

In-situ biodiesel production from a municipal waste water clarifier effluent stream

GC van Tonder

21721548

Dissertation submitted in fulfilment of the requirements for the degree *Magister* in *Chemical Engineering* at the Potchefstroom Campus of the North-West University

Supervisor: Prof S Marx

Co-supervisor C Schabort

November 2014

Abstract

This study investigated *In situ* biodiesel production with supercritical methanol. A micro-algae based feedstock was used and obtained from a local water treatment plant situated just outside of Bethal, South Africa (S 26° 29' 19.362" E 29° 27' 11.552"). The wet feedstock was used as harvested with only the excess moisture being removed.

Characterisation of the feedstock showed that a wide variety of macro-algae, micro-algae, cyanobacteria and bacterial species were present in the feedstock. The main algal species isolated from the feedstock were *Nostoc sp.* and *Chlamydomonas*. The feedstock was found to have a higher heating value (HHV) of 22 MJ.kg⁻¹ and a lower heating value (LHV) of 16.03 MJ.kg⁻¹ with an inherent moisture content of 270g.kg⁻¹ feedstock. The protein and fat content of the feedstock was determined by the Agricultural Research Council (ARC) and found to be 370.1 g.kg⁻¹ and 61.6 g.kg⁻¹ on a moisture free basis respectively. The high protein and fat content gives a theoretical bio-yield of 430 wt%. The low lignin content and high cellulose and hemi-cellulose content indicated that the feedstock would be suitable for energy production.

Three experimental sets were performed to determine the effect certain reaction parameters will have on the bio-char, bio-oil and biodiesel yields. The first set entailed hydrothermal liquefaction without the addition of methanol. The second set involved *in situ* biodiesel production with supercritical methanol, while both supercritical methanol and an acid catalyst were used during *in situ* biodiesel in the third set.

For the first set of experiments the effect of temperature (240°C to 340°C in intervals of 20°C) on the crude bio-oil and bio-char yields were investigated. The highest bio-char yield was found to be 336g g char.kg⁻¹ biomass at 280°C, while the highest crude bio-oil yield was 470.7 g crude bio-oil per kg biomass at 340°C. In the second set of experiments the dry biomass loading was kept constant at 500 g.kg⁻¹ and the temperature varied (240°C to 300°C in intervals of 20°C) along with methanol to dry biomass ratio (1:1, 3:1 and 6:1). The optimum bio-oil yield of 597.1 g bio-oil per kg biomass for this set was found at 500 g.kg⁻¹ biomass loading, 300°C and 3:1 methanol to dry biomass ratio. The highest bio-char yield was found to be 382.6 g bio-char.kg⁻¹ biomass for a 1:1 methanol to dry biomass weight ratio set with 500 g.kg⁻¹ biomass loading at 280°C.

An increase in methanol ratio also led to an increase in crude bio-oil yields however the 3:1 methanol to dry biomass mass ratio was found to give the highest bio-oil yield and the purest biodiesel, with less unsaturated FAME. The 6:1 methanol to dry biomass mass ratio did

however increase the FAME yield, which tends to show completion of the *in situ* production of biodiesel. This was also seen in the amount fatty acid methyl esters (FAME) present in the crude bio-oil as the degree of transesterification starts to increase with an increase in methanol. The FAME content was determined using gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC-MS).

During the last set of experiments the temperature (260°C to 300°C in intervals of 20°C) and methanol to dry biomass ratio (1:1, 3:1 and 6:1) was varied at a constant catalyst loading of 1 wt% of the dry biomass. The optimum yields achieved were 627 g crude bio-oil per kg biomass and 376 g bio-char per kg biomass at 300°C and 280°C, respectively. These yields were achieved at 500 g.kg⁻¹ biomass loading and 6:1 methanol ratio. Compared to the experiments where no catalyst was used, a slight increase in the yield was observed with the addition of an acid catalyst. This might be due to the base metals present in the feedstock that can lead to saponification during transesterification without the addition of an acid catalyst.

An overall improvement in the extraction of crude bio-oil was observed with *in situ* production compared to hydrothermal liquefaction. During *in situ* liquefaction, the bio-oil yield increased by 150 g crude bio-oil per kg biomass higher, while the bio-char yields did not significantly vary at the optimum point of 280°C this finding has a significant value for green coal research.

The highest HHV for the bio-char of 27 MJ.kg⁻¹ +/- 0.17 MJ.kg⁻¹ was found at 280°C and a 3:1 methanol ratio. The HHV of the bio-char decreases with an increase in temperature as more of the hydrocarbons are dissolved and form part of the bio-crude make-up. The highest HHV recorded for the crude bio-oil was 42 MJ.kg⁻¹ at a 6:1 methanol ratio, a temperature of 300°C and an acid catalyst. The crude bio-oil HHV, which increased with an increase in temperature, is well within the specifications of the biodiesel standard (SANS, 1935).

The highest FAME yield of 39.0 g.kg⁻¹ was obtained using a 6:1 methanol ratio and a temperature of 300°C in the presence of an acid catalyst. The crude oil contained 49.0 g.kg⁻¹ triglycerides with alkenes (C₁₃, C₁₅ and C₁₇) making up the balance. The purest biodiesel yield was achieved at 3:1 methanol to dry biomass mass ratio, as it had the lowest yield unsaturated methyl esters.

The overall FAME yield increased with an increase in methanol ratio. The derivatised FAME yields were the highest during hydrothermal liquefaction (55.0 g.kg⁻¹ biomass). The *in situ*

production of biodiesel from waste water clarifier effluent stream was found to be possible. Further investigation is needed into sufficient harvesting methods, including the optimum harvesting location, as this will result in fewer impurities in the stream and subsequent higher yields.

Keyword:

In situ biodiesel production, micro-algae, hydrothermal liquefaction

I, G.C van Tonder, hereby declare to be the sole author of the report entitled:

***In situ* biodiesel production from municipal waste water treatment plant clarifier effluent stream**

For the fulfilment of the requirements for the degree of Master of Engineering in the School of Chemical and Minerals Engineering of the North-West University, Potchefstroom Campus.

Cornie van Tonder
Potchefstroom

Acknowledgements

Trust in the LORD with all thine heart; and lean not unto thine own understanding.

⁶ *In all thy ways acknowledge him, and he shall direct thy paths.*

– Proverbs 3:5 & 6 –

"But Jesus beheld them, and said unto them, with men this is impossible;

but with God all things are possible."

– Matthew 19:26 –

A special mention to the following people without whom this study would not have been possible:

- Our Heavenly Father for providing me with the opportunity to be able to do this study. Without His grace none of this would have been possible.
- Prof. Sanette Marx for giving clear guidance and for her unconditional support.
- Corneels Schabort who has guided my post graduate development for years and giving me optimal support and insights.
- Dr. Roelf Venter for being readily available over the past year, giving support and ideas on how I should proceed.
- Mrs. Eleanor de Koker for always being willing to help.
- My parents, Ockie and Chrisna, for their support and prayers throughout my studies.
- My brother, Ernst, for his support and friendship throughout my studies.
- Kit Richards, for the friendship, support and words of encouragement.
- Gideon van Rensburg, for his assistance on the gas chromatography and advice in the lab.
- Wentzel Jordaan for always being available for advice and arranging time off from work.
- Jan Kroeze and Adrian Brock, for helping with the lab set-up and fixing of defects.
- Nico Lemmer for helping with the TGA and bombcalorie analysis.
- My Eskom colleagues for their patience and understanding when I needed time off to complete this study.

1. Contents

Nomenclature	ix
List of figures	x
List of Tables	xii
1. Introduction	1
1.1 Background and motivation	1
1.2 Micro-algae as feedstock for the production of renewable fuel.....	3
1.3 Biomass conversion processes	3
1.3.1 Biochemical conversion processes	3
1.3.2 Thermochemical conversion processes	4
1.4 Biofuels	4
1.4.1 Biodiesel production	4
1.6 Operation at local water treatment plant	8
1.7 Problem statement	9
1.8 Aims and objectives.....	10
1.9 Scope of investigation.....	10
1.10 References	12
2. Literature survey	16
2.1 Biomass.....	16
2.3 Biofuel Industrial Strategy	17
2.2 Algae	20
2.2.1 Classification.....	20
2.2.2 Cyanobacteria (Blue-green algae) (<i>Phylum Cyanophyta</i>).....	22
2.3 Micro-algae at municipal waste treatment plant	23
2.4. Algae as a feedstock for biofuels	23
2.5 Biodiesel.....	25
2.6 Production of biodiesel	28
2.7 Hydrothermal processing of biomass.....	34
2.7.1 Liquefaction.....	34
2.8 Reaction parameters for liquefaction	36
2.9 Concluding remarks.....	40
2.10 References.....	42
3. Experimental.....	50
3.1 Harvesting of feedstock	50
.....	50
3.2 Classification of algae in feedstock.....	52

3.2.1 Characterisation of feedstock	53
3.3 Materials.....	54
3.4 Hydrothermal and thermochemical liquefaction experimental method	55
3.5 Analytical methods	59
3.5.1 Proximate analysis	59
3.5.2 Gas chromatography analysis (GC)	59
3.5.4 GC coupled to mass spectrometry (GC-MS) analysis	60
3.6 References	61
4. Results and Discussions	62
4.1 Classification of algae in feedstock.....	62
4.2 Experimental Error	66
4.3 Effect of reaction variables on crude bio-oil yield	66
4.3.1 <i>In situ</i> biodiesel production	66
4.3.1.1 The effect of temperature and solvent on bio-oil composition and yield.....	66
4.3.1.2 The effect of temperature and solvent on bio-char yield	73
4.3.1.3 The effect of an acid catalyst on the bio-oil yield and bio-char yield.....	75
4.3.1.4 The effect of time spent in supercritical fluid on the bio-oil yield	77
4.3.2 Hydrothermal liquefaction	80
4.4 Summary	84
4.5 References	85
5. Conclusion and recommendations	88
5.1 Conclusion.....	88
5.2 Recommendations.....	88
APPENDIX A - Calculations	90
APPENDIX B – Raw data	94

Nomenclature

Abbreviations	
BOCLE	Ball-on-cylinder lubricity evaluator
BTL	Biomass to liquid
CV	Calorific value
DCFR	Discounted cash flow rate
FFA	Fatty
FAME	Fatty acid methyl esters
FID	Flame Ionisation detector
GA	Gas compound
GC	Gas chromatography
GC - MS	Gas chromatography – mass spectrometry
HC	Hydrocarbons
HFRR	High frequency reciprocating rig
HHV	Higher heating value
L	Litre
MGDG	Monogalactosyl-diacylglycerol
ROA	Return on Asset
NPV	Net present value
USA	United States of America
TG	Triglycerides
TSS	Total suspended solids
TMSH	Trimethyl Sulfonium Hydroxide
VO	Volatiles
WTP	Water treatment plant

List of figures

Figure 1-1 Estimated energy reserves (— Coal — Gas — Oil).....	1
Figure 1-2 Transesterification (Marchetti <i>et al.</i> , 2007)	5
Figure 1-3 Production of biodiesel from micro-algae (adapted from Demirbas & Demirbas, 2011)	6
Figure 1-4 Production of biodiesel from micro-algae, through direct transesterification of wet algae under super critical methanol (a), microwave assisted transesterification (b), and downstream separation and purification of crude biodiesel (adapted from Patil <i>et al.</i> , 2012)8	
Figure 1-5 Open pond on Sewage plant situated in Bethal, South Africa.....	9
Figure 1-6 Evaporation pond on the sewage plant situated in Bethal, South Africa	9
Figure 2-1 Van Krevelen diagram, showing a comparison between the H/C ratios of coal and biomass (taken from IRF, 2013).....	17
Figure 2-2 Fresh water cyanobacteria (Vincent, 2009)	22
Figure 2-3 Typical representation of biodiesel production process (adapted from Marchetti <i>et al.</i> , 2007).....	29
Figure 2-4 Transesterification (Marchetti <i>et al.</i> , 2007)	29
Figure 3-1 Evaporation pond at the sewage plant situated in Bethal, South Africa	50
Figure 3-2 Sieves for removal of excess moisture.....	51
Figure 3-3 Coning and quartering method adapted from Patnaik (2004).	52
Figure 3-4 Experimental setup for liquefaction	56
Figure 3-5 Representation of experimental method.....	57
Figure 3-6 Representation of experiments done.....	58
Figure 3-7 GC setup	60
Figure 4-1 Examples of cell morphologies observed in mixed culture. All images were taken using 100x objective lens.	63
Figure 4-2 Microscope image of floating organism (10x magnification)	64
Figure 4-3 Micro-algae cell morphologies after plating	65
Figure 4-4 Plating of micro-algae	65
Figure 4-5 Influence of temperature and methanol to dry biomass ratio on the crude bio-oil during liquefaction with supercritical methanol at a biomass loading of 500 g.kg ⁻¹ and residence time of 30 min. (Dry biomass loading = 50%; Residence time = 30 min (— Hydrothermal liquefaction — 6:1 methanol ratio — 3:1 methanol ratio — 1:1 methanol ratio)	67
Figure 4-6 Influence of methanol to dry biomass ratio on the FAME yield and triglyceride yields at 300°C (— C12 — C14 — C14:1 — C16:0 — C16:1 — C18:0 — C18:1 — C18:2).....	69

Figure 4-7 Influence of temperature on the FAME yields for the 3:1 methanol to dry biomass ratio (— C12 — C14 — C16:0 — C16:1 — C18:0 — C18:1 — C18:2)	70
Figure 4-8 Distillation curve for the 1:1 methanol ratio at 300°C	71
Figure 4-9 Distillation curve for the 3:1 methanol ratio at 300°C	71
Figure 4-10 Effect of temperature on the HHV for the 1:1 and 6:1 methanol to dry biomass mass (— 1:1 — 6:1)	72
Figure 4-10 Influence of temperature and methanol to dry biomass ratio on the bio-char yield during liquefaction with supercritical methanol at a biomass loading of 500 g.kg ⁻¹ and residence time of 30 min. (— Hydrothermal liquefaction — 6:1 methanol ratio — 3:1 methanol ratio — 1:1 methanol ratio)	73
Figure 4-11 Influence of and an acid catalyst on the bio-oil yield during liquefaction with supercritical methanol at a biomass loading of 500 g.kg ⁻¹ and residence time of 30 min. (— Hydrothermal liquefaction — 6:1 methanol ratio — Acid catalyst)	74
Figure 4-12 Influence of temperature on the FAME yield and triglyceride yields at 300°C (— C12 — C14 — C16:0 — C16:1 — C18:0 — C18:1 — C18:2 — C18:3).....	75
Figure 4-13 Influence of acid an acid catalyst on the bio-char yield during liquefaction with supercritical methanol at a biomass loading of 500 g.kg ⁻¹ and residence time of 30 min. (— Hydrothermal liquefaction — 6:1 methanol ratio — Acid catalyst)	76
Figure 4-15 Influence of acid an acid catalyst on the bio-char yield during liquefaction with supercritical methanol at a biomass loading of 500 g.kg ⁻¹ and residence time of 30 min. (— 240°C — 260°C — 280°C — 300°C — 320°C — 300°C).....	77
Figure 4-16 Effect of time spent in supercritical fluid for the different methanol to dry biomass mass ratios (a) 1:1, (b) 3:1, (c) 6:1. (— bio-oil — bio-char— bio-gas).....	78
Figure 4-17 Influence of temperature product distribution for hydrothermal liquefaction at a biomass loading of 500 g.kg ⁻¹ and residence time of 30 min (— Biochar — Bio-oil — Biogas)	80
Figure 4-18 Influence of temperature on the FAME yield and triglyceride yields at 300°C (— C12 — C14 — C16:0 — C16:1 — C18:0 — C18:1 — C18:2).....	82
Figure 4-19 Distillation curve for the hydrothermal liquefaction at 260°C.....	83

List of Tables

Table 1-1 Liquefaction previously done on micro-algae.....	7
Table 2-1 Comparison of properties of biodiesel, produced from micro-algae oil and diesel fuel (Rawat <i>et al.</i> , 2013)	25
Table 2-2 Properties of biodiesel from vegetable oils adapted from Demirbas and Demirbas (2011)	27
Table 2-3 Comparison of diesel and biodiesel standards (adapted from Balat & Balat, 2010)	28
Table 2-4 Alkali-catalysed transesterification reaction used for biodiesel production (Balat & Balat, 2010)	30
Table 2-5 Acid-catalysed transesterification reaction used for biodiesel production (Balat & Balat, 2010)	31
Table 2-6 Summary of some studies performed on <i>in situ</i> transesterification with supercritical alcohol	32
Table 3-1 Compositional analysis of feedstock on a dry basis.....	53
Table 3-2 Information on chemicals used.....	54
Table A-0-1 Experimental data Bio-oil/Biodiesel.....	92
Table A-0-2 Experimental error Bio-char	93

1. Introduction

In this chapter a brief overview of the content of this study is presented. The background and motivation for this study are given in Section 1.1. The suitability of micro-algae as a feedstock for biodiesel production is briefly discussed in Section 1.2, after which the following two sections give an outline of biodiesel production routes. The operations at the local water treatment plant where the algae were harvested are presented in Section 1.5. The problem statement and objectives of this study are listed in Section 1.6.

1.1 Background and motivation

The biofuel industry has been earmarked by many as a major potential source for job creation and economic development. The current global need for biofuels is driven by relieving the pressure on fossil fuels, finding an environmentally friendly fuel, upliftment of the agricultural sector and promotion of sustainable development (Goyal *et al.*, 2008; Demirbas, 2009; Rawat *et al.*, 2013).

According to the World Coal Institute (2014) it is estimated that at the current utilisation rate, coal, oil and natural gas will last 112, 46 and 54 years respectively, which creates a desperate need to preserve fossil fuels. Figure 1.1 shows an estimation of fossil fuel reserves, adapted from Bloomberg (2013).

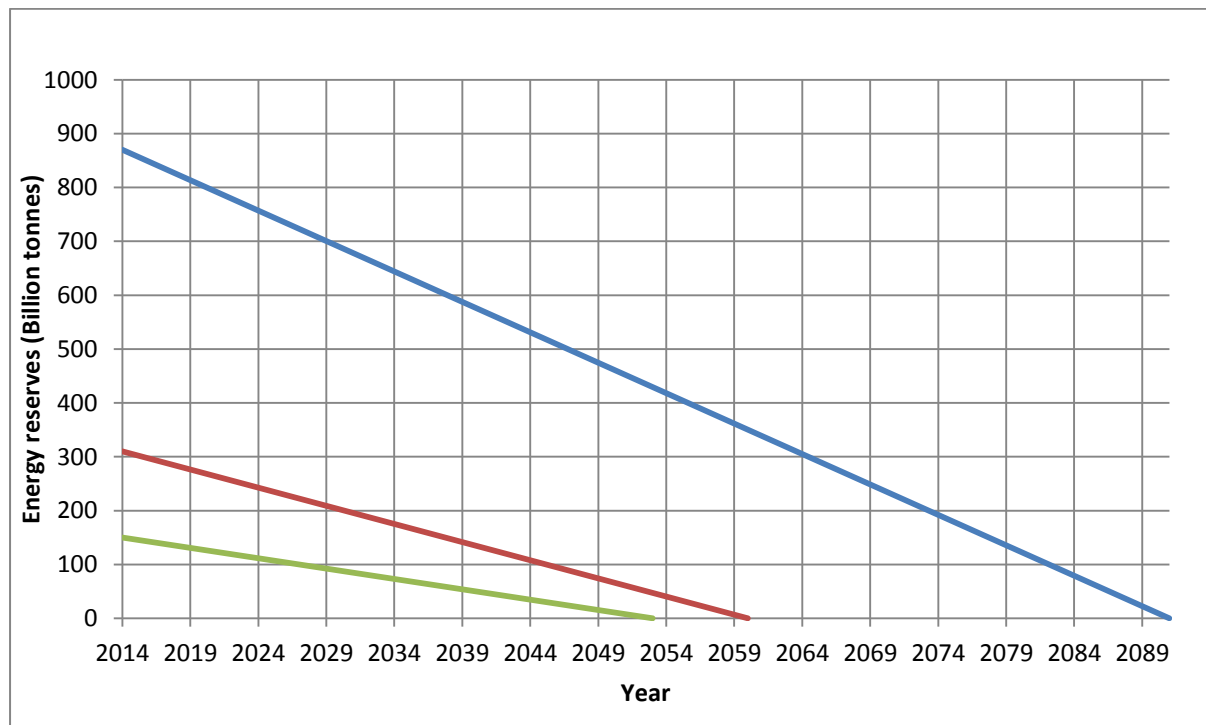


Figure 1-1: Estimated energy reserves (—Coal —Gas —Oil)

Another constraint on fossil fuels is that the majority of the global oil reserve, estimated to be 63 %, is held in the Middle East (Demirbas & Demirbas, 2011). This region is known for its economical and geopolitical instability. Furthermore, each developing country dependent on oil as an energy source is affected by the trading prices of oil. Independence for developing countries from globally held oil would essentially mean more stable economic growth that is less dependent on the global economy.

Bloomberg (2013) estimated that within the next two decades the global middle class will grow by two-thirds. The growth in middle class relates to 3 billion more people demanding energy, which will result in depletion sooner than estimated by the World Coal Institute. Thus to avoid an irreversible environmental impact and reaching the peak-oil demand sooner, alternative and renewable energy sources will have to start filling the gap.

The current alternative energy sources available are hydro, solar, wind, geothermal and bio-renewables. Each of these energy sources should be evaluated on its own merit to determine how sustainable it would be as a long term replacement for fossil fuels. A closer look at biofuel production processes in the past has led to many concerns, because many of the processes are not economically viable, nor are they thermodynamically sound (Rawat *et al.*, 2013). Biomass energy is currently seen as the fourth large primary energy source in the world (Wu *et al.*, 2012).

The Biofuel Industrial Strategy of South Africa proposes the use of sugar cane, sugar beets, sunflowers, canola and soybean crops for bioethanol and biodiesel production (Department of Energy, 2007). Sunflower, canola and soybean crops have been proposed for the production of biodiesel, while sugar cane and sugar beets the proposed crops for bio-ethanol production. The disadvantages of using first generation feedstock, as proposed by the Biofuel Industrial Strategy of South Africa, is the highest carbon footprint compared to other biofuel generations, biofuels obtained from first generation processes can only be blended with other fuels and most importantly the food versus fuel argument (Liew *et al.*, 2014).

The food versus fuel argument has been in play for many years, with studies showing that crops set out for human consumption will take up more arable land than can be provided (Gressel, 2008; Demirbas, 2009). Economic considerations for using crops set out for human consumption will lead to a higher demand for those crops, which in turn will influence the trading prices of these crops. Higher prices will in return bring forth pressure on both the biofuel industry as well as the consumers. The need thus arises to find a feedstock that is

not set out for human consumption, is economically feasible to use and can produce biofuels through a thermodynamically sound process route.

Third generation crops like micro-algae and aquatic weeds might be more economically feasible than conventional crops with little arable land needed and a high reproductive capacity making it possible for more frequent harvesting (Quinn & Davis, 2014; Christi, 2007).

1.2 Micro-algae as feedstock for the production of renewable fuel

Algae technology has the potential to be a vital tool in the reduction of greenhouse gas emissions (Demirbas & Demirbas, 2011). According to Rawat *et al.* (2013) micro-algae's high cellular concentration of lipids has attracted a lot of attention as a source for the production of carbon-neutral biodiesel. Micro-algae can be seen as the ultimate alternative for fossil fuel resources for the production of liquid based fuels (Rawat *et al.*, 2013).

Algae, like other plants, both terrestrial and aquatic, converts solar energy into chemical energy through photosynthesis. The chemical energy is stored in the underlying plant constituents, like oils, carbohydrates and proteins. Micro-algae is described as one of the most effective plants in the world when it comes to the efficiency of photosynthesis, thus making it one of the best possible sources for biodiesel production (Demirbas & Demirbas, 2011).

1.3 Biomass conversion processes

Biomass conversion processes are divided into two groups, namely biochemical conversion and thermochemical conversion processes. The choice of biomass production route is mainly based on the characteristics of the biomass and the conversion product requirements (Saxena *et al.*, 2009). Biochemical conversion processes consist mainly of transesterification and fermentation, which yields biodiesel, bio-ethanol, bio-butanol, bio-methanol and biogas. Thermochemical conversion processes consist of gasification, pyrolysis, liquefaction and torrefication. The main product yields of these processes are bio-oil, bio-char and bio-gas (Chen *et al.*, 2014; Tekin *et al.*, 2014).

1.3.1 Biochemical conversion processes

Biochemical conversion processes make use of micro-organisms to produce products from biomass, including gas, water and waste (Küçük & Demirbas, 1997; Demirbas, 2009). These processes include fermentation in which ethanol, carbon dioxide and water are produced, as

well as anaerobic digestion in which biomass is decomposed by bacteria without the presence of oxygen. The primary products are methane and carbon dioxide.

1.3.2 Thermochemical conversion processes

1.3.2.1 Liquefaction

Liquefaction entails heating a biomass in the presence of a catalyst to a high temperature (240 – 340°C). Liquefaction attempts to liquefy the feed without going through the gas phase. Hydrothermal liquefaction is the attempt to liquefy the biomass in the presence of heated water and high pressure (5 – 22 MPa). Peterson *et al.* (2008) defines liquefaction as the stage before the critical point of water is reached, thus below 374°C and 22 MPa. Liquefaction is then followed by pyrolysis and gasification. Generally in hydrothermal liquefaction the presence of a catalyst is not required but can be used to aid the process (Chen *et al.*, 2014; Peterson *et al.*, 2008; Goyal *et al.*, 2008; Erzengin & Küçük, 1998).

The process can then yield a mixture of gas (between 2-10%), bio-char (between 5 - 60%) and oil (up to 50%) (Goyal *et al.*, 2008).

1.4 Biofuels

Biofuels include bio-ethanol, biodiesel, bio-butanol, bio-methanol, vegetable oils, pyrolysis oils, bio-hydrogen and bio-gas (Demirbas, 2000). Biomass based liquid fuels that have the potential to replace carbon based fuels are biodiesel and bio-ethanol. Currently the primary feedstock for bioethanol is sugarcane (about 60%) and maize, while plant oil such as soya oil is used in the production of biodiesel.

1.4.1 Biodiesel production

Biodiesel is a diesel equivalent mono alkyl ester based oxygenated fuel. Production of biodiesel recently tends to use non-edible vegetable oil, waste oil and grease (Demirbas, 2009).

1.4.1.1 Methods of lipid conversion to biodiesel

Conversion of lipids to biodiesel is generally achieved by transesterification with low hydrocarbon alcohols in the presence of a catalyst, which is usually an acid, base or enzyme catalyst (Rawat *et al.*, 2013). Separation and transesterification through *in situ* methods can also be utilised to convert lipids to biodiesel.

Biomass derived liquid extraction consists of a multiple step process that requires a combination of polar and non-polar solvents, as well as ultra-sonication under high pressure at ambient temperature (Rawat *et al.*, 2013).

Rawat *et al.* (2013) further stated that the esterification processes are energy intensive and thus less cost effective. Accordingly, alternative processes for dewatering and lipid extraction are needed for this biodiesel production method. Lertsathapornsuk *et al.* (2008) showed that the alternative for speeding up the reaction time of transesterification through conventional means (convection, conduction and radiation) from the reactor, is that a much faster and less energy intensive reaction can be achieved by microwave assisted transesterification of oil. In the case of Lertsathapornsuk *et al.* (2008) microwave assisted transesterification was performed on palm oil. This gave much higher yields over shorter times.

Thermochemical conversion processes such as pyrolysis, liquefaction and direct burning methods have also been suggested as viable alternatives for biodiesel production. Production through thermochemical processes produce biodiesel via the production of bio-oil (Rawat *et al.*, 2013).

1.4.1.2 Transesterification

Marchetti *et al.* (2007) state that in transesterification different types of oils react with an alcohol to produce esters and glycerol. The alcohol (methanol or ethanol) is usually in excess. Figure 1-2 shows the reaction route of transesterification.

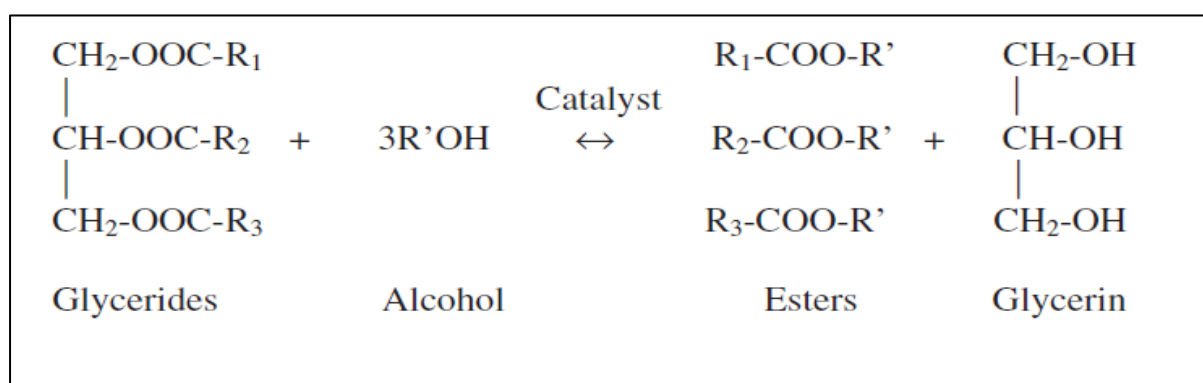


Figure 1-2: Transesterification (Marchetti *et al.*, 2007)

Various reaction pathways exist for transesterification, these include homogeneous catalytic method, heterogeneous catalytic method, enzymatic method and most recently super critical methanol and ethanol for *in situ* transesterification reactions (Leung & Lueng, 2010; Jain & Sharma, 2010; Aarthy *et al.*, 2014; Reddy *et al.*, 2014).

1.4.1.3 Biodiesel production process with micro-algae as feedstock

Brink (2011) stated that there are five major steps in biodiesel production from micro-algae, i.e. cultivation, harvesting, extraction of oils, conversion via transesterification and separation and purification. Brink (2009) further states that cultivation and harvesting are the biggest financial constraints in the production of biodiesel. Some of the harvesting and cultivation methods evaluated include open pond systems, photo-bioreactors for cultivation, as well as some harvesting methods, including different types of flocculation methods, filtration, as well as centrifugal methods (Rawat *et al.*, 2013; Brink, 2009). Some of these methods have been proven to be energy intensive, thus making the overall process costly. Rawat *et al.* (2013) evaluated current technologies for upscaling of the process, and found several shortfalls. There is subsequently a need for evaluation of current methods to find the best optimal solution and defining of a process flow that is energy efficient on a large scale.

Demirbas and Demirbas (2011) defined the basic process for production of biodiesel from micro-algae, as seen in Figure 1-4.

