M. Hodgson, J. A. Gallagher, J. M. Jones, and I. S. Donnison, “Seasonal variation in the chemical composition of the bioenergy feedstock *Laminaria digitata* for thermochemical conversion,” *Bioresource Technology*, vol. 102, no. 1, pp. 226–234.

[118] F. Gelin, J. W. de Leeuw, J. S. Sinninghe DamstÃ©, S. Derenne, C. Largeau, and P. Metzger, “Scope and limitations of flash pyrolysis-gas chromatography/mass spectrometry as revealed by the thermal behaviour of high-molecular-weight lipids derived from the green microalga *Botryococcus braunii*,” *Journal of Analytical and Applied Pyrolysis*, vol. 28, no. 2, pp. 183–204, 1994.

[119] F. Gelin, J. P. L. A. Gatellier, J. S. S. Damsté, P. Metzger, S. Derenne, C. Largeau, and J. W. de Leeuw, “Mechanisms of flash pyrolysis of ether lipids isolated from the green microalga *Botryococcus braunii* race A,” *Journal of Analytical and Applied Pyrolysis*, vol. 27, no. 2, pp. 155–168, 1993.

[120] F. Gadel and A. Bruchet, “Application of pyrolysis-gas chromatography-mass spectrometry to the characterization of humic substances resulting from decay of aquatic plants in sediments and waters,” *Water Research*, vol. 21, no. 10, pp. 1195–1206, 1987.

[121] H. R. Schulten and G. Gleixner, “Analytical pyrolysis of humic substances and dissolved organic matter in aquatic systems: structure and origin,” *Water Research*, vol. 33, no. 11, pp. 2489–2498, 1999.

[122] T. B. R. Siddhartha Gaur, *Thermal Data for Natural and Synthetic Fuels*. New York: Marcel Dekker Inc.

[123] D. Meier and O. Faix, “State of the art of applied fast pyrolysis of lignocellulosic materials — a review,” *Bioresource Technology*, vol. 68, no. 1, pp. 71–77, 1999.

[124] D. H. Miller, D. T. A. Lamport, and M. Miller, “Hydroxyproline Heterooligosaccharides in Chlmaydomonas,” *Science*, vol. 176, pp. 918–20, 1972.

[125] K. Roberts, “Crystalline Glycoprotein Cell Walls of Algae: Their Stucture, Composition and Assembly,” *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, vol. 268, no. 891, pp. 129–146, 1974.

[126] S. H. Imam, M. J. Buchanan, H. C. Shin, and and W. J. Snell, “The Chlamydomonas cell wall: characterization of the wall framework,” *Journal of Cell Biology*, vol. 101, pp. 1599–1607, 1985.

[127] H. Takeda, “Classification of Chlorella strains by cell wall sugar composition,” *Phytochemistry*, vol. 27, no. 12, pp. 3823–3826, 1988.

[128] D. Domozych, M. Ciancia, J. U. Fangel, M. D. Mikkelsen, P. Ulvskov, and W. G. T. Willats, “The cell walls of green algae: a journey through evolution and diversity,” *Frontiers in Plant Science*, vol. 3.

[129] M. Blumreisinger, D. Meindl, and E. Loos, “Cell wall composition of chlorococcal algae,” *Phytochemistry*, vol. 22, no. 7, pp. 1603–1604, 1983.

[130] M. M. Phukan, R. S. Chutia, B. K. Konwar, and R. Kataki, “Microalgae Chlorella as a potential bio-energy feedstock,” *Applied Energy*, vol. 88, no. 10, pp. 3307–3312.

[131] A. Wijanarko, Dianursanti, A. Y. Sendjaya, H. Hermansyah, A. B. Witarto, M. Gozan, B. T. Sofyan, K. Asami, K. Ohtaguchi, R. W. Soemantojo, and S. K. Song, “Enhanced *Chlorella vulgaris* Buitenzorg growth by photon flux density alteration in serial photobioreactors,” *Biotechnology and Bioprocess Engineering*, vol. 13, no. 4, pp. 476–482, 2008.

[132] K. Roberts, M. Gurney-Smith, and G. J. Hills, “Structure, composition and morphogenesis of the cell wall of *Chlamydomonas reinhardi*: I. Ultrastructure and preliminary chemical analysis,” *Journal of Ultrastructure Research*, vol. 40, no. 5–6, pp. 599–613, 1972.

[133] I. S. M. David H. Miller Derek T. A. Lamport, and Maureen Miller, “THE CHEMICAL COMPOSITION OF THE CELL WALL OF *CHLAMYDOMONAS GYMNOGAMA* AND THE CONCEPT OF A PLANT CELL WALL PROTEIN,” *J Cell Biol.* , vol. 1, no. 63(2), pp. 420–429. .

[134] D. R. Davies and A. Plaskitt, “GENETICAL AND STRUCTURAL ANALYSES OF CELL-WALL FORMATION IN *CHLAMYDOMONAS-REINHARDI*,” *Genetical Research*, vol. 17, no. 1, p. 33–&, 1971.

[135] B. Klein, “STRUKTURBASIERTES SCREENING ZUR GEWINNUNG VON CO-ENZYM Q10 AUS PHOTOTROPHEN MIKROORGANISMEN,” Universitaet Erlangen-Nuernberg/Germany, 2010.

[136] B. C. Klein, S. J. Bartel, K. H. Darsow, I. Naumann, C. Walter, R. Buchholz, and H. A. Lange, “IDENTIFICATION OF COENZYME Q10 FROM *PORPHYRIDIUM PURPUREUM* (RHODOPHYTA) BY MATRIX-ASSISTED LASER DESORPTION IONIZATION CURVED FIELD REFLECTRON MASS SPECTROMETRY1,” *Journal of Phycology*, vol. 47, no. 3, pp. 687–691.

[137] C. K. Veena, A. Josephine, S. P. Preetha, and P. Varalakshmi, “Beneficial role of sulfated polysaccharides from edible seaweed *Fucus vesiculosus* in experimental hyperoxaluria,” *Food Chemistry*, vol. 100, no. 4, pp. 1552–1559, 2007.

[138] I. Gomez, A. Wulff, M. Y. Roleda, P. Huovinen, U. Karsten, M. Liliana Quartino, K. Dunton, and C. Wiencke, “Light and temperature demands of marine benthic microalgae and seaweeds in polar regions,” *Botanica Marina*, vol. 52, no. 6, pp. 593–608, 2009.

[139] H. Hop, T. Pearson, E. N. Hegseth, K. M. Kovacs, C. Wiencke, S. Kwasniewski, K. Eiane, F. Mehlum, B. Gulliksen, M. Wlodarska-Kowalczuk, C. Lydersen, J. M. Weslawski, S. Cochrane, G. W. Gabrielsen, R. J. G. Leakey, O. J. Lønne, M. Zajaczkowski, S. Falk-Petersen, M. Kendall, S.-Å. Wängberg, K. Bischof, A. Y. Voronkov, N. A. Kovaltchouk, J. Wiktor, M. Poltermann, G. di Prisco, C. Papucci, and S. Gerland, “The marine ecosystem of Kongsfjorden, Svalbard,” *Polar Research*, vol. 21, no. 1, pp. 167–208, 2002.

