THE UNIVERSITY
of ADELAIDE

DIRECT CONVERSION OF MICROALGAE BIOMASS TO BIOCRUDE WITH HYDROTHERMAL LIQUEFACTION

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THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

SCHOOL OF CHEMICAL ENGINEERING

FACULTY OF ENGINEERING, COMPUTER & MATHEMATICAL SCIENCES

THE UNIVERSITY OF ADELAIDE, AUSTRALIA

MAY, 2015

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Executive Summary

Microalgae are generally considered as a promising biomass source for applications including production of advanced biofuels, chemicals, wastewater treatments, various organic substances or a combination of any of the above. The advantages of microalgae includes faster growth rates than terrestrial plants, ability to use non-arable land for mass production, and their ability to grow in poor quality water, as well as their ability to remove pollutants from wastewater streams. However, one of the major challenges of microalgae for commercialisation to is its economic downstream production and conversion to biofuels and chemicals. When harvested, microalgae contains up to ~90% water content, thus economic conversion of high moisture content biomass to valuable products such as biofuels remains a challenge. Hydrothermal liquefaction (HTL) involves processing of high moisture content biomass in hot compressed water, avoiding the drying step for biomass feedstocks. Therefore HTL is advantageous as it avoids the energy intensive drying process in contrast to processes that involve drying of biomass prior to processing into biofuels.

HTL products comprise biocrude, solid residues, aqueous and gas phases. The primary product biocrude is upgradable to liquid transportation fuels. The aqueous phase contains essential nutrients that could be recycled to microalgae cultivation ponds. The gas phase contains about 98mol% carbon dioxide (CO_2) and 2mol% hydrocarbon gases. The gas phase can also be directly recycled to the microalgae cultivation ponds for pH control and supply of CO_2, thus, reducing production costs. Aside from the feedstock selection, maximising yield and improving the quality of biocrude, achieving an energy positive, low carbon footprint and economic viability are important challenges facing a HTL-microalgae-biofuels system. In addition, the biocrude must meet refining specifications, for example, it needs to be high in carbon and hydrogen, low nitrogen, oxygen, sulphur, and have a high energy density similar to conventional crude oils.

This thesis was on optimisation studies to quantify process parameters. Process parameters such as reaction temperature, reaction time, feedstock, catalyst selection, solvent type for maximum yield and improved quality biocrude have been investigated. A halophytic microalga *Tetraselmis* sp. was investigated in this study. The investigation was performed at different reaction temperatures 310^{o}C, 330^{o}C, 350^{o}C, and 370^{o}C at reaction times of 5min, 15min, 30min, 45min, and 60min with a fixed 16%w/w microalgae biomass solid content feedstock. This investigation showed that the operating conditions to achieve maximum biocrude yield and ideal quality biocrude for refining were different. The obtained biocrude yields were in the range 35wt% to 68wt% (ash free dry weight), the maximum achieved at a reaction temperature of 350^{o}C and a 5min reaction time. High yields of biocrude were achieved by short reaction times, whilst better quality biocrude were achieved at long reaction times. The biocrude was found to contain undesired high amounts of heteroatoms, particularly nitrogen and oxygen and metallic impurities. Thus the resultant biocrude cannot be directly applied as transportation fuels. For transportation purposes, the biocrude will need to be upgraded to remove the heteroatoms or used for boiler firing or blended with fossil crude. The biocrude exhibited higher heating value (HHV) of 28MJ/kg to 39MJ/kg similar in nature to petroleum crude. The C, H, N, S, and O content were in the range 67%w/w to 79%w/w (carbon), 6%w/w to 9.5%w/w (hydrogen), ~4%w/w to 6.5%ww/w (nitrogen), 0.5%w/w to 0.9%w/w (sulphur) and 6%w/w to 21.6%w/w for oxygen.

Due to the high concentration of nitrogen in the resultant biocrude, an alternative to direct HTL involving pretreatment of biomass with the extraction of protein prior to further processing of the resultant residue to biocrude was performed. The influence of process conditions during both pre-treatment for protein extraction and HTL of the pre-treated *Tetraselmis* sp. for biocrude production was investigated. The pretreatment step was shown to extract protein which could be used as valuable by-products. The extraction was shown to improve the quality of the resultant treated microalgae biomass, as the amounts of nitrogen content were reduced. HTL of the pretreated microalgae led to a maximum biocrude yield of 65wt% at 310^{o}C, 30min which was more than a 50% improvement in yield as compared to HTL of untreated microalgae at the same reaction conditions. To achieve a similar biocrude yield using the untreated biomass required a much higher reaction temperature of 350^{o}C. Using recycled HTL aqueous phase as reaction media led to a 25wt% higher biocrude yield. HTL of pre-treated microalgae biomass led to 32% to 46% nitrogen reduction in the resultant biocrude. A maximum of 15wt% protein extract was obtained during microalgae pre-treatment at 150^{o}C, 20min. A similar energy input was required in biocrude production from untreated route and the combined pre-treatment and HTL.

The effect of seawater as reaction media during hydrothermal liquefaction of microalgae was investigated. Different seawater concentrations containing varying amounts of dissolved salt

were employed and the results compared to deionised water. It was found that seawater could improve the biocrude yield, however, with no substantial enhancement in properties.

Products yields and properties from the HTL of freshly harvested microalgae biomass slurry and diluted pulverised microalgae biomass were compared. The results show higher biocrude yields could be obtained from fresh biomass slurry than diluted pulverised biomass. In addition, lower yields in solid residue and aqueous phase were obtained from fresh biomass slurry compared to diluted pulverised biomass. However, there was no substantial difference in the properties of biocrude from the different feedstocks.

Improving the quality of HTL biocrude without the application of a catalyst or hydrogen was performed with vacuum distillation. In addition to the primary product (distilled biocrude), the distillate contained a substantial amount of solid residue and water. Vacuum distillation of HTL biocrude led to 62wt% to 67wt% and 70wt% to73wt% of distilled-biocrude from *Spirulina* sp. and *Tetraselmis* sp. biocrudes, respectively. It was shown that the higher heating value (HHV) increased from 32MJ/kg to ~40MJ/kg following distillation. About 83% carbon and 78% energy was recovered in the distilled biocrudes from HTL biocrudes feed. The results show improved quality biocrude as there were significant reductions in oxygen and metallic contents, and boiling point range in the distilled biocrudes.

A concept for integrating anaerobic digestion (AD) and hydrothermal liquefaction for waste management to recover residual carbon was investigated by processing the AD digestate via HTL. The results show the feasibility to recover residual carbon from organic waste producing valuable product biocrude. An average biogas yield of $0.5m^3/kgVS_{added}$ was obtained following anaerobic digestion at different hydraulic retention times (HRTs). The HTL of the resultant effluents seem to vary with HRTs, and a maximum biocrude yield of 42wt% was achieved. It was found that up to 76% and ~ 70% carbon and energy can be recovered in the biocrude. Importantly, the resultant biocrude was found to have very low nitrogen content similar to that of petroleum crude.

The outcome of this study will provide fundamental data for a commercial development of HTL microalgae biofuels. One of the major outcomes of this work is the feasibility to produce high yields in biocrude at a very short reaction time (5min) and importantly under subcritical condition. The high yields obtained from *Tetraselmis* sp. will significantly

improve the life-cycle analysis and the viability of HTL microalgae biofuels. The demonstrated ability of microalgae pretreatment prior to HTL is a potential means to simultaneously to obtain a co-product alongside the primary product biocrude. The revenue derived from the co-products will also improve the economics of HTL. Moreover, the idea of using recycled pretreatment process water as HTL reaction media is a significant contribution as it leads to a higher biocrude yield. The use of natural seawater as a reaction media is novel as it avoids employing organic solvents, saving associated cost and freshwater. It was found that the use of catalyst for upgrading of biocrude, improving its HHVs and molecular weight is not necessarily required, since similar upgraded biocrude yields and fuel properties were achieved following vacuum distillation of biocrude.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Acknowledgements

I would like to acknowledge my supervisors Associate Prof David Lewis, Prof Peter Ashman and Dr Senthil Chinnasamy. I am especially grateful for all their understanding, patience, constant guidance, valuable and constructive criticism and assistance throughout my PhD.

I highly appreciate the financial support received from the Australian Research Council's Linkage Projects funding scheme (LP100200616) with industry partner SQC Pty Ltd and the Australian Renewable Energy Agency (ARENA) advanced biofuels readiness program. I would like to acknowledge the financial support I have received in the form of a Postgraduate Scholarship from the Education Trust Fund of the Federal Republic of Nigeria.

I wish to thank past and current members and PhD colleagues of the Microalgal Engineering Research Group: Kwong Shui Andrew Lee, Sanaz Orandi, Sophie Fan Sing, Stephen Pahl, Amir Foghani, Andrew Ward, Mason Erkelens, Steven Amos, Theo Kalaitzadis, Wynand Van den Berg, Quang Doan. I would like to thank past and present School of Chemical Engineering Office members of staff: Elaine Minerd, Mary Barrow, Gemma Higgs, Monica Dinan and Sue Earle for all their administrative assistance during my PhD Programme. I am grateful to Dr Michelle Picard for the editorial assistance.

The laboratory and technical assistance provided by members of staff, Aban Infrastructure Pvt. Ltd, Biotechnology Division India was greatly appreciated. I would especially thank Prof S Sivasenka and his team at the National Centre for Catalysis Research, India Institute of Technology, Chennai, India. Special thanks to members of staff of the Research and Development, Chennai Petroleum Cooperation Ltd, India. I am also grateful to members of staff at the Central Leather Research Institute, Pilot Plant, Chennai, for the technical and laboratory assistance.

Finally, my heart goes to my wife, and beautiful daughter Cherish Eboibi for their love, emotional support and encouragement throughout my PhD period. I thank Cherish for her patience and understanding of me not being fully with her during this gruelling process. I would like to thank my parents Mr and Mrs Lucky Eboibi (JP), grandmother, brothers and sisters. I am grateful for all your immeasurable love and support. I am also grateful to all friends and well-wishers for their care and support, thank you.

Preface

This thesis is submitted as a portfolio of publications according to the "Specifications for Thesis 2014" of the University of Adelaide. The journals in which the papers were published or submitted have high impact factors in the field of Chemical Engineering. Data on the impact factors of the journals are listed below:

Journal Title	Impact Factors	
	2015	5 year
Bioresource Technology	5.03	5.60
RSC Advances	3.70	3.70
Fuel Processing Technology	3.02	3.70
Environmental Progress & Sustainable Energy	1.27	1.55

The main intellectual contribution of this thesis has been published in the following five journal papers:

1. Eboibi, BE, Lewis, DM, Ashman, PJ & Chinnasamy, S 2014, 'Effect of operating conditions on yield and quality of biocrude during hydrothermal liquefaction of halophytic microalga *Tetraselmis* sp', *Bioresource Technology*, 170, 20-29.

2. Eboibi, BE, Lewis, DM, Ashman, PJ & Chinnasamy, S 2014, 'Hydrothermal liquefaction of microalgae for biocrude production: Improving the biocrude properties with vacuum distillation', *Bioresource Technology*, 174, 212-221.

3. Eboibi, BE, Lewis, DM, Ashman, PJ & Chinnasamy, S 2015, 'Influence of process conditions on pre-treatment of microalgae for protein extraction and the production of biocrude during hydrothermal liquefaction of pre-treated *Tetraselmis* sp.', *RSC Advances*, 5, 20193-20207.

4. Eboibi, BE, Lewis, DM, Ashman, PJ & Chinnasamy, S 'Effect of seawater and wet feedstock on product distribution and properties during hydrothermal liquefaction of microalgae', *Fuel Processing Technology*, Under review.

5. Eboibi, BE, Lewis, DM, Ashman, PJ & Chinnasamy, S 'Integrating anaerobic digestion and hydrothermal liquefaction for renewable energy production: An experimental investigation', *Environmental Progress & Sustainable Energy*, Under review.