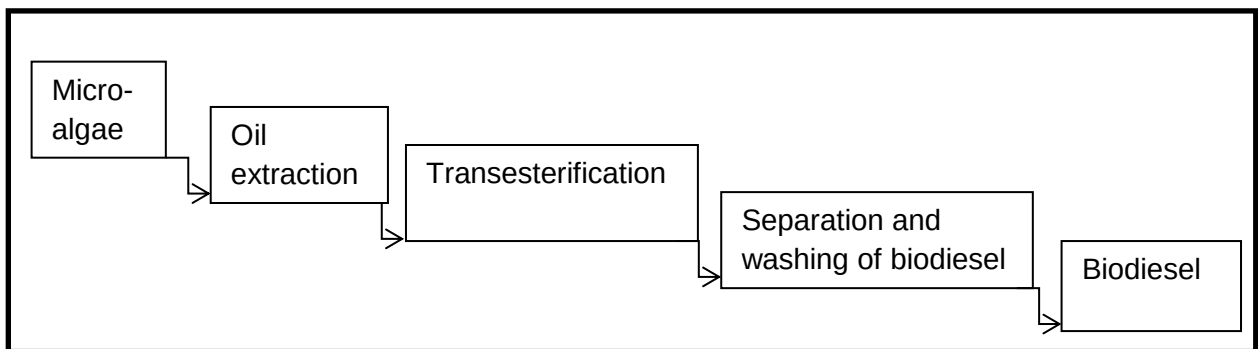


Figure 1-3: Production of biodiesel from micro-algae (adapted from Demirbas & Demirbas, 2011)

Barnard (2009) compared the theoretical bio-oil production potential for algae and other terrestrial crops and found that algae can produce as much as nine times more oil than conventional crops.

1.5 Liquefaction of Micro-algae

Table 1.1 shows the bio-oil yields obtained from micro-algae species in the past. It can be noted that for most of these experiments the optimum yields were achieved at a temperature of 300°C.

Table 1-1: Liquefaction previously done on micro-algae

Micro-Algae	Temperature (°C)	Oil yield (g.kg ⁻¹)	Reference
<i>Boreyococcus braunii</i>	200	780	Inoue <i>et al.</i> , 1994
<i>Boreyococcus braunii</i>	300	640	Sawayama <i>et al.</i> , 1995
<i>Spirulina sp.</i>	300 - 425	783	Matsui <i>et al.</i> , 1997
<i>Mycrocystis auruginosa</i>	300	156.0	Barnard, 2009
<i>Cyclotella meneghinia</i>	300	153.3	Barnard, 2009
<i>Cyclotella meneghinia</i>	300	160.3	Barnard, 2009
<i>Chlorella vulgaris</i>	350	360	Ross <i>et al.</i> , 2010
<i>spirulina</i>			
<i>Nannochloropsis occulata</i>	350	350	Biller and Ross, 2011
<i>Nannochloropsis sp.</i>	260	550	Li <i>et al.</i> , 2014
<i>Chlorella sp.</i>	220	829	Li <i>et al.</i> , 2014
<i>C. Pyrenoidosa</i>	280	350	Gai <i>et al.</i> , 2014

Two well-known biodiesel production processes are direct transesterification of wet algae under super critical methanol and microwave assisted transesterification (Patil *et al.*, 2012) (see Figure 1-4). Patil *et al.* (2012) compared these two biodiesel production processes. The variable compared was methanol to wet algae weight ratio and reaction time. It was found that the biodiesel yields for the two methods varied between 251.2 g.kg⁻¹ biomass and 857.5 g.kg⁻¹ biomass for the supercritical method and between 403.5 g.kg⁻¹ biomass to 801.3 g.kg⁻¹ biomass for the microwave assisted method. Note that for the supercritical method wet algae are used, while dry algae are used for the microwave assisted transesterification.

Compared to the yields shown in Table 1-1, most of the supercritical methods have higher yields than hydrothermal liquefaction ranging from 600 g.kg⁻¹ biomass to 900 g.kg⁻¹ (Reddy *et al.*, 2014; Shin *et al.*, 2012).

***In situ* transesterification of micro-algae and microwave assisted transesterification**

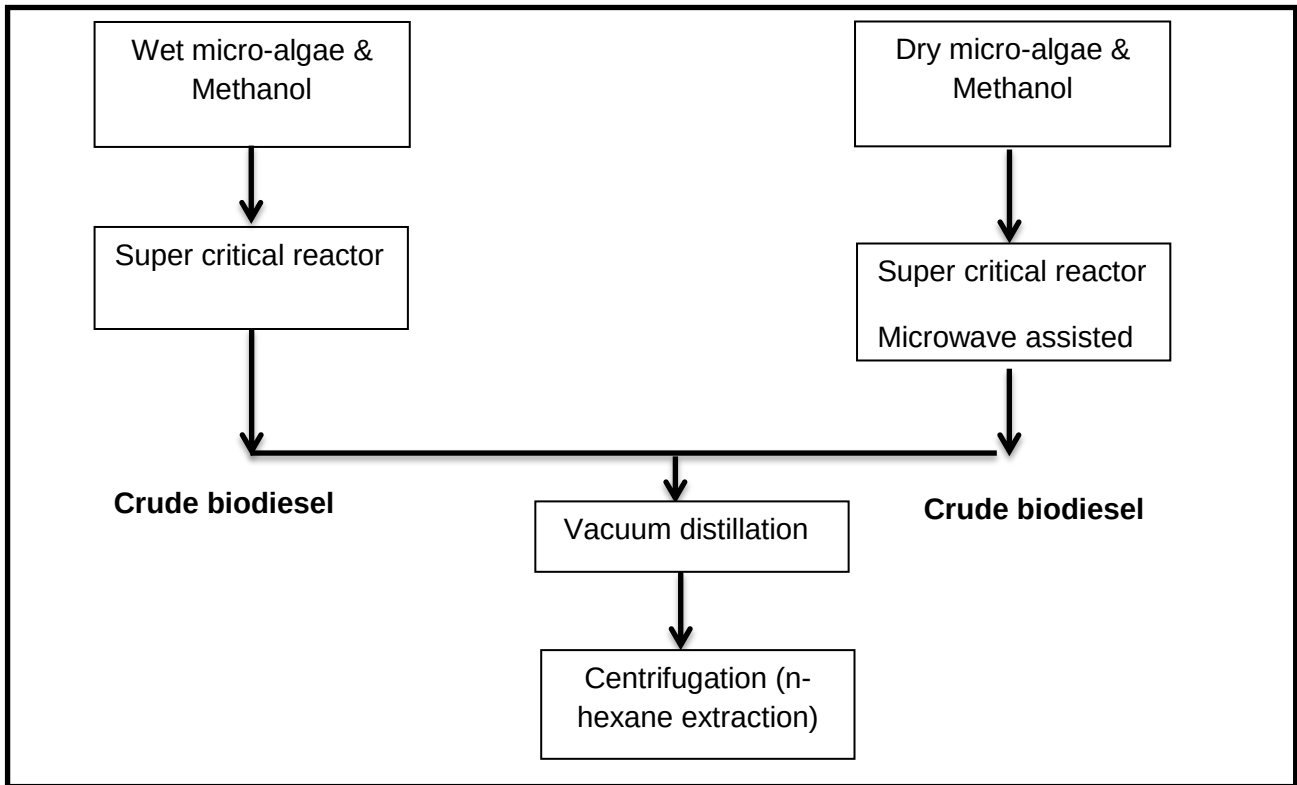


Figure 1-4: Production of biodiesel from micro-algae, through direct transesterification of wet algae under super critical methanol (a), microwave assisted transesterification (b), and downstream separation and purification of crude biodiesel (adapted from Patil *et al.*, 2012)

1.6 Operation at local water treatment plant

As can be seen in Figure 1.5, there is a high occurrence of natural algae growth at the waste water treatment plant situated just outside of Bethal (S 26° 29' 19.362" E 29° 27' 11.552"), South Africa. Currently the algae growing in the pond is backwashed over a weir, and into an evaporation pond on the plant (Figure 1.6).



Figure 1-5: Open pond on Sewage plant situated in Bethal, South Africa



Figure 1-6: Evaporation pond on the sewage plant situated in Bethal, South Africa

1.7 Problem statement

Alternative energy sources should be investigated to find a sustainable solution to the current world energy crisis. Biomass has the potential to be a sustainable alternative fuel source. A feedstock is needed that does not compete for arable land and is not set out for human consumption. Micro-algae have the potential to be a sustainable feedstock. Further

investigation is required to see whether the feedstock obtained from the local water treatment plant can be used as is for the production of FAME.

1.8 Aims and objectives

The aim of this study is to evaluate the effluent from a clarifier on a municipal waste water plant as a possible feedstock for *in situ* biodiesel production.

The following objectives were defined to achieve the aim of the study:

- Classification of feedstock obtained from the water treatment plant in order to quantify the theoretical bio-oil yield that can be produced versus what is actually being obtained. It is important to see what quantities of biomass constituents are present within the feedstock. High protein and lipid content will give a theoretical indication of the suitability of the feedstock for biofuel production.
- Investigating hydrothermal batch liquefaction of the feedstock. The aim of performing batch liquefaction experiments is to use it as a control group to compare the effect addition of a polar protic solvent like methanol will have on the oil yield and the oil characterisation.
- Investigating in-situ biodiesel production with thermochemical liquefaction (varying temperature, methanol ratio and biomass loading). The objective here is to see whether it biodiesel can successfully be produced through *in situ* thermochemical liquefaction, and if so find optimum reaction parameters in which it can be performed.
- Investigating the effect of an acid catalyst on the oil and FAME yield during thermochemical *in situ* biodiesel production. The objective here is to see whether or not a catalyst will affect the reaction rate, and the degree of conversion of triglycerides.

1.9 Scope of investigation

To reach the aims and objectives as discussed in Section 1.8, the following is required from the subsequent chapters in this report:

- **Chapter 2 – Literature study**

The aim of the literature review is to define the theoretical background and review work previously done on biodiesel production. The literature review will also aim to critically evaluate the current energy situation in South Africa and the steps taken to implement a sustainable biofuels sector by government. This study will form the basic understanding of

the projects and the boundaries where it functions. The following main areas will be addressed:

- Review of biomass and suitability of biomass for biofuel production.
- Review of algae including classification, constituents, energy reserves in algae and suitability of algae as a potential feedstock for biofuel production.
- Review of cyanobacteria (blue-green algae).
- Review of the occurrence of algae at municipal waste water treatment plants and harvesting methods of the algae.
- Critical review of the Biofuels Industrial Strategy of South Africa and the implementation of the strategy.
- Review of biodiesel and biodiesel production methods.
- Review of hydrothermal and thermochemical liquefaction and the effect of operating parameters on the various product yields.

• **Chapter 3 – Experimental**

The aim of this chapter is to define the experimental setup chosen, to achieve the aims and objectives as defined in Section 1.8, and the standards that govern it. The following are addressed in this chapter:

- Description of experimental procedure and experimental setup.
- Description of parameters varied during the experiments.
- Classification of feedstock and harvesting of the feedstock.
- Materials used in the study.
- Analytical methods used to analyse products obtained from experiments.

• **Chapter 4 – Results and discussion**

The results are discussed in this chapter, and will be interpreted and compared to literature as given in chapter 2 of this document.

• **Chapter 5 – Conclusion and recommendation**

This chapter will give conclude on the suitability of using an effluent stream from a waste water clarifier system to produce biofuels, and recommend a way forward in order to make it more economically feasible.

1.10 References

- Aarty, M., Saravanan, P., Gowthaman, M.K., Rose, C. & Kamini, N.R. 2014. Enzymatic transesterification for production of biodiesel using yeast lipases: An overview. *Chemical Engineering Research and Design*, 92(8):1591-1601.
- Barnard, A. 2009. Extraction of oil from algae for biofuel production by thermochemical liquefaction. Potchefstroom: NWU. (Dissertation – M.Eng)
- Biller, P. & Ross, A.B. 2011. Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. *Bioresource Technology*, 102(1): 215 – 225.
- Brink J. 2009. The cultivation and harvesting of micro-algal biomass from the Hartbeespoort Dam for the production of biodiesel. Potchefstroom: NWU. (Thesis – PhD)
- Bloomberg. 2013. Sustainability. www.bloomberg.com/sustainability/energy/ Date of access: 30 November 2014.
- Chen, W., Lin, B., Huang, M. & Chang. 2014?. Thermochemical conversion of microalgal biomass into biofuels: A review. *Bioresource Technology* (In press).
- Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances*, 25: 294 – 306.
- Demirbas, A. 2000. Mechanisms of liquefaction and pyrolysis reactions of biomass. *Energy Conversion and Management*, 41(6): 633 – 646.
- Demirbas, A. 2009. Biofuels securing the planet's future needs. *Energy Conversion and Management*, 50(9): 2239 – 2249.
- Demirbas, A. & Demirbas, M.F. 2011. Importance of algae oil as a source of biodiesel. *Energy Conversion and Management*, 52(1): 163 –170.
- Department of Energy **see** South Africa. Department of Energy. South Africa. Department of Energy. 2007. Biofuel Industrial Strategy of South Africa. Pretoria.
- Erzengin, M. & Küçük, M.M. 1998. Liquefaction of sunflower stalk by using supercritical extraction. *Energy conversion management*, 39(11): 1203 – 1206.

- Gai, C., Zhang, Y., Chen, W., Zhou, Y., Schideman, L., Zhang, P., Tommaso, G., Kuo, C. & Dong, Y. 2014. Characterisation of aqueous phase from the hydrothermal liquefaction of *Chlorella pyrenoidosa*. *Bioresource Technology*, (In press).
- Goyal, H.B. Seal, D. & Saxena, R.C. 2008. Bio-fuels from thermochemical conversion of renewable resources: A review. *Renewable and Sustainable Energy Reviews*, 12(2): 504 – 517.
- Gressel, J. 2008. Transgenics are imperative for biofuel crops. *Plant Science*, 174(3): 246 – 263.
- Inoue, S., Dote, Y., Sawayama, S., Minowa, T., Ogi, T. & Yokoyama, S. 1994. Analysis of oil derived from liquefaction of *Botryococcus Braunii*. *Biomass and Bioenergy*, 6(4): 269 – 274.
- Jain, S. & Sharma, M.P. 2010. Kinetics of acid catalysed transesterification of *Jatropha curcas* oil. *Bioresource Technology*, 101(20): 7701 – 7706.
- Küçük, M.M. & Demirbas, A. 1997. Biomass conversion processes. *Energy Conversion and Management*, 38(2):151-165.
- Lertsathapornsuk, V., Pairintra, R., Aryunsuk, K. & Krisnangkura, K. 2008. Microwave assisted in continuous biodiesel production from waste frying palm oil and its performance in a 100 kW diesel generator. *Fuel Processing Technology*, 89(12): 1330 – 1336.
- Leung, D.Y.C., Wu, X. & Leung, M.K.H. 2010. A review on biodiesel production using catalysed transesterification. *Applied Energy*, 87(4): 1083 – 1095.
- Li, H., Liu, Z., Zhang, Y., Li, B., Lu, H., Duan, N., Liu, M., Zhu, Z. & Si, B. 2014. Conversion efficiency and oil quality of low-lipid high-protein and high-lipid low-protein microalgae via hydrothermal liquefaction. *Bioresource Technology*, 154(1): 322 – 329.
- Liew, W.H., Hassim, M.H. & Ng, D.K.S. 2014. Review of evolution, technology and sustainability assessments of biofuel production. *Journal of Cleaner Production*, 71: 11 – 29.
- Marchetti, J.M., Miguel, V.U. & Errazu, A.F. 2007. Possible methods for biodiesel production. *Renewable and Sustainable Energy Reviews*, 11(6): 1300 – 1311.
- Matsui, T., Nishihara, A., Ueda, C., Ohtsuki, M., Ikenaga, N. & Suzuki, T. 1997. Liquefaction of micro-algae with iron catalyst. *Fuel*, 76(11): 1043 – 1048.

- Patil, P.D., Gude, V.G., Mannarswamy, A., Cooke, P., Nirmalakhandan, N., Lammers, P. & Deng, S. 2012. Comparison of direct transesterification of algal biomass under supercritical methanol and microwave irradiation conditions. *Fuel*, 97(1): 822 – 831.
- Peterson, A.A., Vogel., F., Lachance, R.P., Fröling, M., Antal., M.J. & Tester, J.W. 2008. Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies. *Energy and Environmental Science*, 1: 32 – 65.
- Quinn, J.C. & Davis, R. 2014. The potentials and challenges of algae based biofuels: A review of the techno-economic, life cycle, and resource assessment modelling. *Bioresource technology*, (In press).
- Rawat, I., Kumar, R.R, Mutanda, T. & Bux, F. 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy*, 103(3) 444 – 467.
- Reddy, H., Mupanneni, T., Patil, P.D., Ponnusamy, S., Cooke, P., Schaub, T. & Deng, S. 2014. Direct conversion of wet algae to crude biodiesel under supercritical ethanol conditions. *Fuel*, 115(1): 720 – 726.
- Ross, A.B., Biller, P., Kubacki, M.L., Li, H., Lea-Langton, A. & Jones, J.M. 2010. Hydrothermal processing of microalgae using alkali and organic acids. *Fuel*, 89(9): 223 – 243.
- Sawayama, S., Inoue, S., Dote, Y. & Yokoyama, S. 1995. CO₂ fixation and oil production through microalgae. *Energy Conversion and Management*, 36(6): 729 – 731.
- Saxena, R.C., Adhikari, D.K. & Goyal, H.B. 2008. Biomass-based energy fuel through biochemical routes: A review. *Renewable and Sustainable Energy Reviews*, 13(1):167 – 178.
- Shin, H.Y., Lee, S.H., Ryu, J.H. & Bae, S.Y. 2012. Biodiesel production of waste lard using supercritical methanol. *The Journal of Supercritical Fluids*, 61(1): 134 – 138.
- Tekin, K., Karagöz, S. & Bektas, S. 2014. A review of hydrothermal biomass processing. *Renewable and Sustainable Energy Reviews*, 40(9): 673 – 687.
- World Coal Institute. 2014. Coal Facts.
<http://www.worldcoal.org/pages/content/index.aspx?PageID=188> . Date of access: 30 November 2014.

Wu, K.T., Tsai, C.J., Chen, C.S. & Chen, H.W. 2012. The characteristics of torrefied microalgae. *Applied Energy*, 100: 52 – 57.

2. Literature survey

The literature survey on micro-algae and *in situ* biodiesel production routes are presented in this chapter. The literature survey starts by investigating biomass and biomass constituents (Section 2.1), followed by an investigation into micro-algae in Section 2.2.

A critical discussion on the implication of the current biofuels industrial strategy proposed in South Africa is presented in Section 2.3. Section 2.4 provides an insight into the suitability of micro-algae as possible feedstock for biodiesel and other renewable fuels. An investigation into the chemical make-up and production of biodiesel is provided in section 2.5 and 2.6.

The last two sections of this chapter deal specifically with hydrothermal processing, presenting studies previously performed as well as the theoretical influences on reaction parameters of hydrothermal liquefaction.

2.1 Biomass

Biomass is defined as all living matter in which solar energy is stored (Goyal *et al.*, 2008). The energy in biomass is stored in chemical bonds. When the adjacent oxygen, hydrogen and carbon bonds are broken by decomposition, combustion or digestion, the stored chemical energy are released (McKendry, 2002). This is done through biomass conversion processes include thermochemical conversion and biochemical conversion. The products formed in these conversion methods can further be used in the production of biofuels. Erzengin and Küçük (1998) state the advantages for the use of biofuels as more environmentally friendly, has a high hydrogen content, low contamination, and is a renewable supply. Zabaniotou and Innodou (2008) stated that biomass makes up 63 % of renewable energy.

From an environmentally friendly perspective, biomass has attracted attention over the years as an alternative to mainstream petrochemical raw materials (Bhaskar *et al.*, 2008). Balat *et al.* (2009) explain that it is more economical to produce energy from biomass in comparison to other forms of fuel. Demirbas (2000) further classify biomass as one of the most abundant resources and the only renewable energy resource.

Biomass comes from photosynthesis, which is the reaction between CO₂, sunlight and water. Photosynthesis produces carbohydrates which are seen as the building blocks of biomass. Less than 1 % of the solar energy used during photosynthesis is stored as chemical energy. The energy is stored within the structural components making up the

biomass. This energy is the driving force behind the use of biomass for biofuel production. Extraction of this energy results in CO₂ and water, which result in a cyclic relationship seeing as the CO₂ is then available for further photosynthesis reactions (McKendry, 2002).

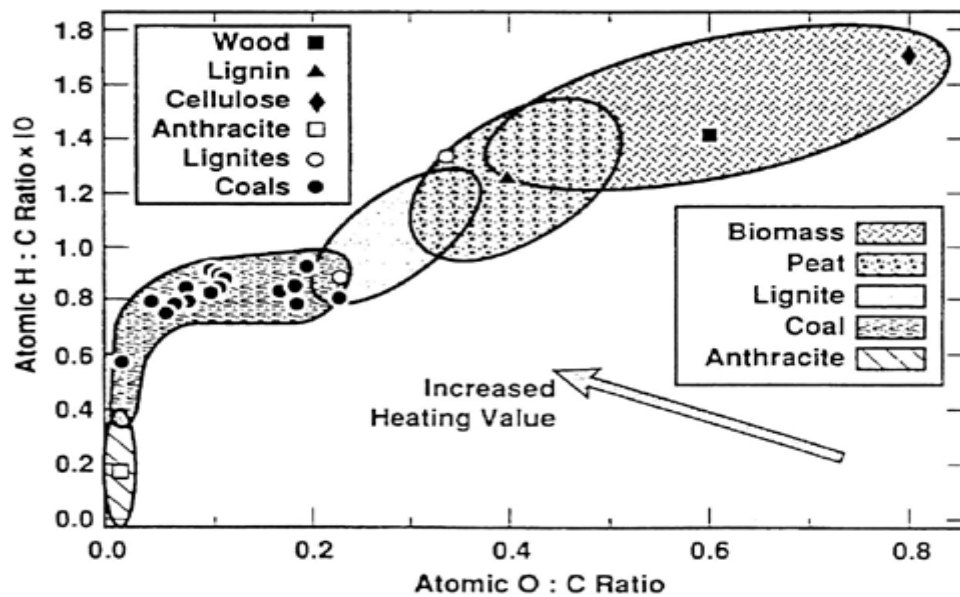


Figure 2-1: Van Krevelen diagram, showing a comparison between the H/C ratios of coal and biomass (taken from IRF, 2013)

Figure 2-1 shows a Van Krevelen diagram, which depicts the differences between the hydrogen to carbon ratio (H/C) and the oxygen to carbon ratio (O/C) of biofuels and coal. The lower the oxygen to carbon ratio, the higher the calorific value of the substance. A big difference between most biomass sources and fossil fuels is the carbon content. Biomass contain on average 50% carbon, which is much lower than that found in fossil fuels (IFR, 2013).

The carbon to oxygen ratio is one of the many factors that should be considered in biomass processing for biofuels. Another relationship of importance is that the higher the calorific value of the biomass the lower the ash content, which means less downstream processing, is required.

2.3 Biofuel Industrial Strategy

The driving force behind the South African Biofuels Industrial Strategy is job creation and agricultural support, with the biofuel industry having the potential to make up a large part of the gross domestic product (GDP) in South Africa. Internationally the dependence on global

oil supplies has a great and detrimental effect on developing countries (The energy collective, 2014).

In South Africa a 2 % (400 million litres per annum) penetration of biofuels (both diesel and petrol) in the national liquid fuel supply was proposed in 2007. In 2014 a position paper was issued by the Department of Energy proposing a mandatory blending ratio of 2 % for ethanol and 5% for biodiesel to be implemented by October 2015 (Department of Energy, 2014). The proposed biomass crops are sugar cane, sugar beets, canola, soybean crops and sunflowers (Department of Energy, 2007). Certain crops like maize and *Jatropha* were not considered due to concerns raised by the public for food security and biodiversity.

The strategy further proposed that the target market penetration would only require 1.4 % of arable land. Worldwide the use of biofuels has become a matter of interest. The European Union (EU) implemented a 5.75 % renewable energy penetration for transport fuels in 2010, with a projected increase to 10 % by 2020 (Apostolakou *et al.*, 2009). The projected use of liquid based fuels by 2020 for the United States and Europe is 325 metric tonnes. The proposed market penetration of 10 % by the EU thus relates to 32.5 metric tonnes of biofuels to be produced in 2020 (Apostolakou *et al.*, 2009).

Up to 2009 Europe was able to produce 2.4 metric tonnes of biodiesel per year and dominated this field worldwide due to the implementation of tax exemptions and EU directives (Apostolakou *et al.*, 2009).

South Africa, like many other countries, is dependent on global oil reserves for petroleum fuel products. This raises many concerns as the majority of these reserves are held in the Middle East, where political and geopolitical concerns are substantial (Demirbas & Demirbas, 2011).

Fluctuations in the trading price of crude oil led to rapid increases in the fuel price in South Africa. The fluctuating price of crude oil is a result of changes in supply and demand (The energy collective, 2014).

The petrol price in South Africa is linked to the price of crude oil in international markets, traded in US dollars. This in turn, influences everything from fuel prices to commuting costs. Independence would go a long way towards the sustainability of developing countries (The energy collective, 2014).

The South African energy supply is mainly coal based with a large reliance on lower grade coal (sub-bituminous). South Africa further imports crude oil and a small natural gas reserve

along the South Coast. About 64 % of South Africa's liquid fuel demand is met by crude oil and the remaining 36 % is made up of synthetic fuel produced locally, mainly from natural gas and coal (Department of Energy, 2014).

As previously stated, the crops for biodiesel production in South Africa are mainly soya beans and sunflower seeds. The Department of Energy (DOE) did an economic evaluation on the pricing and manufacturing of biofuels crops. The feasibility was analysed in terms of supply and demand, cost and pricing.

It was found that due to high protein content, low fibre content and the amino acid profile, soyameal (obtained from the crushing of soya beans) is more valuable than sunflower oil cake. In South Africa approximately 0.8 million tonnes of soya meal are imported annually. In the case of sunflower cake, however, the supply was found to satisfy local demand.

The fact that soya meal is imported, economically justifies the use of soya beans for biodiesel production, with soya meal as by-product. This could have great applications in the South African economy and reduce the dependency on international resources. The glycerol produced as a by-product from the production process of biodiesel can further be marketed for revenue. However crude glycerol has limited use without clean-up which needs to be investigated in order to make the by-product more economically viable.

The economical evaluation of the DOE estimated that for a plant capacity of 100 000 tons per annum, the capital investment for soya beans is R1 135 million and for sunflower oilseed it is estimated at R1 041 million.

It was then further calculated from historical data (2010) that a business model for soya beans would have a return on asset (ROA) of 1.2 % at the proposed levy price. An additional 117 c/l would be needed to obtain an ROA of 15 %. In the case of sunflower seed an additional 567 c/l would be required to achieve a ROA of 15 %. Soya beans are thus the economically preferred feedstock. All of these factors, as well as the basic fuel price and the ZAR/USD exchange rate, led to a conclusion that the basic price of biodiesel should be R10.61 per litre.

It has been shown that soya beans have the potential of producing 655 L of biodiesel per hectare (Christi, 2007). The Industrial Biofuel Strategy of South Africa suggests that for soya beans and sunflower there will be arable land available, seeing as South Africa has underutilised farm land. For a proposed plant of 113 000 m³ with 655 L/ha a total of 172 519

ha would be needed, which in a sense does not seem feasible, considering the large impact on the initial capital expenditure. For the proposed penetration of 2 % by the Industrial Biofuel Strategy of South Africa, which is equivalent to 400 000 million litres per year, many more of these plants and larger ones would be needed. This makes the gross arable land required 4 times more.

The job creation incentive has largely been accepted with open arms. The potential exists in South Africa for a larger market penetration, seeing as there is a high dependency on crude oil (60%). If the Industrial Biofuel Strategy of South Africa is managed well and have enough government incentives to further the sectors growth the fossil fuel dependency can be alleviated. Third generation studies, which are still in the developmental stages, have shown that production of biodiesel can be as much as 70 times higher than in the case of first generation crops (Demirbas, 2009).

Certain algae strains have shown the potential to produce 47 000 L/ha (Christi, 2007). The higher production potential per hectare could have a substantial impact on the required land use thus making the suggested market penetration by the Industrial Biofuel Strategy of biodiesel more likely in an economic sense. Many challenges regarding the cultivation, harvesting and extraction of oil have to be overcome before biofuels production from algae will become a reality.

2.2 Algae

Henderson *et al.* (2007) defines micro-algae as photosynthetic, aquatic plants that utilise inorganic nutrients such as nitrogen and phosphorous. Algae can further be classified as unicellular and multi-cellular organisms. Unicellular algae are known as micro-algae and multi-cellular algae are known as macro-algae (Chen *et al.*, 2009).

The distinction between algae and other chlorophyll containing plants is algae's reproductive abilities. This can be further explained by the fact that algae reproduce through gametes that are produced by the organism itself (Bold & Wynne, 1978).

2.2.1 Classification

Micro- and macro-algae are classified by pigmentation, life cycle, cellular structure, type and number of flagella (Hu *et al.*, 2008). The criteria mentioned are the determining factors for

the classification of the algae (Prescott, 1969). The following are classes of macro- and micro-algae:

Macro-algae are divided into three groups, namely (Hu *et al.*, 2008):

- Brown seaweed (*Phaeophyceae*)
- Green seaweed (*Chlorophyceae*)
- Red seaweed (*Rhodophyceae*)

Micro-algae are divided into the following classes (Hu *et al.*, 2008):

- Diatoms (*Bacillariophyceae*)
- Green algae (*Chlorophyceae*)
- Golden algae (*Chrysophyceae*)
- Yellow-green algae (*Xanthophyceae*)
- Dinoflagellates (*Dinophyceae*)
- Pico-plankton (*Prasinophyceae* and *Eustigmatophyceae*)
- Blue-green algae also known as Cyanobacteria (*Cyanophyceae*)

Khan *et al.* (2009) stated that green algae, diatoms, golden algae and cyanobacteria are the most common groups of algae species and are abundantly available. These species are regarded as great importance, because starches and triacylglycerols are stored in the plant as energy and are found in fresh and dirty water environments (Khan *et al.*, 2009).

Algae produce fats in two stages (Tiffany, 1958). The first stage entails the formation of glycerine and a fatty acid, which is achieved by the reduction of the oxygen content in the sugars. The combination of the glycerine and the fatty acid then combines to form a fat (triglycerols). Algae produce fatty acids into glycerol based membrane lipids. These lipids constitute 50 – 200 g.kg⁻¹ of the dry cell weight (Hu *et al.*, 2008, Barreiro *et al.*, 2013). Hydrocarbons are another form of lipids. To produce proteins algae need mineral salts containing nitrogen, sulphur and phosphorus (Hu *et al.*, 2008). Saturated fatty acids that occur in algae cells are lauric acid (C₁₂), myristic acid (C₁₄), palmitic acid (C₁₆), stearic acid (C₁₈). With palmitic acid being the most commonly found FA in micro-algae (Hu *et al.*, 2008)

Vieler *et al.* (2007) states that in plants the lipid composition is dominated by characteristic glyceroglycolipids and one phospholipid. These lipids of plant cells are responsible for 50 % of total membrane lipids and are enriched in monogalactosyl-diacylglycerol (MGDG) (Vieler *et al.*, 2007).