[140] K. Zacher, R. Rautenberger, D. Hanelt, A. Wulff, and C. Wiencke, “The abiotic environment of polar marine benthic algae,” *Botanica Marina*, vol. 52, pp. 483–490, 2009.

[141] H. Svendsen, A. Beszczynska-Møller, J. O. Hagen, B. Lefauconnier, V. Tverberg, S. Gerland, J. B. Ørbøk, K. Bischof, C. Papucci, M. Zajaczkowski, R. Azzolini, O. Bruland, C. Wiencke, J.-G. Winther, and W. Dallmann, “The physical environment of Kongsfjorden–Krossfjorden, an Arctic fjord system in Svalbard,” *Polar Research*, vol. 21, no. 1, pp. 133–166, 2002.

[142] D. Hanelt, K. Bischof, and C. Wiencke, *The coastal ecosystem of Kongsfjorden, Svalbard. Synopsis of biological research performed at the Koldewey Station in the years of 1991-2003*, 492nd ed. Ber. Polarforsch. Meeresforsch., 2004, pp. 14–25.

[143] “Porphyridium purpureum - Info.” [Online]. Available: http://www.straininfo.net/strains/821039/browser. [Accessed: 12-Nov-2012].

[144] J. A. S. GmbH, “Frontier Mutli-Functional Pyrolysis System 2020.” Joint Analytical Systems GmbH, 2209.

[145] F. L. Ltd., “Double Shot Pyrolyser Model Py2020iD Operation Manual,” 2008.

[146] K. R. W. Thomas J. Stark Linda C. Doherty, “Application Note - Acquisition and analysis of combined GC/MSD and GC/FID data,” *Agilent Technologies Company*, vol. (. 1999.

[147] D. M. O. Faix I.Fortmann, “Thermal degradation products of wood,” *Holz als Roh- und Werkstoff*, vol. 48, pp. 281–285, 1990.

[148] O. Faix, I. Fortmann, J. Bremer, and D. Meier, “Thermal degradation products of wood,” *European Journal of Wood and Wood Products*, vol. 49, no. 7, pp. 299–304, 1991.

[149] J. Odermatt, D. Meier, K. Leicht, R. Meyer, and T. Runge, “Approaches to applying internal standards for the quantification of paper additives by Py-GC/MSD,” *Journal of Analytical and Applied Pyrolysis*, vol. 68–69, pp. 269–285, 2003.

[150] H. Pakdel and C. Roy, *Chemical characterization of wood oils obtained in a vacuum pyrolysis process development unit*. 1987, pp. 203–214.

[151] T. Nakai, S. N. Kartal, T. Hata, and Y. Imamura, “Chemical characterization of pyrolysis liquids of wood-based composites and evaluation of their bio-efficiency,” *Building and Environment*, vol. 42, no. 3, pp. 1236–1241, 2007.

[152] C. Branca, P. Giudicianni, and C. Di Blasi, “GC/MS Characterization of Liquids Generated from Low-Temperature Pyrolysis of Wood,” *Industrial & Engineering Chemistry Research*, vol. 42, no. 14, pp. 3190–3202, 2003.

[153] M. K. W. Rosemary Buffington, *Detectors for Gas Chromatography - A practical primer*. Hewlett-Packard Co., 1991.

[154] H. S. Siegfried H. Kaegler, “Neue Mineraloelanalyse Band 2: Chromatographie.” Urban-Verlag, 2006.

[155] R. C. Brown and C. Stevens, Eds., *Thermochemical Processing of Biomass: Conversion into Fuels, Chemicals and Power*. Wiley-Blackwell, 2011, p. 348.

[156] E. H. Harris, *he Chlamydomonas sourcebook : a comprehensive guide to biology and laboratory use*. San Diego: Academic Press, 1989, p. 780.

[157] D. Corporation, “SE 400 and SE 500 Solvent Evaporators - Technical Information,” Salt Lake City Technical Center.

[158] A. Friedl, E. Padouvas, H. Rotter, and K. Varmuza, “Prediction of heating values of biomass fuel from elemental composition,” *Analytica Chimica Acta*, vol. 544, no. 12, pp. 191–198, 2005.

[159] F. M. Jackson, L. Michaelson, T. C. M. Fraser, A. K. Stobart, and G. Griffiths, “Biosynthesis of triacylglycerol in the filamentous fungus *Mucor circinelloides*,” *Microbiology*, vol. 144, no. 9, pp. 2639–2645, 1998.

[160] E. Grima, A. Medina, A. Giménez, J. Sánchez Pérez, F. Camacho, and J. García Sánchez, “Comparison between extraction of lipids and fatty acids from microalgal biomass,” *Journal of the American Oil Chemists’ Society*, vol. 71, no. 9, pp. 955–959, 1994.

[161] G. Griffiths, H. E. Jones, C. L. Eaton, and A. K. Stobart, “Effect of n-6 polyunsaturated fatty acids on growth and lipid composition of neoplastic and non-neoplastic canine prostate epithelial cell cultures,” *The Prostate*, vol. 31, no. 1, pp. 29–36, 1997.

[162] V. Méchin, C. Damerval, and M. Zivy, “Total Protein Extraction with TCA-Acetone,” vol. 355, 2006, pp. 1–8.

[163] “E 1131-03 ASTM Standard Test Method for Compositional Analysis by Thermogravimetry.”

[164] W. Lampert and U. Sommer, *Limnoökologie*, 2nd ed. Stuttgart: Georg Thieme Verlag, 1999, p. 440.

[165] P. Christen and R. Jaussi, *Biochemie Eine Einfuehrung mit 40 Lehreinheiten*. Berlin Heidelberg: Springer Verlag, 2005, p. 636.

[166] A. Demirbas, “Combustion characteristics of different biomass fuels,” *Progress in Energy and Combustion Science*, vol. 30, no. 2, pp. 219–230, 2004.

[167] P. Ruparez, “Mineral content of edible marine seaweeds,” *Food Chemistry*, vol. 79, no. 1, pp. 23–26, 2002.

[168] S. Wang, X. M. Jiang, X. X. Han, and J. G. Liu, “Combustion Characteristics of Seaweed Biomass. 1. Combustion Characteristics of *Enteromorpha clathrata* and *Sargassum natans*,” *Energy & Fuels*, vol. 23, no. 10, pp. 5173–5178, 2009.

[169] J. M. G. L. W. W. Linda E. Graham, *Algae*, 2nd ed. San Fransisco: Pearson Education Inc., Pearson Benjamin Cummings, 2009.

[170] Z. Shuping, W. Yulong, Y. Mingde, L. Chun, and T. Junmao, “Pyrolysis characteristics and kinetics of the marine microalgae *Dunaliella tertiolecta* using thermogravimetric analyzer,” *Bioresource Technology*, vol. 101, no. 1, pp. 359–365.