Chapter 1

Introduction

1.1 Biofuels

The overdependence on fossil energy has led to volatile crude oil prices and contributed to global warming. These factors have contributed to the increasing research in renewable and sustainable advanced biofuels to compliment fossil fuels. Microalgae are perceived as a sustainable source of biomass for commodity products such as biofuels and bioenergy. This thesis was on the optimisation of direct conversion of renewable microalgae feedstock to biocrude. The resultant biocrude is upgradable to transportation fuels using existing convectional crude oil refineries. This thesis is by publication and a series of published papers are presented.

Biofuels in form of transportation fuels are an alternative energy source to fossil fuels derived from biomass. Biomass has been the major source of energy since the ‘stone ages’ worldwide before the discovery of petroleum crude. With increased development and industrialisation, fossil fuels have become more popular and are now the major source of energy due to their higher energy density, availability and relative cheapness. However, due to an over dependence on fossil fuels and concerns about global warming, there has been a drive for advances in renewable energy sources.

The main focus of renewable energy researchers has been on environmental issues such as the mitigation of global warming by reducing carbon dioxide (CO_2) emissions, and also as an alternative to fossil fuels in terms of quality, availability and affordability. Most of the renewable energy sources such as geothermal, hydroelectric, tidal and wind have mainly focussed on the electricity market, while research on biofuels (~66%) has mainly focussed on the worldwide energy demand for transportation of (Schenk *et al*., 2008). Though there have been some recent developments in electric cars, the application of batteries in airplanes, ships and heavy vehicles is not viable due to safety concerns arising out of the use of lithium based batteries as well as concerns regarding cost (Biller, 2013). Therefore it is necessary to improve the technological development of sustainable biofuels sources and their applications.

The potential advantages of biofuels include its use as a suitable source for the mitigation of carbon dioxide. Though CO_2 is emitted during combustion, it is however removed from the atmosphere during the growth of biomass (Biller and Ross, 2011). Other important merits of developing sustainable biofuel sources are the provision of foreign exchange earnings, energy security, socio economic factors, and global competiveness in fuel markets (Demirbas *et al*., 2008; Valdez, 2013).

Biomass is broadly defined and it includes a range of components with varying compositions. The main components are carbohydrate, protein, lignin and lipids (McKendry, 2002a; Aktar and Amin, 2002). Biomass is considered as one of the most significant sources of renewable energy, providing a more suitable sustainable future energy system (Toor *et al*., 2011; Yan *et al*., 1999). Biomass sources such as energy crops, agricultural crops, and wood have been considered as feedstock for biofuels and chemicals. However, work in this area has been limited due to the fact that these crops compete with food production for arable land and are impacted by climatic and environmental factors (Yu *et al*., 2011a). In order to avoid these negative effects, contemporary research in this area focuses on biofuels that are sourced from biomass that does not compete with food or land. The emphasis in the development of biofuels is now on more effective eco-friendly products using biomass that is normally discarded; including agricultural waste, forestry waste, switch grass and, most recently, microalgae. The production of biofuel from these sources comes with the potential of reducing greenhouse gas emissions (Alfonso *et al*., 2009; Miao and Qinqyu, 2004). In the last decade, microalgae biomass has received increasing interest as a promising renewable biomass source for the production of next generation liquid biofuel due to its versatility (Patil *et al*., 2008; Williams *et al*., 2010).

The potential of microalgae as a source of bio-fuels include faster growth rate of which their exponential growth rates exceed that of other biomass sources (Brennan and Owende, 2010; Spolaore *et al*., 2006). CO_2 reduction in the atmosphere is achieved by capturing emitted CO_2 from fossil fuels (Ross *et al*. 2011). Microalgae are a non-food based resource that could be grown on non-arable land, hence avoiding competition with land use. They have high oil yields and require lower amounts of freshwater in comparison with terrestrial biomass, and freshwater is not required if marine algae strains are used (Wijffels *et al*., 2010; Williams *et al*., 2010). The microalgae oil yield is higher than terrestrial oil plants palm and rape seed oils that produces 6,000 and 1500l/ha/yr (Demirbas and Demirbas, 2011). In addition microalgae cultures have high oil productivity compared with oilseed crops. For example, microalgae cultivated in open ponds yields 12,000 litres of biodiesel per hectare as compared to 1190 litre/hectare of rapeseed (Schenk *et al*., 2008).

Several pathways for the production of liquid biofuels from different biomass sources have been under investigation (Petrus and Noordermeer, 2006; Jena *et al*., 2011; McKendry, 2002a&b). The different pathways are classified into two main categories, namely: biochemical and thermochemical processes. The well-established technologies for biomass conversion processes are the biochemical, including fermentation and anaerobic digestion. Fermentation and transterification are the two main pathways to biofuels that are commercially pursued (Peterson *et al*., 2008). Fermentation is performed in an anaerobic environment for the conversion of glucose and fructose to ethanol and CO_2 by the activity of yeast. About 10vol% of the resultant ethanol can be blended with conventional gasoline and applied to existing combustion engine modification. Anaerobic digestion (AD) is applied for treating organic waste such as animal manure, sewage and industrial effluents and solid substrates (energy crops, agricultural residues and food wastes). AD involves the breakdown and stabilization of complex organic matter by a consortium of microorganisms for the production of biogas (Raposo *et al*., 2012; Caramiello *et al*., 2013). The resultant biogas is commonly combusted leading to the production of a renewable electricity and heat which can ideally replace fossil fuels energy sources.

One of the drawbacks of the fermentation and anaerobic digestion process is that it requires long hydraulic retention times (days); and does not provide complete usage of the feedstock. Thus substantial amounts (up to 50%) of organic matter commonly referred to as digestate or effluents are left in the by-product. The digestate is usually high in bacteria, virus and nutrients and these could be an issue if not properly handled.

The thermochemical processes are the conventional gasification, pyrolysis and hydrothermal liquefaction, which can also be used to convert biomass to biofuels. Gasification involves the reaction of solid or liquid organic material such as biomass, coal or oil with air, oxygen and or steam at elevated temperatures to produce gas product referred to as syngas or producer gas (Barreiro *et al*., 2013a; Huber *et al*., 2006). The produced gas mostly contains CO, CO_2, H_2, CH_4 and N_2 (Hirano *et al*., 1998) can also be used in the production of fuels and chemicals e.g. diesel by the Fisher Tropsch process.

Pyrolysis refers to thermal decomposition of biomass in the absence of oxygen to bio-oil, solid biochar and gas fraction at 370-700^{o}C and atmospheric pressure (Toor *et al*., 2011). Pyrolysis is divided into fast pyrolysis, slow pyrolysis and flash pyrolysis (Marcilla *et al*., 2013). Fast pyrolysis mainly involves the production of a substantial amount of liquid (bio-oil) and gaseous fuels. It is operated at high heating rate (within range of 1°C/s to 200°C/s) and at high operating temperature and short reaction times in order of seconds with fine biomass particle size (<1mm). Slow pyrolysis involves lower heating rates of 5°C/min to 80°C/min and long reaction times of 5min to 30min, to improve the production of tars and chars for co-combustion with coal. Flash pyrolysis produces mostly liquid products due to the rapid high heating rate (>1000°C/s) under a few second of reaction times using fine particle size of <0.2mm. It has been suggested to be viable route for the conversion of biomass to liquid fuels as future replacement of fossil fuels. This is primarily due to the achievable high-biomass to liquid conversion rate of ~75.5% (Barreiro *et al.*, 2013a). However, in addition to the high process temperature, the resultant liquid fuel, bio-oil is acidic, unstable, highly oxygenated, and has a low higher heating value (Chiaramonti *et al.*, 2003; Czernik and Bridgwater, 2004). Also the bio-oil contains water which is one of the main challenges. Therefore, the biocrude needs upgrading via hydrogenation/catalytic cracking in order to reduce the impurities.

A related thermal process for liquid biofuel production is the conventional transterification, especially applied for lipid/oil-rich biomass. Transterification involves the extraction of triglycerides (fatty acids) from biomass with subsequent transterification of the fatty acids or natural oils to biodiesel (Peterson *et al.*, 2008). However, the transterification pathway is costly as it normally involves energy–and-resource-intensive steps for: dewatering of the biomass and drying of the dewatered biomass before solvent extraction (Brown *et al.*, 2010; Levine *et al.*, 2010; Patil *et al.*, 2011).

One of the disadvantages of conventional themochemical processes are limitations to biomass feedstock <20wt% moisture content, while, biomasses especially microalgae have a typically high moisture content of 80wt% to 90wt% (Patil *et al.*, 2008). Due to the biomass characteristic of high moisture content, the conventional thermochemical process such as gasification and pyrolysis are uneconomic for the production of transportation fuel (Amin, 2009) Therefore, wet thermochemical processes such as hydrothermal liquefaction (HTL) are more suitable for high moisture content biomass feedstock, especially for microalgae. Importantly, HTL can process wet biomass, thereby avoiding the intensive energy associated with a drying step. In a HTL process the whole wet-biomass slurry can be decomposed and converted in hot compressed water to high energy density hydrocarbon biofuels for aviation and ground transportation (Brown *et al*., 2010; Peterson *et al*., 2008; Toor *et al*., 2011). HTL offers enhanced reaction rates and efficient product separation of products. In addition, the HTL route provides an additional benefit as nutrients such as nitrogen and phosphorus, and mineral elements (e.g. Fe, Ca, Mg, and K) in the wastewater can potentially be recycled to the cultivation pond to cultivate additional microalgae biomass (Jena *et al.*, 2011; Biller *et al*., 2012; Tsukahara *et al*., 2001) reducing production costs (Frank *et al*., 2013). Moreover, HTL has the potential to produce valuable by-products with simultaneous production of liquid transportation fuels (Biller *et al*., 2013; Charkraborty *et al*., 2012; Miao *et al*., 2012). A detailed literature review on hydrothermal liquefaction of biomass is presented in chapter 2.

1.2 Aim and structure of the thesis

The overall aim of this thesis was to investigate the direct conversion of microalgae biomass to biocrude using hydrothermal liquefaction. It is desirable to obtain maximum biocrude yield at low process temperature and at the shortest possible reaction time. As processes with short reaction times require smaller reactor volumes, and therefore, lower capital cost commercial operations. In addition, maximum yields at low process temperature will reduce the energy consumption. Importantly, it is highly desirable for the resultant biocrude to be of good quality, allowing direct utilization as a heavy fuel or upgrading in convectional refineries. Hence biocrude with lower nitrogen, oxygen, and metallic content is favourable as it will avoid/reduce refining issues. Additionally, biocrude of low boiling point range fractions is more preferable compared to high molecular weight boiling point fraction because the former has higher commercial value. To achieve this aim of hydrothermal liquefaction of biomass to biocrude an optimization study was performed at different reaction conditions. This is a significant research and the information could be useful to the scientific and industrial community toward envisaged commercialization of HTL microalgae biofuels. The associated objectives as outlined below were investigated.

The main aim of this study was to determine the optimum temperature and time for the maximum yield and quality of biocrude. In order to achieve this aim, the microalgae *Tetraselmis* sp. biomass was investigated at a reaction temperature of 310^{o}C, 330^{o}C, 350^{o}C and 370^{o}C with a reaction times of 5min, 15min, 30min, 45min and 60min with a fixed 16%w/w solid content feedstock. The resultant biocrudes were analysed for elemental and metallic composition. The carbon and nitrogen distribution including the energy recovery in the biocrude at each operating condition was determined. The results of this work are presented in Chapter 3.

The investigation of HTL of microalgae biomass for biocrude production was followed by vacuum distillation of the resultant biocrudeis presented in Chapter 4. Limited information was available on the properties of HTL-microalage-biocrude following distillation. The objective of this study was to investigate the feasibility of improving the quality of biocrude without a catalyst or hydrogen as normally applied during upgrading of biocrude. The effect of biocrude distillation on its elemental carbon, hydrogen, nitrogen, energy value and metallic contents were investigated. The properties of the resultant biocrude were examined and conclusion drawn regarding the quality of the biocrude by elemental analysis, ICP-MS and simulated distillation.