Sharma *et al.* (2012) further elaborated that micro-algae lipids include neutral lipids, polar lipids, wax esters, sterols and hydrocarbons. Micro-algae lipids also include prenyl derivatives like carotenoids. These lipids produced can be categorised in two groups namely storage lipids (non-polar) and structural lipids (polar). Storage lipids are in the form of triglycerols and can be made up of saturated fatty acid (FA) and unsaturated FA. The storage lipids can be utilised to produce biodiesel (Sharma *et al.*, 2012).

Structural lipids have a high content of polyunsaturated FA (Sharma *et al.*, 2012). Polyunsaturated FA can lead to unstable biodiesel. The polar lipids (phospholipids) are structural components of the cell membranes of micro-algae.

2.2.2 Cyanobacteria (Blue-green algae) (*Phylum Cyanophyta*)



Figure 2-2: Fresh water cyanobacteria (Vincent, 2009)

Cyanobacteria are Gram-negative oxygenic photosynthetic prokaryotes (Henderson *et al.*, 2007). Henderson *et al.* (2007) further states that cyanobacteria have potential applications in a variety of areas, with the largest potential in the agricultural industry, more specifically as nutrient supplements. Cyanobacteria contain chlorophyll along with the *phycobiliproteins* *phycocyanin* and *allophycocyanin*, which are responsible for giving cyanobacteria their characteristic blue-green colour (Vincent, 2009).

Gupta *et al.* (2013) stated that cyanobacteria are autotrophic that exhibits metabolic capabilities and survival mechanism that include nitrogen fixation, chromatic adaption. Cyanobacteria can also adapt by forming symbiotic associations with eukaryotic hosts. Cyanobacteria lacks internal organelles, histone proteins and a discrete nucleus (Gupta *et al.*, 2013). Gupta *et al.* (2013) reported the oil content of some cyanobacteria strains from 10 to 180 g.kg⁻¹.

2.2.3.1 Life cycle of cyanobacteria

In the life cycle of cyanobacteria the cells only grow until nitrogen depletion leads to building of heterocyst (specific cells designed for nitrogen fixation and only occur in some forms of cyanobacteria). When temperature drops as summer ends, the lower amount of light decreases the further growth of cells. At this stage, some of the cells turn derivatives into resting cells, namely akinetes (thick walled dormant cells). This is seen as a survival cell for cyanobacteria). The cells now sink to the bottom of the water body where nutrients are soaked up during the colder months of the year. When ambient conditions become more favourable the cells germinate, and rise to the top due to the buoyancy provided by the gas vesicles (Hense & Beckman, 2005).

2.2.3.2 Eco-physiology of cyanobacteria

Photosynthesis of cyanobacteria takes place by using water as an electron donor and producing oxygen. This is the case for most algae species. However some species of cyanobacteria also have the ability to produce elemental sulphur by using hydrogen sulphide as the electron donor in photosynthesis (Vincent, 2009). Vincent (2009) further states that cyanobacteria can tolerate low oxygen concentrations, which makes cyanobacteria adaptable in cases where hypoxia is common due to eutrophication.

Many cyanobacteria species are regarded as public health threats due to their ability to produce toxins such as microcystin, anatoxin-a and saxitoxin (Li & Watanabe, 2001).

2.3 Micro-algae at municipal waste treatment plant

Water treatment plants provide a nutrient rich growth medium for algae, which leads to a higher occurrence of algae, commonly known as eutrophication. The presence of high concentrations of algae lead to problems such as hypoxia, clogging of filters and odours (Hoko & Makado, 2011). Management and removal of algal species play a vital role in the efficiency of water treatment plants.

2.4. Algae as a feedstock for biofuels

Demirbas and Demirbas (2011) stated that algae are the fastest growing plants in the world with roughly 50 wt% oil content. Algae technologies could provide a key tool for reducing greenhouse gas emissions from coal fired power plants and other carbon intensive industrial processes (Demirbas & Demirbas, 2011).

Most major land based cultivated biomass consist of carbohydrates, proteins and lipids, while for micro-algae protein is the major component. The main considerations for suitable biomass are the materials and thermodynamics (Ginzburg, 1993). Ginzberg (1993) further explained that the heat of formation of hydrocarbons should be taken into consideration. The desirability of each constituent can be determined when comparing to the heat of formation of typical biomass constituents. Compared to the heat of formation of conventional hydrocarbons, carbohydrates are the least desirable biomass constituent. Carbohydrates only contain a third of the energy compared to hydrocarbons. The heat of formation value is much higher for lipids and proteins due to the chemical makeup of these two constituents (Proteins is made up of peptide chains and lipids consist of non-polar compounds with an aliphatic character), making micro-algae a desirable feedstock.

Rawat *et al.* (2013) stated the advantages of using micro-algae as a potential feedstock as follows:

- Short harvesting life, which allows for continuous harvesting of the biomass.
- High solar energy yields, which results in superior lipid productivity.
- Neutral lipids are generally obtained with a high level of saturation.
- Rapid growth rates and short generation times.
- Some micro-algae species have doubling times as short as 3.5 hr.
- Micro-algae gives a theoretical yield of 47,000 – 308,000 L/ha year in comparison the palm oil, which can produce 5950 L/ha year of biodiesel.
- Less water is required for cultivation of micro-algae than in other terrestrial plants.
- Uses non-arable land.
- An estimated 2 % of the land needed to produce the correct amount of biodiesel from crop based sources.
- Growth can remove the nitrate and phosphates from waste water.
- Cost of upstream processing of micro-algae relatively smaller than that of land based crops.
- Micro-algae is carbon-neutral and utilises 1.83 kg of carbon dioxide per kg of dry mass produced.
- Biodiesel produced from micro-algae has similar properties than that of petro-diesel.

Demirbas and Demirbas (2011) stated the possible disadvantages for the use of micro-algae as follows:

- Can produce unstable biodiesel with many polyunsaturates
- Biodiesel from algae performs poorly compared to the alternatives

Table 2-5 shows that micro-algae diesel produced thus far compares well to normal diesel standards (SANS 1953). The HHV of the crude bio-oil obtained from micro-algae are well within specification of biofuel standards (35 – 50 MJ.kg⁻¹) (Barreiro *et al.*, 2013; Chen *et al.*, 2014). Rawat *et al.* (2013) reported properties of biodiesel produced from micro-algae (see Table 2-1).

Table 2-1: Comparison of properties of biodiesel, produced from micro-algae oil and diesel fuel, (Rawat *et al.*, 2013)

Properties	Biodiesel from Micro-Algae
Density	0.864
Viscosity	5.2
Flashpoint (° C)	115
Solidifying point (° C)	-12
Acid value (mg KOH/g)	0.375
Heating value (MJ/kg)	41
H/C ratio	1.81
Cetane number	-

2.5 Biodiesel

Apostolakou *et al.* (2009) defines biodiesel as the mono-alkyl esters of long chain fatty acids derived from renewable lipids. Biodiesel is produced from the process of transesterification of animal fats or vegetable oils (Marcetti *et al.*, 2007). Apostolakou *et al.* (2009) further states that the transesterification process uses short chain alcohols along with a suitable catalyst to produce esters and glycerol as a by-product.

Biodiesel has become an attractive alternative to fossil fuel based petroleum products. . The drawback of using plant oils is that it has a high viscosity and low volatility, which lead to

poor combustion in diesel engines (Basha *et al.*, 2009). Transesterification is used to lower the viscosity of the vegetable oil to produce a higher grade biodiesel from bio-oil. This is achieved through removing the glycerides and combining oil esters with alcohol. The lower viscosity product results in better combustion (Basha *et al.*, 2009).

Biodiesel has the advantage that emissions from combustion are lower than that of crude oil based diesel. Biodiesel does not produce polyaromatic hydrocarbons (PAH) and nitrated polyaromatic hydrocarbons (nPAH). Both PAH and nPAH's are known to be carcinogenic (Barnard, 2009). Apostolakou *et al.* (2009) stated the advantages of biodiesel as follows:

- 1) It is made from renewable resources that are not dependent on international markets, but can be produced locally.
- 2) Less carbon dioxide is emitted as well as particulates and sulphur dioxide.
- 3) Produces 78 % less carbon dioxide.
- 4) It is safer to handle.
- 5) Biodiesel is biodegradable and non-toxic.

Apostolakou *et al.* (2009) further states the disadvantages of biodiesel as follows:

- 1) High price.
- 2) Increased NO_x emissions.
- 3) The impact on the durability of diesel engines.

Table 2-2 shows the properties for biodiesel obtained from certain crops. In comparison to diesel the largest difference is the viscosity.

Table 2-2: Properties of biodiesel from vegetable oils adapted from Demirbas and Demirbas (2011)

Crop (MJ/kg)	Kinematic Viscosity (mm²/s)	Cetane Number	Cloud point °C	Pour point °C	Flash point °C	LHV
Peanut	4.9	54	5	-	176	33.6
Soya bean	4.5	45	1	-7	178	33.5
Babassu	3.6	63	4	-	127	31.8
Palm	5.7	62	13	-	164	33.5
Sunflower	4.6	49	1	-	183	33.5
Tallow	-	-	12	9	96	-
Diesel	3.06	50	-	-16	76	43.8
20 % Biodiesel blend	3.2	51	-	-16	128	43.2

Table 2-3 shows a comparison between the biodiesel standards and the diesel standards (Balat & Balat, 2010)

Table 2-3: Comparison of diesel and biodiesel standards (adapted from Balat & Balat, 2010)

Property	Diesel	Biodiesel
Standard	ASTM D975	ASTM D6751
Composition	HC (C10 – C21)	FAME (C12 – C22)
Kin. Viscosity (mm ² /s) at 313 K	1.9 – 4.1	1.9 – 6.0
Specific gravity	0.85	0.88
Flash point (K)	333 - 353	273 – 443
Cloud point (K)	258 – 278	270 – 285
Pour point (K)	238 – 258	258 – 289
Water (vol %)	0.05	0.05
Carbon (wt%)	87	77
Hydrogen (wt%)	13	12
Oxygen (wt%)	0	11
Sulphur (wt%)	0.05	0.05
Cetane number	40 – 55	48 – 60
HFRR (μm)	685	314
BOCLE scuff (g)	3500	>7000

2.6 Production of biodiesel

In 2009 it was stated that current production plants of biodiesel in the world have a production capacity of 40 kiloton per year and that it would be possible to increase this capacity to 800 kiloton per year (Apostolakou *et al.*, 2009). Marchetti *et al.* (2007) gives the process route for biodiesel production as shown in Figure 2.3.

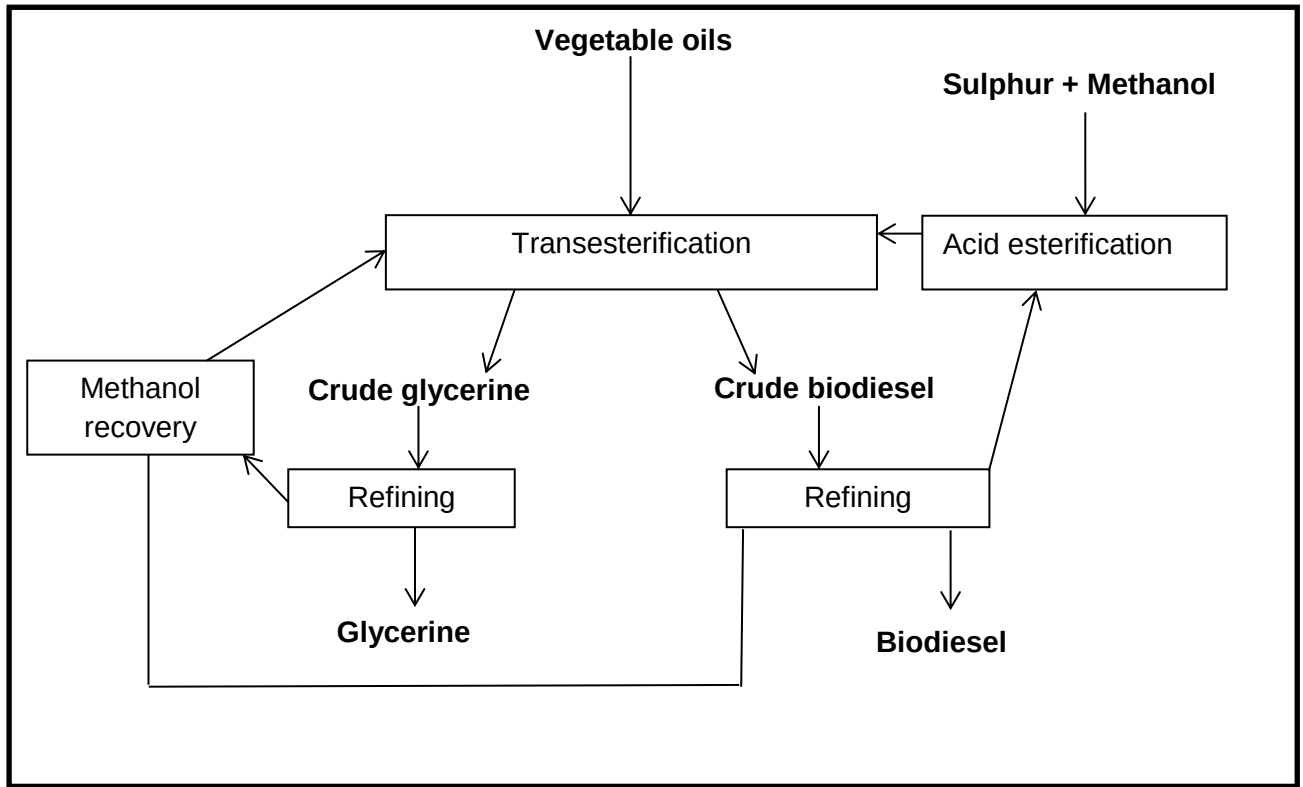


Figure 2-3: Typical representation of biodiesel production process (adapted from Marchetti *et al.*, 2007)

Figure 2-4 gives a representation of the transesterification reactions (Marchetti *et al.*, 2007). For the transesterification reaction approximately 100 kg of oil reacts with 10 kg of the short chain alcohol and produces 100 kg biodiesel and 10 kg glycerol (Apostolakou *et al.*, 2009). Apostolakou *et al.* (2009) further states that biodiesel can also be produced through other methods including super critical conditions in the absence of a catalyst and biochemical routes with the use of enzymes.

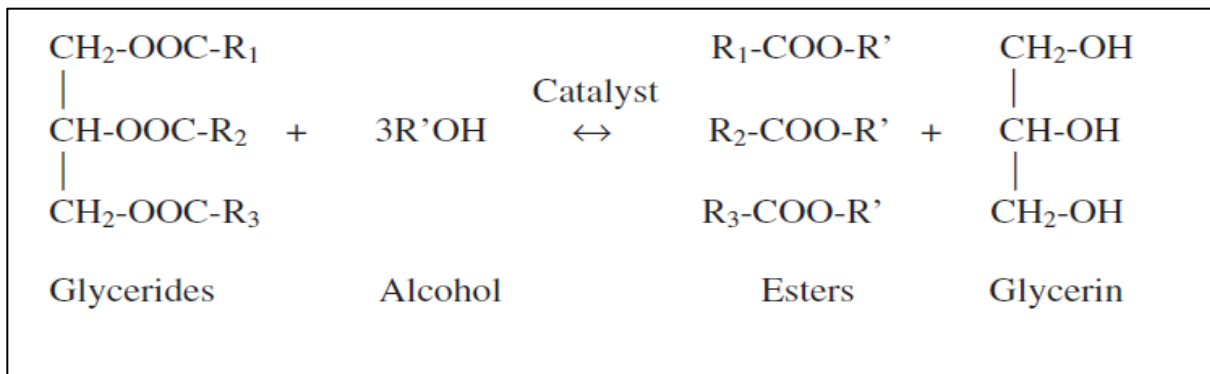


Figure 2-4: Transesterification (Marchetti *et al.*, 2007)

The process turns glyceryl esters into methyl esters. As can be seen from Figure 2-4, the addition of methanol or ethanol removes the glycerol structure from the triglycerides and replaces it with a methyl group. It should also be noted that the reaction is reversible, thus the reaction will continue until equilibrium is reached between the products and the reagents. This results in an amount of unreacted triglycerides (Marchetti *et al.*, 2007).

To avoid this from happening, an excess amount of alcohol is used to shift the reaction to the product side to achieve as high as possible conversion of the triglycerides. In industrial processes an excess of three moles of alcohol is used (Fukuda *et al.*, 2001).

Suitable catalysts (alkali or acid catalysts) yield different reaction conditions, with alkali-catalysed transesterification being the most suitable for industrial processes. The alkali-catalysed reaction is approximately 4000 times faster than that of the acid-catalysed reaction (Fukuda *et al.*, 2001). The alkaline catalyst that is utilised can be potassium or sodium hydroxide (Marchetti *et al.*, 2005). Typical reaction conditions for alkali-catalysed transesterification are at 60°C, atmospheric pressure and a reaction time of 90 minutes. Separation of the catalyst and excess methanol from the product stream is usually achieved by water washing of the biodiesel (Marchetti *et al.*, 2007).

Tables 2-4 and 2-5 shows biodiesel yields from various feed stocks, using alkali transesterification and acid transesterification respectively.

Table 2-4: Alkali-catalysed transesterification reaction used for biodiesel production (Balat and Balat, 2010)

Feed oil	Catalyst	Yield (%)
Soybean	KOH	95
Sunflower	NaOH	97.1
Cottonseed	NaOCH ₃	96.9
Rice bran	NaOCH ₃	83.3
Palm	NaOH	95
Palm kernel	KOH	96
Jatropha	KOH	97.6
Waste frying	KOH	95.8

Table 2-5: Acid-catalysed transesterification reaction used for biodiesel production (Balat & Balat, 2010)

Feed oil	Catalyst	Yield (%)
Soybean	H ₂ SO ₄	>90
Soybean	HCl	65
Rice bran	HCl	>90
Corn	p-toluenesulphonic acid	97.1
Canola	AlCl ₃	98

Production of biodiesel through *in situ* methods have gained a lot of attention recently (Patil *et al.*, 2012; Reddy *et al.*, 2014; Levine *et al.*, 2013). *In situ* methods also known as direct transesterification include the simultaneous extraction of triglycerides and transesterification of the feedstock (Leung & Lueng, 2010; Jain & Sharma, 2010; Aarthy *et al.*, 2014; Reddy *et al.*, 2014). These methods include the uses microwave assisted transesterification, ultrasound assisted transesterification and supercritical methanol transesterification (Borugadda & Goud, 2012). Table 2-6 shows a summary of results of direct transesterification with supercritical methanol and ethanol found in literature.

Table 2-6: Summary of some studies performed on *in situ* transesterification with supercritical alcohol

Production method	Biodiesel yield obtained	Comment	Reference
Super critical ethanol	67 %	Reaction temperature of 265°C and residence time of 20 mins, Diesel had a CV value of 43 MJ/kg. Research showed that an increase in temperature favours the yield of FAEE, but only up to 270°C, after which it starts to drop significantly. Feedstock was algae (<i>Nannochloropsis Salina</i>)	Reddy <i>et al.</i> , 2014
Super critical methanol	89.91 %	Temperature was at 335°C, with a molar ratio of 45:1 (MeOH to oil) at a reaction time of 15 mins and an agitation speed of 500 rpm. The feedstock here was waste lard	Shin <i>et al.</i> , 2012
Super critical methanol	80%	A temperature of 255°C, with a ratio of wet algae to methanol (wt/vol) of 1:9, and a reaction time of 25 min, the production cost following their process was estimated as \$0.26/gal	Patil <i>et al.</i> , 2012
Supercritical methanol	90.4% for Krating oil and 84.6% for Jatropha oil.	The highest yield for the krating oil was achieved at 260°C and 16 MPa while for the Jatropha oil the maximum yield was obtained at 320°C and 15 MPa and a residence time of 5 min and a molar ratio of methanol to oil of 40:1	Samniang <i>et al.</i> , 2014

Production method	Biodiesel yield obtained	Comment	Reference
Supercritical methanol	95%	Palm oil was used in this study, the study compared the yields for supercritical and subcritical conditions, and found that the yield significantly increased with supercritical conditions. This can be due to the lipid extraction during direct liquefaction. The study also utilised a heterogeneous base catalyst. The maximum yield was obtained with 3% catalyst load and at a residence time of 60 min and a methanol to oil molar to ratio of 24:1.	Asri <i>et al.</i> , 2013
Supercritical methanol, supercritical ethanol	-	Yields obtained for the supercritical methanol and supercritical ethanol was 41.6% and 53.8% respectively. The feedstock used was <i>E. spectabilis</i> was used as feedstock. The yields were achieved at 310°C. The author also investigated the use of supercritical acetone, which is not a transesterification method. 71.4% of the biomass was converted into liquid in this study.	Aysu <i>et al.</i> , 2014

2.7 Hydrothermal processing of biomass

Peterson *et al.* (2008) defines hydrothermal processing as a biomass conversion method at high temperatures (above 200°C) and pressures ranging from 4 MPa to 20 MPa. The high temperatures result in a biofuel that is free from any biological active matter. Some other advantages are high energy efficiency, separation efficiency, the possibility to use wet feedstock, no microbial cultures or enzymes needed, higher H/C ratio oil, better solubility of fatty acids, and protein derivatives as solvents approaches critical conditions, and better reaction rates at supercritical conditions, which lead to a reduction in the interphase mass transfer resistance (Peterson *et al.*, 2008)

Barreiro *et al.* (2013) and Chen *et al.* (2014) list the hydrothermal processes, namely fast pyrolysis, liquefaction and gasification. Chen *et al.* (2014) also mentions torrefication as a hydrothermal process, torrefication takes place at low temperatures (200°C) and is used to produce a biochar for green coal applications. Liquefaction is characterised as being below the critical temperature and pressure of water, which is at 374°C and 22 MPa, respectively. When water reaches the critical point, the density of water decreases drastically without a change or liquid to gas phase (Peterson *et al.*, 2008).

During gasification the biomass is heated between 600°C and 1300°C and syngas is produced as primary product (Barreiro *et al.*, 2013; Chen *et al.*, 2014). The syngas is then converted into alkanes using Co, Fe or Ru based catalyst. Fast pyrolysis entails heating biomass for short time periods to between 400°C and 650°C and oxygenated hydrocarbons are produced as primary product. Liquefaction is similar to fast pyrolysis, just with a lower temperature range between 250°C and 340°C (Barreiro *et al.*, 2013; Chen *et al.*, 2014, Yokoyama *et al.*, 1987; Karaca & Bolat, 2000; Xu & Etcheverry 2008).

2.7.1 Liquefaction

Demirbas (2000) stated that fragments produced by hydrolysis during liquefaction are degraded to smaller compounds. The smaller compounds are later rearranged through condensation, polymerisation and cyclisation, which essentially lead to the formation of gas and a residue as new compounds. Chen *et al.* (2014) gives the various products that form as gas, volatile organic compounds, residue, heavy oil and water soluble oil. Initially the biomass is broken down into fragments at low temperatures. As the temperature increases the reaction tends to be less favourable for the production of long-chained compounds. At

high temperatures the degradation of heavy oil and water soluble oil can also be noted. This can be explained by the hydrogen donor present in the system favouring the molecular rearrangement and stabilisation of free radicals or by the hydrocracking of heavier molecules

Barreiro *et al.* (2013) and Chen *et al.* (2014) stated that the reaction pathways during liquefaction cannot be described by single reaction steps seeing as the constituents of biomass each interact with the other to form chemicals such as acids, alcohols, aldehydes, ketones, esters, heterocyclic derivatives and phenolic compounds

. The basic process steps are as follows (Chornet & Overend, 1985):

- i. *Polymeric structures in the biomass material are solubilised;*
- ii. *Solubilised polymers are split into smaller units;*
- iii. *Smaller units are stabilised by either increased solvolytic action or addition of an external reducing agent*

Another proposed reaction route is the formation of preasphaltene, asphaltene and a residue through the decomposition of the feedstock. This is then followed by the conversion of these components into gas and oil through hydrogenation/hydrocracking or into a gas or a residue by polycondensation (Wang *et al.*, 2007).

The glycerol present in lipids of the biomass are converted into methanol, acetadehyde, propionaldehyde, acrolein, allyl alcohol, ethanol and formaldehyde, carbon monoxide, carbon dioxide and hydrogen gas during hydrothermal liquefaction. While the fatty acids can be converted into long-chain hydrocarbons. Proteins, on the other hand, hydrolyse with hydrothermal liquefaction. The peptide bonds undergo decarboxylation that produce amines and carbonic acid these products can then repolymerise to form long chain hydrocarbons. The products of the decarboxylation reactions can also form aromatic ring components like phenols, pyrrole or idole. Carbohydrates do not break down directly to crude bio-oil, the polar compounds that are believed to form during hydrothermal liquefaction can then produce long chain hydrocarbons. The water soluble components formed from carbohydrates are organic acids, aldehydes, benzene and alcohols (Barreiro *et al.*, 2013).

Barreiro *et al.* (2013) states that crude bio-oil is a viscous and energy-dense liquid that is produced from lipids, proteins, carbohydrates and algaenans. The crude 5% to 95% of that of bio-oil can have an energy content of 75 to 95% of that of conventional carbon based fuels. The oxygen content found in crude bio-oil is much lower than that presence in the feedstock. The main problem that crude bio-oil has is that it has a high nitrogen content,

which leads to high NO_x emissions (Barreiro *et al.*, 2013; Chen *et al.*, 2014). The aqueous phase produced as by product from hydrothermal liquefaction contains high amounts of organic matter and nutrients (Barreiro *et al.*, 2013).

According to Barreiro *et al.* (2013) the gas main constituents of the gas products are CO₂, H₂, and smaller fractions of N₂, C₂H₄ and C₂H₆. Trace amounts of CO present in the gas phase indicates that the deoxygenation of the feedstock takes place by means of decarboxylation rather than decarbonylation. The CO might also be attributed to the water-gas shift reaction (Barreiro *et al.*, 2013).

2.8 Reaction parameters for liquefaction

A number of studies investigated have shown the influence of various liquefaction conditions on liquefaction product yields and distribution. (Barreiro *et al.*, 2013, Chen *et al.*, 2014; Elliot *et al.*, 2014; Yang *et al.*, 2014; Zhang *et al.*, 2013; Duan *et al.*, 2013; Eboibi *et al.*, 2014). The main conditions investigated are the residence time, temperature, solvent and catalyst. Brief explanations of each of the findings are given below.

2.8.1 Influence of temperature on bio-oil and bio-char yields

As temperature increases, the crude bio-oil yield increases until a maximum is reached, after which the yield starts to decline (Yang *et al.*, 2014; Zhang *et al.*, 2013; Duan *et al.*, 2013; Barreiro *et al.*, 2013, Chen *et al.*, 2014; Aysu & Kucuk, 2013). The optimum temperature in most cases ranges between 280°C to 320°C. In the case of Yang *et al.* (2014) liquefaction was performed with *E. Prolifera* as feedstock. It can be seen that above 270°C there is an inversely proportional relationship between the water soluble oil (WSO) and the bio-oil yield.

Barreiro *et al.* (2013) also noted that as the temperature increase the bio-gas yield starts to increase. This increase in bio-gas indicates the degradation of the char initially formed, seeing as the bio-oil yield starts to increase along with the gas yield while the bio-char yield starts to decrease at higher temperatures.

The bio-oil yield is expected to increase with temperature to just below the supercritical point of water, after which a decrease in bio-oil yield should be observed due to light cracking of oils to gas and bio-char during pyrolysis (360°C and upwards). The critical temperature of water is 374.15°C. As water starts to heat up and compress the characteristics changes drastically with a lower permittivity which leads to an increase in the solubility of hydrophobic compounds (Barreiro *et al.*, 2013). Barreiro *et al.* (2013) further explained that at the subcritical conditions of water the hydrogen bonds become less and weaker, thus giving

higher availability for H^+ and OH^- ions, which will promote reactions like hydrolysis (Yang *et al.*, 2014; Zhang *et al.*, 2013; Duan *et al.*, 2013; Barnard, 2009; Barreiro *et al.*, 2013; Chen *et al.*, 2014).

According to Barreiro *et al.* (2013) temperature is a critical liquefaction condition as the crude bio-oil yield and oil composition is affected by temperature. According to Aysu and Kucuk (2013) hemicellulose starts to decompose before cellulose and lignin as it is thermally less stable. The decomposition of cellulose and hemicellulose will lead to an increase of bio-oil yield at temperatures above 250°C. Lignin, on the other hand, only starts to decompose at temperatures higher than 300°C but is followed by repolymerisation reactions (Aysu & Kucuk, 2013). Barreiro *et al.* (2013) states that as the temperature increase the nitrogen content in the bio-oil also increases, which might indicate the degradation of proteins at higher temperatures, seeing as proteins is the primary source of nitrogen in crude bio-oil.

The effect of temperature on FAME yields during thermochemical liquefaction with supercritical methanol is similar to that of hydrothermal liquefaction. The higher yield of FAME at higher temperatures is explained by the fact that at higher temperature (above methanol's critical point) the oils and the methanol would mix into one miscible fluid (Asri *et al.*, 2013; Sun *et al.*, 2014). Asri *et al.* (2013) further elaborates, stating that at a higher temperature the transesterification reaction rate would increase, which correlates with Arrhenius law. Arrhenius law state that intrinsic rate constant is a function of reaction temperature.

2.8.2 The influence of catalyst on bio-oil and bio-char yield

Chen *et al.* (2014) states that the most commonly used catalyst for liquefaction are sodium carbonate (Na_2CO_3) and potassium hydroxide (KOH). Some studies have shown that homogeneous catalysts like Na_2CO_3 and KOH can improve the crude bio-oil yield (Ross *et al.*, 2010; Jena *et al.*, 2011; Biller & Ross, 2011). According to Jena *et al.* (2011) base catalyst has a negative impact on the production of bio-char. The base catalyst, however, gave better organic decomposition, resulting in higher oil yields. Chen *et al.* (2014) states that the bio-char yield can be increased or decreased depending on the feedstock. Barreiro *et al.* (2013) states that the base catalysts increase the pH of the system, which in turn inhibits the dehydration of the feedstock and promotes decarboxylation reactions. Base catalysts can also aid the water-gas shift reaction by supporting the formation of H_2 and CO_2 . The increased quantities of H_2 will act as a reducing agent, which will increase the HHV of the crude bio-oil (Barreiro *et al.*, 2013). According to Jena *et al.* (2011) Na_2CO_3 had a favourable effect on the oil yield during liquefaction due to the formation of sodium formate,

which comes from the carbohydrates, lipids and proteins present in the biomass. The subsequent formation of OH^- and HCOO^- ions further favours hydrocarbon degradation.