[171] P. Pan, C. W. Hu, W. Y. Yang, Y. S. Li, L. L. Dong, L. F. Zhu, D. M. Tong, R. W. Qing, and Y. Fan, “The direct pyrolysis and catalytic pyrolysis of *Nannochloropsis sp* residue for renewable bio-oils,” *Bioresource Technology*, vol. 101, no. 12, pp. 4593–4599, 2010.

[172] P. McKendry, “Energy production from biomass (part 1): overview of biomass,” *Bioresource Technology*, vol. 83, no. 1, pp. 37–46, 2002.

[173] P. MacArtain, C. I. R. Gill, M. Brooks, R. Campbell, and I. R. Rowland, “Nutritional Value of Edible Seaweeds,” *Nutrition Reviews*, vol. 65, no. 12, pp. 535–543, 2007.

[174] P. T. Williams and P. A. Horne, “The role of metal salts in the pyrolysis of biomass,” *Renewable Energy*, vol. 4, no. 1, pp. 1–13, Feb. 1994.

[175] J. S. Burlew and W. Carnegie Institution of, *Algal culture : from laboratory to pilot plant*. Washington, D.C. Carnegie Institution of Washington, 1953.

[176] T. Happe and J. D. Naber, “Isolation, characterization and N-terminal amino acid sequence of hydrogenase from the green alga *Chlamydomonas reinhardti*i,” *European Journal of Biochemistry*, vol. 214, no. 2, pp. 475–481, 1993.

[177] M. B. COVINGTON, “Omega-3 Fatty Acids,” *American Family Physician*, vol. 70, no. 1, pp. 133–140, 2004.

[178] G. Habermehl, P. Hammann, H. C. Krebs, and W. Ternes, *Naturstoffchemie Eine Einfuehrung*, 3rd ed. Berlin: Springer Verlag, 2008, p. 698.

[179] S. Teras, G. Barcela-Coblijn, M. Benet, R. Ã lvarez, R. Bressani, J. E. Halver, and P. V Escriba, “Oleic acid content is responsible for the reduction in blood pressure induced by olive oil,” *Proceedings of the National Academy of Sciences*, vol. 105, no. 37, pp. 13811–13816, 2008.

[180] Q. Hu, “Hand book opf Microalgal Culture, Biotechnology and Applied Phycology,” A. Richmond, Ed. Blackwell Sience Ltd, a Blackwell Publishing company, 2007, pp. 83–94.

[181] U. Räisänen, I. Pitkänen, H. Halttunen, and M. Hurtta, “Formation of the main degradation compounds from arabinose, xylose, mannose and arabinitol during pyrolysis,” *Journal of Thermal Analysis and Calorimetry*, vol. 72, no. 2, pp. 481–488, 2003.

[182] V. A. Alvarez and A. Vazquez, “Thermal degradation of cellulose derivatives/starch blends and sisal fibre biocomposites,” *Polymer Degradation and Stability*, vol. 84, no. 1, pp. 13–21, 2004.

[183] S. Maiti, S. Purakayastha, and B. Ghosh, “Thermal characterization of mustard straw and stalk in nitrogen at different heating rates,” *Fuel*, vol. 86, no. 10â€“11, pp. 1513–1518, 2007.

[184] L. Sanchez-Silva, D. Lopez-Gonzalez, J. Villasenor, P. Sanchez, and J. L. Valverde, “Thermogravimetric-mass spectrometric analysis of lignocellulosic and marine biomass pyrolysis,” *Bioresource Technology*, vol. 109, pp. 163–172.

[185] A. Williams, “The role of alkoxyl radicals in the nitrite pyrolysis,” University of Leeds (Department of Physical Chemistry), 1959., Leeds,, 1959.

[186] S. J. Qin, Y. Z. Sun, and Y. G. Tang, “Early Hydrocarbon Generation of Algae and Influences of Inorganic Environments During Low Temperature Simulation,” *Energy Exploration & Exploitation*, vol. 26, no. 6, pp. 377–396, 2008.

[187] H. N. Bhilwade, N. Tatewaki, H. Nishida, and T. Konishi, “Squalene as Novel Food Factor,” *Current Pharmaceutical Biotechnology*, vol. 11, no. 8, pp. 875–880.

[188] M. Spanova and G. Daum, “Squalene – biochemistry, molecular biology, process biotechnology, and applications,” *European Journal of Lipid Science and Technology*, vol. 113, no. 11, pp. 1299–1320.

[189] Y. Jiang, K.-W. Fan, R. Tsz-Yeung Wong, and F. Chen, “Fatty Acid Composition and Squalene Content of the Marine Microalga *Schizochytrium mangrovei*,” *Journal of Agricultural and Food Chemistry*, vol. 52, no. 5, pp. 1196–1200, 2004.

[190] A. Banerjee, R. Sharma, Y. Chisti, and U. C. Banerjee, “*Botryococcus braunii*: A renewable source of hydrocarbons and other chemicals,” *Critical Reviews in Biotechnology*, vol. 22, no. 3, pp. 245–279, 2002.

[191] N. Muradov, B. Fidalgo, A. C. Gujar, and A. T-Raissi, “Pyrolysis of fast-growing aquatic biomass - *Lemna minor* (duckweed): Characterization of pyrolysis products,” *Bioresource Technology*, vol. 101, no. 21, pp. 8424–8428, 2010.

[192] S. Tsuge and H. Matsubara, “High-resolution pyrolysis-gas chromatography of proteins and related materials,” *Journal of Analytical and Applied Pyrolysis*, vol. 8, no. 0, pp. 49–64, 1985.

[193] J.-F. Rontani and J. K. Volkman, “Phytol degradation products as biogeochemical tracers in aquatic environments,” *Organic Geochemistry*, vol. 34, no. 1, pp. 1–35, 2003.

[194] C. M. Lakshmanan and H. E. Hoelscher, “Production of Levoglucosan by Pyrolysis of Carbohydrates. Pyrolysis in Hot Inert Gas Stream,” *Product R&D*, vol. 9, no. 1, pp. 57–59, 1970.

[195] D. K. Shen and S. Gu, “The mechanism for thermal decomposition of cellulose and its main products,” *Bioresource Technology*, vol. 100, no. 24, pp. 6496–6504, 2009.

[196] L. I. N. Li and H. Zhang, “Preparing Levoglucosan Derived from Waste Material by Pyrolysis,” *Energy Sources*, vol. 26, no. 11, pp. 1053–1059, 2004.

[197] A. G. Liden, F. Berruti, and D. S. Scott, “A KINETIC MODEL FOR THE PRODUCTION OF LIQUIDS FROM THE FLASH PYROLYSIS OF BIOMASS,” *Chemical Engineering Communications*, vol. 65, no. 1, pp. 207–221, 1988.

[198] F. Shafizadeh, R. H. Furneaux, T. G. Cochran, J. P. Scholl, and Y. Sakai, “Production of levoglucosan and glucose from pyrolysis of cellulosic materials,” *Journal of Applied Polymer Science*, vol. 23, no. 12, pp. 3525–3539, 1979.