In Chapter 5, the influence of operating conditions on pre-treatment of *Tetraselmis* sp. microalgae for protein extraction and on production of biocrude during hydrothermal liquefaction of pre-treated *Tetraselmis* sp. biomass was investigated. The main aim of this study was on the feasibility of producing biocrude from pre-treated microalgae biomass. The main aim of the parametric investigation was to obtain maximum yield of biocrude and extract. The fate of nitrogen distribution during biocrude production and in microalgae pre-treatment was of particular interest. This study investigated the effect of using recycled wastewater from the pre-treatment step as a reaction media. The effect on HTL product yields and properties were quantified.

The investigation on the effect of seawater as reaction media on yields, distribution and properties of product during HTL of microalgae is described in Chapter 6. In addition the effect of freshly harvested microalgae biomass slurry and diluted pulverised microalgae biomass was also examined. The primary objective of this chapter was to provide an alternative reaction media to the commonly employed deionised water, organic solvents for liquefaction. The use of organic solvent, catalyst is aimed to increase yield and/or improve the quality of biocrude, however, their use is uneconomical.

The concept of integrating HTL with a waste treatment facility such as anaerobic digestion is presented in Chapter 7. The objective of this study was to explore the feasibility of recovering residual carbon from sludge also known as digestate, producing a more valuable product biocrude. The experimental investigation was performed for sustainable renewable energy production, improving waste management, and reduction in environmental pollution. The digstate were obtained from anaerobic digestion of manure at different hydraulic retention times (HRTs). The impact of digestate obtained at differing HRTs in biocrude yield was investigated.

The literature review on this area of research is presented in Chapter 2. The aim of Chapter 2 was to provide a better understanding of the previous studies in this research area, identification of areas that have been investigated and important research gaps that require further investigation.

Chapter 2

Literature review

2.1. Hydrothermal liquefaction

Hydrothermal liquefaction is considered as the most promising technology for the direct conversion of biomass to hydrocarbon based biofuels. Hydrothermal liquefaction (HTL) is typically performed at a reaction temperature of 200^{o}C to374^{o}C, and at a pressure of 15MPa to 20MPa in an aqueous environment with or without catalyst in a closed reactor (Peterson *et al*., 2008; Ross *et al*., 2010).

HTL to biocrude is perceived to mimic the natural processes that take place in the production of fossil fuel reserves. These fossil fuel reserves have been established by the decaying and transformation of organic matter under pressure and heat over decades. Petroleum crude and gas is the main product of decaying plant matter (e.g. phytoplankton and zooplankton) while coal is formed from terrestrial plants. Employing high temperatures and pressures to process organic matter or carbonaceous feedstocks through HTL is therefore considered as a natural approach for developing renewable fuels. Just as the yield and quality of petroleum crude depends on the geographical location of fossil fuel reserves and environmental factors, the applied process conditions (for HTL), feedstock composition and reactor configuration strongly influence the quality and yield of the HTL products, particularly biocrude. The primary biomass components include carbohydrates, proteins, and lipids (Toor *et al*., 2011) and the HTL conversion of these components in hot compressed water to biofuel differ due to complex reaction mechanism. During HTL, close to the critical point of water, biomass feedstocks are decomposed and depolymerised to smaller molecules. These molecules reploymerize to form hydrocarbons compounds and gas (Zhang *et al*., 2010; Biller and Ross, 2011). The main reaction mechanism during HTL has been described and summarised as (Alba *et al*., 2011; Demirbas, 2000; Moller *et al*., 2011; Peterson *et al*., 2008) as follows:

1. The hydrolysis of biomass macromolecules into smaller monomers
2. Conversion of these smaller fragments by cleavage, dehydration, decarboxylation into smaller compounds.
3. Recombination of smaller fragments e.g. through condensation and polymerization producing new compounds.

The products from hydrothermal liquefaction are biocrude, solid residue, gas and aqueous phases. One of the main aims of hydrothermal liquefaction is to produce a primary product biocrude with high higher heating value than the feedstock by removal of oxygen. The biocrude is a dark and viscous energy dense liquid (~30 MJ/kg to 40MJ/kg) similar to that of petroleum crude. Biocrude contains complex mixture of numerous large numbers of compounds with a wide range in molecular weight (Elliott *et al.*, 2013). Biocrude can be directly combusted in boilers as heavy fuel, or upgraded by hydrotreating to reduce the nitrogen and oxygen content of the oil making it more suitable as a transportation fuels and renewable chemicals. The gaseous phase mostly contains CO_2 (>93mol%) and <7mol% hydrocarbon gases (H_2, CH_4, C_2H_4, C_2H_6) (Brown *et al.*, 2010; Valdez *et al.*, 2012). This gas phase containing predominately CO_2 can be recycled to the microalgae cultivation pond, while H_2 can be used for further hydrotreating of the resultant biocrude (Barreiro *et al.*, 2013a). Thus the gas phase is attractive from a biorefinery point of view. The HTL aqueous phase is rich in major secondary and micronutrients and it can be recycled to microalgae cultivation ponds to offset nutrients requirements (Jena *et al.*, 2011b; Frank *et al.*, 2013). Apart from ash, HTL solid residue also contains small quantities carbon, nitrogen, hydrogen, and sulphur which may be used as soil amendment.

Hydrothermal liquefaction of biomass to biofuel is considered as a promising technology. However, most research studies in HTL are limited to laboratory scale, primarily, due to uncertainties and challenges. The next section provides a summary of the current state of research on hydrothermal liquefaction of microalgae.

2.2. Previous research on hydrothermal liquefaction of microalgae

Most research on the HTL of microalgae biomass has been performed at subcritical temperature conditions where the primary product is biocrude. A summary of published research investigations on hydrothermal liquefaction of microalgae biomass without catalysts is presented in Table 2. The pioneering investigation in converting microalgae biomass to biocrude via hydrothermal liquefaction was conducted in the early 1990s by Yokoyama's group at the National Institute for Resources and Environment (NIRE) in Japan. This group published four articles on the liquefaction and reported 50wt% and 37wt% of biocrude yield from *Botryoccocus braunii* and *Dunaliella tertiolecta*, respectively, using a batch fed closed vessel. Their first publication reported HTL of *B. braunii* in a 300mL autoclave at 200^{o}C, and 340^{o}C and 60min holding time (Dote *et al.*, 1994). Operating without catalyst, the biocrude yield reported was 40wt% to 58wt% and the maximum yield was obtained at 300^{o}C. Although a similar biocrude yield of 58wt% was achieved at 200^{o}C, it was unsuitable for producing drop-in biofuels because it was highly viscous. This paper reported that the biocrude yield at 200^{o}C and 300^{o}C except 340^{o}C exceeded the microalgae lipid content. This observation confirmed that apart from lipids, carbohydrate and protein fractions of microalgae can also contribute to biocrude yield. The HHV of biocrude produced under optimum condition (300^{o}C) in their study was 50MJ/kg and it contained 0.9%w/w nitrogen and no oxygen. The HHV reported in their study was higher than the values reported in previous studies, which were between 30MJ/kg to 40MJ/kg. There was an increase in nitrogen content observed with increased reaction temperature, indicating increased fractionation of proteins.

After their first publication, they wrote an article on the analyses of biocrude produced from *B. braunii* (Inoue *et al*., 1994). In this paper the recovery of hydrocarbons in the biocrude fractions decreases with increase in reaction temperature, and the addition of catalyst improved the recoveries. Operating at 200^{o}C led to maximum recovery of hydrocarbons while an increase in the process temperature resulted in the formation of smaller molecular weight hydrocarbons. The biocrude contained high molecular weight oxygenated compounds when liquefaction was performed at the highest reaction temperature (340^{o}C), which was attributed to the production of biocrude from non-lipid fractions of the microalgae. Consequently, the biocrude had a lower HHV of 42MJ/kg compared to 50MJ/kg obtained at 200^{o}C with no oxygen. This suggests that biocrude with no/low oxygen is of higher quality. Two further investigations on HTL of *D. tertiolecta* was reported by Minowa *et al*., (1995) and Sawayama *et al*. (1999). They reported that *D. tertioecta* as a high protein (~64wt%, ash free basis) containing microalga with relatively low (~21wt%) lipid content. The biocrude yield from *D. tertiolecta* was 20wt% lower than that of *B. braunii*. It contained ~7 and 11%w/w of nitrogen and oxygen respectively and recorded an energy density of ~36MJ/kg. The energy input for cultivation, harvesting and processing *B. braunii* to biocrude oil was significantly less when compared to *D. tertiolecta*. They concluded that the liquefaction of B. *braunii* resulted in more energy output compared to *D. tertiolecta*. Nevertheless, this paper showed that the total energy input (13.82MJ/kg) utilised for producing microalgae biomass more than doubles the heat input (6.69MJ/kg) required for converting the microalgae to biocrude. The cultivation step consumed more energy (9.78MJ/kg), suggesting the need for the development of new technologies that require less energy. This could make the HTL process of microalgae environmentally sustainable

A long-term concern besides the upstream for selecting suitable microalgae feedstock is maximising yields of biocrude and improving their qualities. Accordingly, the effect of process parameters on the yield and quality of biocrude during hydrothermal liquefaction of microalgae has been the focus of most HTL microalgae biofuels studies. Brown *et al*., (2010) investigated the influence of operating temperature on hydrothermal liquefaction of *Nannochloropsis* sp., focusing on biocrude and gas production at a constant holding time of 60min. The study was conducted at 200°C to 500°C with 50°C incremental steps. It was reported that the optimum condition was 350°C leading to maximum biocrude yield of 43wt%. The biocrude was characterised with 79% carbon content, a higher heating value of 39MJ/kg, and a maximum energy recovery of 78%. Higher operating temperatures increased gasification reactions resulting to 1.8mmol/g of gas phase yield with a HHV of ~ 4.2MJ/kg.

Zou *et al*., (2010b) studied the effect of temperature (300°C to 380°C), and 10min to 100min reaction time on HTL products yield using a stainless steel 100mL capacity and magnetic stirrer. The scope of this study was the effect of reaction temperature, holding time and biomass to water ratio on biocrude yield, solid residue and aqueous phase. The maximum biocrude yield of 36.9wt% was achieved at 360°C, 30min, however, with a low HHV of 26.62MJ/kg compared to 39MJ/kg and 50MJ/kg of previous studies (Brown *et al*., 2010; Dote *et al*., 1994). The solid residue and aqueous phase yield was 14.4wt% and 48.7wt%, respectively. It was found that a biomass to water ratio of 1:10 was more suitable for high biocrude yield, as a decreased in biocrude yield was observed with an increased in biomass to water ratio. This suggests that liquefaction with higher solid content could improve the energy balance, although additional energy usage is utilized to concentrate the biomass slurry. It also means that using lot of water is uneconomical as more energy is utilized which could also be more expensive for downstream wastewater treatment.