A decrease in the oil yield content with an increase in the catalyst loading was found by Barnard (2009). This is explained by the reaction taking place between the fatty acids in algae and the base catalyst. This acid-base reaction produces a sodium salt that is soluble in water. The sodium based salt present (produced during saponification of the FFA) will be removed in the washing process of the oil, thus reducing the oil yield. NiO and $\text{Ca}_3(\text{PO}_4)_2$ as catalyst led to a decrease in oil yield according to Jena *et al.* (2011).

Heterogeneous catalyst has also shown an increase in the yield. Duan and Savage (2011) investigated six different heterogeneous catalyst and showed that there is an improvement in the crude bio-oil yield. Barreiro *et al.* (2013) however states that heterogeneous catalyst have several drawbacks like sintering dissolution, poisoning or intraparticle diffusion limitations. Biller and Ross (2011) states that the biggest effect of a catalyst is not on the bio-oil yield itself but rather on the deoxygenation and denitrogenation of the bio-oil. Aysu and Kucuk (2013) state that alkali salts coming from the base catalyst can aid to hydrolyse biomass constituents like cellulose and hemicellulose, which would essentially mean higher conversion of the organic matter through the condensation, cyclisation and repolymerisation of smaller compounds formed from deoxygenation, dehydration and decarboxylation.

Homogeneous catalysts like acetic and formic acid have been reported to increase the oxygen content of the bio-oils (Barreiro *et al.*, 2013). Biller and Ross (2011) also reported that for the liquefaction of proteins and carbohydrates that the absence of a catalyst is favourable. Barreiro *et al.* (2013) further reports that when a reducing agent like H_2 are present literature shows that the use of a catalyst reduces the crude bio-oil yield.

The effect of catalysts on *in situ* biodiesel production can occur in a high occurrence of organic salts in the feedstock which can lead to saponification to take place during transesterification. Thus the addition of an acid catalyst might have a positive effect on the FAME yield. Other *in situ* transesterification methods have proven that the addition of an acid catalyst can give an increase in methyl ester yields (Ehimen *et al.*, 2009).

2.8.3 The influence of biomass loading on bio-oil and bio-char yield

Barreiro *et al.* (2013) stated that a higher biomass loading can result in lower amounts water available to stabilise and dissolve the biomass. A reduction in biomass loading however reduces the energy required to heat the water. Savage *et al.* (2012) and Jena *et al.* (2011) both showed an increase in crude bio-oil with an increase in biomass loading and a reduction in the organic matter dissolved in the aqueous product. Jena *et al.* (2011) showed an increase of 7% in crude bio-oil with an increase of 10 wt% to 20 wt% of biomass loading. For an increase of biomass loading from 20 wt% to 50 wt% no visible effect is noticed by Jena *et al.* (2011).

Water acts as a solvent during liquefaction, thus playing a vital role in the solubility of the system. Not enough water present would thus result in less biomass being dissolved. An optimum value for biomass is still lacking in literature.

2.8.4 The influence of methanol to dry biomass ratio on bio-oil and bio-char yield (solvent)

Yuan *et al.* (2011) investigated the effect of three different solvents, namely ethanol, methanol and dioxane on bio-oil and bio-char yields from liquefaction. From their results it can be seen that dioxane gave the lowest bio-oil yield, while at higher temperatures (357°C) methanol gave the highest bio-oil yield. Lee *et al.* (2008) found a similar trend for the liquefaction of micro-algae. Yuan *et al.* (2011) found that as the temperature increase for different solvents the conversion rates are different. For dioxane the conversion rate of biomass to liquid product is lower than that for ethanol and methanol. While methanol and ethanol had a much higher conversion rate initially at higher temperatures the rate starts to decrease (Yuan *et al.*, 2011). Yuan *et al.* (2011) attributes this trend to methanol and ethanol being polar protic solvents. The hydrogen bonds could lead to hydrogen donation. Thus the intermolecular hydrogen bonds might decompose into hydrogen free radicals. The free radicals are then reactive for hydrocracking of long chain polymers like proteins, lipids and carbohydrates (Yuan *et al.*, 2014). Yang *et al.* (2014) also states that solvents can donate a H⁺- or OH⁻-group, which would result in higher bio-oil yields. Higher quantities of solvent present would increase the stabilisation of the fragmented biomass thus inhibiting repolymerisation.

Aysu and Kucuk (2013) further elaborates on the effect of polar protic solvents, stating that in the case of cellulose the hydrogen bonds present in the solvent will break down the cellulose chain and eventually the crystalline structure at high temperatures and pressures.

The glycosidic bonds of cellulose and hemicellulose are polar and as such they decompose very rapidly in polar protic solvents (Aysu & Kucuk, 2013). On the other hand dipolar aprotic solvents have a higher decomposition rate of the carbonyl groups present in biomass constituents like lignin (Aysu & Kucuk, 2013). Aysu and Kucuk (2013) further stated that the advantage of using solvents with lower critical temperature would essentially mean that the interphase mass transfer limitations are inhibited.

Minami and Saka (2006) stated that an *in situ* biodiesel production method with supercritical methanol is possible without a catalyst due to the methanolysis ability of methanol. Normal liquefaction will break the triglycerides (TG) present into fatty acids (FA) and glycerine. The FA, in the presence of methanol, can then directly be converted to fatty acid methyl esters (FAME).

The higher the methanol ratio the higher the FAME yield is expected to be, as the simultaneous breaking down of TGs and the production of FAME is taking place. According to Minami and Saka (2006) super critical methanol will be more likely to have gaseous properties. The supercritical fluid properties would thus lead to an improved mass transfer state. Initially it would be expected that multi liquid phase would be present due to the difference in solubility for the fluids present in the reactor.

2.8.5 The influence of residence time on bio-oil and bio-char yield

Chen *et al.* (2014) states that residence time and temperature should be considered simultaneously as a higher temperature with a shorter time will yield a higher crude bio-oil yield. Barreiro *et al.* (2013) states the average residence in literature as 60 minutes.

Jena *et al.* (2011) showed an increase in bio-oil yield as the residence time increase to 60 minutes at 350°C and increase above 60 minutes showed a decrease in the bio-oil. Chen *et al.* (2014) attributed this decrease to lighter hydrocarbons converting to gaseous products. There is a need to find the optimal ratio for temperature and residence to find the most economical value. Some studies have reported much higher yields at higher temperature with shorter residence time than other studies on the same feedstock that used longer residence times and lower temperatures.

2.9 Concluding remarks

Micro-algae can be utilised as a feedstock for biodiesel production, seeing as micro-algae species consist of high lipid contents, proteins and carbohydrates, all of which are favourable

components for the production of biofuels (Hu *et al.*, 2008; Barreiro *et al.*, 2013; Chen *et al.*, 2014).

Micro-algae species have also shown that it has higher yields per hectare than first and second generation crops as proposed by the current Biofuel Industrial Strategy of South Africa (Christi, 2007). The Biofuels Industrial Strategy of South Africa is mainly driven out of a job creation point of view and giving some incentive for large producers. The main aim however of the strategy is to produce biofuels from sugar cane, sugar bagasse, sun flower oil and soya beans for the purpose of blending with conventional hydrocarbon fuels and not as a replacement or alternative for fossil fuels. The first generation crops proposed by the Biofuels Industrial Strategy of South Africa, however, proved that it might be ineffective seeing, as in the long term it will not be viable as an alternative due to the immense amounts of arable land needed. However, soya beans for biodiesel production have proven that it might be economically feasible to implement in the short term, which creates the need to find alternatives for long term replacement of conventional fuels (Department of Energy, 2014).

Algae has a potential feedstock showed significant potential however some challenges still exist before large scale production would be able to take place. These include harvesting, cultivation and handling of the high moisture. In terms of challenges for biodiesel production, some strains of algae have been known to produce unstable unsaturated FAME, which causes it performing poorly compared to other alternatives. However, some studies found that the biodiesel produced from micro-algae is well within SANS 1953 standards, meaning that the production of biodiesel can be industrially viable.

Possible biodiesel production methods from algae include direct lipid extraction, after which it is transesterified and *in situ* production methods like thermochemical liquefaction, ultrasonication and microwave assisted transesterification (Leung & Leung, 2010). For this study thermochemical liquefaction was chosen as the *in situ* production. Studies showed that high amounts of biomass can be converted and transesterified with thermochemical liquefaction (Reddy *et al.*, 2014; Shin *et al.*, 2012; Patil *et al.*, 2012; Samniang *et al.*, 2014; Asri *et al.*, 2013; Aysu *et al.*, 2014).

The effects of hydrothermal liquefaction have been well investigated, and it was found that higher temperatures favours the conversion of biomass into bio-oil and temperatures above 250°C start to aid in the decomposition of certain biomass constituents like cellulose, hemicellulose and lignin. The addition of a solvent seems to be favourable on the bio-oil yield, especially polar protic solvents. The effect of an acid catalyst on the bio-oil yield is

uncertain as it has not been investigated thoroughly. The same goes for the effect of residence time where literature is contradicting each other and residence should be evaluated in terms of temperature rather than a parameter on its own.

2.10 References

Aarty, M., Saravanan, P., Gowthaman, M.K., Rose, C. & Kamini, N.R. 2014. Enzymatic transesterification for production of biodiesel using yeast lipases: An overview. *Chemical Engineering Research and Design*, 92(8): 1591 – 1601.

Apostolakou, A.A., Kookos, I.K., Marazioti, C. & Angelopoulos, K.C. 2009. Techno-economic analysis of a biodiesel production process from vegetable oils. *Fuel Processing Technology*, 90: 1023 – 1031.

Asri, N.P., Machmudah, S., Suprpto, W., Budikarjono, K., Roesyadi, A. & Goto, M. 2013. Palm oil transesterification in sub- and supercritical methanol with heterogeneous base catalyst. *Chemical Engineering and Processing: Process Intensification*, 72: 63 – 67.

Aysu, T. & Kukuk, M.M. 2013. Liquefaction of giant fennel (*Ferula orientalis* L.) in supercritical organic solvents: Effects of liquefaction parameters on product yields and character. *The Journal of Supercritical Fluids*, 83: 104 – 123.

Aysu, T., Demirbas, A., Bengu, A.S. & Kucuk, M.M. 2015. Evaluation of *Eremurus spectabilis* for production of bio-oils with supercritical solvent. *Process Safety and Environmental Protection*, 94: 339 – 349.

Balat, M., Balat, M., Kirtay, E. & Balat, H. 2009. Main routes for the thermo-conversion of biomass into fuels and chemicals. Part 1: Pyrolysis systems. *Energy Conversion and Management*, 50: 3147 – 3157.

Balat, M. & Balat, H. 2010. Progress in biodiesel processing. *Applied Energy*, 87: 1815 – 1835.

Barnard, A. 2009. Extraction of oil from algae for biofuel production by thermochemical liquefaction. Potchefstroom: NWU. (Dissertation – M.Eng)

Barreiro, D.L., Prins, W., Ronsse, F. & Brilman, W. 2013. Hydrothermal liquefaction (HTL) of microalgae of biofuel production: State of the art review and future prospects. *Biomass and Bioenergy*, 53: 113 – 127.

Basha, S.D., Francis, G., Makkar, H.P.S., Becker, K. & Sujatha, M. 2009. A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *Jatropha curcas* L. germplasm from different countries. *Plant Science*, 176: 812 - 823.

Biller, P. & Ross, A.B. 2011. Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. *Bioresource Technology*, 102(1): 215 – 225.

Bold, H.C. & Wynne, M.J. 1978. *Introduction to the Algae: Structure and Reproduction*. New Jersey: Prentice-Hall, Inc. 706 p.

Brink J. 2009. The cultivation and harvesting of micro-algal biomass from the Hartbeespoort Dam for the production of biodiesel. Potchefstroom: NWU. (Thesis – PhD)

Bhaskar, T., Sera, A., Muto, A. & Sakato, Y. 2008. Hydrothermal upgrading of wood biomass: Influence of the addition of K₂CO₃ and cellulose/lignin ratio. *Fuel*, 87: 2236 – 2242.

Bold, H.C. & Wynne, M.J. 1978. *Introduction to the Algae: Structure and Reproduction*. New Jersey: Prentice-Hall, Inc. 706 p.

Borugadda, V.B. & Goud, V.V. 2012. Biodiesel production from renewable feedstock: Status and Opportunities. *Renewable and Sustainable Energy Reviews*, 16: 4763 – 4784.

Chen, P., Min, M., Chen, Y., Wang, L., Li, Y & Chen, Q. 2009. Review of the Biological and engineering aspects of algae to fuels approach. *International Journal of Agriculture and Biological Engineering*, 2(4): 1 – 30.

Chen, W., Lin, B., Huang, M. & Chang. 2014?. Thermochemical conversion of microalgal biomass into biofuels: A review. *Bioresource Technology*, (In press).

Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances*, 25: 294 – 306.

Chornet, E. & Overend, R.P. 1985. Biomass Liquefaction: An Overview. (In Overend, R.P., Milne, T.A. & Mudge, L.K., ed. *Fundamentals of Thermochemical Biomass Conversion*. New York: Elsevier Applied Science Publishers. 967 – 1001 pp.

Department of Energy **see** South Africa. Department of Energy.

South Africa. Department of Energy. 2007. *Biofuel Industrial Strategy of South Africa*. Pretoria.

Department of Energy **see** South Africa. Department of Energy.

South Africa. Department of Energy. 2014. Pretoria.

Demirbas, A. 2000. Mechanisms of liquefaction and pyrolysis reactions of biomass. *Energy Conversion and Management*, 41(6): 633 – 646.

Demirbas, A. 2009. Biofuels securing the planet's future needs. *Energy Conversion and Management*, 50(9): 2239 – 2249.

Demirbas, A. & Demirbas, M.F. 2011. Importance of algae oil as a source of biodiesel. *Energy Conversion and Management*, 52(1): 163 –170.

Duan, P. Xu, Y. & Bai, X. 2013. Upgrading of crude duckweed bio-oil in subcritical water. *Fuel*, 27(8): 4729 – 4738.

Duan, P. & Savage, P.E. 2011. Upgrading of crude algal bio-oil in supercritical water. *Bioresource Technology*, 102: 1899 – 1906.

Eboibi, B.E., Lewis, D.M., Ashman, P.J. & Chinnasamy, S. 2014. Effect of operating conditions on yield and quality of biocrude during hydrothermal liquefaction of halophytic microalgae *Tetraselmis* sp. *Bioresource Technology*, 170: 20 – 29.

Ehimen, E.A., Sun, Z.F. & Carrington, C.G. 2009. Variables affecting the in situ transesterification of microalgae lipids. *Fuel*, 89: 677 – 684.

Elliot, D.C., Biller, P., Ross, A.B., Schmidt, A.J. & Jones, S.B. 2014. Hydrothermal liquefaction of biomass: Developments from batch to continuous process. *Bioresource Technology*, (In press).

Erzengin, M. & Küçük, M.M. 1998. Liquefaction of sunflower stalk by using supercritical extraction. *Energy Conversion and Management*, 39(11): 1203 – 1206.

Fukuda, H., Kondo, A. & Noda, H. 2001. Biodiesel fuel production by transesterification of oils. *Journal of Bioscience and Bioengineering*, 92(5): 405 – 416.

Ginzburg, B. 1993. Liquid fuel (oil) from halophilic algae: A renewable source of non-polluting energy. *Renewable Energy*, 3(2-3): 249 – 252.

Goyal, H.B. Seal, D. & Saxena, R.C. 2008. Bio-fuels from thermochemical conversion of renewable resources: A review. *Renewable and Sustainable Energy Reviews*, 12(2): 504 – 517.

Gressel, J. 2008. Transgenics are imperative for biofuel crops. *Plant Science*, 174(3): 246 – 263.

Gupta, V., Ratha, S.K., Sood, A., Chaudhary, V. & Prasanna, R. 2013. New insights into the biodiversity and applications of cyanobacteria (blue-green algae) – Prospect and challenges. *Algal Research*, 2: 79 – 97.

Henderson R., Parsons, S.A. & Jefferson, B. 2007. The impact of algal properties and pre-oxidation on solid–liquid separation of algae. *Water Research*, 42: 1827 – 1845.

Hense, I. & Beckmann, A. 2005. Towards a model of cyanobacteria life cycle – effects of growing and resting stages on bloom formation of N₂-fixing species. *Ecological Modelling*, 195: 205 – 218.

Hoko, Z. & Makado, P.K. 2011. Optimization of algal removal process at Morton Jaffray water works, Harare, Zimbabwe. *Physics and Chemistry of the Earth*, 36: 1141–1150.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M. & Darzins, A. 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspective and advances. *The Plant Journal*, 54: 621 – 639.

IFR. 2013. <http://www.handbook.ifrf.net/handbook/cf.html?id=23>

Date of access: 30 November 2014.

Jain, S. & Sharma, M.P. 2010. Kinetics of acid catalysed transesterification of *Jatropha curcas* oil. *Bioresource Technology*, 101(20): 7701 – 7706.

Jena, U. Das, K.C. & Kastner, J.R. 2011. Effect of operating conditions on thermochemical liquefaction on biocrude production from *Spirulina plantensis*. *Bioresource technology*, 102 (10): 6221 – 6229.

Karaca, F. & Bolat, E. 2000. Coprocessing of a Turkish lignite with a cellulosic waste material 1. The effect of coprocessing on liquefaction yields at different reaction temperatures. *Fuel Processing Technology*, 64: 47 – 55.

Karaca, F. & Bolat, E. 2002. Coprocessing of a Turkish lignite with a cellulosic waste material 2. The effect of coprocessing on liquefaction yields at different reaction pressures and sawdust/lignite ratios. *Fuel Processing Technology*, 75: 109 – 116.

Khan, S.A., Hussain, M.Z., Prasad, S. & Barnerjee, U.C. 2009. Prospects of biodiesel production from microalgae in India. *Journal of Renewable and Sustainable Energy Reviews*, 13(9): 2361 – 2372.

Küçük, M.M. & Demirbas, A. 1997. Biomass conversion processes. *Energy Conversion and Management*, 38:151-165.

Lee, H., Lee, Y., Lim, J. & Song, E. 2008. Transesterification of RBD palm oil using supercritical methanol. *The Journal of Supercritical Fluids*, 44: 356 – 363.

Leung, D.Y.C., Wu, X. & Leung, M.K.H. 2010. A review on biodiesel production using catalyzed transesterification. *Applied Energy*, 87(4): 1083 – 1095.

Levine, R.B., Bollas, A. & Savage, P.E. 2013. Process improvement for supercritical in situ transesterification of carbonized algal biomass. *Bioresource Technology*, 136: 556 – 564.

Li, D., Chen, L., Xu, D., Zhang, X., Ye, N., Chen, F. & Chen, S. 2012. Preparation and characteristic of bio-oil from marine brown alga. *Bioresource Technology*, 104: 737 – 742.

Li, R. & Watanabe, M.M. 2001. Fatty acid profiles and their chemotaxonomy in planktonic species of *Anabaena* (Cyanobacteria) with straight trichomes. *Phytochemistry*, 57: 727 – 731.

- Lui, W., Wang, C. & Zhengyu Y. 2009. The preparation of high calorific fuel (HCF) from water hyacinth by deoxy-liquefaction, *Bioresource technology*, 100:6451 – 6456.
- Marchetti, J.M., Miguel, V.U. & Errazu, A.F. 2007. Possible methods for biodiesel production. *Renewable and Sustainable Energy Reviews*, 11(6): 1300 – 1311.
- Minami, E. & Saka, S. 2006. Kinetics of hydrolysis and methyl esterification for biodiesel production in two-step supercritical methanol process. *Fuel*, 85: 2479 – 2483.
- McKendry, P. 2002. Energy production from biomass (Part one): An overview of biomass. *Biomass technology*, 83: 37 - 46.
- Mussatto, S.I., Dragone, G., Gulmaraes, P.M.R., Silva, J.P.A., Carnelro, L.M., Roberto, I.C., Vicente, A., Domingues, L. & Teixeira, J.A. 2010. Technological trends, global market, and challenges of bio-ethanol production. *Biotechnology Advances*, 28: 817 – 820.
- .
- Patil, P.D., Gude, V.G., Mannarswamy, A., Cooke, P., Nirmalakhdan, N., Lammers, P. & Deng, S. 2012. Comparison of direct transesterification of algal biomass under supercritical methanol and microwave irradiation conditions. *Fuel*, 97(1): 822 – 831.
- Peterson, A.A., Vogel., F., Lachance, R.P., Fröling, M., Antal., M.J. & Tester, J.W. 2008. Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies. *Energy and Environmental Science*, 1: 32 – 65.
- Prescott, G.W. 1969. *The Algae: A Review*. London: Thomas Nelson and Sons Ltd. 436 p.
- Qu, Y., Wei, X. & Zhong, C. 2003. Structural analysis of bio-oils from sub- and supercritical water liquefaction of woody biomass. *Energy*, 32: 196 – 202.
- Rawat, I., Kumar, R.R, Mutanda, T. & Bux, F. 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy*, 103(3) 444 – 467.
- Reddy, H., Mupanneni, T., Patil, P.D., Ponnusamy, S., Cooke, P., Schaub, T. & Deng, S. 2014. Direct conversion of wet algae to crude biodiesel under supercritical ethanol conditions. *Fuel*, 115(1): 720 – 726.

- Ross, A.B., Biller, P., Kubacki, M.L., Li, H., Lea-Langton, A. & Jones, J.M. 2010. Hydrothermal processing of microalgae using alkali and organic acids. *Fuel*, 89(9): 223 – 243.
- Samniang, A., Tipachan, C. & Kajorncheappun-ngam, S. Comparison of biodiesel production from crude *Jatropha* oil and Krating oil by supercritical methanol transesterification. 2014. *Renewable Energy*, 68: 351 – 355.
- Savage, P.E., Valdez, P.J., Nelson, M.C., Wang, H.Y. & Lin, X.N. 2012. Hydrothermal liquefaction of *Nannochloropsis* sp. Systematic study of process variables and analysis of the product fractions. *Biomass and Bioenergy*, 46: 317 – 331.
- Sharma, K.K., Schumann, H. & Schenk, P.M. 2012. High lipid induction in microalgae for biodiesel production. *Energies*, 5: 1532 – 1553.
- Shin, H.Y., Lee, S.H., Ryu, J.H. & Bae, S.Y. 2012. Biodiesel production of waste lard using supercritical methanol. *The Journal of Supercritical Fluids*, 61(1): 134 – 138.
- Sun, Y., Reddy, H.K., Muppaneni, T., Ponnusamy, S., Patil, P.D., Li, C., Jiang, L. & Deng, S. 2014. A comparative study of direct transesterification of camelina oil under supercritical methanol, ethanol and 1-butanol conditions. *Fuel*, 135: 530 – 536.
- Tiffany, L.H. 1958. *Algae: The Grass of Many Waters*. Springfield: Charles C Thomas. 199 p.
- The Energy Collective. 2014. Are declining oil prices increasing the risk to OPEC, U.S. Energy Security or clean fuels supplies?. www.theenergycollective.com/jemillerep/2146151/ Date of access: 2 November 2014.
- Toor, S.S., Rosendahl, L. & Rudolf, A. 2011. Hydrothermal liquefaction of biomass: A review of subcritical water technologies. *Energy*, 36: 2328 – 2342.
- Vieler, A., Wilhelm, C., Goss, R., Süß, R. & Schiller, J. 2007. The lipid composition of the unicellular green alga *Chlamydomonas reinhardtii* and the diatom *Cyclotella meneghiniana* investigated by MALDI-TOF MS & TLC. *Chemistry and Physics of Lipids*, 150: 143 – 155.
- Vincent, W.F. 2009. Cyanobacteria. Protists, In *Encyclopaedia of Inland Waters* (pp. 226 – 232). Quebec City, QC. Encyclopaedia of Inland Waters.
- Wang, G., Li, W., Li, B., Chen, H. & Bai, J. 2007b. Direct liquefaction of sawdust under syngas with and without catalyst. *Chemical Engineering and Processing*, 46: 187 – 192.
- World Coal Institute. 2014. Coal Facts.

<http://www.worldcoal.org/pages/content/index.aspx?PageID=188> . Date of access: 30 November 2014.

Xu, C. & Etcheverry, T. 2008. Hydro liquefaction of woody biomass in sub- and super-critical ethanol with iron-based catalysts. *Fuel*, 87: 335 – 345.

Xu, C. & Lancaster, J. 2008. Conversion of secondary pulp/paper sludge powder to liquid oil products for energy recovery by direct liquefaction in hot-compressed water. *Water Research*, 42: 1571 – 1582.

Yang, Y.F., Feng, C.P., Inamori, Y. & Maekawa, T. 2004. Analysis of energy conversion characteristics in liquefaction of algae. *Resources, Conservation and Recycling*, 43: 21 – 33.

Yin, S. & Tan, Z. 2012. Hydrothermal liquefaction of cellulose to bio-oil under acidic, neutral and alkaline conditions. *Applied Energy*, 92(1), 234-239.

Yokoyama, S., Suzuki, A., Murakami, M., Ogi, T., Koguchi, K. & Nakamura, E. 1987. Liquid fuel production from sewage sludge by catalytic conversion using sodium carbonate. *Fuel*, 66: 1150 – 1155.

Yuan, X., Wang, J., Zeng, G., Huang, H., Pei, X. Li, H., Liu, Z. & Cong, M. 2011. Comparative studies of thermochemical liquefaction characteristics of micro-algae using different organic solvents. *Energy*, 36: 6406 – 6412.

Zabaniotou, A. & Ionnidou, O. 2008. Evaluation of utilization of corn stalks for energy and carbon material production by using rapid pyrolysis at high temperature. *Fuel*, 87(6): 834 – 843.

Zhang, J., Chen, W.T., Zhan, P., Luo, Z. & Zhang, Y. 2013. Hydrothermal liquefaction of *Chlorella pyrenoidosa* in sub- and supercritical ethanol with heterogeneous catalyst. *Bioresource Technology*, 133: 389 – 397.

3. Experimental

The experimental work conducted in this study is discussed in this chapter. The harvesting and pretreatment methods of the feedstock are discussed in Section 3.1 and characterisation of the feedstock is given in Section 3.2. A description of the materials used in this study is provided in Section 3.3. The experimental method used is provided in Section 3.4 with subsections for each of the three experimental sets. A discussion of the analytical methods used in this study is presented in Section 3.5.

3.1 Harvesting of feedstock



Figure 3-1: Evaporation pond at the sewage plant situated in Bethal, South Africa

Algae samples were harvested from a municipal waste water treatment plant, situated in Bethal, which is located in Mpumalanga (S 26° 29' 19.362" E 29° 27' 11.552"). The vertical rake sampling method was used. The stream harvested is downstream from the main sewage clarifier. Before the point of harvest the algae medium grows in an open pond system, after which it overflows over a weir and then flows into an evaporation pond. The samples were then harvested from the evaporation pond (Figure 3-1) at the point of entry from the open pond.

The samples were then sieved using a 0.5 mm mesh to remove all excess moisture before drying commenced (see Figure 3-2). The filter cake was oven dried overnight at 105°C, to remove all surface moisture.

The total moisture content was determined after drying to be 944.5 g.kg⁻¹ of the wet biomass sample. With the surface moisture being 827.2 g.kg⁻¹ and the inherent moisture content of the biomass being 117.3 g.kg⁻¹ of the wet biomass sample.



Figure 3-2: Sieves for removal of excess moisture.

The dried biomass was coned and quartered (see Figure 3.3) to obtain a homogeneous feedstock for use in experiments (Patnaik, 2004).

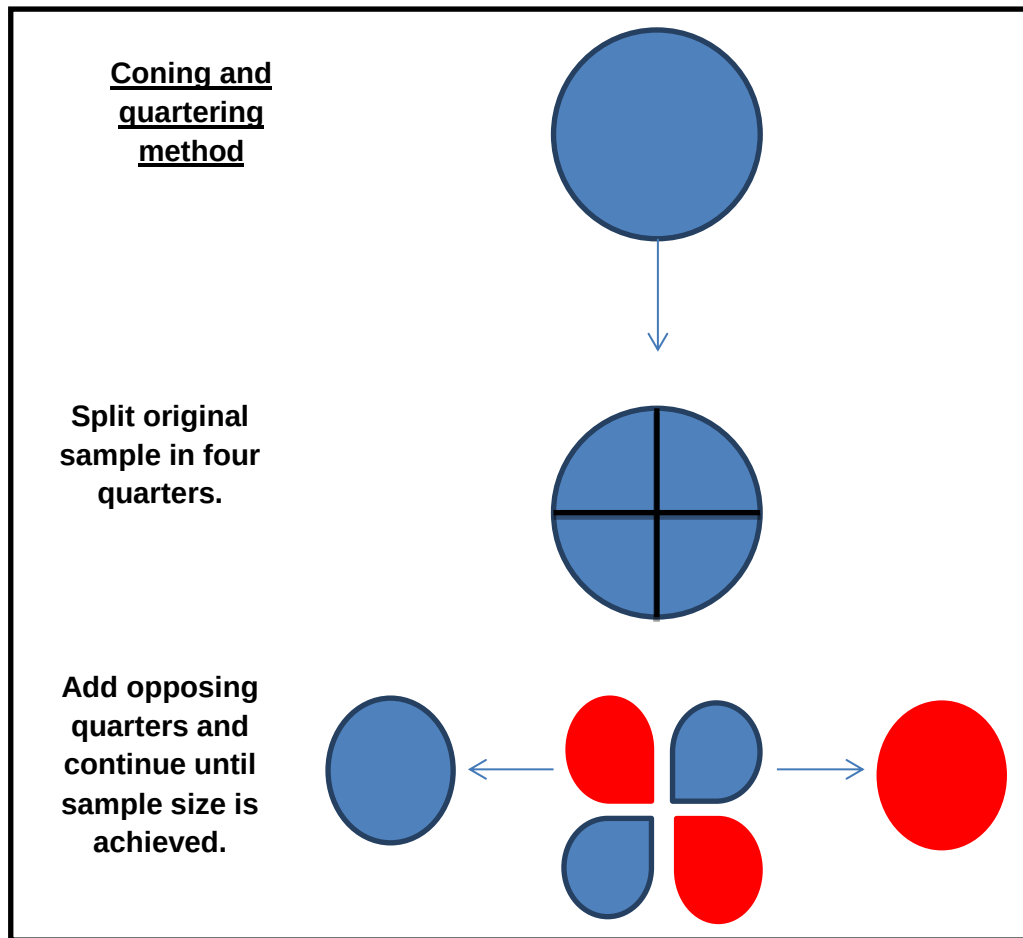


Figure 3-3: Coning and quartering method adapted from Patnaik (2004).

3.2 Classification of algae in feedstock

Classification of the feedstock was done by Dr. Rob van Hille at the Centre for Bioprocess Engineering (CeBER) at the University of Cape Town (UCT). The following procedure was followed for the classification of the algae species:

A protocol of enrichment and isolation of species was followed during the classification process, because of the diverse nature of the waste water. DNA was extracted from the floating macro- and micro-algae and the species present were identified using 18 rRNA gene sequencing.

Micro algal species were then isolated from the bulk liquid using a selective growth media. The isolated species were identified using 18S rRNA gene sequencing.

3.2.1 Characterisation of feedstock

The ash and inherent moisture of the dried feedstock were determined using thermogravimetric analysis (TGA).