[199] R. Fahmi, A. V Bridgwater, L. I. Darvell, J. M. Jones, N. Yates, S. Thain, and I. S. Donnison, “The effect of alkali metals on combustion and pyrolysis of Lolium and Festuca grasses, switchgrass and willow,” *Fuel*, vol. 86, no. 10â€“11, pp. 1560–1569, 2007.

[200] X. Zhu and Q. Lu, “Biomass,” in *Biomass*, M. N. B. Momba, Ed. Sciyo, p. 202.

[201] H. R. Schulten, U. Bahr, and W. Görtz, “Pyrolysis field ionization mass spectrometry of carbohydrates : Part b: Polysaccharides,” *Journal of Analytical and Applied Pyrolysis*, vol. 3, no. 3, pp. 229–241, 1982.

[202] H. R. Schulten and C. Sorge, “Pyrolysis methylation—mass spectrometry of whole soils,” *European Journal of Soil Science*, vol. 46, no. 4, pp. 567–579, 1995.

[203] D. J. Nowakowski, C. R. Woodbridge, and J. M. Jones, “Phosphorus catalysis in the pyrolysis behaviour of biomass,” *Journal of Analytical and Applied Pyrolysis*, vol. 83, no. 2, pp. 197–204, 2008.

[204] D. J. Nowakowski, C. R. Woodbridge, and J. M. Jones, “Phosphorus catalysis in the pyrolysis behaviour of biomass,” *Journal of Analytical and Applied Pyrolysis*, vol. 83, no. 2, pp. 197–204, 2008.

[205] T. Bruton, H. Lyons, Y. Lerat, M. Stanley, and M. B. Rasmussen, “A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland,” 2009.

[206] K. D. Maher and D. C. Bressler, “Pyrolysis of triglyceride materials for the production of renewable fuels and chemicals,” *Bioresource Technology*, vol. 98, no. 12, pp. 2351–2368, 2007.

[207] J. Hatanaka, Y. Kimura, Z. Lai-Fu, S. Onoue, and S. Yamada, “Physicochemical and pharmacokinetic characterization of water-soluble Coenzyme Q10 formulations,” *International Journal of Pharmaceutics*, vol. 363, no. 1â€“2, pp. 112–117, 2008.

[208] T. Maller, T. Battner, A.-F. Gholipour, and W. Kuhn, “Coenzyme Q10 supplementation provides mild symptomatic benefit in patients with Parkinson’s disease,” *Neuroscience Letters*, vol. 341, no. 3, pp. 201–204, 2003.

[209] “Market Coenzyme Q10.” [Online]. Available: http://www.prweb.com/releases/coenzyme_Q10/CoQ10/prweb4223894.htm. [Accessed: 19-Nov-2012].

[210] L. Fele Ailnik and A. Jazbinaek, “Recovery of renewable phenolic fraction from pyrolysis oil,” *Separation and Purification Technology*, vol. 86, no. 0, pp. 157–170.

[211] P. de Wild, “BIOMASS PYROLYSIS FOR CHEMICALS,” University of Groningen, The Netherlands, 2011.

[212] R. M. Gallivan and P. K. Matschei, “Fractionation of oil obtained by pyrolysis of lignocellulosic materials to recover a phenolic fraction for use in making phenol-formaldehyde resins,” 1980.

[213] C. Amen-Chen, H. Pakdel, and C. Roy, “Separation of phenols from Eucalyptus wood tar,” *Biomass and Bioenergy*, vol. 13, no. 1â€“2, pp. 25–37, 1997.

[214] R. N. Patel, S. Bandyopadhyay, and A. Ganesh, “Extraction of cardanol and phenol from bio-oils obtained through vacuum pyrolysis of biomass using supercritical fluid extraction,” *Energy*, vol. 36, no. 3, pp. 1535–1542.

[215] J. Wang, H. Cui, S. Wei, S. Zhuo, L. Wang, Z. Li, and W. Yi, “Separation of Biomass Pyrolysis Oil by Supercritical CO2 Extraction,” *Smart Grid and Renewable Energy*, vol. 1, no. 2, pp. 98–107, 2010.

[216] “Levoglucosan pricing.” [Online]. Available: http://www.weiku.com/products/13430124/Levoglucosan.html. [Accessed: 03-Nov-2012].

[217] D. Radlein, “Study of levoglucosan production – A review,” in *Fast Pyrolysis of Biomass: A Handbook, Vol. 2*, A. V Bridgwater, Ed. CPL Press UK, 2002, pp. 205–241.

[218] E. M. Prosen, D. Radlein, J. Piskorz, D. S. Scott, and R. L. Legge, “Microbial utilization of levoglucosan in wood pyrolysate as a carbon and energy source,” *Biotechnology and Bioengineering*, vol. 42, no. 4, pp. 538–541, 1993.

[219] N. M. Bennett, S. S. Helle, and S. J. B. Duff, “Extraction and hydrolysis of levoglucosan from pyrolysis oil,” *Bioresource Technology*, vol. 100, no. 23, pp. 6059–6063, 2009.

[220] H. Q. Luyen, J. Y. Cho, H. W. Shin, N. G. Park, and Y. K. Hong, “Microalgal growth enhancement by levoglucosan isolated from the green seaweed Monostroma nitidum,” *Journal of Applied Phycology*, vol. 19, no. 2, pp. 175–180, 2007.

[221] N. K. Kochetkov, A. F. Sviridov, M. S. Ermolenko, and N. D. Zelinsky, “Synthesis of macrolide antibiotics. 1. Synthesis of the C1-C6 segment of 14-membered macrolide antibiotics,” *Tetrahedron Letters*, vol. 22, no. 43, pp. 4315–4318, 1981.

[222] A. F. Sviridov, V. S. Borodkin, M. S. Ermolenko, D. V Yashunsky, and N. K. Kochetkov, “Stereocontrolled synthesis of erythronolides A and B in a (C5-C9) + (C3-C4) + (C1-C2) + (C11-C13) sequence from 1.6-anhydro-Î²-D-glycopyranose (levoglucosan). Part 1. Synthesis of C1-C10 and C11-C13 segments,” *Tetrahedron*, vol. 47, no. 12-13, pp. 2291–2316, 1991.

[223] C. R. Vitasari, G. W. Meindersma, and A. B. de Haan, “Water extraction of pyrolysis oil: The first step for the recovery of renewable chemicals,” *Bioresource Technology*, vol. 102, no. 14, pp. 7204–7210.

[224] L. Moens, “Isolation of Levoglucosan from Lignicellulosic pyrolysis oilderived from wood or waste newsprint,” U.S. Patent US00541993.

[225] D. S. Scott, J. Piskorz, D. Radlein, and P. Majerski, “Process for the production of anhydrosugars from lignin and cellulose containing biomass by pyrolysis,” U.S. Patent US005395455A1993.

[226] G. P. Ghimire, H. C. Lee, and J. K. Sohng, “Improved Squalene Production via Modulation of the Methylerythritol 4-Phosphate Pathway and Heterologous Expression of Genes from Streptomyces peucetius ATCC 27952 in Escherichia coli,” *Applied Environmental Microbiology*, vol. 75, no. 22, pp. 7291–7293, 2009.