Table 1: Summary of work on HTL of microalgae without catalysts

Microalgae	**Process parameters**				**Biocrude yield and properties**					**References**
	Reactor capacity	**T°C**	**Time (min)**	**Solid content (wt%)**	**Optimum condition**	**Max. biocrude yield, wt%**	**Carbon %**	**Nitrogen %**	**HHV (MJ/kg)**	
Nannochloropsis sp., *Chlorella* sp.	100 mL	220-300	30-90	15-25	220°C, 90min, 20wt% solid	82.9	75.6	0.3	34.2	Li *et al*., (2014)
Chlorella protothecoides, *Scenedesmus* sp.	4.1mL	250-400	10-60	15	350°C, 20min	48	NR	NR	NR	Valdez *et al*., (2014)
Scenedesmus ob., Phaeodactylum tri., N. gadiatana.,S. Almeriensis, T.suecica, C. vulgaris, P.purpureum, D.tertiolecta.	43mL	250, 375	5	5-7	375 °C, 5min	58.1	72-74	5.8-7.1	34.9-37.2	Barreiro *et al*., (2013b)
Chlorella sp, *Spirulina* sp.	~2L	250-350	3-5	1-10	350°C, 3min, 10wt% solid	41.7	70.1	7.7	33.8	Jazwari *et al*., (2013)
Nannochloropsis sp.		250-400	10-90	5-35	350°C, 40min	50	75	5	NR	Valdez *et al*., (2012)
*Scenedesmus*sp., *Spirulina* sp.	2L	300	30		300°C, 30min	45	72.6, 72.2	6.5, 8.1	35.3-35.8	Vardon *et al*., (2012, 2011)
Desmodesmus sp.	45mL	175-450	5, 60		375°C, 5min	49	75.4	5.7	36	Alba *et al*., (2011)
Spirulina sp.	1.8L	200-380	30-120	10-50	350°C,60min	39.9	73.7	6.30	35.2	Jena *et al*., (2011a)
Chlorella vu., N. occulata, P. cruetum, Spirulina sp,	75mL	350	60	11	350°C,60min	35	73.6-72.5	4.1- 7	35.1-39	Biller and Ross, (2011)
Chlorella pyrenoidosa	2L	200-300	10-120	20	280°C,120min	39.4	NR	NR	35.4	Yu *et al*., (2011a&b)
Dunaniliella tertiolecta	100mL	300-380	10-100	10	360°C, 30min	36.9	56.1	4.97	26.6	Zou *et al*., (2010b)
Nannochloropsis sp.	35mL	200-500	60	20	350°C, 60min	43	76	3.9	39	Brown *et al*., (2010)
Dunaniliella tertiolecta	100mL	250-340	5, 60	20	300°C, 60min	43.8	71.3	6.9	33.3	Minowa *et al*., (1995)
Botryococcus braunii	300mL	200-340	60	NR	300°C, 60min	57	84.2	0.9	50	Dote *et al*., (1994)

Moreover, Jena *et al.*, (2011a) reported 20wt% solid concentration to be more suitable for maximum biocrude yield of 39.9wt% with HHV of ~35.3MJ/kg at 350°C, 60min holding time. Interestingly, there was increased deoxygenation at higher reaction temperatures, but an increase in nitrogen content in biocrude, mostly due to the onset of protein decomposition. A trade-off therefore exists between high oxygen and nitrogen content biocrude, the decision could be dependent on the desired end-product and its application. It was reported that the solid loading and reaction time had no substantial effect on either the oxygen or nitrogen content and HHV. However, the biocrude yield increased with about 10wt% when the holding time was increased to 60min. Jena *et al.*, (2011a) investigated the effect of solids concentrations (10%w/w to 50%w/w). Their results showed operating temperatures from 200-380°C and with reaction times up to 120min.

In a similar investigation, Yu *et al.*, (2011a, b) studied the HTL of a low-lipid (0.1wt%) microalga *Chlorella pyrenoidosa*. High-lipid microalgae are often preferred to low-lipid microalgae for maximum yield of biocrude. Researchers are motivated to explore the feasibility of producing maximum biocrude yield from low-lipid microalgae, as the biomass productivity of low-lipid microalgae is usually higher than that of high-lipid species. The influence of temperature (200°C to 300°C) and reaction time (10min to 120 min) on biocrude yield was examined in a 2L reactor and 20%w/w solids loading. It was shown that the maximum refinable biocrude yield was produced at 280°C, however, with a long holding time of 120min. Recent studies have demonstrated that the maximum yield in biocrude can be achieved at short holding times of ~5min. Shorter holding time helps in reducing the reactor size and energy use which leads to reduction in the production cost of biocrude. Thus, a reduction in production cost improves the economic viability of microalgae biofuels produced through HTL process. Using a batch reactor, Faeth *et al.*, (2013) obtained 66wt% biocrude yield in a minute at 350°C when the sand bath was set to 600°C. Alba *et al.*, (2011) achieved 49.4wt% biocrude yield under 5min but at 375°C. Elliott *et al.*, (2013) reported, 38wt% to63.6wt% yield of biocrude in a continuous reactor. The continuous system was based on a throughput of 1.5l/h of slurry using up to 35%w/w solid content. However, more studies are required to optimise the HTL conversion process in a continuous-scale reactor.

Yu *et al.*, (2011b) observed that there was a general increase in carbon, nitrogen and energy recovery in the biocrude oil fraction due to an increase in reaction temperature, but the reverse was observed in the solid residue. A maximum of 65.4% energy was recovered in the biocrude at 280^{o}C, 120min, whereas 65% to 70% and 35% to 40% of nitrogen and carbon, respectively, fractionated in the aqueous phase. The carbon distribution in the gas phase, mostly in the form of CO_2, was however, less than 10%. The higher heating value at the above operating condition was 35.4MJ/kg; however, it increased to 38.5MJ/kg at 300^{o}C, 30min, suggesting further deoxygenation.

Biller and Ross (2011) compared the yields and composition of biocrude following HTL of different model compounds represented by lipids, proteins and carbohydrate fractions of microalgae with microalgae strains rich in proteins, carbohydrates and lipids. The microalgae investigated included *Porphridium cruentum* (rich in carbohydrates), *Spirulina* sp. (high protein low lipid strain), *Chlorella vulgaris* (high protein medium lipid strain) and the marine alga *Nannochloropsis oculata* (high lipid strain) strain. It was reported that biocrude production from microalgae was generally higher than the model compounds except sunflower. Biocrude yield recorded the following trend lipids>proteins>carbohydrates of which high-lipid strains produced maximum biocrude yields (35wt% to 36wt%) compared to ≤ 27wt% for high carbohydrate and high protein containing microalgae strains. One of the key points made in this paper is that microalgal strain rich in lipids produced maximum yield of biocrude , which is in contrast with most studies that have reported maximum biocrude yield from low-lipid strains. For example, Yu *et al*., (2011 a, b) obtained 39.4wt% biocrude yield from *C. pyrenoidosa* with poor lipid content. Zou *et al*., (2010 a) reported 36.9wt% biocrude yield from lipid extracted *D. tertiloecta*. This suggests that biocrude from microalgae is not only formed from the lipid fraction but also from protein and carbohydrate fractions, due to the interaction of various components in cross-linked reactions (Toor *et al*., 2011; Torri *et al*., 2012). Biocrude was found to contain high amounts of protein derivatives particularly N-heterocyclic compounds: whereas the carbohydrates produced cyclic ketones and phenols and the lipids mainly formed fatty acids and hydrocarbons.

Researchers from the University of Twente, Netherlands reported a detailed study on HTL of *Desmodesmus* sp. in two different publications (Alba *et al*., 2011; Torri *et al*., 2012). The effect of operating conditions with varying temperature (175^{o}C to 450^{o}C) and holding time (5min and 60min) at 20%w/w solid content was the focus of their first publication (Alba *et al*., 2011) while the second study (Torri *et al*., 2012) was primarily on the molecular characterization of the biocrude produced under different operating conditions. It was found that the HTL treatment at 375^{o}C and 5min resulted in maximum biocrude yield of 49.4wt%. The HHV of biocrude and energy recovery reported in the study were 35.4MJ/kg and 75%, respectively. This study also examined, the extent of cell rupture under different process conditions using scanning electron microscopy at pre and post liquefaction. A significant increase in biocrude yield was observed when the HTL temperature was increased from 225^{o}C to 250^{o}C at 5min holding time. This coincided with most substantial visual disruption of cells. HTL at 175^{o}C did not show any cell rupture and the cells appeared to be intact similar to that of the fresh microalgae. At 220^{o}C the cells appeared clustered, but the cells were still largely unbroken. Further increase in temperature to 225^{o}C, resulted in a strong cell clustering impact with deformed cells. The majority of cells were ruptured at 250^{o}C, and no individual cells were recognizable above 250^{o}C. This study concluded that the thermal disruption of cells in the HTL process above 250^{o}C enhanced the conversion efficiency leading to an increase in biocrude yield and a large decrease in solid residues.

In this study conducted by Alba *et al.*, (2011), the elemental composition and product distribution as a function of process temperature was also examined. It was found that nitrogen fractionation in the biocrude behaved differently. A significant increase in nitrogen content of biocrude was observed when the HTL temperature was increased from 200^{o}C to 225^{o}C for a short holding period (5min) while a long reaction time of 60min was required to observe significant increase in nitrogen content of biocrude at lower temperature (200^{o}C). The conditions reported in the study for protein decomposition and nitrogen release were in agreement with previous studies (Jena *et al.*, 2011a; Yu *et al.*, 2011b). It was found that nitrogen content continually increased with increasing reaction temperature, while the oxygen content reduced. It was concluded that the process conditions are mostly dependent on desired end product; that if lipid-rich biocrude is desired, the reaction temperature should not be higher than 250^{o}C. However, if “petroleum crude-like” biocrude is desired, then process temperatures of up to 375^{o}C could be applied. In a following publication (Torri *et al.*, 2012) the resultant biocrude fraction was subjected to detailed chemical characterization using different analytical techniques. The turnout of biocrude molecular compounds and HTL possible reaction pathways under various process conditions was revealed. In summary, it was shown that biocrude obtained at lower reaction temperature (<250^{o}C) mostly consists of lipids and some short-chain algaenan and hydrophobic protein fragments. At harsher operating conditions, proteins and carbohydrate begin to breakdown resulting in increased quantities of cyclic dipetides, amino acids, furans and asphaltene-like compounds (e.g. alkylpyrrolidinones, pyrazines). It was concluded that the increase of these compounds in the oil led to increased biocrude mass yield with increased temperature and an increase in nitrogen content in the biocrude.

Valdez *et al*., (2012) investigated the effects of process temperature on HTL products, particularly biocrude during the hydrothermal treatment of *Nannochloropsis* sp. Operating temperatures of 250^{o}C to 400^{o}C with 50^{o}C steps, a reaction time of 10min to 90min, and biomass loading of 5%w/w to 35%w/w was investigated. It was found that the maximum biocrude yield of 50wt% was achieved at 350^{o}C, 20min; increasing the microalgae loading increased the biocrude yield from 36wt% to 46wt% with 15%w/w loading was adjudged the optimum. There were also a decrease in solid yields and an increase in gas phase yields. In agreement with previous reports (e.g. Yu *et al*., 2011b; Brown *et al*., 2010), the gas yield mostly consist of 93mol% CO_2 the rest accounting for some flammable gases such as H_2, CH_4, C_2H_4, and C_2H_6 increases as both time and temperature increase. At 250^{o}C, <1wt% of microalgae biomass was converted to gas phase but the yields increased to 13wt% at 400^{o}C. It was reported that regardless of operating temperature and holding time, the yields of aqueous phase was 51wt%. This is in contrast to previous reports (e.g. Alba *et al*., 2011) suggesting that increasing temperature reduces the aqueous phase yield from 41.5wt% to 15.3wt% at 250 and 400^{o}C, respectively, leading to increase in biocrude yields. Valdez *et al*., (2012) reported that up to 84% nitrogen was fractionated in the aqueous phase, accounting for about two-thirds of initial nitrogen content of the microalgae. The N content was found to be both time and temperature dependent, and an increase in reaction temperature led to an increase in nitrogen content in the biocrude. The high nitrogen content in the biocrude is undesired and reaffirms the need to develop techniques capable of biocrude denitrogenation.

Jazrawi *et al*., (2013) investigated the hydrothermal processing of *Chlorella* sp. and *Spirulina* sp. using a pilot plant continuous system. Process temperatures (250^{o}C to 350^{o}C), biomass loadings (1wt% to 10wt%), pressures (150bar to 200bar) and short residence time (3min to 5min) were investigated. It was found that severe operating conditions with higher biomass loadings resulted in increased biocrude yields. The harsh reaction conditions seem to favour lower oxygen content, carbon recovery, and formation of lower molecular weight in the biocrude. Unfortunately, there was increase nitrogen content in the biocrude, a common issue with batch studies (Jena *et al.*, 2011a; Brown *et al.*, 2010). Hydrothermal treatment of *Chlorella* sp. with 10%w/w biomass loading led to the maximum biocrude yield of 41.7wt% at 350°C and 3min holding time. The HHV at this condition was 33.8MJ/kg similar to 32.5MJ/kg with 5wt% solid concentration, suggesting that biomass loading had no substantial effect in biocrude energy density. It was concluded that the composition of the biocrude oil differs between short and long holding times.