The compositional analysis of feedstock was done by the Irene laboratories of the Agricultural Research Council (ARC) and the values of the most important components are given in Table 3.1.

Table 3-1: Compositional analysis of feedstock on a dry basis

Analysis	Method	Unit	Value
Ash	ASTM 048	wt%	20.03
*Protein	Not SANAS accredited	wt%	27.06
Fat (ether extraction)	ASTM 044	wt%	4.50
Neutral detergent fibre, NDF	ASTM 060	wt%	26.13
Acid detergent fibre, ADF	Not SANAS accredited	wt%	24.57
Acid detergent lignin, ADL(Lignin) ^a	Not SANAS accredited	wt%	13.06
Total carbohydrates	ASTM 075	wt%	36.68
Energy	ASTM 053	MJ/kg	16.03
Cellulose ^b	-	wt%	11.51
Hemicellulose ^c	-	wt%	1.56

^a Lignin = ADL, ^b Cellulose = ADF - ADL, ^c Hemicellulose = NDF – ADF

From the compositional analysis it can be seen that the feedstock is a high protein low lignin biomass feed. According to Barreiro *et al.* (2013) and Chen *et al.* (2014) most crude bio-oil yields are made up of lipids, proteins and carbohydrates. The theoretical yield however cannot be determined as the reaction pathways during hydrothermal processes and the interaction between constituents is unclear.

3.3 Materials

Table 3.2 shows the various chemicals used during this study. Chemicals were used as received from the supplier.

Table 3-2: Information on chemicals used

Chemical	Purity (%)	Supplier	Cas no	Purpose
Chloroform	99.0	ACE	67-66-3	Extraction of organic matter after liquefaction/Solvent for GC MS analysis
Acetone	99.0	Sigma-Aldrich	75-09-2	Extraction of organic matter after liquefaction
n-Hexane	99.0	Rochelle chemicals	110-54-3	Extraction of FAME
Dichloromethane	99.0	Sigma-Aldrich	75-09-2	Dissolving oil as preparation for GC analysis
Methanol	99.0	Rochelle Chemicals	67-56-1	Solvent used during liquefaction/Reagent for transesterification reaction
Trimethyl sulfonium hydroxide	0.25 M in methanol	Sigma-Aldrich	17287-03-5	Derivatisation agent for GC analysis of bio-oil composition
Sulphuric acid	98	ACE	7664-93-9	Catalyst for liquefaction
Nitrogen (gas)	99 (UHP)	AFROX	7727-37-9	Reaction atmosphere for liquefaction

3.4 Hydrothermal and thermochemical liquefaction experimental method

The experimental setup for the liquefaction is similar to that mentioned in literature (Liu *et al.*, 2009; Reddy *et al.*, 2014; Rawat *et al.*, 2013; Patil *et al.*, 2012; Li *et al.*, 2014). The autoclave is constructed from 316 stainless steel with a design pressure of 200 bar (see Figure 3.5)

A typical experimental run was done according to the procedure shown in Figure 3-5. First the biomass is weighed and then loaded into the autoclave along with water, methanol or acid catalyst depending on the experimental set. The autoclave is closed and bolted shut with the magnetic stirrer on top. The bolts are torqued to 70 N.m. The autoclave is then pressurised to 20 bar with ultra-high purity (UHP) nitrogen and left at this pressure for 10 minutes to detect any leaks. If no leaks are observed, the gas is slowly released to purge the system. The system is purged 5 times in total.

After the system has been purged the heating jackets are put on the autoclave and the magnetic stirrer and the temperature controllers are switched on. Once the temperature inside the autoclave reaches the set point the temperature inside the autoclave is maintained for 30 minutes. The autoclave is then allowed to cool down to room temperature. The cooling down of the autoclave is assisted with an electrical fan.

Once the autoclave has reached room temperature the gas is vented before the autoclave is opened. After the autoclave is opened, 100 ml of chloroform is added and the autoclave is closed again, where after the magnetic stirrer is switched on for 10 minutes. This is done to dissolve any residual organic matter that is suspended in the autoclave.

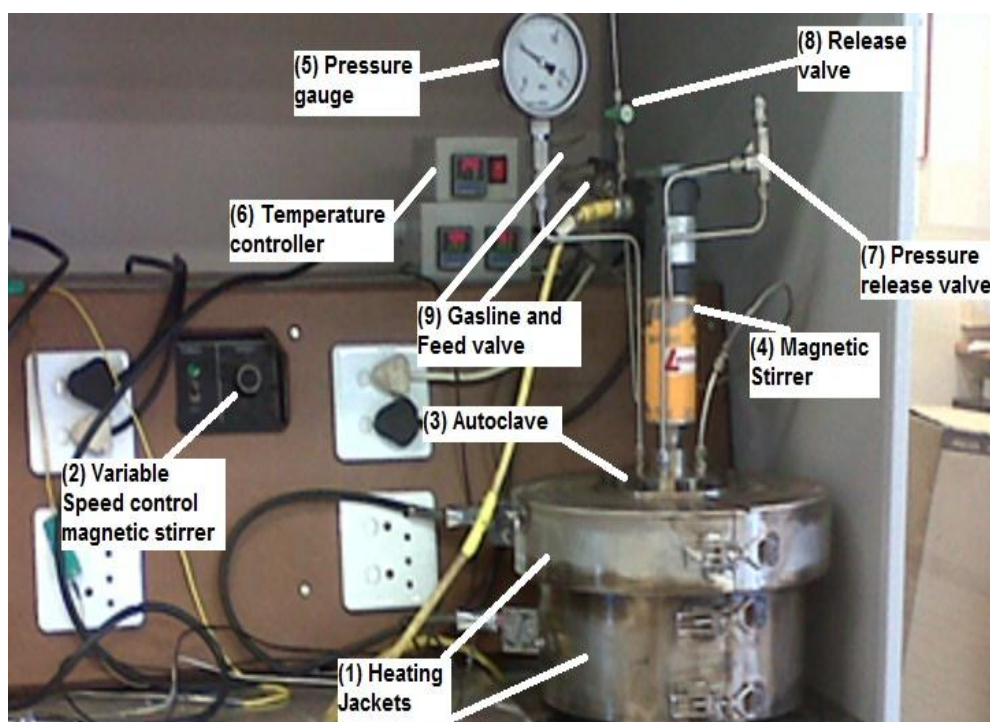


Figure 3-4: Experimental setup for liquefaction

Figure 3-5 shows a representation of the experimental method after liquefaction.

Batch hydrothermal liquefaction (addition of water) of the feedstock was performed as a control to determine the amount of oil that can be extracted from the feedstock. Hydrothermal liquefaction was done at various temperatures to compare yields obtained to previous studies and to validate the experimental method.

A similar process was followed for the sub-critical and supercritical methanol production of biodiesel with the only difference being the addition of methanol as reagent during liquefaction. Figure 3-6 gives a representation of the experiments done and the products obtained in each case.

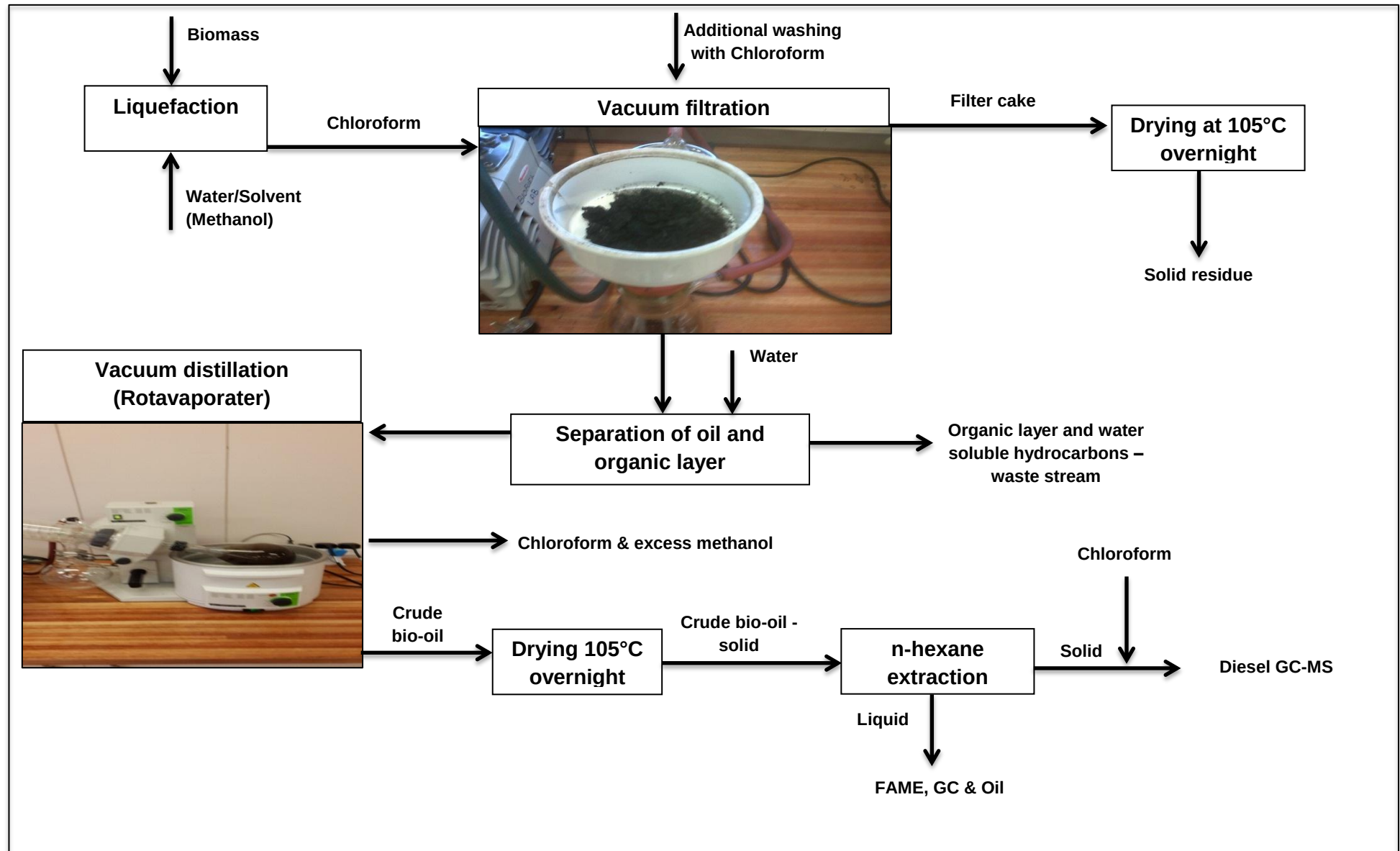


Figure 3-1: Representation of experimental method

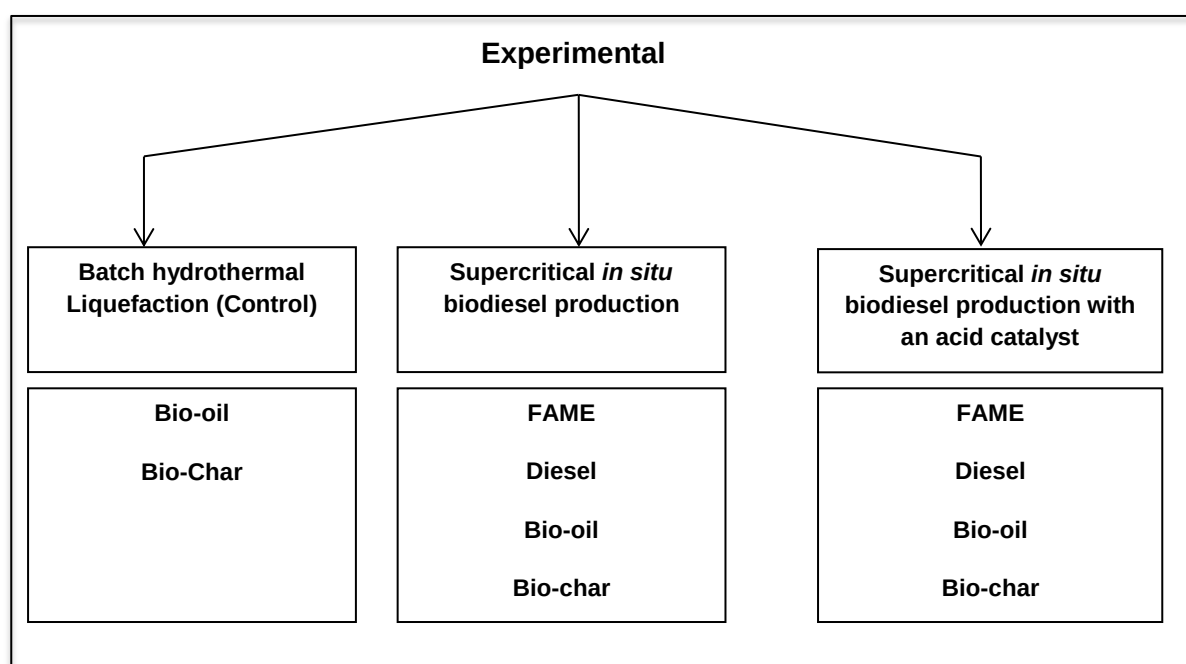


Figure 3-6: Representation of experiments done

3.4.1 Batch hydrothermal liquefaction

During the batch hydrothermal liquefaction the reaction temperature was varied from 240°C to 320°C in intervals of 20°C at a reaction time of 30 minutes and a biomass loading of 500 g.kg⁻¹. The heating and cooling rate of the reactor was 2.15 K.min⁻¹.

3.4.2 *In situ* biodiesel production in supercritical methanol

3.4.2.1 Methanol mass ratio

The methanol to dry biomass mass ratio was investigated (1:1, 3:1, 6:1) over the range of 240°C to 320°C in intervals of 20°C. The biomass loading and residence time were kept constant at 500 g.kg⁻¹ and 30 min respectively. The heating and cooling rate was at 2.15 K.min⁻¹.

3.4.2.2 Reaction temperature

The reaction temperature was varied from 240°C to 320°C in intervals of 20°C. The heating and cooling rate was kept constant at 2.15 K.min⁻¹. The biomass loading was kept at 500 g.kg⁻¹. The effect of the reaction temperature was investigated at three different methanol mass ratios (1:1, 3:1, 6:1).

3.3.1.2 *In situ* biodiesel production in supercritical methanol and acid catalyst

The effect of the addition of an acid catalyst was investigated at 500 g.kg⁻¹ and 1000 g.kg⁻¹ biomass loading in solvent at three different methanol mass ratios (1:1, 3:1, 6:1) and at a single temperature (300°C). The catalyst loading was kept constant at 10 g.kg⁻¹ of dry biomass mass, and the catalyst used was sulphuric acid (H₂SO₄). The residence time, heating and cooling rate was kept constant at 30 minutes and 2.15 K.min⁻¹.

3.5 Analytical methods

3.5.1 Proximate analysis

A thermogravimetric analyser (TGA) was used to determine the inherent moisture content, volatile matter and ash content of the feedstock under a O₂ atmosphere. The certified standard used for the proximate analysis through thermogravimetric analysis is ASTM 7582. A representative sample weighing less than 1 gram was inserted into a proximate analyser. The temperature was then increased to 130°C. The crucible was weighed after three hours and the differential weight of the crucible recorded as the inherent moisture content. The temperature was then ramped up to 450°C to determine the volatile matter (VM). Lastly the temperature was ramped up to 950°C and the crucible weighed again. The fixed carbon was then calculated according to Equation 3.1 (ASTM D3172 standard):

(3.1)

$$FC\%(db) = 100 - (VM\%(db) + ASH\%(db))$$

Where FC% is the fixed carbon mass percentage, VM% is the volatile matter mass percentage, ASH% is the ash mass percentage on a dry basis.

3.5.2 Gas chromatography analysis (GC)

GC analysis was used to determine the composition of the bio-oil and biodiesel samples. An Agilent 7890 GC was used, with an Agilent auto injector, a HP-5MS (100m) capillary column and a flame ionisation detector (FID). Figure 3-7 shows the GC (1) with the auto injector (2).

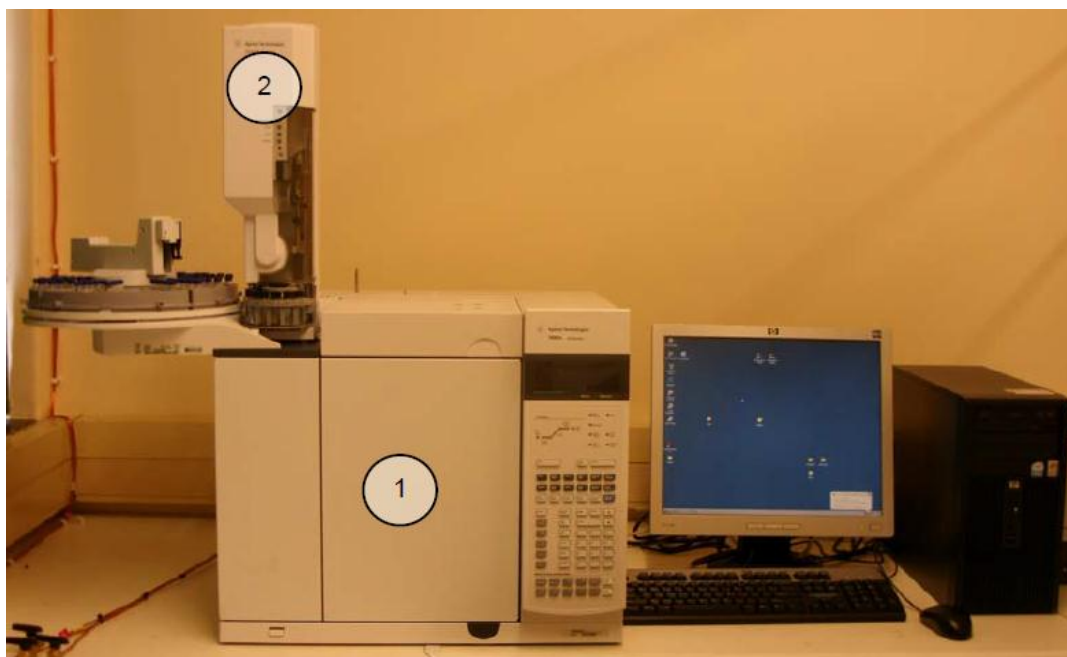


Figure 3-7: GC setup

The GC analysis conditions were: inlet temperature of 275 °C; injection volume of 0.2 μL (auto injection); oven programming of 90°C for 4 min, 90 to 300°C at 10°C.min⁻¹, 300°C for 20 min; FID detector at 350°C; H₂ flow rate of 40 mL.min⁻¹, make-up He flow rate of 10 mL.min⁻¹ and air flow rate of 400 mL.min⁻¹.

3.5.4 GC coupled to mass spectrometry (GC-MS) analysis

GC-MS was used to qualitatively determine other hydrocarbons present in the oil sample. The GC-MS used in this study was an AGILENT 5975 μs .

3.6 References

- Barreiro, D.L., Prins, W., Ronsse, F. & Brilman, W. 2013. Hydrothermal liquefaction (HTL) of microalgae of biofuel production: State of the art review and future prospects. *Biomass and Bioenergy*, 53: 113 – 127.
- Chen, W., Lin, B., Huang, M. & Chang. 2014?. Thermochemical conversion of microalgal biomass into biofuels: A review. *Bioresource Technology*, (In press).
- Li, H., Liu, Z., Zhang, Y., Li, B., Lu, H., Duan, N., Liu, M., Zhu, Z. & Si, B. 2014. Conversion efficiency and oil quality of low-lipid high-protein and high-lipid low-protein microalgae via hydrothermal liquefaction. *Bioresource Technology*, 154(1): 322 – 329.
- Lui, W., Wang, C. & Zhengyu Y. 2009. The preparation of high calorific fuel (HCF) from water hyacinth by deoxy-liquefaction. *Bioresource technology*, 100 (24): 6451 – 6456.
- Patil, P.D., Gude, V.G., Mannarswamy, A., Cooke, P., Nirmalakhandan, N., Lammers, P. and Deng, S. 2012. Comparison of direct transesterification of algal biomass under supercritical methanol and microwave irradiation conditions. *Fuel*, 97(1): 822 – 831.
- Patnaik, P. 2004. *Dean's analytical chemistry handbook*. 2nd edition. New York: McGraw-Hill. 1193 p.
- Rawat, I., Kumar, R.R, Mutanda, T. & Bux, F. 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy*, 103(3) 444 – 467.
- Reddy, H., Mupanneni, T., Patil, P.D., Ponnusamy, S., Cooke, P., Schaub, T. & Deng, S. 2014. Direct conversion of wet algae to crude biodiesel under supercritical ethanol conditions. *Fuel*, 115(1): 720 – 726.

4. Results and Discussions

In this chapter an in-depth discussion is given of all the results obtained from the hydrothermal liquefaction and *in situ* biodiesel production with supercritical methanol. Section 4.1 provides results obtained from the classification of the feedstock, while Section 4.2 gives the experimental error for the bio-oil and bio-char yields.

The results are presented in Section 4-3. The results for *in situ* biodiesel production are presented in Section 4.3.1, with subsequent sections for the effect of temperature, solvent ratio and acid catalyst. Section 4.3.1.4 shows the effect of time spent passed the supercritical point of methanol on the bio-oil yield. In Section 4.3.2 the results for hydrothermal liquefaction are presented.

Section 4.4 presents a summary and concluding remarks on the results obtained in this study.

4.1 Classification of algae in feedstock

Classification of the feedstock was done by Dr. Rob van Hille at the Centre for Bioprocess Engineering Research (CeBER) at the University of Cape Town (UCT). It was found during initial investigation that the feedstock was more complex than was originally thought. Initial investigation under light microscopy showed the following morphologies as seen in Figure 4.1.

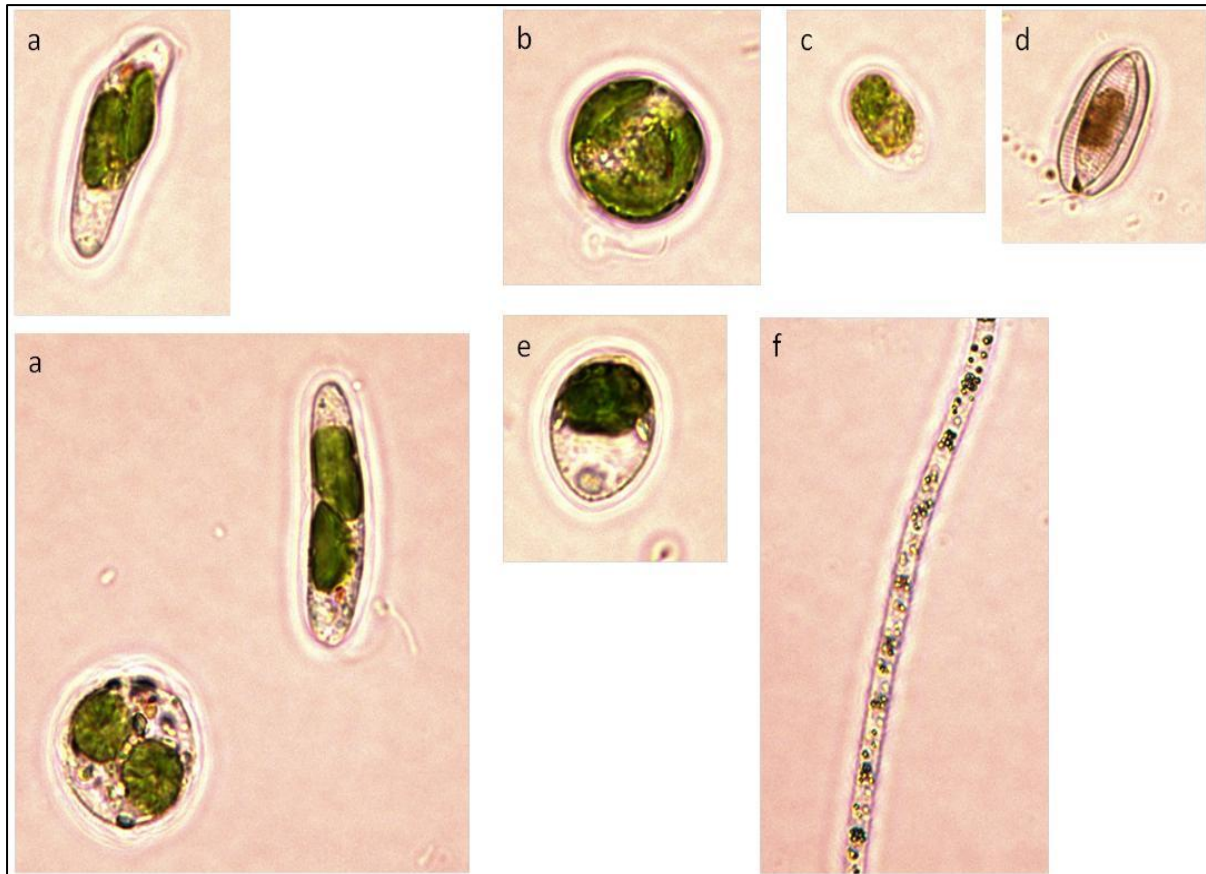


Figure 4-1: Examples of cell morphologies observed in mixed culture. All images were taken using 100x objective lens.

Where:

- (a) – was found to be able to slowly change shape from spherical form to the elongated form, indicating the absence of a rigid cell wall. It could thus be a protozoan that feeds on micro-algae and accumulates the pigment of the ingested cells.
- (b), (c) and (e) are all unicellular green algae, representing three distinct species, based on relative cell and chloroplast size.
- (d) is a fresh water diatom.
- (f) is a multicellular algal or cyanobacteria specie.

A multicellular macro algal floating organism also observed under light microscopy is shown in Figure 4-2.

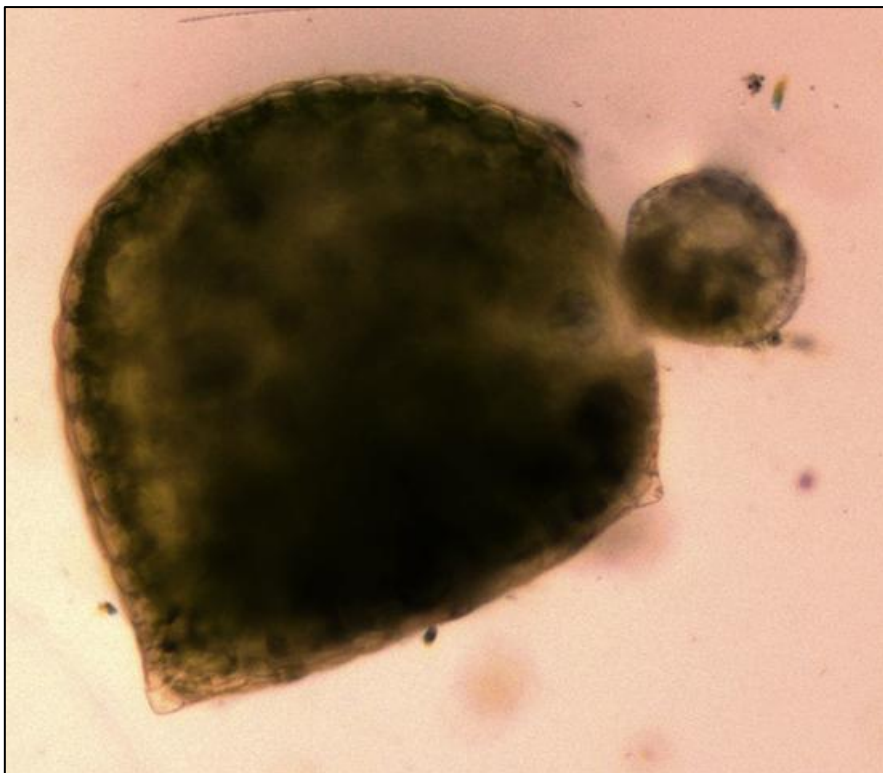


Figure 4-2: Microscope image of floating organism (10x magnification)

The plated micro-algae (see Figures 4-3 and 4-4) were proven to be *Nostoc* sp. and *Chlamydomonas*. In Figure 4-3 the elongated morphologies is a typical representation of *Nostoc*. *Nostoc* is a genus of cyanobacteria.

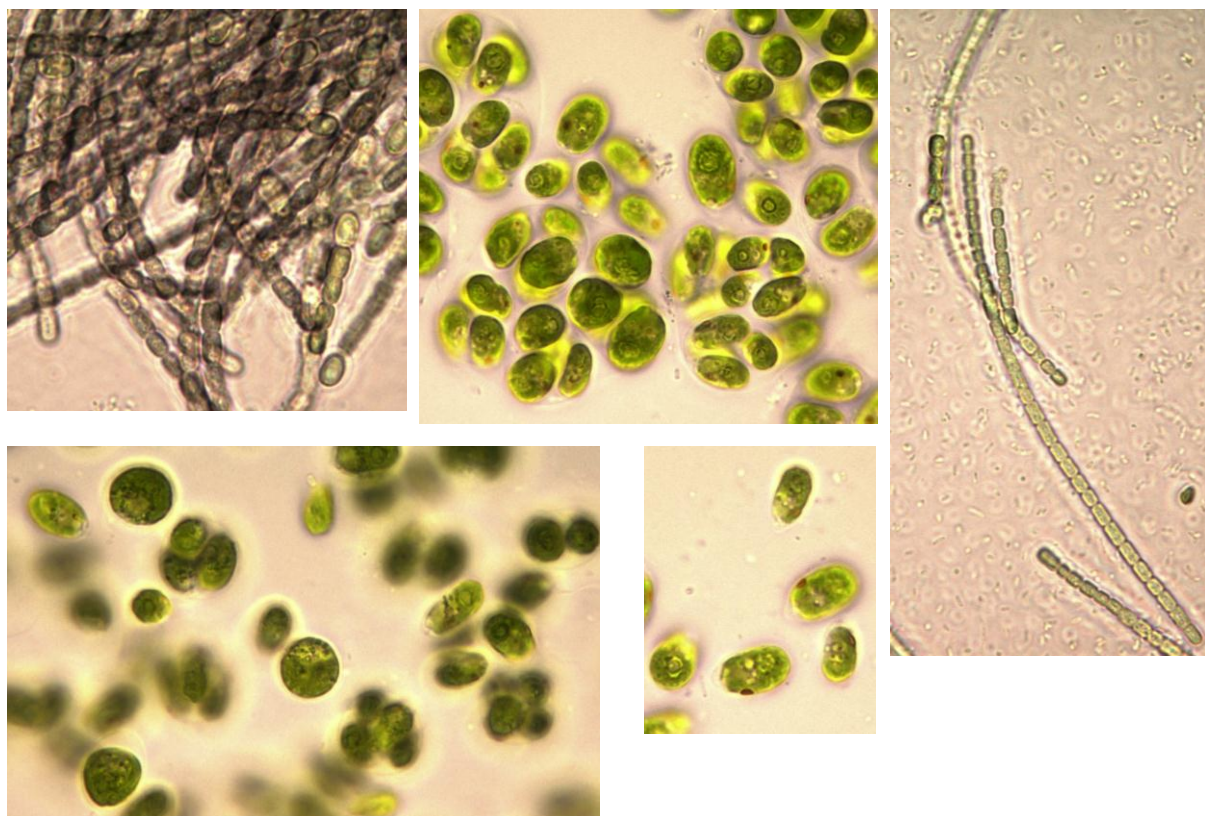


Figure 4-3: Micro-algae cell morphologies after plating

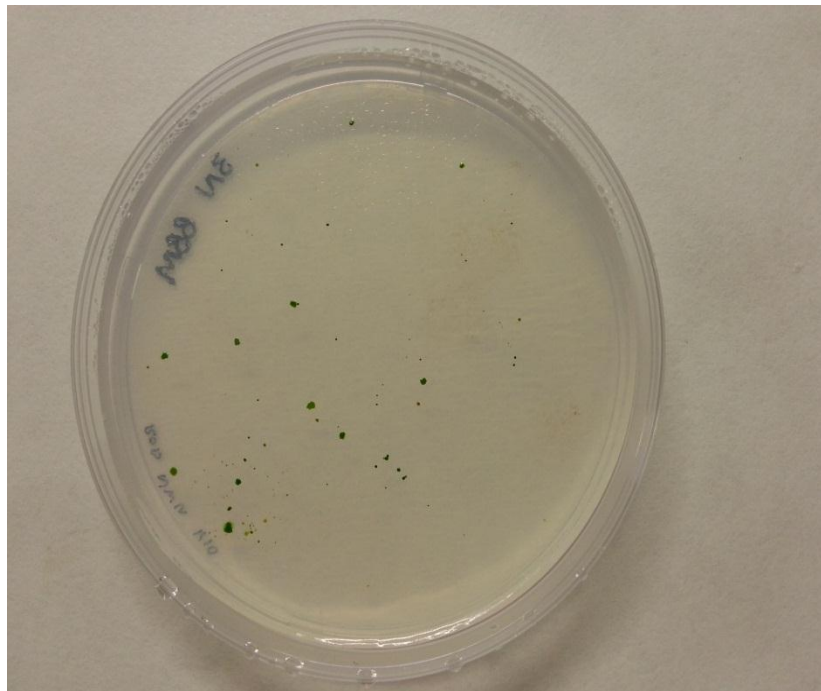


Figure 4-4: Plating of micro-algae

4.2 Experimental Error

The experimental error for this study was determined by repeating the same experiment 5 times at fixed conditions. The experimental error for the thermochemical experiments was calculated on both the oil/biodiesel yield (post n-hexane extraction) and on the char yield. The error was calculated for a 95% confidence interval. Calculation steps are shown in APPENDIX A of this document. For the bio-oil/FAME yield the experimental error was determined as 8.57 % and for the bio-char yield 5.12 %.