[227] H. Sun, D. Wiesenborn, K. Tostenson, J. Gillespie, and P. Rayas-Duarte, “Fractionation of squalene from amaranth seed oil,” *Journal of the American Oil Chemists’ Society*, vol. 74, no. 4, pp. 413–418, 1997.

[228] H.-P. He, Y. Cai, M. Sun, and H. Corke, “Extraction and Purification of Squalene from Amaranthus Grain,” *Journal of Agricultural and Food Chemistry*, vol. 50, no. 2, pp. 368–372, 2001.

[229] S. J. Johannes Lehmann, “Biochar for Environmental Management: Science and Technology.” Earthscan Ltd, London, UK, p. 404, 2009.

[230] S. Sohi, E. Lopez-Capel, E. Krull, and R. Bol, “Biochar, climate change and soil: A review to guide future research,” *CSIRO Land and Water Science Report 05/09*, 2009.

[231] S. P. Sohi, E. Krull, E. Lopez-Capel, and R. Bol, “A Review of Biochar and Its Use and Function in Soil,” in *Advances in Agronomy*, vol. Volume 105, L. S. Donald, Ed. Academic Press, 2010, pp. 47–82.

[232] K. Kebelmann and A. Hornung, “The effect of the particle size of Chlorella vulgaris Beijerinck (CCAP211/11B) biochar derived from intermediate pyrolysis on the elution of nutrients during soxhlet extraction,” in *Proceedings of the Bioten Conference on Biomass Bioenergy and Biofuels 2010*, 2010.

[233] L. Mišurcová, “Chemical Composition of Seaweeds,” in *Handbook of Marine Macroalgae*, John Wiley & Sons, Ltd, pp. 171–192.

[234] D. (ed. Soto, “Integrated mariculture- A global review FAO FISHERIES and aquaculture TECHNICAL PAPER 529,” Rome, 2009.

[235] C. Wang, R.-C. Yu, and M.-J. Zhou, “Effects of the decomposing green macroalga Ulva (Enteromorpha) prolifera on the growth of four red-tide species,” *Harmful Algae*, vol. 16, no. 0, pp. 12–19.

[236] F. Liu, S. Pang, T. Chopin, S. Gao, T. Shan, X. Zhao, and J. Li, “Understanding the recurrent large-scale green tide in the Yellow Sea: temporal and spatial correlations between multiple geographical, aquacultural and biological factors,” *Marine Environmental Research*, no. 0.

[237] S. J. Pang, F. Liu, T. F. Shan, N. Xu, Z. H. Zhang, S. Q. Gao, T. Chopin, and S. Sun, “Tracking the algal origin of the Ulva bloom in the Yellow Sea by a combination of molecular, morphological and physiological analyses,” *Marine Environmental Research*, vol. 69, no. 4, pp. 207–215.

[238] J. Vasquez and N. Guerra, “The use of seaweeds as bioindicators of natural and anthropogenic contaminants in northern Chile,” *Hydrobiologia*, vol. 326–327, no. 1, pp. 327–333, 1996.

[239] M. Caliceti, E. Argese, A. Sfriso, and B. Pavoni, “Heavy metal contamination in the seaweeds of the Venice lagoon,” *Chemosphere*, vol. 47, no. 4, pp. 443–454, 2002.

APPENDICES

Appendix A – Publications

The list below details publications arising from this research:

Kebelmann K, Hornung A. **The effect of the particle size of *Chlorella vulgaris* Beijerinck (CCAP211/11B) biochar derived from intermediate pyrolysis on the elution of nutrients during Soxhlet extraction**, Bioten Conference. Birmingham, United Kingdom, 2010

Kebelmann K, Hornung A, Griffiths G, Karsten U. **Intermediate pyrolysis and product identification by TGA and Py-GC/MS of green microalgae and their extracted protein and lipid components**, Biomass and Bioenergy (2012).

Kebelmann K, Hornung A, Griffiths G, Karsten U. **Thermo-chemical behaviour and chemical product formation from Polar macroalgal biomass during intermediate pyrolysis**, Fuel (2012).
Under revision

Appendix B – Validation report analytical pyrolysis system

WGD Wissenschaftlicher Gerätebau & Dienstleistung GmbH

WGD GmbH, Niendorf 05, 23999 Insel Poel, Germany

Validation - Report

Gaschromatograph - MS - Pyrolyser

GC 5890, MSD 5971/2, PY-2020iD

Aston University

This document includes 32 pages

Date of operation: 19-25th July 2011

WGD
Wissenschaftlicher Gerätebau
& Dienstleistung GmbH
Niendorf 5
23999 Insel Poel

25.07.2011

Serviceengineers
Dr. Günter Richard / Dr. Lutz Kebelmann

Wissenschaftlicher Gerätebau & Dienstleistung GmbH
Geschäftsführerin Anja Peters
Steuernummer 060/122/03420
Ust-IDNr DE 247323822
Handelsregister HRB 9115

Bankverbindung
Konto 352 401 131 3
Bankleitzahl 160 500 00
Mittelbrandenburgische Sparkasse
IBAN Nr. DE 98 1605 0000 3524011313
SWIFT Code: WELA DE D1 PMB

Telefon +49 38425 42304
Fax +49 38425 42305
E- mail: wgd-gmbh@hotmail.de

Appendix B continued

WGD Wissenschaftlicher Gerätebau & Dienstleistung GmbH

Appendix Gaschromatograph (GC 5890)

Description of the device
Determine the general device status

Typ of equipment:	GC 5890N
Manufacturer:	Hewlett Packard
Year of installation:	2011
Serialnumber:	C128/83

Temperature control of oven	set	actual	valuation
	40°C	39,2°C	ok
	50°C	50,2°C	ok
	100°C	99,99°C	ok
	200°C	199,9°C	ok
	250°C	249,2°C	ok

Time difference for oven prgram	----	- 0,12 min		ok
Splitflow (stand allone)	6,6 ml/min	6,6 ml/min	4-10 ml/min	ok
Septum purge	1-3 ml/min	2,8 ml/min	4-10 ml/min	ok

WGD
Wissenschaftlicher Gerätebau
& Dienstleistung GmbH
Niendorf 5
23999 Insel Poel
25.7.2011

Appendix B continued

WGD Wissenschaftlicher Gerätebau & Dienstleistung GmbH

Appendix Gaschromatograph (Common)

Description of the device
Determine the general device status

Typ of Equipment:	GC 5890N
Manufacturer:	Hewlett Packard
Year of installation:	2011
Serialnumber:	C128/83

Connected accessories devices

1. PC:	Win98 no name Computer L&C 487/11	
2. Software:	G1701AA	
3. other devices:	MSD 5971	3341G00510
	Double shot Pyrolyser	PY8091472iDE
	Autosampler 7673	32227G07739

Environmental conditions:

Relative air humidity	< 75%	ok
Room temperature	15 – 30°C	ok
Phase voltage actual value	230V ±5%	ok

Assessment of the general device status: The units are located in a precise and operational condition.