Barreiro *et al.*'s (2013b) investigation was on the effect of strain specific parameters represented by biochemical composition, cell structure and growth environment of different microalgae on biocrude yields. Two process temperatures (250°C and 375°C) were investigated for each microalga with a 5min holding time. It was found that an increase in temperature led to a substantial increase in biocrude oil yields amongst all strains. This reduced oxygen content in biocrude leading to improvement in HHVs. At 250°C, there were large variations in the biocrude yields (17.6wt% to 44.8wt%) within the species, while at 375°C the difference became less significant (from 45.6wt% to 58.1wt%). At high reaction temperature (375°C), the HHV were in the range of 34.9MJ/kg to 37.2MJ/kg. At mild reaction temperature, strain parameters had an influence on yields. High biocrude yields were obtained from species lacking cell walls, but under severe conditions strain specific parameters had no substantial impact on the biocrude yields. It was found that high carbohydrate content species led to lower biocrude yields in accordance with Biller and Ross, (2011) and Valdez *et al.*, (2012). In this paper (Barreiro *et al.*, 2013b) biocrude yields failed to be correlated with individual strain biochemical composition, which reaffirmed that it is not mandatory for high-lipid content species for HTL when suitable high temperatures are applied. However, some questions surrounding the influence in the quality of biocrude are yet to be established e.g. in terms of hydrocarbon content.

Recently Li *et al.*, (2014) showed that HTL of high lipid-microalgae led to higher biocrude yields but with very poor oil quality when considering the hydrocarbon content. The focus of the study was to examine how lipid and protein content microalgae influence oil yield and its quality; effect of operating temperatures (220°C to 300°C), holding time (30min to 90min) at 15wt% to 25wt% solid content. The biochemical compositions of the microalgae investigated are presented in Table 2. It includes a low-lipid (14.1wt%) high-protein (52.4wt%) *Nannochloropsis* sp. and a high-lipid (59.9wt%) low protein (9.3wt%) *Chlorella* sp.

Table 2: Biochemical composition of microalgae *Nannochloropsis* sp and *Chlorella* sp.

Microalgae Species	**Biochemical composition (%)**				
	Protein	**Lipid**	**Fibre**	**Non-fibrous Carbohydrate**	**Ash**
Nannochloropsis sp.	52.4	14.1	5.3	21.9	6.3
Chlorella sp.	9.3	59.9	12.7	13.2	4.9

Source: Li *et al*., (2014).

It was found that the microalgae composition significantly affected the yield and quality of biocrude, a maximum yield of 82.9wt% the largest so far was obtained from *Chlorella* sp. at 220^{o}C, 90min holding time with 20%w/w solid loading; whereas 55wt% for *Nannochloropsis* sp. at 260^{o}C, 60min at 25%w/w solid content. The HHV at these conditions was 31.5MJ/kg and 34.9MJ/kg for *Nannohloropsis* sp. and *Chlorella* sp, respectively. At 300^{o}C, it increased to ~37MJ/kg for both microalgae, possibly showing further deoxygenation. It was reported, however, that the optimal conditions for biocrude yields were inconsistent with that for the biocrude quality in agreement with previous work (e.g. Jazwari *et al*., 2013). Though, the largest biocrude yield was achieved with *Chlorella* sp., the amount of hydrocarbons were higher in the biocrude obtained from *Nannochloropsis* sp. The hydrocarbon content was 29.8wt% and 17.9wt% for *Nannochloropsis* sp. and *Chlorella* sp. respectively. The amounts of organic acids and fatty acids in the biocrude oil increased with an increase in process temperature and reaction time. Reaffirming the hydrolysis of lipids during hydrothermal liquefaction process conditions, this could be minimized at short holding time and at lower reaction temperature and strongly dependent on species.

Most of the published results discussed herein mainly focus on determining the optimum process conditions for the primary product biocrude. Research studies for a ‘tool’, a process model to predict the overall performance of the HTL are limited, which is an area that requires additional research. Perhaps due to the complexity of substrates, and reaction pathway could have led to difficulties in establishing the reaction kinetics and models for the HTL process. Also there are problems in predicting the detail of chemical conversion, product properties and distribution. In this context, Valdez *et al*., (2013, 2014), investigated the process reaction network providing insights into a kinetic model to predict HTL yields, and conversion rate of lipids, protein and carbohydrate following hydrothermal liquefaction of microalgae. It was shown that HTL reprocessing of individual solid, biocrude and aqueous phase led to additional oil yields with less yield in other fractions, but this might not be economical in terms of extra energy consumption. The proposed kinetic model could account for the trends in how biocrude oil varies with reaction temperature and time; however, the model only operates within the predefined experiment variables applied in determining the model parameters. The model predicted that the yield in biocrude was highest at process temperature >300°C, and < 20min holding time. It was found that at 250°C, the largest kinetic rate constant for oil production was for the conversion of protein to biocrude; at 350°C and 400°C, the rate constant for biocrude from lipids were higher than for protein. This suggests that the process condition suitable to convert lipids to biocrude will also be sufficient to process protein fractions but the end product biocrude will consist of the undesirable high nitrogen content. The rate constant for carbohydrate conversion to biocrude was far too low compared to those of lipids and protein in agreement with Biller and Ross, (2011) who reported less biocrude production from carbohydrates fractions. This indicates that the microalgae of high non-lipidic and non-proteins components might be a poor feedstock for HTL microalgae biofuels. However, Barreiro *et al*., (2013b) reported that the composition of microalgae (lipids, proteins, and carbohydrate) is insufficient to predict biocrude yields following hydrothermal liquefaction, that it is challenging to establish a direct link between the individual biochemical composition of feedstock and the amount of biocrude produced.

2.3. Catalytic hydrothermal liquefaction of microalgae.

Catalytic hydrothermal liquefaction involves incorporating catalyst during conversion of biomass to biocrude, mainly aimed to improve biocrude yields and properties. The biocrude properties include improving HHVs, reduction in heteroatoms content and viscosity. This could enhance quality of the biocrude and its capability for combustion as well as upgrading to transportation fuels. Catalytic HTL lowers the activation energy leading to an increase in a specific product particularly biocrude. It also suppresses char and coke formation, and leads to a high degree of oxygen removal or reduction of a wide range of oxygenated compounds. Most research studies on the effect of catalyst during HTL of microalgae have mostly made use of homogeneous catalysts, of which Na_2CO_3 has mainly been applied. Very few studies have been published using heterogeneous catalyst. A summary of the published reports on catalytic hydrothermal liquefaction is presented in Table 4.

At the NIRE group, Inoue *et al*., (1994) and Dote *et al*., (1994) performed hydrothermal liquefaction of *B. braunii* with/without 5wt% sodium carbonate in closed vessel at 250^{o}C to 400^{o}C, 5min and 60min reaction time. The effect of reaction parameters (reaction temperature, holding time and catalyst) on biocrude yield and properties were investigated. It was reported that Na_2CO_3 showed no clear catalytic influence on either the biocrude yields or their properties, although it had distinct effects during processing of wood and sewage sludge. Working in the same group Minowa *et al*., (1995) reported an increase in biocrude recovery with the use of sodium carbonate catalyst following liquefaction of *D. tertiolecta* at 200^{o}C and 300^{o}C. Maximum biocrude yield of 78wt% was achieved at 200^{o}C reaction temperature with 5% sodium carbonate catalyst. Decreased biocrude yields (25wt%) were observed with further increased reaction temperatures to 340^{o}C. The differences in biocrude yield without catalyst were 30%, 21% and 1% at 200^{o}C, 300^{o}C and 340^{o}C, respectively, with significant reduction in the oxygen content. The authors highlighted that the catalytic effect on biocrude yield were more pronounced at lower processing temperatures.

A similar finding was reported by Yang *et al*., (2004) who performed their investigation under a nitrogen atmosphere with 5% sodium carbonate at 300^{o}C and 340^{o}C at 30min and 60min reaction time on the HTL of *Microcystis viridis.* Though 33wt% maximum biocrude yield with 31MJ/kg was reported, it was found that the catalyst impact was stronger at lower operating temperature and shorter holding time of 30min.

In 1997 Matsui *et al*. performed HTL of *Spirulina* sp. in the operating temperature range 300^{o}C to 425^{o}C at 30min and 60min reaction time using iron catalyst under hydrogen, nitrogen and carbon monoxide either in deionised water, toluene and tetralin solvent. Using the catalyst with either tetralin or toluene in different atmosphere led to increased biocrude yield from 52.3wt% to 66.9wt% at 350^{o}C, 60min. HTL in water media plus iron catalyst and under nitrogen produced a higher crude yield of 78.3wt% also at 350^{o}C. However the biocrude fractions had a lower carbon and oxygen content as well as a lesser HHV (26MJ/kg). The treatment in toluene produced biocrude with higher carbon, lower oxygen content and higher heating value of 32MJ/kg to 33MJ/kg.

Ross *et al*., (2010) incorporating two alkali potassium hydroxide and sodium carbonate, and organic acids, acetic and formic acid separately converted *Chlorella* sp. and *Spirulina* sp. to biocrude in a high pressure batch reactor at 300°C and 350°C, 60min reaction time. The report revealed that the application of organic acids can enhance biocrude flow properties with significant reduction in the boiling point range. It was reported here that alkali catalyst, sodium carbonate led to the maximum biocrude yield with best properties in both microalgae compared to any of the organic acids. A further study by Biller and Ross, (2011) demonstrated that catalytic effect of alkali and acidic catalyst on the biochemical composition of microalgae behaves differently in biocrude yields and quality. The reported data showed the use of alkali catalyst, sodium carbonate had more positive effects in the processing of high carbohydrates microalgae strains.

As a result liquefaction of the high-carbohydrate microalgae *Porphyridium cr.* led to maximum bocrude yields using Na_2CO_3 catalyst compared to either formic acid or water. Applying alkali catalyst with feedstock of high lipids content promoted saponification reactions leading to soap formation, leading to reduced biocrude yields. However, using sodium carbonate with high-lipids microalgae led to production of long-chain alkanes. High biocrude yields were achieved from liquefaction of model protein samples without alkali or organic acids. Based on this report, high carbohydrate microalgae are best treated in alkali catalyst while high-lipid and protein containing microalgae are favourably processed in either formic acid or without catalyst, leading to increase oil flow properties and decrease in boiling point.

Further studies by Zou *et al.*, (2009, 2010a) showed that using different concentrations of 0% to 10% Na_2CO_3 and 0% to 3% H_2SO_4 catalyst loading led to initial increased in biocrude yield and decreased with further increased in catalyst intake. As a result 5% Na_2CO_3 and 2.4% H_2SO_4 were found to be the optimal catalyst input. However, the variation in Na_2CO_3 loading during processing *D. tertiolecta* to biocrude was found to have <5wt% difference in biocrude increase with respect to the different catalyst intake and without catalyst. The process parameter such as operating temperature and reaction time were reported to have a much larger effect on biocrude yields. This paper shows that microalgae with high lipids content are easier to treat and favourable for higher biocrude yields compared to low fat content microalgae. In addition it was reported that it is not only lipids but inclusive of other organic compounds; protein and carbohydrate that are converted to biocrude during HTL.