4.3 Effect of reaction variables on crude bio-oil yield

4.3.1 *In situ* biodiesel production

4.3.1.1 The effect of temperature and solvent on bio-oil composition and yield

The main objective of this study was *in situ* biodiesel production from a waste water clarifier effluent stream. This Section provides the results of the *in situ* investigation, by showing the different bio-oil yields and the oil composition. The effect of temperature and methanol to dry biomass mass ratio on thermochemical liquefaction is also presented in this Section.

The crude bio-oil yield for thermochemical liquefaction (with supercritical methanol) and for hydrothermal liquefaction is shown in Figure 4-4 with the compositional analysis of the crude bio-oil shown in Figure 4-5.

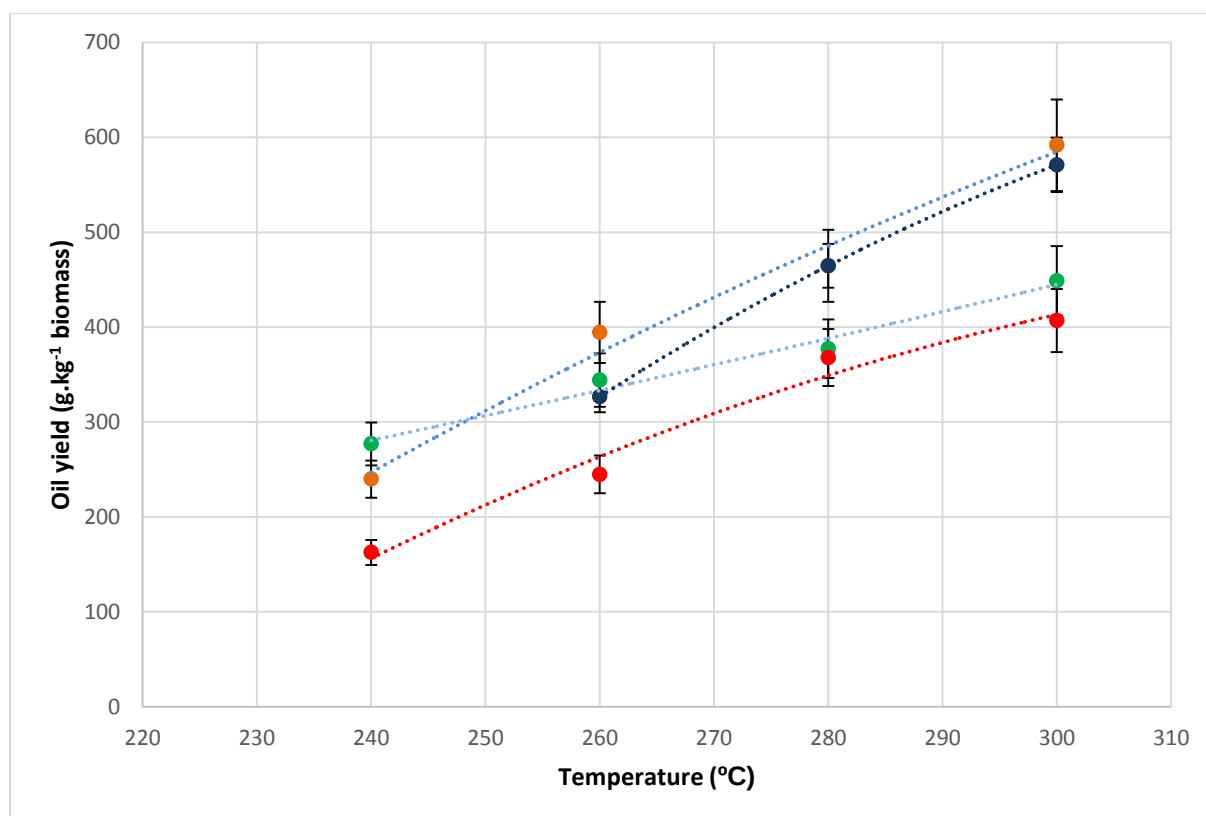


Figure 4-5: Influence of temperature and methanol to dry biomass ratio on the crude bio-oil during liquefaction with supercritical methanol at a biomass loading of 500 g.kg⁻¹ and residence time of 30 min. (● - Hydrothermal liquefaction; ● - 6:1 methanol ratio; ● - 3:1 methanol ratio; ● - 1:1 methanol ratio)

The crude bio-oil yield is shown for 240°C to 300°C for the three different methanol to dry biomass mass ratios (1:1, 3:1, 6:1) as well as for hydrothermal liquefaction. All these experiments were conducted at a constant residence time of 30 minutes with a biomass loading of 500 g.kg⁻¹.

From Figure 4-5 it can be seen that with an increase in temperature there is a linear increase in the crude bio-oil yield. The increase in yield with an increase in temperature is similar to that found in literature (Yang *et al.*, 2014; Zhang *et al.*, 2013; Duan *et al.*, 2013; Barreiro *et al.*, 2013, Chen *et al.*, 2014). The highest yield of 591 g.kg⁻¹ was achieved with a 3:1 methanol to dry biomass ratio at 300°C.

Initially at lower temperatures only carbonisation reactions will occur after which the biomass constituents are broken down into smaller fragments (Chen *et al.*, 2014; Barreiro *et al.*, 2013). Barreiro *et al.* (2013) and Aysu and Kukuc (2013) states that decomposition of hemicellulose and cellulose will start at temperatures higher than 250°C. Thus initially the increase in oil would be due to the recovery of the lipids present in the biomass after which

the increase can be attributed to the degradation of the biomass constituents. The gas yields (taken as the remaining product fraction after bio-oil and biochar yield) declines initially with the increase in temperature which could indicate that the H₂ and N₂ content present are used to aid in the reactions taking place.

After 280°C the increase in bio-oil yield can be attributed to the decomposition of the protein (Barreiro *et al.*, 2013). The compositional analysis of the feedstock (Section 3.2) showed that the feedstock is high in protein and has low lignin content. The biomass constituents degrade by means of deoxygenation, decarboxylation and decarbonylation as the temperature increase the intermolecular forces starts to decrease which then allow for quicker degradation of the feedstock. The compounds formed from the decomposition is then stabilised by the free radicals available and leads to the formation of bio-oil. At higher temperatures higher quantities of free radicals is expected as the hydrogen bonds in the solvents becomes weaker thus higher amounts of H⁺ ions would be present.

Another possible explanation is the critical properties of the solvent (Aysu and Kucuk, 2013; Barreiro *et al.*, 2013). For water and methanol as the temperature increase the permittivity decrease which leads to increased solubility. As the temperature increase the hydrogen bonds would become weaker does giving higher availability of H⁺ and OH⁻ which will take part in hydrolysis reactions (Yang *et al.*, 2014; Zhang *et al.*, 2013; Duan *et al.*, 2013; Barreiro *et al.*, 2013; Chen *et al.*, 2014).

From Figure 4-5 it can further be seen that a higher methanol to dry biomass mass ratio results in a higher crude bio-oil yield. The highest bio-yields of 448 g.kg⁻¹, 592 g.kg⁻¹ and 571 g.kg⁻¹ were obtained at a temperature of 300°C for the 1:1, 3:1 and 6:1 methanol to dry biomass ratio, respectively. The maximum operating temperature of 300°C was used due to the properties of methanol resulting in very high pressures at temperatures above 300°C. If the bio-oil yields at a 3:1 and a 6:1 methanol to dry biomass ratio are compared at higher temperatures, the difference is negligible.

The increased yield with the increase of solvent can be explained by the hydrogen bonds present in methanol (polar protic solvent). These hydrogen bonds would essentially result in free hydrogen radicals (H⁺) forming by decomposition of the intermolecular hydrogen bonds. These free radicals would then aid in hydrocracking of the proteins, lipids and carbohydrates (Yuan *et al.*, 2011; Yang *et al.*, 2014; Aysu & Kucuk, 2013). Thus hydrogen donation would essentially result in higher bio-oil yields. Methanol would also aid in the decomposition of the glycosidic bonds present in cellulose. The interactions between the polar protic solvent and the biomass constituents would explain why higher yields are obtained compared to normal hydrothermal liquefaction. As the temperature increase, the decomposition of certain

biomass constituents becomes more apparent. Barreiro *et al.* (2013) stated that higher temperatures will favour the decomposition of proteins while Aysu and Kucuk, (2013) stated that hemicellulose and cellulose start to decompose at temperatures above 250°C. This could explain the difference between the 3:1, 6:1 and 1:1 mass ratio yields. As the temperature starts to aid in the decomposition of the biomass constituents (reducing the strength of the intermolecular forces) the amount of hydrogen available to aid in hydrolysis and hydrocracking can induce a higher rate of decomposition. This is seen in the trend after 260°C where the higher methanol mass ratio shows a higher rate of conversion, while with the 1:1 ratio the rate of conversion slightly starts to decline (Aysu & Kucuk, 2013; Yuan *et al.*, 2011).

The higher bio-oil yields for lower temperature for the 1:1 ratio might be explained by the temperatures being below the point of decomposition. At 240°C it will be expected that the effect of methanol ratios will be negligible as the temperature is still too low for liquefaction to take place (Barreiro *et al.*, 2013; Chen *et al.*, 2014; Aysu & Kucuk, 2013, Yuan *et al.*, 2011). As stated earlier up until 280°C it is expected that mainly carbonisation reactions occur along with the recovery of the lipid content with only small amounts of degradation of biomass constituents taking place.

The rate of biomass conversion for the 6:1 ratio between 280°C and 300°C is slightly higher than the rate of conversion for the 3:1 ratio. This would indicate that the additional amount of methanol allow for quicker decomposition of the biomass constituent. However, the difference in bio-oil yield between the 6:1 and 3:1 ratio would suggest that at a certain point the amount of solvent present does not have an effect anymore on the yield, thus a point of saturation would be reached. Meaning that additional methanol pass the point of saturation would not necessarily increase the bio-oil yield seeing that at lower ratios the amount of hydrogen bonds present to aid the reactions taking place is sufficient. The 6:1 ratio would however have a large effect on the composition of the bio-oil.

Figure 4-6 shows the compositional analysis of the crude bio-oil obtained at 300°C for the three different methanol ratios.

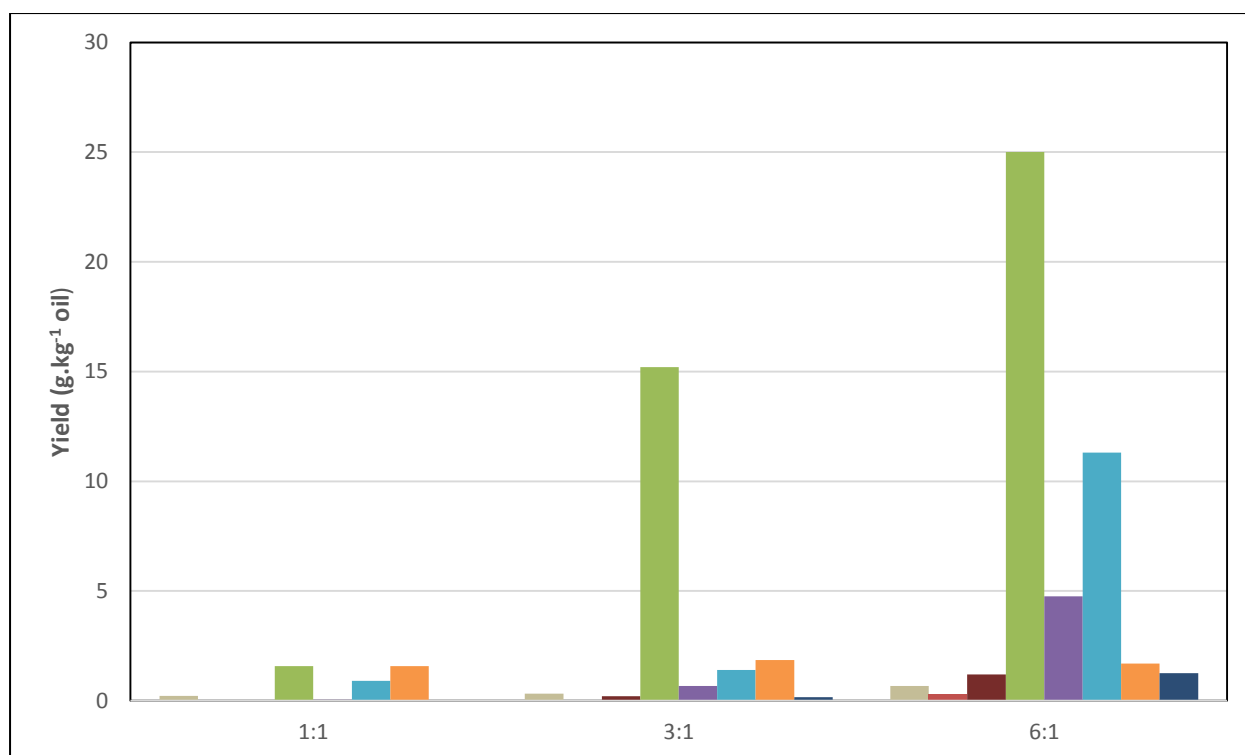


Figure 4-6: Influence of methanol to dry biomass ratio on the FAME yield and triglyceride yields at 300°C (— C12 — C14 — C14:1 — C16:0 — C16:1 — C18:0 — C18:1 — C18:2)

From Figure 4-6 it can be seen that as the methanol to biomass mass ratio increase the amount of FAME formed also increases (Minama and Saka, 2006; Ehimen *et al.*, 2009). It can also be noted that complete *in situ* biodiesel production was only achieved with a methanol to dry biomass mass ratio of 6:1. The highest quality biodiesel was obtained at the 3:1 methanol ratio with a value of 15.2 g.kg⁻¹ and low yields of unsaturated FAME. As the methanol ratio is increased from 3:1 to 6:1 the amount of unsaturated fatty acids (C_{16:1}, C_{18:2}) increases drastically, as well as the quantity of C₁₈ formed. The higher quantities of C_{16:1} to C_{18:2} would indicate that some carbon groups attached to C₁₆ to form the C₁₈ chains.

The higher yield with the addition of methanol might be due to the excess amounts of methanol present to aid in the transesterification of the triglycerides (Minama and Saka, 2006; Ehimen *et al.*, 2009). The change in properties would then be ascribed to the extra hydrogen present and the different reactions taking place during liquefaction. The hydrogen will aid in the stabilisation of the components formed (Yang *et al.*, 2014; Zhang *et al.*, 2013; Duan *et al.*, 2013; Barreiro *et al.*, 2013, Chen *et al.*, 2014).

Figure 4-7 shows the effect of temperature on the FAME yield. The result shown is for the 3:1 methanol to biomass mass ratio at a constant residence time of 30 min.

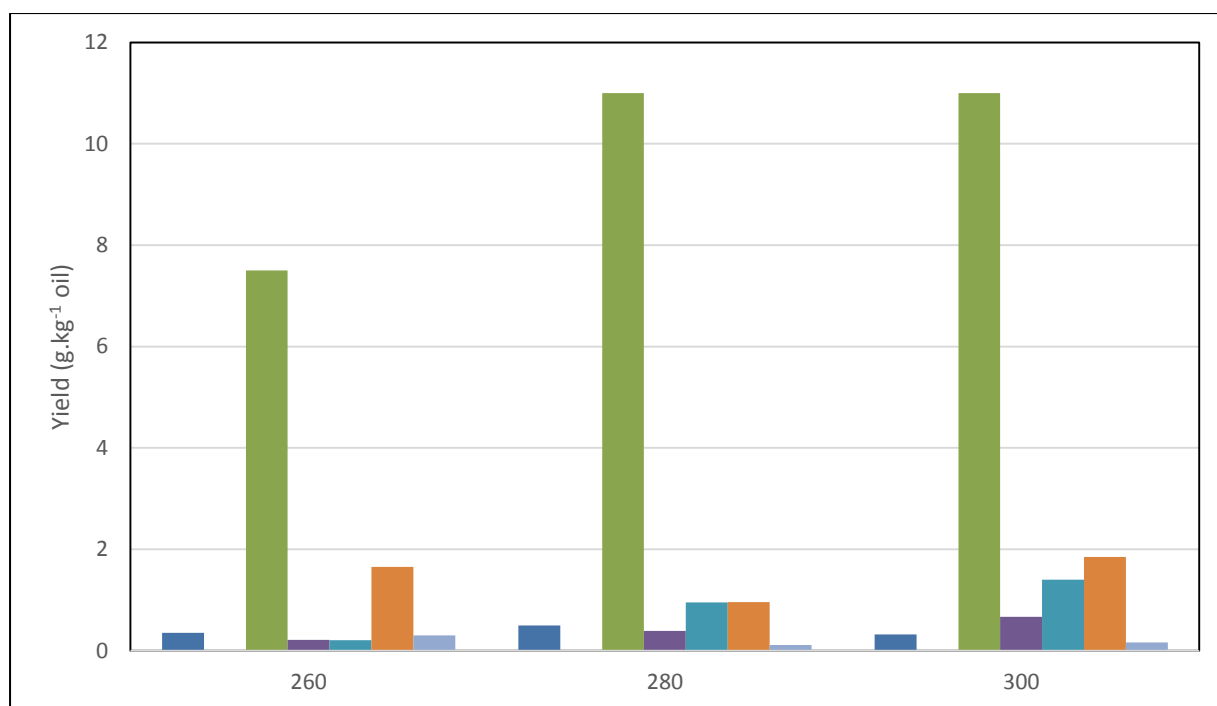


Figure 4-7: Influence of temperature on FAME yield for the 3:1 methanol to dry biomass ratio (— C12 — C14 — C16:0 — C16:1 — C18:0 — C18:1 — C18:2)

From Figure 4-7 it can be seen that with an increase in temperature the FAME yields increase due to the carbonization reactions as mentioned earlier up until 280°C after the methyl palmitate yield starts to decline slightly. The highest FAME present is methyl palmitate which comes primarily from the algal oils (Hu *et al.*, 2008).

The higher yield of FAME at higher temperatures can be explained by the fact that at higher temperature (above methanol's critical point) the oils and the methanol would mix into one miscible fluid (Asri *et al.*, 2013; Sun *et al.*, 2014). According to Arrhenius law as temperature increase the reaction rate would increase (Asri *et al.*, 2013). Thus if the increased reaction rate is combined with the fact that at higher temperatures higher amounts of oil is produced it would explain the higher yields of FAME at higher temperatures.

At 260°C the primary FAME present is methyl palmitate. From 260°C to 280°C there is a decrease in the methyl oleanate but an increase in methyl stearate, which could indicate that some of the double bonds broke or two carbons were removed and is in the higher methyl palmitate yields or evaporated into the gas phase. The decrease in methyl palmitate between 280°C and 300°C may be attributed to the condensation of carboxylic components from the biogas that would also explain the increase in methyl stearate.

Figures 4-8 and 4-9 shows a boiling point curve set up from the GC-MS results for the 1:1 and 3:1 methanol to dry biomass mass ratios.

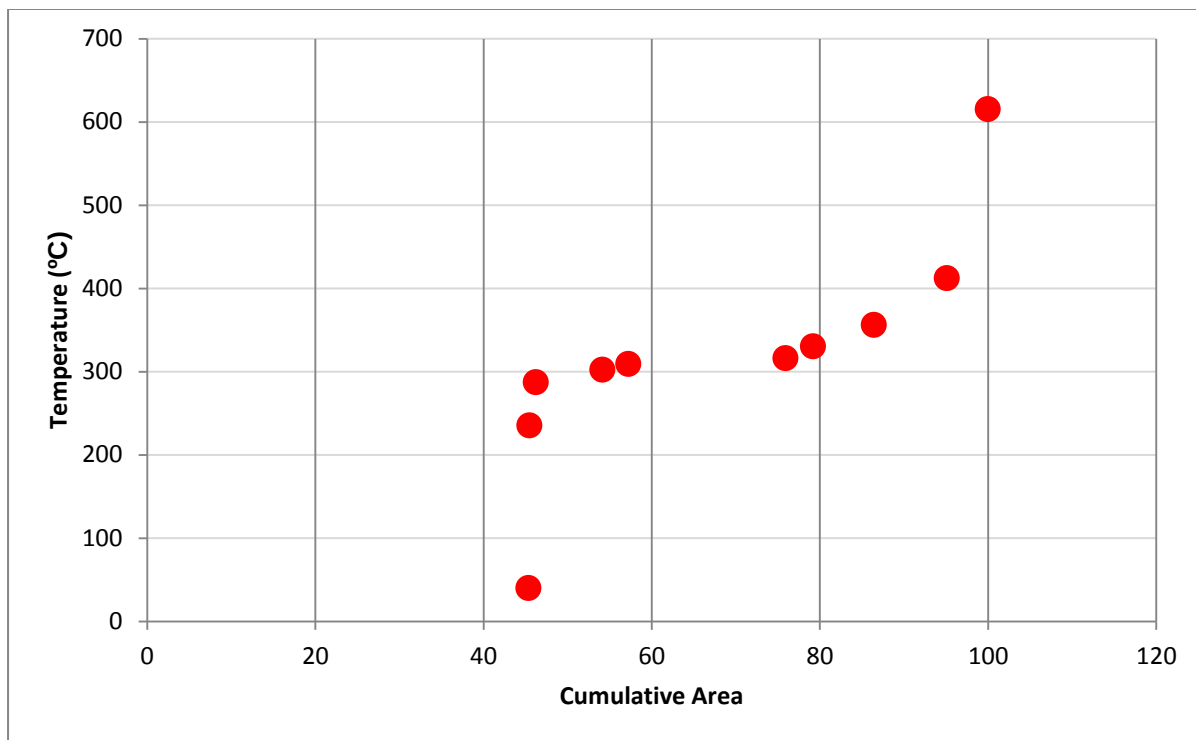


Figure 4-8: Distillation curve for the 1:1 methanol ratio at 300°C

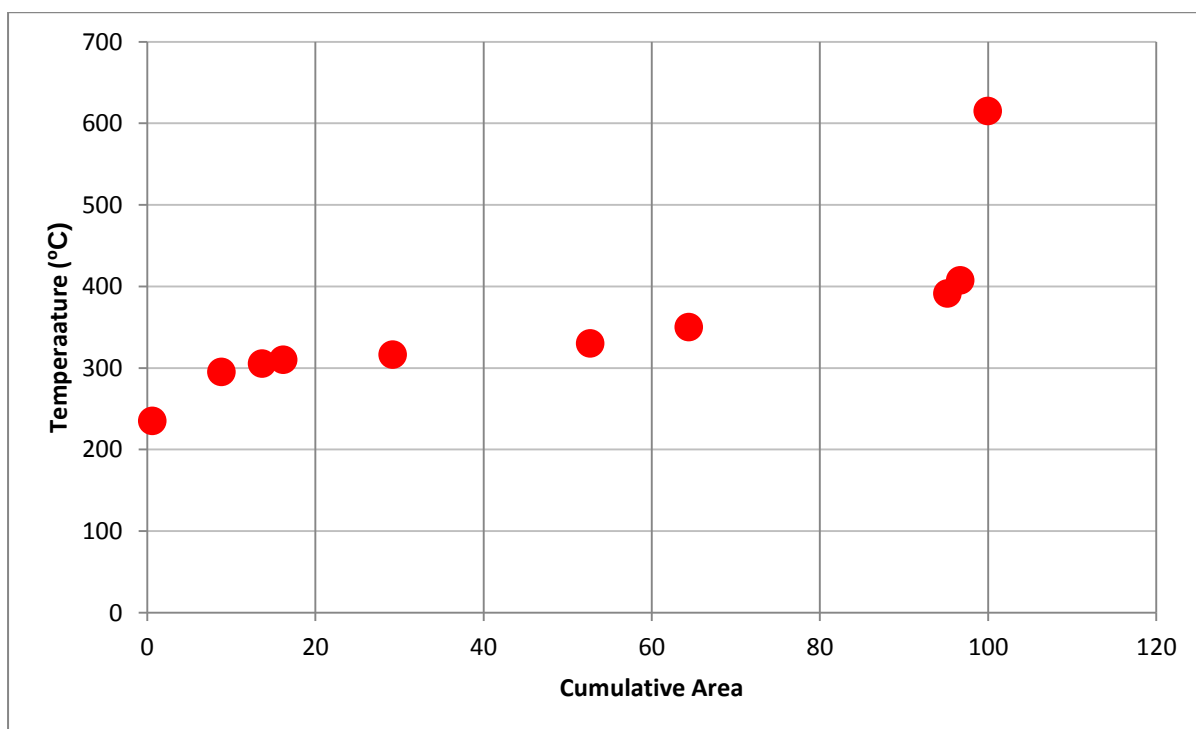


Figure 4-9: Distillation curve for the 3:1 methanol ratio at 300°C

From these Figures it is evident that the highest concentration of components present in the bio-oil yield is between a boiling point of 270°C and 420°C which indicates the presence of long chain carbon bonds still present in the bio-oil. The GC-MS results also revealed the presence of C₁₅ and C₁₇ alkenes present which might indicate that the bio-oil could be used as a renewable diesel.

A similar trend is seen in Figure 4-9 than seen in Figure 4-8, with the highest concentration of components present in the bio-oil being between a boiling point of 270°C and 420°C.

Figure 4-10 shows the effect of temperature and methanol to dry biomass mass ratio on the HHV of the bio-oil.

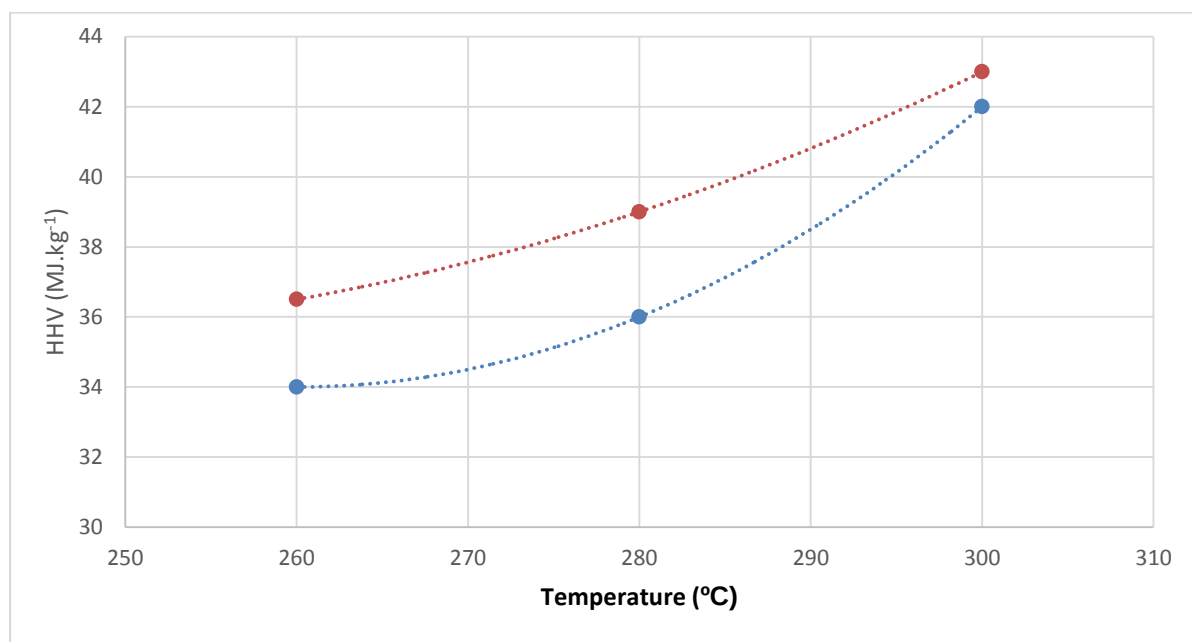


Figure 4-10: Effect of temperature on the HHV for the 1:1 and 6:1 methanol to dry biomass mass ratios (● 1:1; ● 6:1)

From Figure 4-10 it can be seen that as the temperature of conversion increase the HHV increases. This can only be due to deoxygenation of the biomass constituents (Barreiro *et al.*, 2013, Chen *et al.*, 2014). The same explanation would be valid for the increase in HHV for the increase in methanol to dry biomass mass ratio.

4.3.1.2 The effect of temperature and solvent on bio-char yield

The bio-char yield for thermochemical liquefaction (with supercritical methanol) and for hydrothermal liquefaction is shown in Figure 4-11.