Date of validation: July 2011

Next validation: July 2012

WGD
Wissenschaftlicher Gerätebau
& Dienstleistung GmbH
Niendorf 5
23999 Insel Poel

Appendix B continued

WGD Wissenschaftlicher Gerätebau & Dienstleistung GmbH

Appendix Double shot pyrolyser (PY2020iD)

Description of the device
Determine the general device status

Typ of Equipment:		GC 5890N
Manufacturer:		Hewlett-Packard
Year of installation:		2011
Serialnumber:		C128/83
Peripheral:	Double shot pyrolyser 2020iD	PY8091472 i DE

	set	actual	
Temperature Stability (10 min) at 200 degrees	< 1°C	0,5°C	OK
Temperature control range furnace	50°C	49,5°C	OK
	100°C	101,0°C	OK
	200°C	199,5°C	OK
	500°C	499,5°C	OK
	700°C	701,0°C	OK
Temperaure control range needle	25-400°C	+/- 1°C	OK
Current Stability	< 1,2 V	0,6 V	OK
Pressure test (2 min)	2 bar	< 0,1 bar	OK
Stability test electronic	stable	stable	OK
Software test (all parameter)	stable	stable	OK

WGD
Wissenschaftlicher Gerätebau
& Dienstleistung GmbH
Niendorf 5
23999 Insel Poel
25.7.2014

Appendix B continued

WGD Wissenschaftlicher Gerätebau & Dienstleistung GmbH

Appendix Massenselektiv Detektor (MSD 5971)

Description of the device
Determine the general device status

Typ of Equipment:		GC 5890N
Manufacturer:		Hewlett Packard
Year of installation:		2011
Serialnumber:		C128/83
Peripheral:	MSD 5971	3341G00510

Level PFTBA			ok
Diff-pump oil	changed		ok
Foreline pump changed			ok
	set	actual	
RFPA-voltage for masses 100	< 100	89	ok
und 600	< 600	560	ok

Appendix:	Standard Spectra Auto Tune	ok
	System Verification - Tune	ok
	Air and Water Check	ok
	Installation Checklist	ok
	Familiarization Checklist	ok

ok = specification of the producer

WGD
Wissenschaftlicher Gerätebau
& Dienstleistung GmbH
Niendorf 5
23999 Insel Poel
25.7.2011

Appendix B continued

WGD Wissenschaftlicher Gerätebau & Dienstleistung GmbH

Maintenance Schedule MSD 5971

(additional for your information)

Inspection and maintenance criteria	Equipment ok	actual additional measures
		no action required
All components check for external damage	v	
Remove dust from inside the machine	v	
Fans and motors	v	
Autotune on the current status	v	
Cleaning ion source	v	
Filament	v	
Vacuum system maintenance	v	
Exchange Foreline pump	v	
Diffusion pump oil control / change	v	
Interface nut	v	
Seals	v	
Level PFTBA	v	
Recommissioning	v	
Function test and correctur RFPA-values	v	

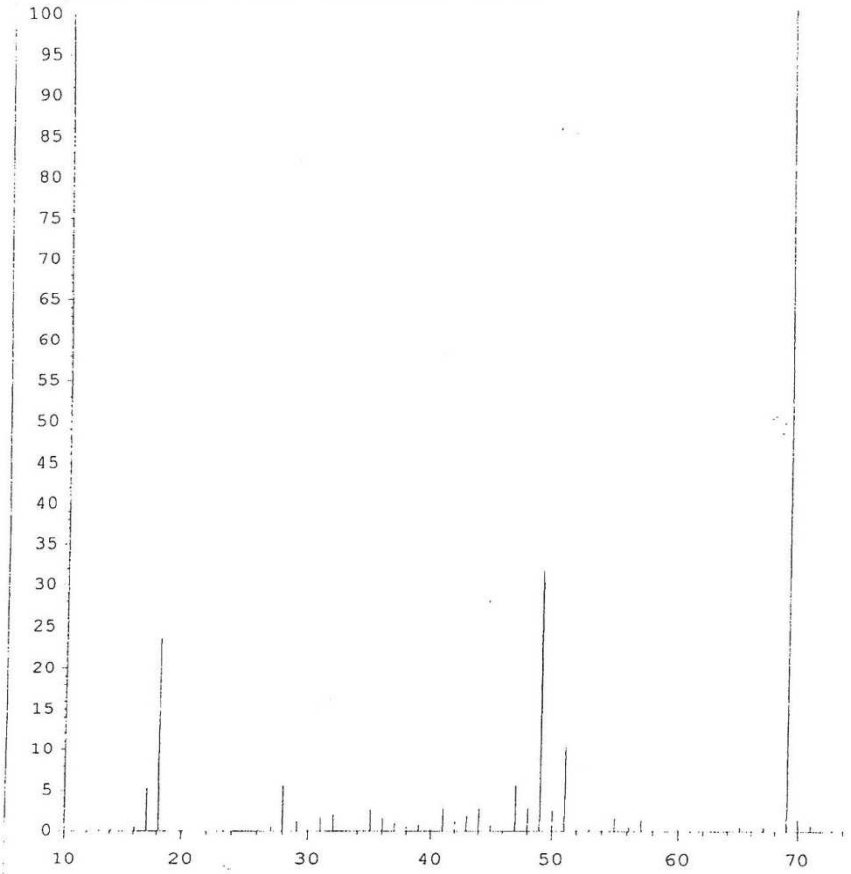
System ok no action required.

WGD
Wissenschaftlicher Gerätebau
& Dienstleistung GmbH
Niendorf 5
23999 Insel Poel
25.7.2011

Appendix B continued

5971 Air and Water Check

Instrument: Instrument #1
Tue Jul 19 12:35:49 2011 D:\MSDCHEM\1\5971N\
Scan: 10.00 - 75.00 Samples: 8 Thresh: 0 Step: 0.10
59 peaks Base: 69.00 Abundance: 816064