Table 3: Summary of previous work on catalytic HTL of microalgae

Microalgae	Process parameters					Biocrude yield and properties					Reference
	T^{o}C	Time (min)	Solid content, wt%	Catalysts	Atmosphere	Biocrude yield, wt%	Carbon %	Hydrogen %	Nitrogen %	HHV (MJ/kg)	
Spirulina sp.	300-350	30-60	10-20	5% Na_2CO_3, NiO, $Ca_3(PO_4)_2$	N_2	51.6	72-75.9	5.4-6.4	4.7-6.4	35-38.4	Jena *et al*., (2012)
Nannochloropsis oc., *Chlorella vulgaris.*	350	60	11	Co/Mo, Ni/Al, Pt/Al	Air	18.1-38.9	74-77.0	8.9-10.2	3.6-5.7	34.5-42.0	Biller *et al*., (2011)
Chlorella vulgaris, *Nannochloropsis oc*., *Porphyridium cr*., *Spirulina* sp.	350	60	11	1M HCOOH, Na_2CO_3	Air	5-20	68-75.4	3.8-5.7	3.2-7	22.8-39	Biller and Ross, (2011)
Nannochloropsis sp.	350	60	NR	50% Pd/C, Pt/C, Ru/C, $NiSiO_2$-Al_2O_3, Co/Mo/γ-Al_2O_3, zeolite	He/H_2	35-57	69-76.2	10.2-11.1	3.5-4.3	35.4-40.1	Duan and Savage, (2011a)
Spirulina sp., *Chlorella* sp.	300 and 350	60	11	1M Na_2CO_3, KOH, HCOOH, HCOOH,	Air	11.6-23	69-73.7-	9.3-11.4	4.6-7.1	34.1-37.8	Ross *et al*., (2010)
Dunaniliella tert. cake	280-380	10-90	10	5% Na_2CO_3, 2.4% H_2SO_4	Air	36.9, 25.8	63.6	7.7	3.7	30.7	Zou *et al*., (2010a, 2009)
Microcystis viridis	300-340	30, 60	NR	5% Na_2CO_3	N_2	33	57.9-63.3	6.4-8.0	3.7-7.5	30-31	Yang *et al*., (2004)
Spirulina sp.	300-425	30, 60	20	1-2mmol $Fe(CO)_5$-S	H_2, N_2, CO	66.9	69-64.2	8.6-8.3	8.4-7.6	33.2-30.5	Matsui *et al*., (1997)
Dunaniliella tert.	250-340	5, 60	20	5% Na_2CO_3	N_2	43.8	71-76.3	8.4-9.6	6.9-7.4	34.2-37.8	Minowa *et al*., (1995)
Botryococcus braunii	200-340		20	5% Na_2CO_3	N_2	44	NR		NR	NR	Inoue *et al*.,(1994)
Botryococcus braunii	200-340	60	NR	5% Na_2CO_3	N_2	64	81-84.8	13.8-14.9	0.3-0.9	47-50	Dote *et al*., (1994)
Jet Fuel	-	-	-	-	-	-	86.2	12.3	0.5	47	Roussis *et al*., (2012)
Petrocrude	-	-	-	-	-	-	83-87	10-14	0.1-2	>40	Speight, (1999)

A larger increase in biocrude yield using 5% Na_2CO_3 was reported by Jena *et al.*, (2012) who investigated effect of Na_2CO_3, $Ca_3(PO_4)_2$ and NiO on hydrothermal liquefaction of *Spirulina platensis*. Though there were no substantial difference in water soluble and solid residues among all catalysts and uncatalyzed reactions but the use of $Ca_3(PO_4)_2$ and NiO catalysts led to lower biocrude yields with increased in gas productions compared to using sodium carbonate and water alone. It was clear in the reported data that 5% Na_2CO_3 catalyst, process temperature and holding time had positive influence on biocrude yield. The differences in biocrude yield compared to non-catalytic runs were up to 6.3wt% to 7.6wt% and 8wt% to 11.7wt% at 300^{o}C and 350^{o}C, respectively, at 30min and 60min reaction time. However, there were no substantial difference in nitrogen 4.74wt% to 6.3wt% and oxygen 10.2wt% to 12.7wt% content in biocrudes from all experimental conditions, except the lowest oxygen content 6.5wt% using NiO. Compared to previous reports that applied 5% Na_2CO_3 this paper seems to have reported highest biocrude increased, though it is not clear but this could be due to the higher protein and carbohydrate contents of the microalgae specie investigated.

Apart from the 11wt% biocrude increase with Na_2CO_3 reported by Jena *et al.*, (2012), the use of homogeneous catalyst seems to have no much positive effect on biocrude yields and properties, especially if the extra catalyst cost is considered (Biller *et al.*, 2011). Hence increasing biocrude yield and improve properties such as the reduction of the heteroatoms content still remain an interesting research area. Consequently, there have been further research studies employing heterogeneous catalysts in hydrothermal liquefaction of microalgae (also presented in Table 3) with envisaged potential advantages over homogeneous catalysts. Although mass transfer of reactants with the catalyst may be difficult to achieve with heterogeneous catalyst but it is easier to recover heterogeneous catalyst than the homogeneous catalysts which are more/or less reactant as well (Biller and Ross, 2012). Though, catalysts stability and poisoning in hydrothermal conditions remains a challenge with heterogeneous catalysts (Akiya and Savage, 2009, and Savage, 2002) but much more with homogeneous catalysts.

Recently, Yu *et al.*, (2014) investigated influence of five heterogeneous and two homogeneous catalysts on HTL of *C. pyrenoidosa*. The scope of the investigation was to compare the effect of the different commercial catalysts on improving yields quality of biocrude. The tested heterogeneous catalysts were Pd/C, Pd/Al_2O_3, Pt/C, Pt/Al_2O_3, Raney-Ni, whereas NaOH and Na_2CO_3 for alkaline homogeneous catalysts. The experiments were performed in 100mL magnetic drive and moveable Parr reactor using 30%w/w dry microalgae plus 5wt% each of different catalyst at 240°C and 280°C under 30min holding time. It was reported that at 240°C the effect of all catalysts showed no substantial effect on biocrude yield except sodium hydroxide which resulted in a significant increase in biocrude oil yield from 31.1% to 41% compared to the uncatalyzed run. There were increased biocrude yield at 280°C of which the maximum 50wt% was achieved with heterogeneous catalyst (Raney-Ni) followed by Pd/Al_2O_3 (46.6wt%), whereas 46wt% and 42wt% were achieved for sodium hydroxide and carbonate (Figure 1). The difference between the maximum and lowest yield was ~11wt% similar to Jena *et al.*, (2012) with the use of sodium carbonate compared to uncatalyzed runs. This could imply that the impact of catalysts on biocrude yield seems to be limited perhaps due to the instability of metal catalyst during hydrothermal conditions.

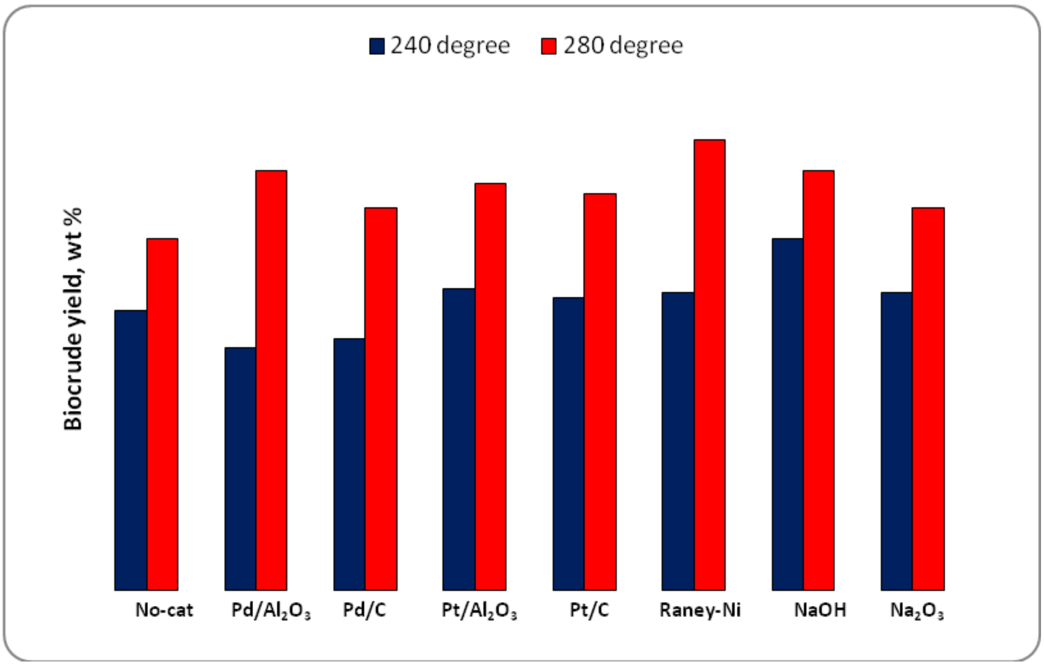

Figure 1: Effect of heterogeneous and homogeneous catalyst on biocrude yield from HTL of microalgae with respect to temperature. Source: Yu *et al.*, (2014).

Despite an increase in biocrude yields with respective catalysts; there was no substantial improvement in the elemental composition compared to that with uncatalyzed reactions. The catalytic effects on the quality of biocrude were less effective at higher temperature compared to low temperature. The use of heterogeneous catalyst e.g. Pd/Al_2O_3 led to substantial increase in hydrocarbons and large decrease in nitro-and oxygen compounds in biocrudes compared to the homogeneous catalyst; NaOH and Na_2CO_3 which resulted to large increase in N and O heterocylic compounds. It was reported that even though the alkaline homogeneous catalyst, NaOH increased biocrude yield it could also increase the complexity due to repolymerzation of relatively small organic compounds to larger organic compounds.

As a result, biocrudes produced from heterogeneous catalysts were of higher quality compared to homogeneous catalysts. However, the biocrude still requires further upgrading due to presence of heteroatoms. Furthermore, the micrographs of recovered metal catalysts shows the deposit of carbon and inorganics on their surface, blocking the pores and this blockage was more pronounced at higher operating temperature. Thus the development of specific catalyst of long life span that could withstand the hydrothermal conditions, to enhance conversion and improve biocrude quality is necessary.

Previous studies on the effect of heterogeneous catalyst on HTL were reported by the Philip Savage Group, US and Andrew Ross Group of the University of Leeds, UK. Duan and Savage, (2011a) investigated the effect of six different catalysts supported on carbon, Al_2O_3, and a zeolite. The metal catalyst investigated were Pd, Pt, Ru, Ni, and Co-Mo. The experiments were conducted at 350^{o}C, 60min with/without $H_{2.}$ It was reported that there was a general increased in biocrude yields at this condition from HTL of *Nannochloropsis* sp. The application of palladium supported on carbon (Pd/C) achieved the maximum biocrude yield of 57wt% compared to 37wt% from uncatalyzed liquefaction. This finding is in contrast to Yu *et al.*, (2014) who reported the lowest biocrude yield of ~42wt% with Pd/C catalyst among all tested catalysts. The difference could be due to variation in microalgae composition and operating temperature. Nevertheless, it suggests that Raney-Ni could be more effective to increase biocrude yields.

There was no significant difference in biocrude yields (45wt% to 50wt%) from all other catalysts. The report highlighted that biocrude yields from HTL without H_2 were insignificant to the catalysts type. It was also noted that the biocrude from application of Pd/C, Pt/C, Ru/C and CoMo/γ-Al_2O_3were lighter in colour and less viscous compared to biocrude from zeolite catalyst or with water alone. Whereas the biocrude produced with nickel catalyzed liquefaction exhibited a dark colour. The energy density, CHNSO and H/C atomic ratios of catalysed biocrudes were largely insensitive to the catalyst type either with/without hydrogen gas. Suggesting it is of no benefit to use hydrogen in liquefaction. Though the addition of hydrogen led to decrease in nitrogen content in most cases except Pd/C indicating a possible denitrogenation but was negligible. It was only Ni/SiO_2-Al_2O_3 that led to complete desulfurization of biocrude but this was alleged to be due to the higher metal loading, while the total sulfur removal could be due to adsorption. The published data shows that the metal catalysts had various effects on gas yields and composition. The use of Ni and Ru increased methane and hydrogen gas whereas zeolite was reported to be the only catalyst to produce nitrogen, the reason being due to the catalytic decomposition of ammonia.