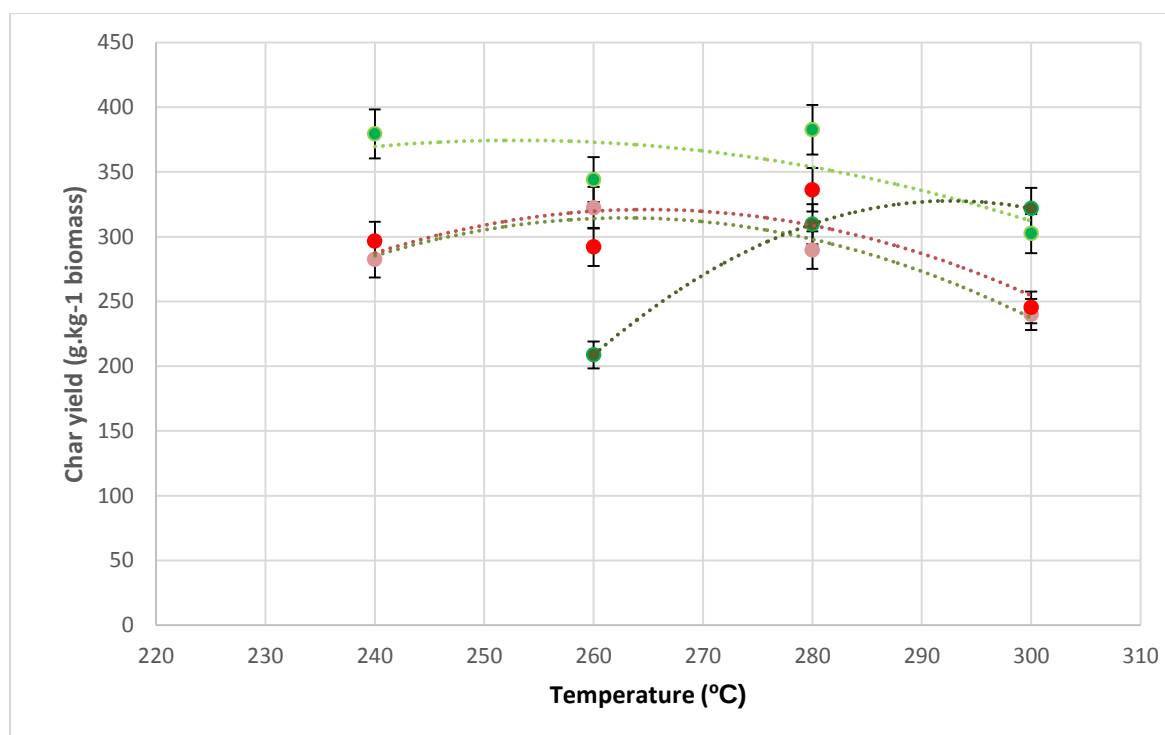


Figure 4-11: Influence of temperature and methanol to dry biomass ratio on the bio-char yield during liquefaction with supercritical methanol at a biomass loading of 500 g.kg⁻¹ and residence time of 30 min. (● 6:1 methanol ratio; ● hydrothermal liquefaction; ● 3:1 methanol ratio; ● 1:1 methanol ratio)

The bio-char yield is shown for 240°C to 300°C in intervals of 20°C for the three different methanol to dry biomass mass ratios (1:1, 3:1, 6:1) as well as for hydrothermal liquefaction all experiments were conducted at a constant residence time of 30 minutes.

From Figure 4-11 it can be seen that the char yield initially increase up to 280°C after which it starts to decline, except in the case of the 6:1 ratio. The yield for the 1:1 methanol to dry biomass mass ratio was the highest, which indicates a decrease in bio-char yield with an increase in the methanol ratio.

According to literature, the bio-char is made up from the lignin content and the ash content of the biomass (Barreiro *et al.*, 2013; Chen *et al.*, 2014, Huan *et al.*, 2013). The bio-char yield obtained in this study is much higher than literature states. Barreiro *et al.* 2013 states that for algae biomass the bio-char yield is usually around 100 g.kg⁻¹ of the biomass. However, Huang *et al.* (2013) found a high bio-char yield in the case of liquefaction of sewage sludge and *spirulina sp.* (approximately 454 g.kg⁻¹ and 254 g.kg⁻¹ respectively), which correlates with the findings in this study. Huang *et al.* (2013) found that some of the hemicellulose also formed char.

Bio-char is formed as a result of repolymerisation taking place if the hydrogen free radicals are not sufficient to stabilise the smaller fragments formed during carbonisation reactions. Repolymerisation reactions can also lead to the reformation of long chain polymers (Barreiro *et al.*, 2013; Chen *et al.*, 2014; Aysu & Kucuk, 2013). A lower bio-char yield would thus be expected with the addition of methanol, seeing that there is more hydrogen available for stabilisation of the components formed, thus inhibiting repolymerisation and aiding in the decomposition of the biomass constituents. The decrease in bio-char yield from 280°C would then be explained by the degradation of the biomass constituents.

4.3.1.3 The effect of an acid catalyst on the bio-oil yield and bio-char yield

The effect of an acid catalyst (H_2SO_4) on the bio-oil yield is shown in Figure 4-12.

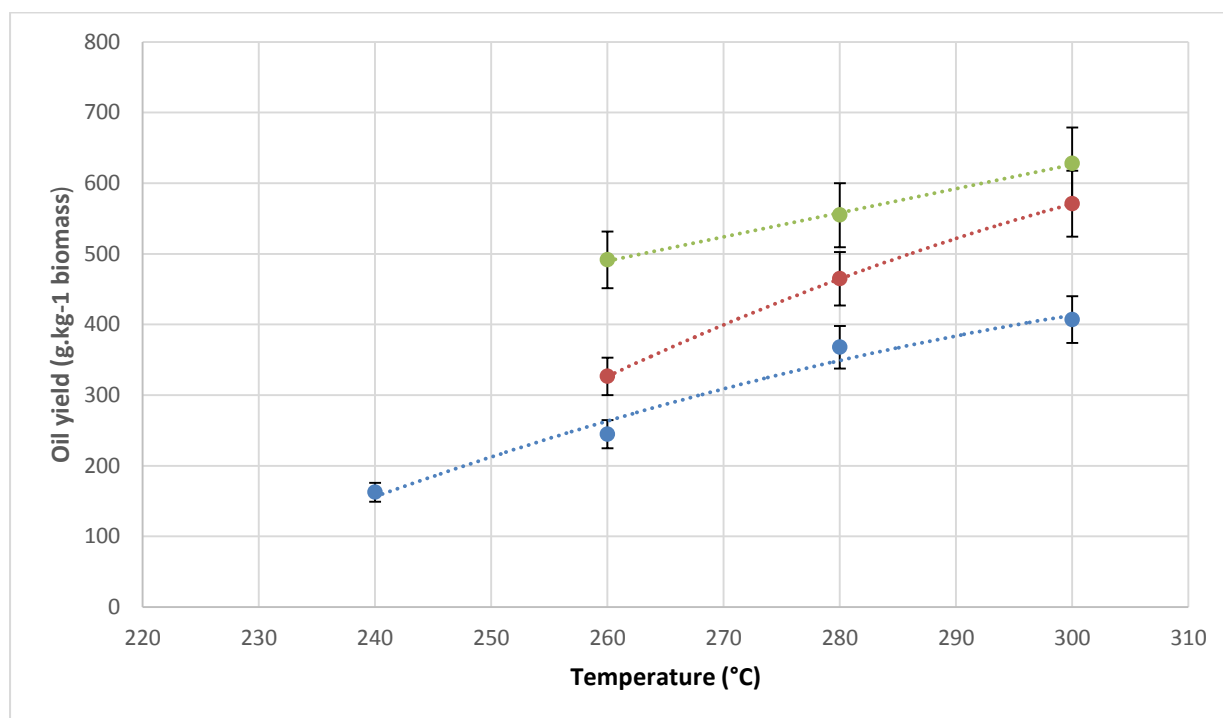


Figure 4-12: Influence of and an acid catalyst on the bio-oil yield during liquefaction with supercritical methanol at a biomass loading of 500 g.kg⁻¹ and residence time of 30 min. (● hydrothermal liquefaction; ● 6:1 methanol ratio; ● Acid catalyst)

The bio-oil yield for the set with an acid catalyst, for the 6:1 methanol ratio as well as for hydrothermal liquefaction is presented in Figure 4-12. The set with an acid catalyst was performed at three different temperatures namely, 260°C, 280°C and 300°C. From Figure 4-12 it can be noted that for all temperatures the bio-oil yield with a catalyst was significantly higher than for the 6:1 and hydrothermal liquefaction bio-oil yield.

For *in situ* biodiesel production, Ehimen *et al.* (2009) found that the addition of an acid catalyst is favourable for bio-oil yield. The dissolution of the acid would aid in the stabilisation of the fragments formed from liquefaction as well as inhibit the formation of saponification products formed by the transesterification of FFA, if there is a high occurrence of organic salts in the feedstock (Ehimen *et al.*, 2009). To inhibit possible reactions between methanol and FFA would be a possible explanation for the increased yield as products of saponification would be lost in the aqueous phase.

Barreiro *et al.* (2013) states that the role of acid catalyst in liquefaction is still unsure as some studies reported that the acid catalyst is not recovered, which would mean it takes part as a reagent rather than a catalyst.

The effect of an acid catalyst (H_2SO_4) on the bio-oil composition is shown in Figure 4-13.

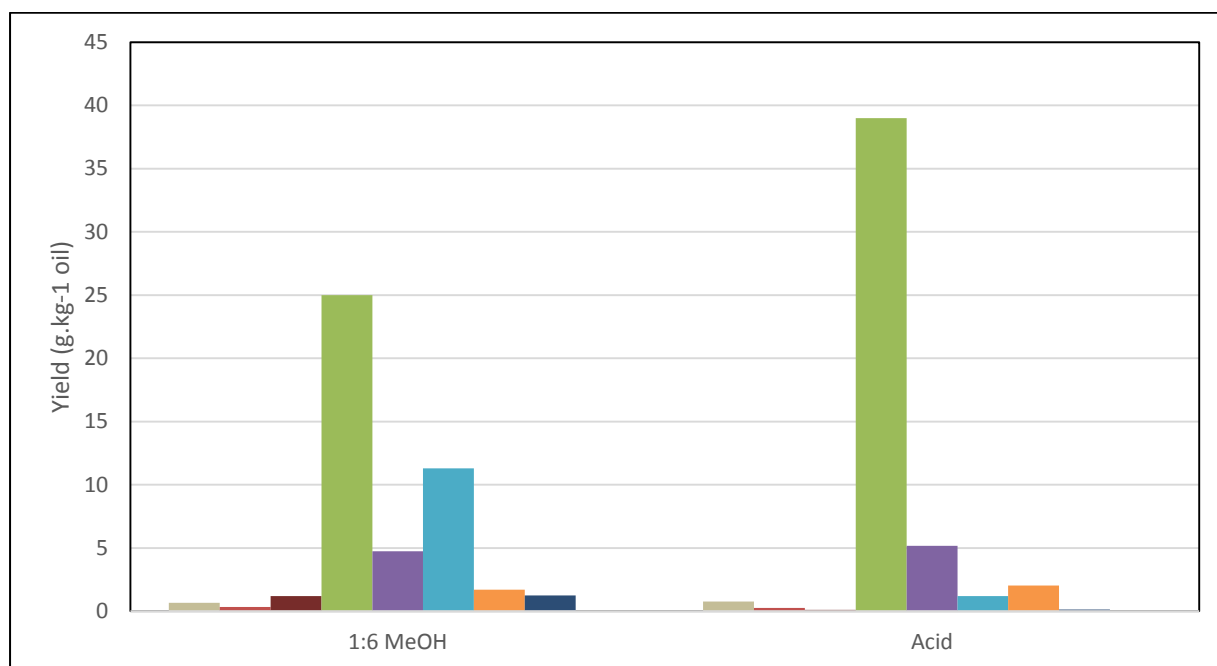


**Figure 4-13: Influence of temperature on the FAME yield and triglyceride yields at 300°C (— C12
— C14 — C16:0 — C16:1 — C18:0 — C18:1 — C18:2 — C18:3)**

From Figure 4-13 it can be seen that the methyl palmitate yield increases with the addition of an acid catalyst while there is a decrease in the methyl stearate. The increase in methyl palmitate indicates that two carbons. The carbons are lost by evaporating into the gas phase or repolymerizing into bio-char. This would be due to the reactions taking place during liquefaction thus the catalyst might aid in the hydrolysis reactions hence the decrease in the unsaturated FAME yields.

The effect of an acid catalyst (H_2SO_4) on the bio-char yield is shown in Figure 4-14.

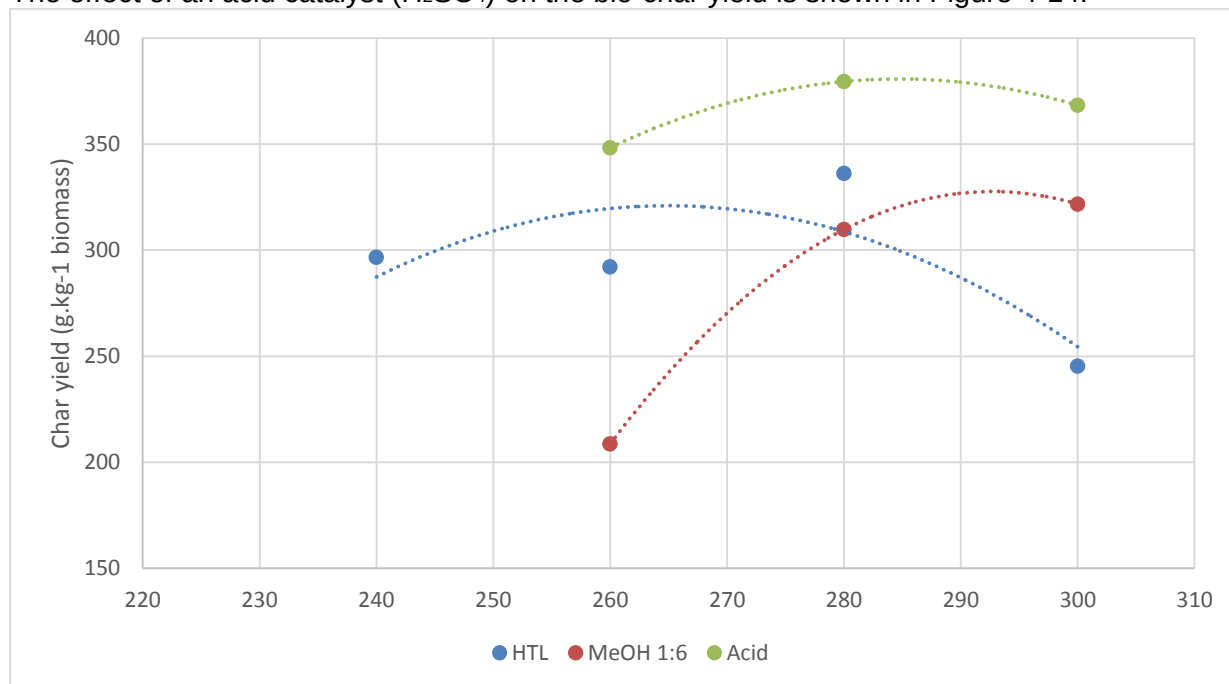


Figure 4-14: Influence of an acid catalyst on the bio-char yield during liquefaction with supercritical methanol at a biomass loading of 500 g.kg^{-1} and residence time of 30 min. (● Hydrothermal liquefaction; ● 6:1 methanol ratio; ● Acid catalyst)

The bio-char yield for the set with an acid catalyst, for the 6:1 methanol ratio as well as for hydrothermal liquefaction is presented in Figure 4-14.

From Figure 4-14 it can be seen that the bio-char yield is higher at all temperatures with the presence of an acid catalyst. The bio-char follows a similar pattern than for the other experimental sets. Higher bio-char yield would suggest that repolymerisation of the smaller compounds produced by liquefaction reactions or condensation of the gas compounds that repolymerise if there is not sufficient hydrogen present to stabilise these compounds (Barreiro *et al.*, 2013; Chen *et al.*, 2014; Aysu and Kucuk, 2013).

4.3.1.4 The effect of time spent in supercritical fluid on the bio-oil yield

Figure 4-15 illustrates the time spent for each temperature range above the supercritical point of methanol.

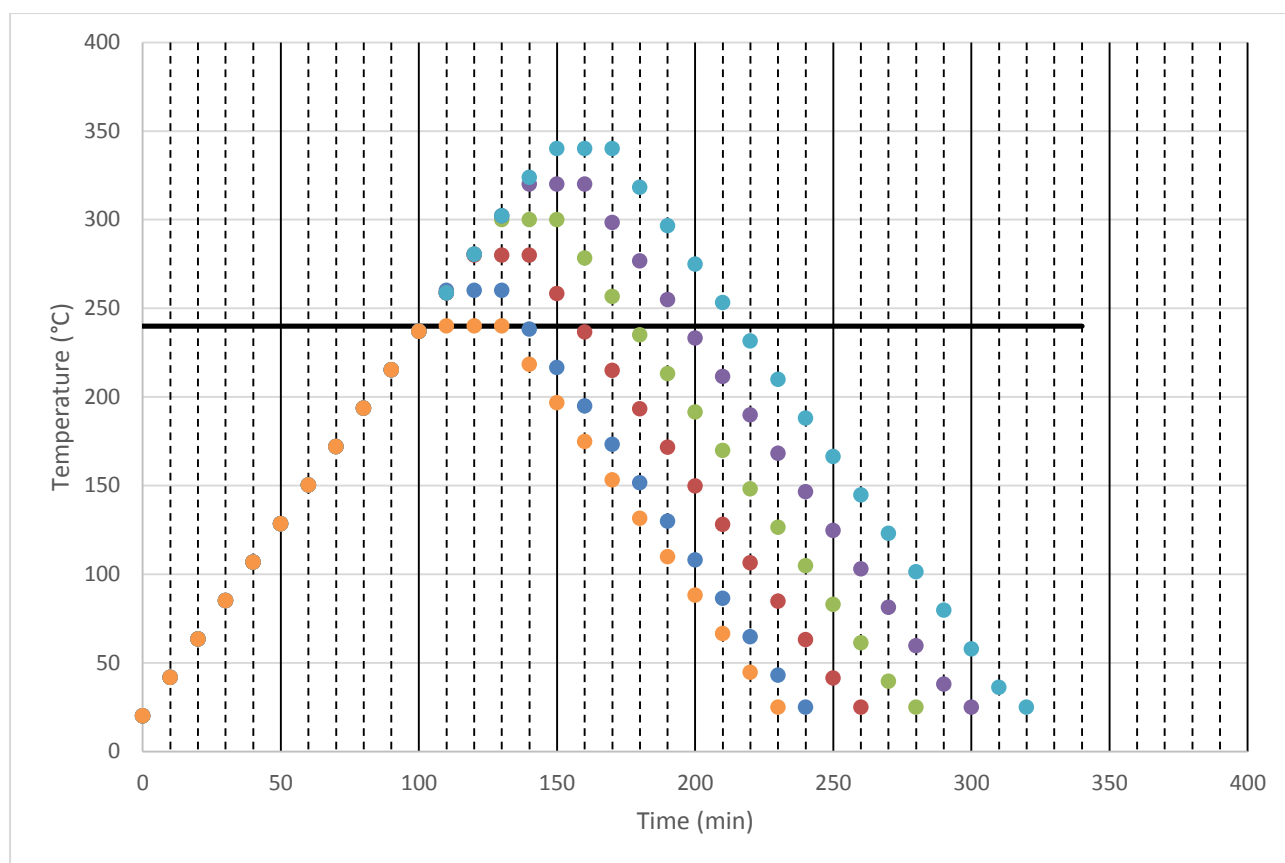


Figure 4-15 Influence of acid an acid catalyst on the bio-char yield during liquefaction with supercritical methanol at a biomass loading of 500 g.kg^{-1} and residence time of 30 min. (— 240°C — 260°C — 280°C — 300°C — 320°C — 340°C , — critical temperature of methanol)

In literature, the effect of residence time has been debated as some studies show that an increase in residence increases the yields obtained and other studies show that at higher temperatures longer residence times effect the bio-oil yield negatively (Barreiro *et al.*, 2013; Chen *et al.*, 2014; Jena *et al.*, 2011).

Figure 4-15 shows the time spent above the critical point of methanol (240°C) for each temperature set (240°C - 340°C in intervals of 20°C). The temperature profile for each set shown in Figure 4-15 was calculated using the heating rate of 2.17 K.min^{-1} and the ambient temperature of 20°C . The new temperature after each minute was then determined up until the set temperature was reached. The temperature was then kept constant at the residence time of 30 minutes, after which the reactor was allowed to cool down to room temperature at 2.17 K.min^{-1} . From Figure 4-15 the time spent above the critical point of methanol was determined and used to plot the effect on the bio-oil yield (shown in Figure 4-16 for each of the three methanol to dry biomass mass ratios). The yields shown in Figure 4-16 is the bio-char, bio-oil and bio-gas yields determined under thermochemical liquefaction as presented in Section 4.3.1.

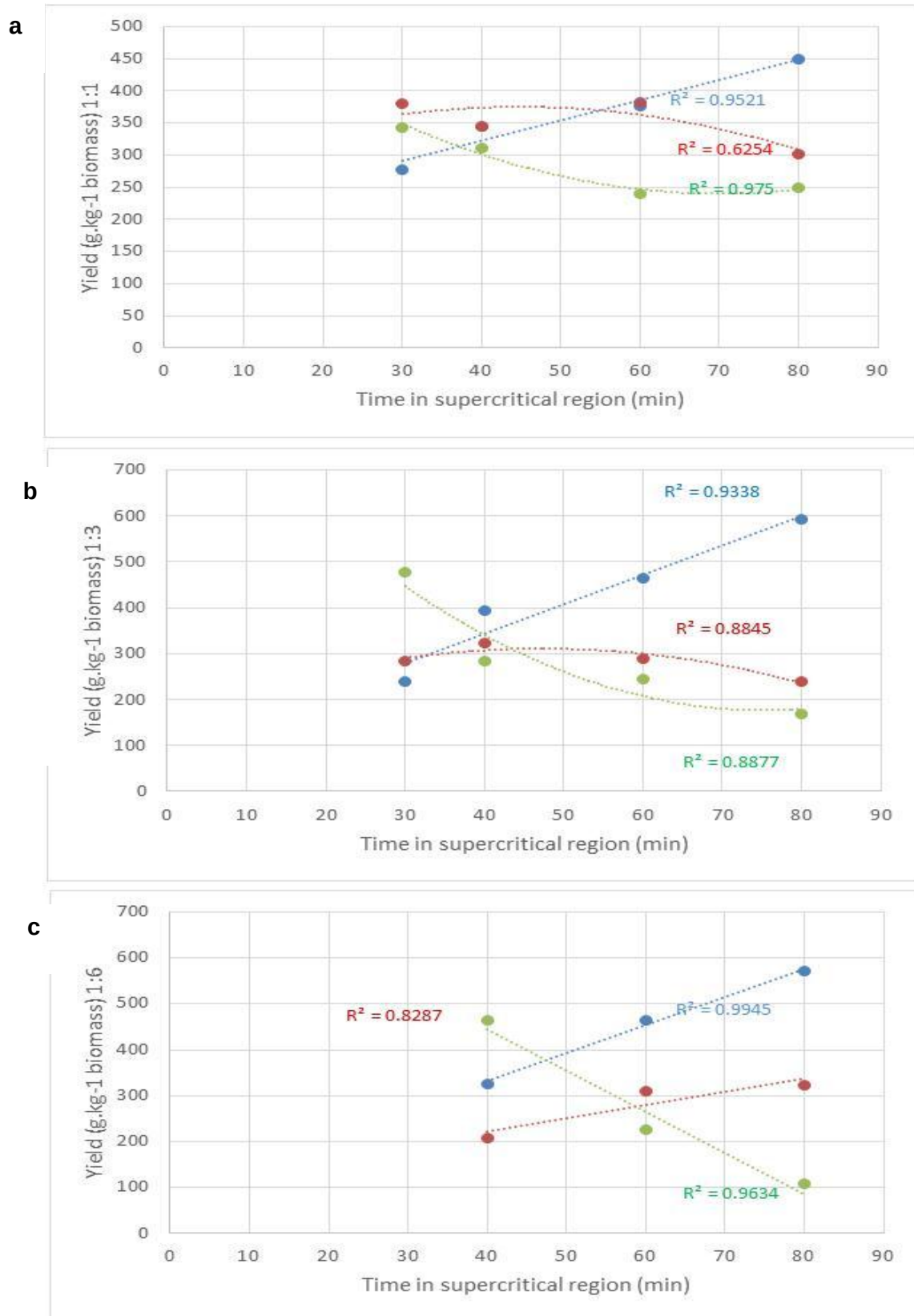


Figure 4-16: Effect of time spent in supercritical fluid for the different methanol to dry biomass mass ratios (a) 1:1, (b) 3:1, (c) 6:1. (● bio-oil; ● bio-char; ● bio-gas)

Figure 4-16 shows that the time spent in supercritical conditions does affect the product distribution. The bio-oil yield shows a linear increase with the time spent passed the supercritical point of methanol. For the 3:1 and 1:1 methanol to dry biomass mass ratios (presented in Figure 4-16a and b) the bio-char yield decrease with an increase in time, the bio-gas yields follows a similar pattern with a slight increase in the bio-gas yield at 80 minutes for the 1:1 methanol ratio. The 6:1 methanol ratio (Figure 4-16c) shows a linear increase for the bio-char, with a sharp decrease in bio-gas yield.

According to Minami and Saka (2006) as methanol approaches its critical point the permittivity and dielectric constant is reduced. The lower permittivity would essentially resulting higher solubility by the supercritical fluid. This supercritical region would thus aid by reducing the internal mass transfer limitations. According to literature (Asri *et al.*, 2013; Sun *et al.*, 2014) the supercritical methanol and oil would form a single miscible fluid which will aid in the conversion of the biomass. This might be a possible explanation for the trends seen in Figure 4-16. As the time increases for the solvent to interact with the oil and simultaneously aid in the conversion of the biomass.

4.3.2 Hydrothermal liquefaction

Hydrothermal liquefaction was performed as a control set for the *in situ* biodiesel production. This Section provides the results for the hydrothermal liquefaction.

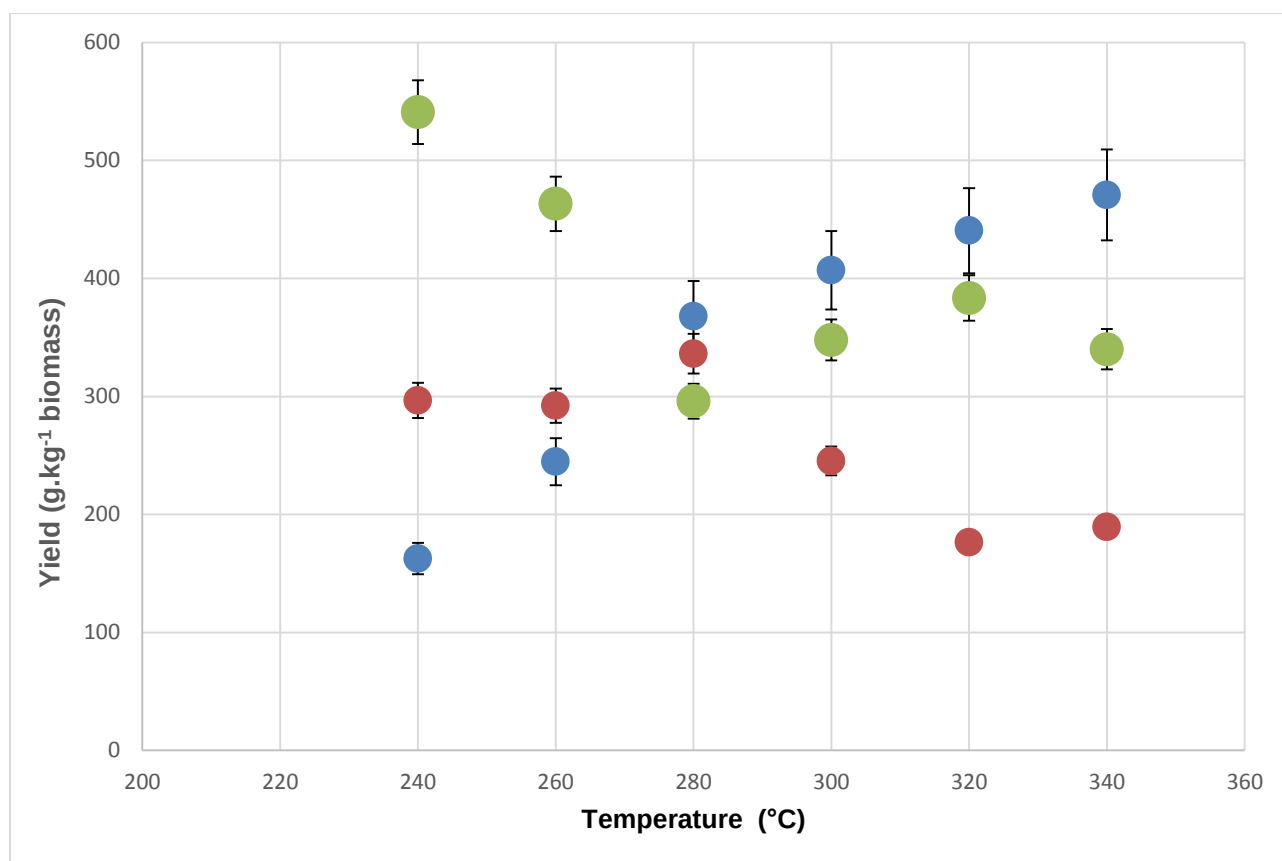


Figure 4-17: Influence of temperature on product distribution for hydrothermal liquefaction at a biomass loading of 500 g.kg⁻¹ and residence time of 30 min (● Biochar, ● Bio-oil, ● Biogas)

The product distribution from 240°C to 340°C is shown in Figure 4-17. All the runs were at a constant residence time of 30 minutes.

From Figure 4-17 it can be seen that the bio-oil yield increase as temperature increases up to 340°C. The bio-char yield increases up until 280°C, after which it starts to decline. The optimum value for the bio-char at 280°C is 363.2 g.kg⁻¹ biomass. The gas yield initially declines as the bio-oil and bio-char yield increases, after which it increases. This trend coincide with the findings of previous studies on hydrothermal liquefaction (Yang *et al.*, 2014; Zhang *et al.*, 2013; Duan *et al.*, 2013; Barreiro *et al.*, 2013, Chen *et al.*, 2014).

A closer look at the product yields, in Figure 4-17, gives a possible reaction mechanism. The increase in bio-oil can be divided into three regions, namely: below 280°C, between 280°C and 320°C and above 320°C. In the first region the increase in bio-char and bio-oil increases more or less linearly. The optimum bio-char yield is then reached at 280°C. The first region indicates that only carbonisation takes place, which will favour the bio-char yield. The optimum yield for bio-char at 280°C coincide with literature (Barreiro *et al.*, 2013) stating that charring is completed at 280°C. In this first phase only the volatile components will be

driven off and the H_2 will be used in the carbonisation reactions. The lipid content will then be released, which explains the increase in bio-oil yield.