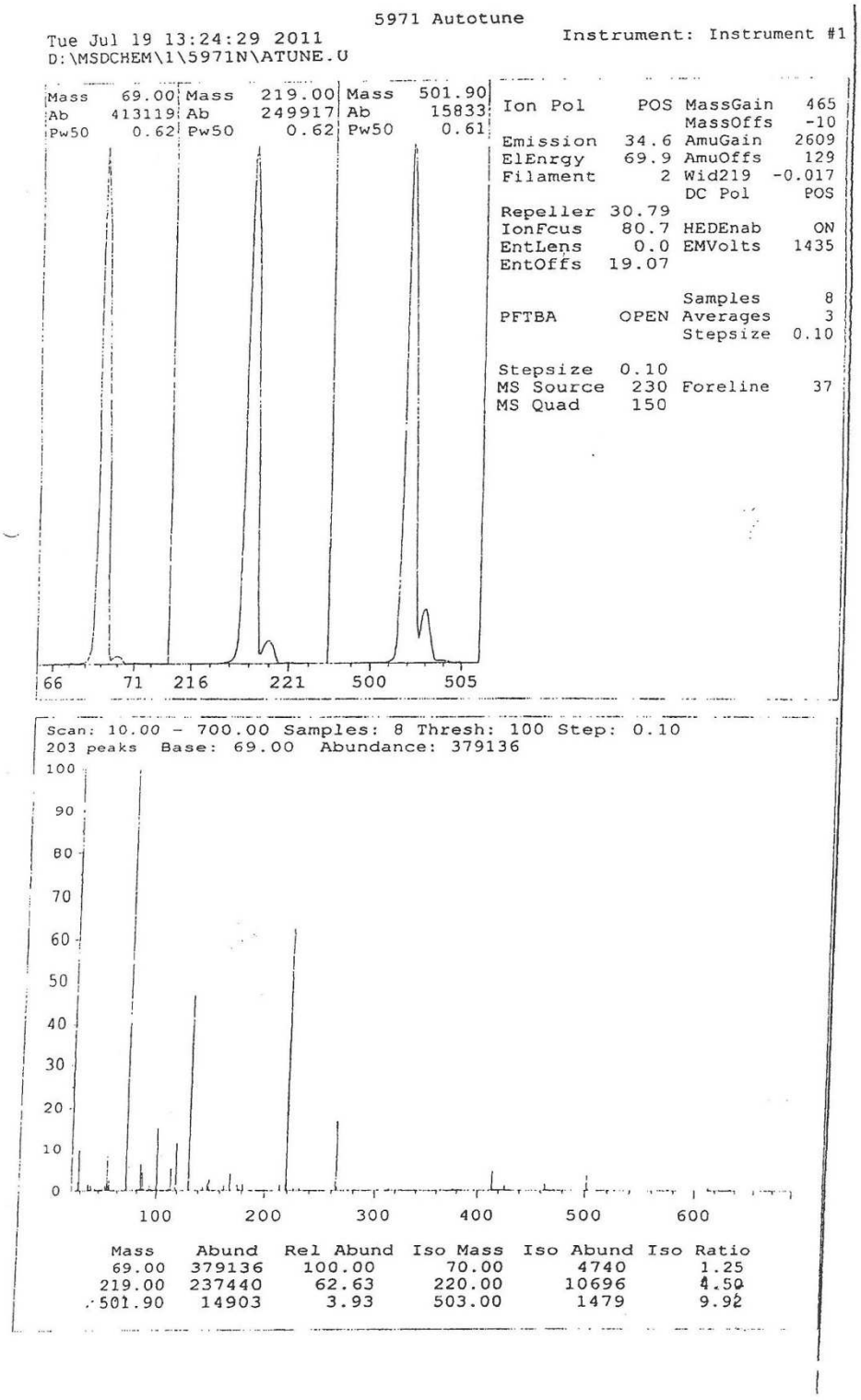
Mass	Abund	Rel Abund	Iso Mass	Iso Abund	Iso Ratio
69.00	816064	100.00	70.00	13752	1.69
18.10	193536	23.72	19.00	601	0.31
28.00	46288	5.67	29.10	9810	21.19

Maximum Sensitivity Quick Tune:air.u

Relative abundances:

18/69 =	23.72	Water%
28/69 =	5.67	Nitrogen%
32/69 =	2.07	Oxygen%
44/69 =	2.84	Carbon Dioxide%
28/18 =	23.92	Nitrogen/Water%

Appendix B continued



Appendix B continued

System Verification - Tune (Detector Optimization) Portion

Instrument Name	: Instrument #1	
DC Polarity	: Positive	
Filament	: 2	
BasePeak should be 69 or 219		Ok
Position of mass 69	69.00	Ok
Position of mass 219	219.00	Ok
Position of mass 502	501.99	Ok
Position of isotope mass 70	70.00	Ok
Position of isotope mass 220	220.00	Ok
Position of isotope mass 503	502.96	Ok
Ratio of mass 70 to mass 69(0.5 - 1.6%)	1.31	Ok
Ratio of mass 220 to mass 219(3.2 - 5.4%)	4.49	Ok
Ratio of mass 503 to mass 502(7.9 - 12.3%)	10.50	Ok
Ratio of 219 to 69 should be > 40% and is	64.06	Ok
Ratio of 502 to 69 should be > 2.4% and is	4.35	Ok
Mass 69 Precursor (<= 3%)	0.42	Ok
Mass 219 Precursor (<= 6%)	0.99	Ok
Mass 502 Precursor (<= 12%)	0.75	Ok
Testing for a leak in the system		
Ratio of 18 to 69 (<20%)	10.36	Ok
Ratio of 28 to 69 (<10%)	2.03	Ok
Electron Multiplier Voltage	1718	Ok

Tune portion of System Verification passed.

System Verification for Instrument #1 Tue Jul 19 13:21:07 2011 1

Appendix C – TAP Medium

Make the following stock solutions:

1. TAP salts

NH_4Cl	15.0 g
$MgSO_4$. 7H2O	4.0 g
$CaCl_2$. 2H2O	2.0 g

Water to 1 litre.

2. Phosphate solution

K_2HPO_4	28.8 g
KH_2PO_4	14.4 g

Water to 100 ml.

3. Hunter's trace elements (see next page)

To make the final medium, mix the following:
2.42 g Tris
25 ml solution #1 (salts)
0.375 ml solution #2 (phosphate)
1.0 ml solution #3 (Hunters trace elements)
1.0 ml glacial acetic acid

Water to 1 litre.

For solid medium, add 15 g agar per litre.

Autoclave.

Appendix C – continued

Hunter Trace Element Solution:

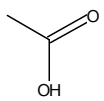
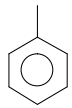
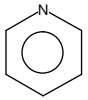
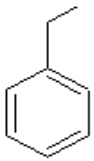
For 1 litre final mix, dissolve each compound in the volume of water indicated.
The EDTA should be dissolved in boiling water, and the FeSO4 should be prepared last to avoid oxidation

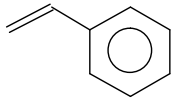
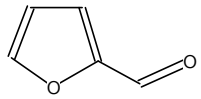
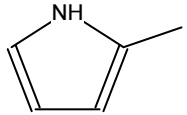
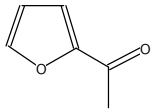
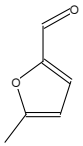
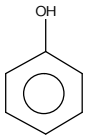
Compound	**Amount**	**Water**
EDTA disodium salt	50 g	250 ml
$ZnSO_4$ 7 H2O	22 g	100 ml
H_3BO3	11.4 g	200 ml
$MnCl_2$ 4 H2O	5.06 g	50 ml
$CoCl_2$ 6 H2O	1.61 g	50 ml
$CuSO_4$ 5 H2O	1.57 g	50 ml
$(NH_4)6Mo_7O_{24}$ 4 H2O	1.10 g	50 ml
$FeSO_4$ 7 H2O	4.99 g	50 ml