The other study on heterogeneous catalysis was conducted by Biller *et al*. (2011), using Co/Mo, Ni/Al and Pt/Al during HTL of *C. vulgaris*, *N. occulata* and model soya oil to biocrude. The experiment was conducted in a 75mL stainless steel unstirred close vessel at 350^{o}C, 1h. The biocrude yields and properties were reported to be different among all catalysts. Compared with deionised water alone, there were general decreased in biocrude yields from *N. occulata;* in 8.8wt%, 16.4wt% and 4.1wt% when Co/Mo, Ni/Al and Pt/Al were used respectively. This decreased in biocrude yields from this microalga is in contrast to increased biocrude yield in previous reports. Following application of various heterogeneous catalyst on different microalgae (Yu *et al*., 2014 and Duan and Savage, 2011a), a minimum of ~8.8wt% biocrude increase has been produced. A possible reason behind the decrease in this paper could be due to percentage composition and/or metal particle size, or the unstirred reaction. There were no substantial increased in biocrude yields from HTL of *C. vulgaris* with the use of cobalt-molybdenium and platinium on alumina supported catalyst. The increased in biocrude yield was only about 3wt% in both cases. Soya-oil led to increase in biocrude yield of 11.8wt% and 13.4wt% using Co/Mo and Pt/Al, respectively, however, with a large decreased of 44.4% with Ni/Al. The general reduction in biocrude yields with Ni/Al catalyst in all feeds was attributed to enhanced gasification reactions; the use of nickel catalyst has been previously reported to favour this outcome (Waldner *et al*., 2007). It was reported that Co/Mo and Pt/Al resulted to slight deoxygenation of the microalgae strains unlike in soya-oil where Ni/Al were more suitable. This finding suggests selection of specific catalysts for efficient deoxygenation of biocrude should be based on the microalgae composition. This paper also shows a general, up to 10% increase in energy density of produced biocrude.

The application of heterogeneous catalysts in HTL of microalgae seems promising at it has been shown to reduce N and O oxygenated compounds reported by Yu *et al*., (2011b). Though the use of heterogeneous catalysts in upgrading of biocrude could be beneficial, particularly to increase carbon recovery and also to improve biocrude quality; oxygenation and denitrogenation and energy density (Elliott *et al*., 2013). However, it is not clear if the addition of catalyst in HTL is very necessary. None of the used catalysts led to any substantial increase in biocrude yields in addition to negligible reduction of the biocrude nitrogen content. Moreover, catalyst stability, recovery and reusability still pose challenges and therefore needs further investigation. Nevertheless, the use of heterogeneous catalysts in upgrading of biocrude to transportation fuels could be of more benefit than in processing of microalgae stage

2.4. Biocrude upgrading

Based on the scientific literature and the data presented in Table 1 and 3, biocrude produced from noncatalytic and catalytic hydrothermal liquefaction tends to be viscous and tar like with high amounts of undesired heteroatoms, though relatively lower in catalytic liquefaction. The HTL of microalgae with both homogeneous and heterogeneous catalyst revealed that the yield in biocrudes and higher heating values were very insensitive to the presence of catalyst. Additionally, biocrude produced cannot be directly used for transportation fuels except in direct combustion and other applications as shown in Figure 2. This led to further investigations in separate treatment of recovered biocrude to higher quality biocrude still containing mixture of liquid hydrocarbons for easier storage and transport which could also be used as transportation fuels. The hydrotreatment of HTL microalgae biocrude is challenging due to the presence of heteroatoms, particularly the high nitrogen content, carboxylic and fatty acids. The previous studies on upgrading of biocrude can be categorized to thermal and catalytic processes. The thermal treatment simply involves processing of the biocrude in a closed vessel without catalyst and either with addition/or no high-pressure hydrogen. A summary of previous work on upgrading of biocrude is presented in Table 4.

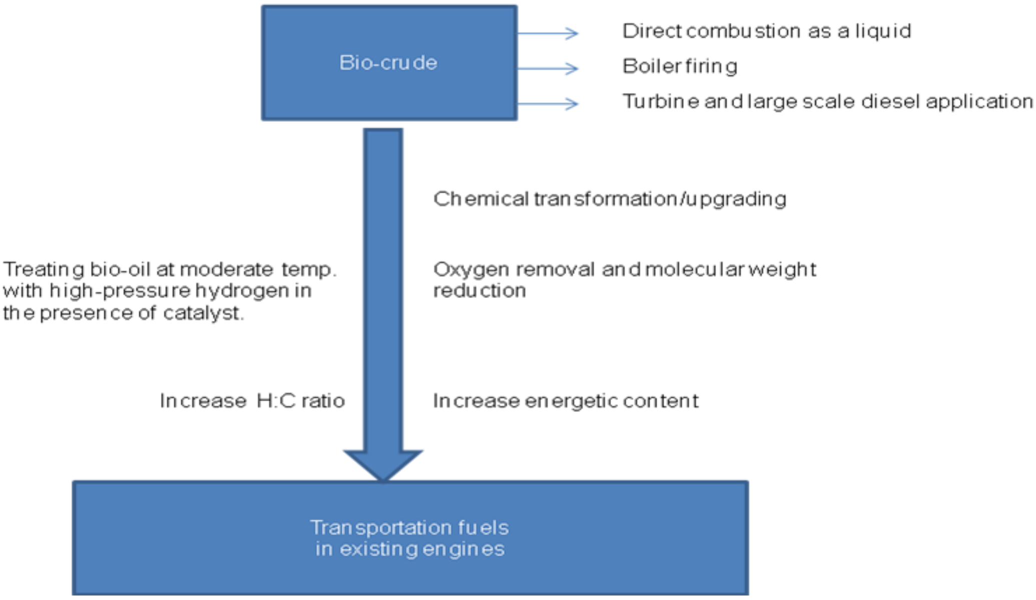

Figure 2: Biocrude upgrading (present work).

Roussis *et al*., (2012) performed hydro treatment of biocrude at sub/supercritical temperatures in 600mL Parr reactor at 350°C to 450°C, 60min holding time. The main aim of the study was to demonstrate that biocrude can be upgraded without the use of expensive catalyst and/or gas. The maximum treated biocrude yield of 86.6wt% was obtained at 350°C, 60min with corresponding 0.4wt% and 2.6wt% solid residue and gas phase yields, respectively. A further increase in reaction temperature led to decreased biocrude yields with increases in solid residue and gas yields. There was a general increase in the carbon content from 77.7%w/w to 84%w/w with no substantial change in hydrogen content. The results showed significant reduction in oxygen content from 5.7%w/w to 0.2%w/w and 1.6%w/w at 400°C and 450°C, respectively, suggesting reduction of total fatty acids in the upgraded biocrude. The higher heating values of upgraded biocrudes were positively affected predominantly due to the increased in carbon and reduction in oxygen content.

The reaction temperatures seem to have important effects during upgrading. Increasing temperature led to a decreased acids concentration, resulting in a beneficial increase in the concentration of saturated compounds e.g. n-alkanes in the resultant biocrudes. Operating at 400°C led to the reduction of acids, maximizing saturated hydrocarbons which are consistent with decarboxylation reaction mechanism. The increase in upgrading temperature increases cracking and the dominance of polymerization reactions which increases gas and coke production. The metallic content reduced with an increase in reaction temperature, which is beneficial to downstream processing, reducing catalyst poisoning and consumption. The study concluded that a lighter, more volatile biocrude characterized with lower densities and free flowing can be produced following thermal treatment. However, the study revealed that the total nitrogen content of the biocrude was not affected by the thermal treatment which remained at 4.2%w/w. The remaining amount of nitrogen in the treated biocrude can be challenging during conventional refining. As a result Roussis *et al.,* (2012) was therefore of the opinion that the reduction of nitrogen content in produced biocrude prior to upgrading is necessary. This could lead total nitrogen removal and enhanced biocrude properties following upgrading.

One of the earlier studies in the upgrading of microalgae biocrude was conducted by the Phillip Savage group at the Department of Chemical Engineering, University of Michigan, USA. Duan and Savage, (2011a) investigated the influence of Pt/C catalyst, pH and hydrogen gas atmosphere in the treatment of biocrude in supercritical water. It was reported that 77wt% yield of treated biocrude was obtained following hydrotreatment. The carbon and hydrogen content increased from 77.3%w/w to 82.8%w/w and 10.5%w/w to 11.3%w/w, respectively. The upgraded crude was generally low in oxygen and nitrogen, free of sulphur with increase higher heating value compared to the initial microalgae biocrude. The hydrogen atmosphere and absence of catalyst led to several desirable changes in higher C, H content and lower S, O and N, but the non-catalytic condition had no substantial effect on the viscosity of produced biocrude. The supercritical treatment with Pt/C and H_2 led to the treated biocrude with lowest total acid number (25.3) compared to 256 of initial microalgae biocrude. It was also reported that the HHVs of treated oil were up to 43MJ/Kg close to those of petrocrude which is encouraging but the process could not totally eliminate the nitrogen content which could pose challenges during refining.

In another related study, Duan and Savage, (2011b) investigated the catalytic hydrotreatment of biocrude produced from *Nannochloropsis* sp. in supercritical water medium. The study was conducted at 400^{o}C, 1h to 8h, and 5wt% to 80wt% Pd/C catalyst loading in hydrogen gas atmosphere. The focus was to obtain optimum conditions for maximum treated biocrude as a function of reaction time and catalyst loading. It was reported that the upgraded biocrude yield strongly depended on holding time, catalyst type and catalyst loading. The treated biocrude yield increased with increasing reaction time from 60min to 240min, the maximum yield of 83wt% obtained at 4h with 20wt% catalyst loading. Increasing catalyst loading of 5wt% to 20wt% led to increase in treated biocrude yield. Treated biocrude yield decreased to

68% with further increase in reaction time to 8h and >20wt% catalyst intake, leading to gas and coke formation. Though Snare *et al*., (2006) suggested that Pd/C catalyst is more active than using Pt/C catalyst during hydrotreatment of biocrude for the removal of fatty acids, based on the data reported by Duan and Savage (2011a&c), 5% Pd/C and 5wt% of Pt/C led to 83wt% and 77wt% treated biocrude yield. It could be inferred that Pd/C and Pt/C have similar catalytic activity during treatment of biocrude in supercritical water media, despite their differences in surface area, micro-pore volume and metal dispersion. The upgraded biocrude generally had lower N-contents which were related to lower fatty acids as shown in GC-MS and FT-IR data. Nevertheless, due to the presence of the oxygen ~3.1%w/w and nitrogen ~3.7%w/w contents in the upgraded biocrude, further decrease of the fatty acids or using other catalyst in biocrude upgrading were suggested before their application to transportation liquids.

Accordingly further investigation in a hydrogen atmosphere using 5% to 20% Pt/C, Mo_2C, HZSM-5 catalyst loading during the catalytic processing of microalga biocrude was performed by same author (Duan and Savage, 2011c). The inclusion of HZSM-5 in their study could probably be due to the catalyst to enhance cracking reactions towards the fragmentation of biocrude heavier components into smaller fuel-range molecule (Peng *et al*., 2009; Souza *et al*., 2008). While the use of Mo_2C were predominantly because of its good catalytic activity in biocrude hydrogenitrigenation, hydrosulfurication and hydrodeoxygenation reactions during hydrotreating of oils (Choi *et al*., 1995; Dhandapani *et al*., 1998). The study was conducted at 430^{o}C to 530^{o}C and 2h to 6h using biocude produced from HTL of *Nannochloropisis* sp. at 340^{o}C, 4h. The reported data revealed that the carbon content of upgraded biocrude was always higher compared to that of initial microalgae biocrude. In the same vain the sulphur content were below detection limit whereas the nitrogen and oxygen contents were generally lower in treated biocrudes than that of the initial biocrude. Even though hydrogen gas was added during the reaction, there was loss of hydrogen atom in the upgraded biocrude with increasing reaction temperature. Higher hydrogen content was only observed at 430^{o}C. The loss in H atom resulted in lower H/C and O/C atomic ratios. The gas phase yields increases with increasing temperature ranging between 2mmol/g to 20mmol/g. The gas phase composition in decreasing concentration was CH_4, C_2H_4, CO_2 and H_2. Under all experimental conditions the hydrogen consumption was higher than 60% with no clear trend in reaction temperature. The optimum condition was

430°C, 6h with 10wt% Mo_2C catalyst loading with 82.0%w/w C; H: 10.7%w/w; N: ~2.6%w/w; S: not detected; O: 4.67%w/w; H/C: 1.57; and HHV: 42.2MJ/kg. The obtained treated biocrude at this condition mainly composed of the alkanes and aromatic compounds. However, based on the optimization data, the catalyst and reaction time had less influence on the nitrogen content, which indicated about 50% reduction relative to initial N-content in microalga biocrude.