In the second region liquefaction will start to take place. Above $250^{\circ}C$, cellulose and hemicellulose would start to decompose through deoxygenation, dehydrogenation, decarbonylation and decarboxylation (Barreiro *et al.*, 2013; Chen *et al.*, 2014). Thus, in this phase depolymerisation would start to occur, which means that oil constituents are formed through stabilisation of the fragments produced. This explains the slight increase in the oil and gas yields. The char yield then decreases as the biomass constituents still present at $280^{\circ}C$ starts to degrade into the oil and gas. The third phase above $320^{\circ}C$, can be seen as a pyrolysis region as the oil yield slightly declines and the char yield increases. During pyrolysis light cracking of the oil into the gas phase takes place, which explains the decrease in bio-oil yield (Barreiro *et al.*, 2013; Chen *et al.*, 2014).

Figure 4-18 presents the triglyceride content present in the bio-oil (the FAME yield shown was derivitised before GC analysis).

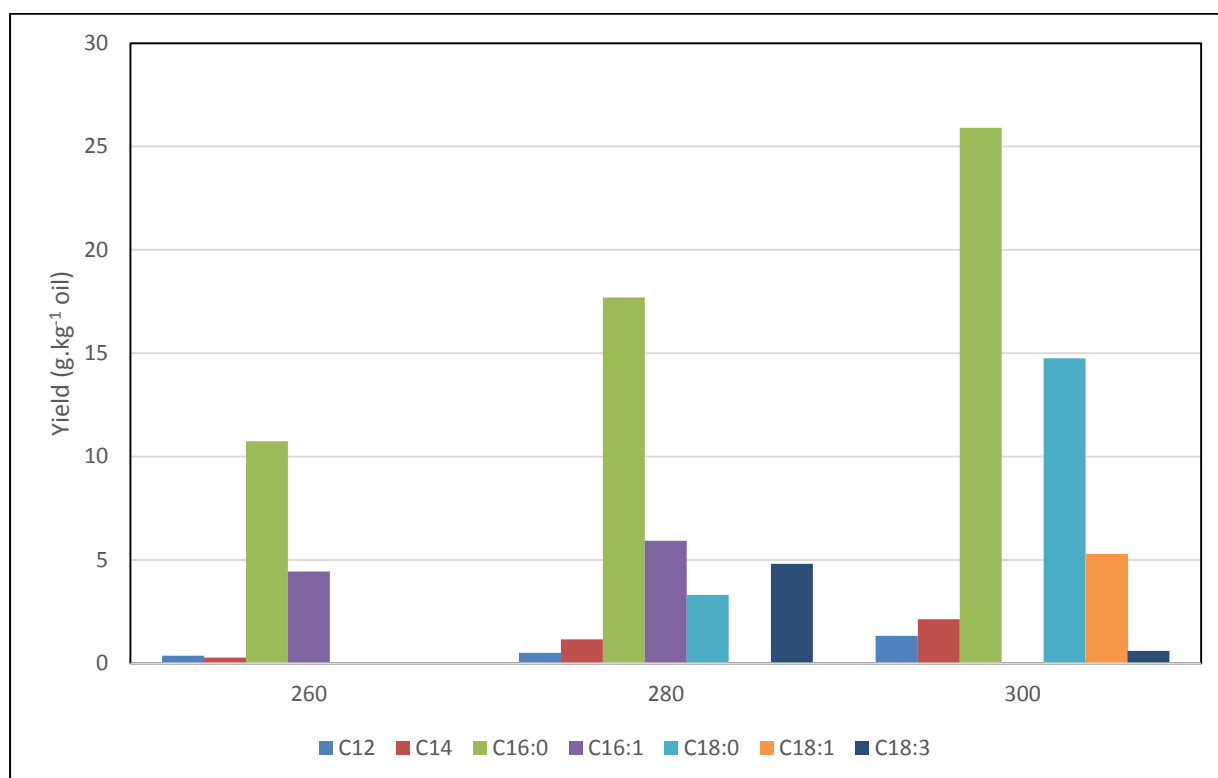


Figure 4-18: Influence of temperature on the FAME yield and triglyceride yields at $300^{\circ}C$ (— C12 — C14 — C16:0 — C16:1 — C18:0 — C18:1 — C18:2)

From Figure 4-18 it can be noted that initially at $260^{\circ}C$ the C_{16} content is high with some $C_{16:1}$ present. Both these fatty acids are associated with algae oil (Hu *et al.*, 2008). At

280°C, the overall fatty acid yield increases. An increase in $C_{16:0}$ and a small increase in $C_{16:1}$ is also noted. At 280°C $C_{18:0}$ and $C_{18:3}$ are also present. Thus between 260°C and 280°C the increase can be ascribed to the recovery of the oil content present in the feedstock as well as the condensation of the gas present to form longer chains ($C_{18:0}$ and $C_{18:3}$).

From 280°C to 300°C it can be seen that there is an increase in the C_{16} and C_{18} yield, with no $C_{16:1}$ chains present. A possible explanation would be that some carbon groups attached to the $C_{16:1}$ to form $C_{18:0}$ and $C_{18:1}$. A sharp decline in the $C_{18:3}$ FA yield can also be seen in this range an explanation for this might be that the double bonds broke due to hydrogenation. There is also an increase in C_{12} and C_{14} FA yields this can be due to decarboxylation and decarbonylation taking place during liquefaction. The combined fatty acid yields are 15.82 g.kg⁻¹ at 260°C, 33.49 g.kg⁻¹ at 280°C and 53.68 g.kg⁻¹ at 300°C. (Barreiro *et al.*, 2013; Chen *et al.*, 2014; Aysu and Kucuk, 2013)

Figure 4-19 shows a boiling curve for the bio-oil obtained from hydrothermal liquefaction at 260°C. The curve was drawn from the results obtained from the GC-MS analysis.

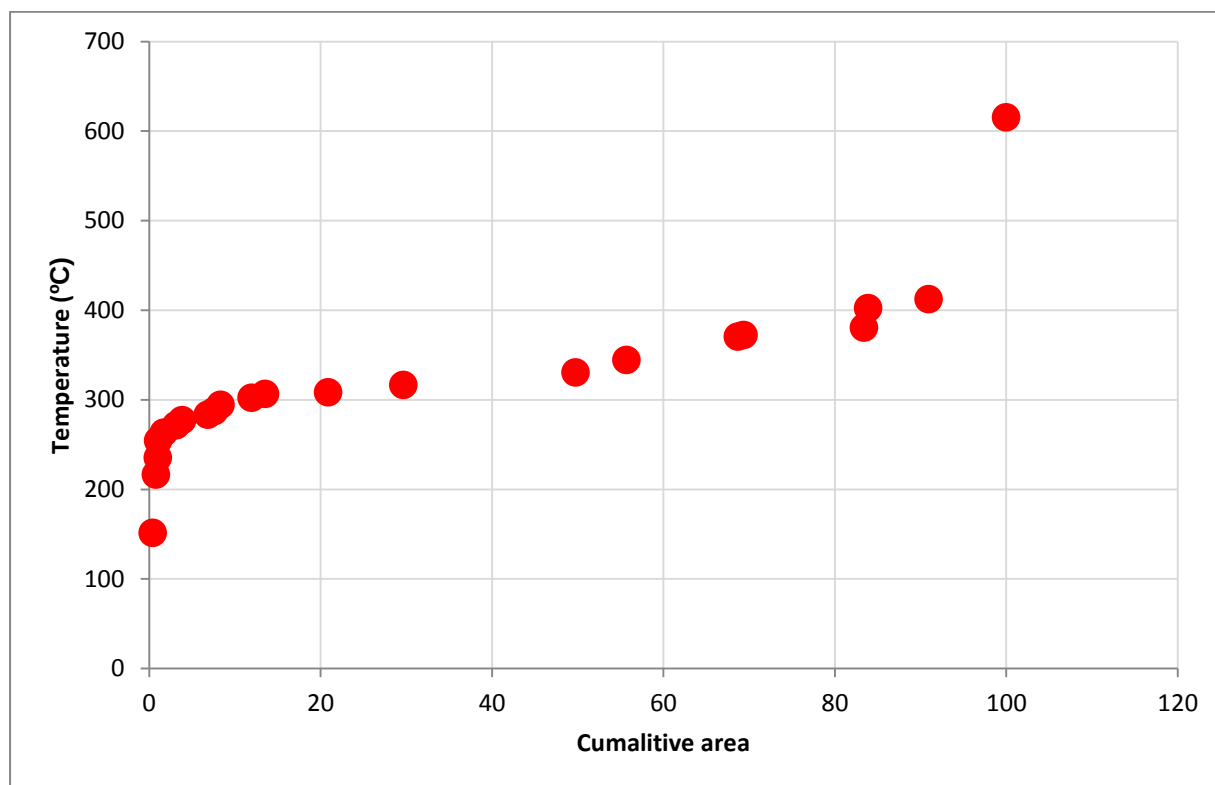


Figure 4-19: Distillation curve for the hydrothermal liquefaction at 260°C

From Figure 4-19 it can be seen that the largest concentration of components present in the bio-oil have a boiling point range between 200°C and 420°C. The GC-MS results showed the presence of some higher chain hydrocarbons. Longer chain hydrocarbons are expected to form when the free radicals present is not sufficient to prevent repolymerisation. The results

howed however is at 260°C thus deoxygenation, decarboxylation and decarbonylation have not taking place, which is evident in the longer chained carbon components found in the GC-MS analysis.

4.4 Summary

In this study the production of fatty acid methyl esters were investigated through an *in situ* production route with hydrothermal liquefaction as the chosen process for lipid removal. The highest overall yield was achieved at 300°C and 6:1 methanol to dry biomass ratio with 10 g.kg⁻¹ biomass acid catalyst and 500 g.kg⁻¹ biomass loading. This yield was 627 g/kg biomass with a C₁₆ yield of 39 g.kg⁻¹ (total FAME yield of 49 g.kg⁻¹ including C₁₂, C₁₄ and C₁₈) and a calorific value of 42 MJ.kg⁻¹. The highest yield obtained is in line with *in situ* biodiesel production studies previously performed (Reddy *et al.*, 2014; Shin *et al.*, 2012; Patil *et al.*, 2012) however some studies have reported higher conversion rates (80% and above). The higher conversion rates can be due to the usage of pure algal species.

For the hydrothermal liquefaction set the effect of temperature on the crude bio-oil and bio-char yields was investigated and found that the highest yield bio-char was 336 g.kg⁻¹ biomass at 280°C and crude bio-oil is 477 g.kg⁻¹. The results showed that before 280°C only carbonisation reactions took place while liquefaction only starts after 280°C.

For the *in situ* biodiesel production set without the use of a catalyst the temperature was varied (240°C to 300°C) along with the methanol to dry biomass mass ratio (1:1, 3:1, 6:1). The optimum bio-crude yield for this set was found at a biomass loading of 500 g.kg⁻¹, 300°C and 3:1 methanol to dry biomass ratio and found to be 591.7 g.kg⁻¹. The highest bio-char yield was found to be 398.5 g bio-char per kg biomass for the 3:1 set with a biomass loading of 500 g.kg⁻¹ at 280°C. The addition of methanol proved to have a positive effect on the bio-oil yield, as well as the FAME yield. These trends are similar to that found in literature (Yang *et al.*, 2014; Zhang *et al.*, 2013; Duan *et al.*, 2013; Barnard, 2009; Yokoyama *et al.*, 1987; Karaca & Bolat, 2000; Xu & Etcheverry, 2008).

The last set the dry biomass loading (50 % dry biomass loading) along with temperature (260°C to 300°C in intervals of 20°C) and methanol to dry biomass ratio (1:1, 3:1 and 6:1) at a constant catalyst loading of 1 wt% of the dry biomass was investigated. The optimum yield achieved here was 627 g crude bio-oil/kg biomass and 376 g bio-char/kg biomass at 300°C and 280°C, respectively. Both were achieved at 500 g.kg⁻¹ biomass loading and 6:1 methanol ratio. Compared to the sets without catalyst loading a slight increase in the yield is

observed with the addition of an acid catalyst, this might be due to the base metals present in the feedstock that can lead to saponification during transesterification.

On the effect of temperature it can be concluded that higher temperatures favours the production of bio-crude. This is as the reaction reaches past the point of carbonisation and the temperature starts to aid in the decomposition of the biomass constituents. This is the same finding throughout literature. At higher temperatures deoxygenation was seen to take place which is evident in the increase of the HHV. The GC-MS results also proofed that at lower temperatures (below 280°C) only carbonisation takes place.

The effect of solvent showed that higher amounts of methanol present leads to higher yields, with a significant increase in yield from no methanol present to a 6:1 methanol to dry biomass mass ratio. 6:1 methanol to dry biomass mass ratio without a catalyst was lower than the 3:1 ratio, which might mean that after a certain point the addition of methanol only effects the oil composition and not the bio-oil yield. The results also showed that the longer time spent in a critical fluid the higher the yield would be. The fatty acid content of the oil in this study showed that there are enough saturated fatty acids present to justify the production of biodiesel. The highest quality biodiesel was obtained at 3:1 methanol ratio.

Further investigation however is needed to see whether or not the biodiesel obtained in this study is up to biodiesel standards. The only parameter quantified is the HHV. The HHV was found to be well within the specification of the SANS biodiesel standard. Another possible route would also be to determine whether the production of renewable diesel from this feedstock is not a more economically viable process. Further investigation into the bio-char will also give light on the way forward.

4.5 References

Asri, N.P., Machmudah, S., Suprpto, W., Budikarjono, K., Roesyadi, A. & Goto, M. 2013. Palm oil transesterification in sub- and supercritical methanol with heterogeneous base catalyst. *Chemical Engineering and Processing: Process Intensification*, 72: 63 – 67.

Aysu, T. & Kukuk, M.M. 2013. Liquefaction of giant fennel (*Ferula orientalis* L.) in supercritical organic solvents: Effects of liquefaction parameters on product yields and character. *The Journal of Supercritical Fluids*, 83: 104 – 123.

Barnard, A. 2009. Extraction of oil from algae for biofuel production by thermochemical liquefaction. Potchefstroom: NWU. (Dissertation – M.Eng)

Barreiro, D.L., Prins, W., Ronsse, F. & Brilman, W. 2013. Hydrothermal liquefaction (HTL) of microalgae of biofuel production: State of the art review and future prospects. *Biomass and Bioenergy*, 53: 113 – 127.

Chen, W., Lin, B., Huang, M. & Chang. 2014?. Thermochemical conversion of microalgal biomass into biofuels: A review. *Bioresource Technology* (2014), <http://dx.doi.org/10.1016/j.biortech.2014.11.050>.

Duan, P. Xu, Y. & Bai, X. 2013. Upgrading of crude duckweed bio-oil in subcritical water. *Fuel*, 27(8): 4729 – 4738.

Ehimen, E.A., Sun, Z.F. & Carrington, C.G. 2009. Variables affecting the in situ transesterification of microalgae lipids. *Fuel*, 89: 677 – 684.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M. & Darzins, A. 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspective and advances. *The Plant Journal*, 54: 621 – 639.

Huang, H., Yuan, X., Zhu, H., Li, H., Liu, Y., Wang, X. & Zeng, G. 2013. Comparative studies of thermochemical liquefaction characteristics of microalgae, lignocellulosic biomass and sewage sludge. *Energy*, 56: 52 – 60.

Jena, U. Das, K.C. and Kastner, J.R. 2011. Effect of operating conditions on thermochemical liquefaction on biocrude production from *Spirulina plantensis*. *Bioresource technology*, 102 (10): 6221 – 6229.

Karaca, F. & Bolat, E. 2000. Coprocessing of a Turkish lignite with a cellulosic waste material 1. The effect of coprocessing on liquefaction yields at different reaction temperatures. *Fuel Processing Technology*, 64: 47 – 55.

Minami, E. & Saka, S. 2006. Kinetics of hydrolysis and methyl esterification for biodiesel production in two-step supercritical methanol process. *Fuel*, 85: 2479 – 2483.

Qu, Y., Wei, X. & Zhong, C. 2003. Structural analysis of bio-oils from sub- and supercritical water liquefaction of woody biomass. *Energy*, 32: 196 – 202.

Shin, H.Y., Lee, S.H., Ryu, J.H. & Bae, S.Y. 2012. Biodiesel production of waste lard using supercritical methanol. *The Journal of Supercritical Fluids*, 61(1): 134 – 138.

Sun, Y., Reddy, H.K., Muppaneni, T., Ponnusamy, S., Patil, P.D., Li, C., Jiang, L. & Deng, S. 2014. A comparative study of direct transesterification of camelina oil under supercritical methanol, ethanol and 1-butanol conditions. *Fuel*, 135: 530 – 536.

Toor, S.S., Rosendahl, L. & Rudolf, A. 2011. Hydrothermal liquefaction of biomass: A review of subcritical water technologies. *Energy*, 36:2328 – 2342.

Xu, C. & Etcheverry, T. 2008. Hydro liquefaction of woody biomass in sub- and super-critical ethanol with iron-based catalysts. *Fuel*, 87: 335 – 345.

Xu, C. & Lancaster, J. 2008. Conversion of secondary pulp/paper sludge powder to liquid oil products for energy recovery by direct liquefaction in hot-compressed water. *Water Research*, 42: 1571 – 1582.

Yang, Y.F., Feng, C.P., Inamori, Y. & Maekawa, T. 2014. Analysis of energy conversion characteristics in liquefaction of algae. *Resources, Conservation and Recycling*, 43: 21 – 33.

Yokoyama, S., Suzuki, A., Murakami, M., Ogi, T., Koguchi, K. & Nakamura, E. 1987. Liquid fuel production from sewage sludge by catalytic conversion using sodium carbonate. *Fuel*, 66: 1150 – 1155.

Yuan, X., Wang, J., Zeng, G., Huang, H., Pei, X. Li, H., Liu, Z. & Cong, M. 2011. Comparative studies of thermochemical liquefaction characteristics of micro-algae using different organic solvents. *Energy*, 36: 6406 – 6412.

Zhang, J., Chen, W.T., Zhan, P., Luo, Z. & Zhang, Y. 2013. Hydrothermal liquefaction of *Chlorella pyrenoidosa* in sub- and supercritical ethanol with heterogeneous catalyst. *Bioresource Technology*, 133: 389 – 397.

5. Conclusion and recommendations

5.1 Conclusion

The production of biodiesel through in-situ transesterification from waste water treatment clarifier effluent stream was investigated in this study. The experiments were conducted in a high pressure autoclave with an inert atmosphere. The highest yield obtained was at 300°C with a percentage recovery of oil initially present in the feedstock of 627 g.kg⁻¹ and an acid catalyst. The study found that oil present in the feedstock can successfully be recovered and converted into methyl esters does successful *in situ* biodiesel production where achieved.

Various process parameters were varied to see their effect on the yield. It was found that the effect of temperature follows a similar path than stated in literature. With an increase in temperature the bio-oil yield increases as well. The effect of an acid catalyst was also investigated and it was found that it had a favourable effect on the overall yield. This can be attributed to the biomass constituents present in the stream. Methanol to dry biomass mass ratio was also investigated and it was found that for an increase in ratio there is an increase in yield. The higher heating values were found to be within the specification for biodiesel standards with the highest HHV at 43 MJ/kg at 300 °C for a 6:1 ratio. The high HHV would suggest a possible net energy gain for the system.

5.2 Recommendations

The recommendation can be made to adapt this process to other feedstock that is high protein low lipid feedstock. If the feedstock used in this study is further utilised, better harvesting methods should be investigation to minimise the degree of impurities in the stream. If there is less impurities the weight percentage oil present in the sample would be much higher thus resulting in a more economically feasible process. One such process would be floatation methods of algae from water, seeing as algae have high buoyancy and hydrophobic properties.

If the current operation at the water treatment plant is evaluated, the recommendation can also be made to harvest upstream of the evaporation pond where this feedstock was harvested, seeing as the exposure time of the feedstock to contaminants would be less. However, for this to be successful the cycle life of the algal species needs to be quantified. If harvesting occurs too early the lipid content present in the algae might be lower.

It is also recommended to further investigate the biodiesel and renewable diesel obtained from this study to see if all the parameters are within the acceptable range of biodiesel standards and evaluate the feasibility of utilising these feedstock in a continuous and large scale setting.

APPENDIX A - Calculations

A1. Calculations

Oil yield:

The oil yield was calculated by the following equations:

Determining of the weight of oil content in the feedstock:

$$(A.1) \quad M_{oc} = \left(\frac{\% oc}{100} \right) * M_F$$

Where:

- M_{oc} = Mass of oil content in the feedstock;
- % oc = Percentage oil content as determined by the ARC;
- M_F = Mass of the feedstock.

Determining the oil yield:

$$(A.2) \quad Oil\ weight\ \% = \frac{M_{oil}}{M_{bm}} \times 100$$

Where:

- M_{oc} = Mass of oil content in the feedstock;
- M_{bml} = Mass of the dry biomass.

Char yield:

Determining the oil yield:

$$(A.3) \quad Char\ weight\ \% = \frac{M_{char}}{M_f} \times 100$$

Where:

- M_f = Mass of feedstock;
- M_{char} = Mass of the char as obtained experimentally.

Experimental error:

Calculation of the mean of the data set:

$$(A.4) \quad X = \frac{1}{N} \sum_i^N x_i$$

Where:

- X = Mean of the data set;
- N = Total number of data points;
- x_i = An individual data point.

Calculation of the standard deviation:

$$(A.5) \quad s = \frac{\sqrt{\sum_i^N (x_i - X)^2}}{N-1}$$

Where:

- s = The standard deviation;
- x_i = An individual data point.

Calculation of the standard error of the mean:

$$(A.6) \quad SE = \frac{s}{\sqrt{N}}$$

Where:

- SE = Standard error of the mean;

Calculation of the z critical value for 95 % confidence level:

$$(A.7) \quad z = \frac{X - \mu}{\frac{s}{\sqrt{N}}}$$

Where:

- z = Critical value;
- μ = the mean value for the continuous variable x;

Calculation of the confidence interval:

$$(A.8) \quad \text{confidence interval} = X \pm (SE \times z)$$

Calculation of the experimental error:

$$(A.8) \quad \text{Experimental error} = \frac{2 \times \text{Confidence interval}}{X} \times 100$$

The following table shows the calculations

Table A1: Experimental data Bio-oil/Biodiesel

Bio-Oil/Biodiesel	
Run	Yield (%)
1	13.85
2	14.42
3	16.04
4	16.16
5	16.34
Average	15.537
Stdev	1.158
z-value	1.150
Experimental Error	8.57%

Table A2: Experimental error Bio-char

Char	
Run	Yield (%)
1	29.96
2	27.64
3	29.79
4	29.48
5	29.52
Average	28.968
Stdev	1.290
z-value	2.571
Experimental Error	5.121

Conversion of as received to moisture free:

$$(A.9) \quad X_{mf} = \frac{X_{ar}}{(1-TM\%)}$$

Where:

- X_{mf} = parameter on a moisture free basis;
- X_{ar} = parameter on a as received basis;
- $TM\%$ = Total moisture percentage.

Determination of biomass loading:

$$(A.9) \quad X_{BL} = \frac{M_{bmf}}{(M_{bm}+TM)} \times 100$$

Where:

- X_{BL} = Biomass loading percentage;
- M_{bmf} = Mass of biomass moisture free (excluding inherent moisture)(g);
- TM = Total moisture weight (g);
- M_{bm} = Mass of biomass (as received – including inherent moisture) (g);

APPENDIX B – Raw data

Table B1: Determination of moisture content of feedstock

Sample	Moisture Content (wt%)
1	94.45
2	93.95
3	94.11
4	94.14
5	94.38
Average	94.20

Table B2: Determination of inherent moisture

Sample #	Inherent moisture content (wt%)
1	27
2	29
3	27.2
4	26.5
5	27.2
Average	27.34

Table B3: Determination of ash content and volatile matter

Sample #	Ash (wt% AR)	Volatile matter (wt% AR)	Volatile matter (wt% MF)
1	22.22	75.92	48.92
2	25.22	79.67	50.67
3	25.7	78.5	51.3
Average	24.38	78.03	50.29

Table B4: Hydrothermal liquefaction

Run	Sample weight (g)	Water weight (g)	Bio-oil yield (wt%)	Bio-char yield (wt%)
240°C	27.1	29	16.25	29.96
260°C	40.5	41.5	24.47	29.21
280°C	34.8	33.9	36.78	33.62
300°C	23	24.9	40.69	24.53
320°C	25	24.8	44.04	17.62
340°C	26.9	28.9	47.07	18.93

Table B5: In situ biodiesel production with super critical methanol (1:1 methanol ratio with a 50% biomass loading)

Run	Sample weight (g)	Water weight (g)	Bio-oil yield (wt%)	Bio-char yield (wt%)
240°C	32.3	28.5	27.70	37.93
260°C	23.6	17.15	34.41	34.41
280°C	25.9	18.82	37.72	38.26
300°C	40.5	29.43	44.86	30.24

Table B6: In situ biodiesel production with super critical methanol (3:1 methanol ratio with a 50% biomass loading)

Run	Sample weight (g)	Water weight (g)	Bio-oil yield (wt%)	Bio-char yield (wt%)
240°C	25.7	25.7	23.99	28.25
260°C	66.3	60.5	39.45	32.23
280°C	22.7	23.8	46.47	28.97
300°C	25.7	29.4	59.17	24.00

Table B7: In situ biodiesel production with super critical methanol (6:1 methanol ratio with a 50% biomass loading)

Run	Sample weight (g)	Water weight (g)	Bio-oil yield (wt%)	Bio-char yield (wt%)
240°C	-	-	-	-
260°C	15.1	10	32.66	20.87
280°C	11.5	7	46.46	30.97
300°C	13.2	8	57.1	32.17

Table B8: In situ biodiesel production with super critical methanol (6:1 methanol ratio with a 100% biomass loading)

Run	Sample weight (g)	Water weight (g)	Bio-oil yield (wt%)	Bio-char yield (wt%)
240°C	-	-	-	-
260°C	12.6	-	27.18	35.68
280°C	14.6	-	32.8	42.71
300°C	10	-	41.1	38.47

Table B9: In situ biodiesel production with super critical methanol (6:1 methanol ratio with a 50% biomass loading and acid catalysed)

Run	Sample weight (g)	Water weight (g)	Bio-oil yield (wt%)	Bio-char yield (wt%)
240°C	-	-	-	-
260°C	10.32	9.25	49.13	34.83
280°C	10.125	11	55.48	37.95
300°C	15.6	14.77	62.78	38.83

Table B10: GC Results (showed in g.kg⁻¹ bio-oil)

	C12ME	C14:0ME	C14:1ME	C16:0ME	C16:1ME	C18:0ME	C18:1ME	C18:2ME	C18:3ME	Total
260°C hydrothermal liquefaction	0.35	0.27	.-	10.75	4.44	-	-	-	-	15.81
280°C hydrothermal liquefaction	0.53	1.15	0.10	17.77	5.93	3.3	-	4.81	-	33.60
300°C hydrothermal liquefaction	1.32	2.13	0.5	25.93	-	14.75	5.29	0.59	3.2	53.71
1:1 MEOH 260 C	0	0	0	0.25	0.335	0.334	1.024	0.1028	0	2.38
1:1 MEOH 280 C	0.36	0	0	1.22	0.15	0.40	1.07	0.06	0	3.28
1:1 MEOH 300°	0.221	0	0.1	1.58	0.45	0.90	1.57	0	0	5.279
6:1 MEOH 260 C	0.54	0.21	0.085	20	5.43	2.79	1.35	0.36	0	30.77

Appendix B

	C12ME	C14:0ME	C14:1ME	C16:0ME	C16:1ME	C18:0ME	C18:1ME	C18:2ME	C18:3ME	Total
6:1 MEOH 260 C Derivitised	0.85	0.42	0.58	32.12	7.11	16.79	1.35	0.77	0	50.04
6:1 MEOH 280 C	0.67	0.33	1.2	25	4.75	11.3	1.69	1.25	0	45.86
3:1 MEOH 300 C 100 % Biomass Loading Derivitised	7.486	0	1.171	15.673	5.810	3.846	1.869	0.004	0	35.86
3:1 MEOH 260 C	0.35	0	0	7.5	0.21	0.207	1.65	0.30	0	10.217
3:1 MEOH 280 C	0.5	0	0	11	0.39	0.95	0.98	0.11	0	13.93
3:1 MEOH 300°	0.32	0	0.2	15.2	0.67	1.4	1.85	0.16	0	19.8
6:1 MEOH 300 C acid catalysed	0.77	0.25	0.1	39	5.17	1.2	2.04	0.14	0	49.2

Table B11: G.C MS results for 3:1 methanol ratio set

300°C 3:1 methanol ratio		
Component	Formula	Quality (%)
Hexadenoic acid methyl ester	$C_{17}H_{34}O_2$	98
Dodecenylsuccinic anhydride	$C_{16}H_{26}O_3$	87
9-Undecenal, 2,10-dimethyl-	$C_{13}H_{24}O$	89
Cyclopentadecanone, 2 hydroxy -	$C_{15}H_{28}O_2$	94
Z-9-Pentadecenol	$C_{15}H_{30}O$	89
Oxiraneundecaoic acid, 3 pentyl-methyl ester	$C_{19}H_{36}O_3$	72
Cis-11-Hexadecenal	$C_{16}H_{30}O$	72
Octadec-9-enoic acid		
2,5-furandione, 3-[(2E)-2-dodecenyl]dihydro-	$C_{16}H_{26}O_3$	95
(3E,12Z)-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	89
2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	89
8-hexadecenal 14-methyl- (z)-	$C_{17}H_{32}O$	87
(3 β ,4 α ,5 α ,9 β)-4,14-Dimethyl-9,19-cycloergost-24(28)-en-3-ol	$C_{30}H_{50}O$	81

Table B12: G.C MS results for 300°C without methanol

300°C without methanol		
Component	Formula	Quality (%)
2,5-furandione, 3- [(2E)-2- dodecenyl]dihydro- Culmorin	$C_{16}H_{26}O_3$ -	95 70
(3E,12Z)-1,3,12- Nonadecatriene-5,14- diol	$C_{19}H_{34}O_2$	90
8-hexadecenal 14- methyl- (z)- Dodecenylsuccinic anhydride	$C_{17}H_{32}O$ $C_{16}H_{26}O_3$	90 91
-(2E,6E,10E)- 3,7,11,15-tetramethyl- 2,6,10,14- hexadecatetraen-1-yl] acetate	$C_{28}H_{40}O_4$	91

Only the components with a quality value above 70 % are shown in table 4-12 and 4-13. As can be seen a combination of Alkenes, ketones, alcohol, fatty acids and methyl esters are present in the samples. The absence of methyl esters can be noted for the sample without methanol. The GC MS results are similar to the reaction path described in section 2.8 for the formation of ketones and other aliphatic compounds.