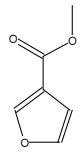
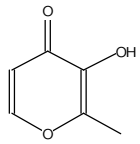
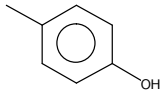
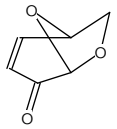
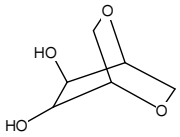
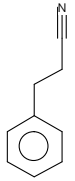
Mix all solutions except EDTA. Bring to boil, then add EDTA solution. The mixture should turn green. When everything is dissolved, cool to 70 degrees C. Keeping temperature at 70, add 85 ml hot 20 % KOH solution (20 grams / 100 ml final volume). Do NOT use NaOH to adjust the pH.
Bring the final solution to 1 litre total volume. It should be clear green initially. Stopper the flask with a cotton plug and let it stand for 1-2 weeks, shaking it once a day. The solution should eventually turn purple and leave a rust-brown precipitate, which can be removed by filtering through two layers of Whatman#1 filter paper, repeating the filtration if necessary until the solution is clear. Store refrigerated or frozen convenient aliquots. Some people shorten the time for formation of the precipitate by bubbling the solution with filtered air. If no precipitate forms, the solution is still usable. However, you might want to check the pH in this case and adjust it to around 7.0 using either KOH or HCl as needed. To prepare sulphur-free trace elements for hydrogen generation, the sulphate salts can be replaced with equimolar chloride salts ($ZnCl_2$ 10.0 g; $CuCl_2$. 2 H_2O 1.00 g; $FeCl_2$. 4 H_2O, 3.60 g).

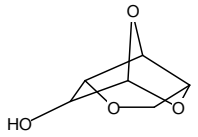
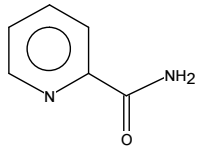
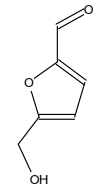
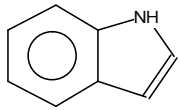
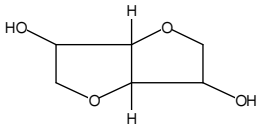
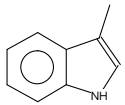
Appendix D – Pyrolysis products by Py-GC/MS at 500 °C

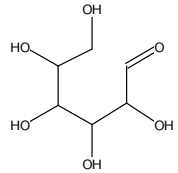
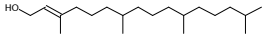
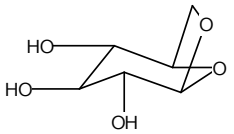
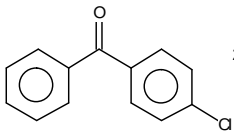
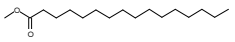
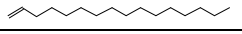
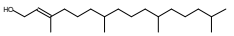
* by retention time of analytical standard ** tentatively assigned

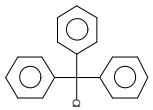
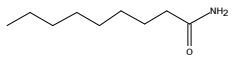
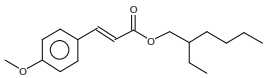
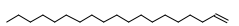
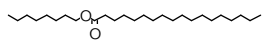
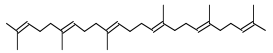
Peak No.	RT (min)	Compound	Formula	Structure	MW	NIST match (%)
1	5.65	Acetic Acid	$C_2H_4O_2$		60	93
2	6.68	Toluene*	C_7H_8		92	-
3	7.17	Pyridine*	C_5H_5N		79	-
4	9.55	Ethylbenzene*	C_8H_{10}		106	-
5	9.88	Pyrrole*	C_4H_5N		67	-

6	11.58	Styrene*	C_8H_8		104	-
7	12.18	Furfural	$C_5H_4O_2$		96	89
8	13.35	1H-Pyrrole, 2 or 3-methyl-	C_5H_7N		81	87
9	13.61	Isomer of 8	-	-	-	82
10	15.46	Ethanone, 1-(2-furanyl)-	$C_6H_6O_2$		110	92
11	18.63	5-Methyl-2-furaldehyde	$C_6H_6O_2$		110	93
12	24.30	Phenol*	C_6H_6O		94	-

13	26.11	3-Furancarboxylic acid, methyl ester	$C_6H_6O_3$		126	90
14	26.85	Maltol	$C_6H_6O_3$		126	92
15	28.39	4-Methylphenol*	C_7H_8O		108	-
16	29.25	Levoglucosenone	$C_6H_6O_3$		126	90
17	33.62	Dianhydromannitol	$C_6H_{10}O_4$		146	89
18	34.97	3-Phenylpropionitrile*	C_9H_9N		131	-

19	35.46	1,4:3,6-Dianhydro-α-D-glucopyranose	$C_6H_8O_4$		144	85
20	36.22	Picolinamide*	$C_6H_6N_2O$		122	-
21	38.34	5-(Hydroxymethyl)-2-furaldehyde	$C_6H_6O_3$		126	87
22	39.02	Indole*	C_8H_7N		117	-
23	40.23	Isosorbide	$C_6H_{10}O_4$		146	87
24	42.25	3-Methylindole*	C_9H_9N		131	-
25	45.15	Heptadecane	$C_{17}H_{36}$		240	85
26	46.80	Anhydrosugar**	-	-	-	82

27	47.47	Anhydrosugar**	-	-	-	83
28	49.99	D-allose**	$C_6H_{12}O_6$		180	87
29	50.99	Phytol	$C_{20}H_{40}O$		296	85
30	51.93	Phenanthrene derivative**	-	-	-	75
31	52.70	Levoglucosan	$C_6H_{10}O_5$		162	84
32	52.83	Isomer of 29	-	-	-	85
33	54.97	2 or 3 or 4-Chlorobenzophenone	$C_{13}H_9ClO$		216	85
34	55.96	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$		270	82
35	58.43	Anhydrosugar**	-	-	-	85
36	59.61	Phenanthrene derivative**	-	-	-	75
37	62.08	1-Hexadecene	$C_{16}H_{32}$		224	85
38	63.15	Trans-phytol	$C_{20}H_{40}O$		296	75
39	65.52	Dipeptide**	-		-	75
40	65.61	Dipeptide**	-		-	70

41	65.78	Triphenylmethyl chloride**	$C_{19}H_{15}Cl$		278	75
42	69.10	Benzoic acid alkyl ester derivative	-	-	-	77
43	69.51	Hexadecanamide**	$C_{16}H_{33}NO$		157	70
44	71.60	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	$C_{18}H_{26}O_3$		290	90
45	72.04	Isomer of 42	-	-	-	85
46	73.78	1-Nonadecene	$C_{19}H_{38}$		266	80
47	74.82	Isomer of 42	-	-	-	80
48	76.11	Heneicosane	$C_{21}H_{44}$		296	83
49	77.11	Benzenedicarboxylic acid, alkyl ester derivative	-	-	-	83
50	78.06	Octadecanoic acid, octyl ester**	$C_{26}H_{52}O_2$		396	75
51	80.32	Squalene	$C_{30}H_{50}$		410	92