Working in same research group, Li and Savage, (2013) reported treatment of biocrude from hydrothermal liquefaction of *Nannochloropsis* sp. The experiment was conducted in a 4mL mini batch reactor at 400°C to 500°C, 0.5h to 4h with 5wt% to 50wt% HZSM-5 catalyst loading. The investigation was on the impact of treatment temperature, holding time and catalyst loading on the yields and composition of treated biocrude and gas phases. The reported data shows reduction in treated biorude from 77wt% to 44wt% with increases in reaction temperature of 400°C to 500°C. Hydrocarbon gas production increases with increasing temperature. The gas production at 400°C, 4h with HZSM-5 was ~2.5mmol/g and higher compared to the 1.4mmol/g and 1.1mmol/g to 1.2mmol/g obtained during thermal and Pt/C catalyst upgrading of microalgal biocrude in previous reports (Roussis *et al.*, 2012 and Duan *et al.*, 2011c). This suggests that biocrude upgrading with zeolite leads to more hydrocarbon gases compared to noble metals or treatment without catalyst. The solid residue decreases with temperature rise, which is in contrast to Roussis *et al.*, (2012) who reported an increase in char content. However, similar amounts were obtained following biocrude treatment.

The reported data show substantial reduction, however, not total removal of nitrogen and oxygen but with below detection limit sulphur content in treated biocrude at all conditions. This led to great reduction in O/C ratios in treated biocrudes compared to initial biocrudes. As a result there was general increased in higher heating values from 38.4MJ/kg to ~44MJ/kg, however there were no substantial difference of the H/C ratios of upgraded biocrude and untreated biocrude. The hydrogen carbon ratios 1.2 at 450°C, 0.9 to 1.4 at 500°C dropped below 1.5 for untreated crude. Catalyst loading was reported to have a more pronounced effect in H/C atomic ratios than retention time. A loading of 5wt% HZSM-5 (1.39) was slightly lower than 1.5 H/C ratio of microalga crude but were higher than 1.1 and 0.9 of treated biocrudes at 4h and 30min, respectively, with 50wt% HZSM-5, all at 500°C reaction temperature.

Table 4: A summary of research on upgrading of resultant biocrude from HTL microalgae

Microalgae	**Process parameters**										**Upgraded Biocrude yield and properties**							**Reference**
	C	**H**	**N**	**S**	**O**	**HHV MJ/kg**	**Biocrude load**	**$T^{o}C$, time**	**Catalyst**	**Atmosphere**	**Max. biocrude yield, wt%**	**C**	**H**	**N**	**S**	**O**	**HHV MJ/kg**	
N. sp.[x]	79.2	10	4.7	0.5	5.7	NR	~53g	125-414, 6-10h	Mo/Co Al support	H_2	79.5	85.0	14.2	<0.1	3.7	0.8	NR	Elliott *et al*., (2013)
N. sp.	78.6	10.4	4.2	0.5	5.3	NR					84.4	85.4	13.3	0.2	<50ppm	1.2	NR	
N. sp (low lipid)	77	10.4	4.2	0.3	8.0	NR					84.6	84.4	13.5	0.3	<50ppm	1.8	NR	
N. sp (high lipid)	77.6	10.6	4.0	0.3	7.2	NR					80.7	84.2	14.0	0.1	<50ppm	1.7	NR	
Nanno. sp.	76.1	9.7	5.3	0.6	8.4	38.4	NR	400-500, 30-8h	50 wt % HZSM-5	H_2	75	84.8	10.7	1.6	ND	2.81	43.4	Li and Savage, (2013)
Nanno. sp.	77.7	11.7	4.2	0.6	5.7	42	NR	350-450	No catalyst	N/A	86.6	83.6	11.7	4.2	0.4	0.2	45	Roussis *et al*., (2012)
Nanno. sp.	77.3	10.5	4.9	0.7	6.5	40.1	0.6	400, 8h	25 wt % Pt/C +pH	H_2, Air	77	80-83	10-11.3	2-2.3	ND	4.31-4.71	41-42.9	Duan and Savage, (2011b)
Nanno. sp.	74.3	9.7	4.5	0.6	11	37.1	0.6	400,1-8h	Pd/C 5-80wt%	H_2	83	81.7	11.5	3.6	ND	3.08	43.51	Duan and Savage, (2011c)
Nanno. sp.	75.9	10.4	4.8	0.5	8.1	39.2	0.4	430-530	Pt/C, Mo_2C, HZSM-5, 5-20wt %	H_2	NR	82-9	7-10.8	2-3.6	ND	1.03-5.3	38-43.5	Duan and Savage, (2011d)

N: *Nannochloropsis;* [x]: lipid extracted, ND: not determined; N/A not applicable; NR: not reported; ppm: parts per million.

Moreover, the elemental carbon, hydrogen and energy recovery in treated biocrudes decreased with increased reaction temperatures. However, lower viscous biocrudes was obtained at higher operating temperatures. Paraffinic biocrude was produced at 400°C containing 95%w/w carbon with 87% energy recovery. Whereas the biocrude obtained at 500°C was more naturally free flowing but with lower yields in biocrude (44wt%) and carbon recovery (70wt%). The energy recovery decreased from 99.3% to 63.8% from 400°C to 500°C.

Furthermore, the report by Elliott *et al.*, (2013) showed promising results after the hydro processing of HTL biorude in fixed bed catalytic reactor. The biocrude was produced from hydrothermal liquefaction of lipid extracted, low lipid and high lipid *Nannochloropisis* sp. at 350°C, 15min with 17wt% to 35wt% solid content with a continuous reactor. The resultant biocrude was hydrotreated with Mo/Co in a hydrogen gas in the same reactor, but the reactor was modified to include the catalyst bed. The yield of upgraded biocrude yield was in the range of 79.5wt% to 84.6wt%. The reported data is interesting as the treated biocrude was almost free of heteroatoms such as oxygen, nitrogen and sulfur. The nitrogen and oxygen content varied between ~0.1%w/w to 0.3%w/w and 0.8%w/w to 1.8%w/w, respectively, the lowest obtained from lipid extracted microalgal biocrude. This suggests that improved decarboxylation and deoxygenation reactions could have taken place. Also some quantities of the N-compounds could have been extracted prior to liquefaction. The sulphur content was also very low <50ppm. It was also reported that the total acid number of the treated biocrude was below detection level, suggesting high removal of oxygen and carboxylic acids in microalgal biocrude. The result of this study was in contrast to previous reports (e.g Duan and Savage, (2011a-c); Li and Savage, (2013) and Roussis *et al*., (2012)) that have been discussed previously. In which about 50% of nitrogen and oxygen content was only removed from the initial biocrude. The variation could be due to reactor configuration, biomass composition and catalyst type. Nevertheless, Elliott *et al*., (2013) report demonstrates the possibility of producing liquid transportation fuels via hydrotreating of biocrude, with almost complete removal of heteroatoms from pretreated microalgae biocrude. However, the upgrading process could be expensive due to the amount of employed catalyst, and catalyst poisoning issues. The extraction of value added coproducts such as pigment, recombinant proteins, and polysaccharides following pretreatment of algae could improve the economics of HTL microalgae biofuels.

In summary, though conditions leading to maximum biocrude yield are preferable, the conditions resulting to lower yields could be desirable if the treated biocrude has better quality such as higher heating values and lower heteroatom contents. In addition, if a long holding time or higher catalyst loading on biocrude promotes hydrocracking reactions of where larger molecules are decomposed to smaller and more volatile molecules, then it could be inferred that the lower yields were due to the loss of the lighter ends during solvent evaporation for biocrude recovery. Though, these previous studies reported significant improvement in quality of the biocrude such as: improved carbon and hydrogen content, energy density, reduced metallic content, and almost free of oxygen and sulphur content, making the treated quality more volatile and less viscous, and having similar properties to fossil fuel, thus favours shipping and storage compared to microalgal biocrude. However in most cases the presence of nitrogen is higher than that of convectional petroleum crude and jet fuel. One of the proposed methods in reducing the nitrogen content in biocrude as mentioned previously is the pre-treatment of microalgae prior to liquefaction (Biller and Ross, 2011; Alba *et al*., 2011).

2.5. Microalgae pre-treatment

As discussed in the previous sections, catalytic and non-catalytic hydrothermal liquefaction, and hydrotreating showed no significant reduction in nitrogen content of the final biocrudes, except Elliott *et al*., (2013). Besides that in a biorefinery system such processes add cost in addition to catalysts being prone to sintering, dissolution and instability in hydrothermal environment, increased energy consumption, and loss of valuable products such as certain lipids and proteins. Although microalgae are a promising source of valuable phytochemicals (Brennan and Owende, 2010), its cultivation for biofuels production is too expensive (Spolaore *et al*., 2006). Hence, the effective use of microalgae with respect to economic sustainability is essential (Tekin and Karagoz, 2013). This has led to experimental studies in the pretreatment of microalgae prior to further processing of the resultant residue via hydrothermal liquefaction. The motivation of integrating microalgae pretreatment with HTL is mostly based on: reducing N-containing compounds, improved quality biocrude, reduction in energy consumption and producing co-products besides the primary product biocrude. This will improve the viability of HTL microalgae biofuels, due to the additional revenue. The summary of published research investigation on pretreatment of microalgae before further processing of residue to biocrude is presented in Table 5.

The pretreatment of microalgae for extraction of components before converting the pretreated microalgae to biocrude was first investigated in the US. Chakraborty *et al.*, (2012) carried-out a two-step sequential hydrothermal liquefaction for extracting value-added polysaccharides from *Chlorella sorokinana* before converting the resultant residue to biocrude. The first of the two steps involved the extraction of polysaccharides by processing microalga biomass at temperatures of 140^{o}C to 200^{o}C, and 10min to 40min reaction times. Polysaccharides were recovered by ethanol precipitation of the treated microalgae slurry. Then the pretreated microalgae were subsequently processed between 220^{o}C to 300^{o}C at 5min to 60min holding time. The optimum pretreatment condition was reported to be 160^{o}C, 20min, producing 32wt% polysaccharides from 45.6wt% present in the initial microalgae biomass. Whereas 240^{o}C, 20min was found to be most suitable with 30wt% maximum biocrude yield, which was 5wt% higher with about 50% less solid residue compared to HTL of untreated microalgae. Interestingly the pre-treatment approach led to a significant reduction (70%) in nitrogen content in produced biocrude. This technique seems promising as it offers the opportunity for the simultaneous production of co-products alongside the primary product biocrude. This will enhanced the revenue in addition to the life-cycle analyses of HTL microalgae fuels. Although integrating the pretreatment in HTL involved additional step in addition to extra usage of organic solvent to precipitate the polysaccharides, but estimating the energy-input indicated no substantial difference compared with the direct liquefaction of untreated microalgae. Moreover, it was reported that equivalent biocrude yield, energy recovery and process efficiency achieved at much lower temperature were similar to those obtained at higher temperature in conventional liquefaction.

Table 5: Summary of biocrude production following microalgae pre-treatment

Microalgae	**Pre-treatment process parameters**						**Biocrude yield and properties from HTL of treated algae**					**Reference**
	Reactor capacity	**T^{o}C**	**Time, min**	**Solid content, wt%**	**Extract & maximum amount, wt%**	**HTL optimum condition**	**Max. biocrude yield, wt%**	**Carbon %**	**Hydrogen %**	**Nitrogen %**	**HHV (MJ/kg)**	
Nannochloropsis oc.	45mL	80-140	12	10	Lipid, 11.3	300^{o}C, 15min	29.4	67-73	11-9.1	5.1-4.9	41-38.2	Biller *et al*., (2013)
C. fritschii					Lipid, 1.4		23.9	64-68	9-8.1	6.9-4.7	35-37.7	
P. ellipsoidea					Lipid, 37.5		49.5	77-74	12-11.6	0.6-0.9	43-44.4	
Chlorella. Sorokiniana	1L	140-200	5-60	10	Polysaccharide, 32.2	240^{o}C, 20min	30	70.3	11-10.8	0.7-1.6	37-20	Miao *et al*., (2012) & Chakraborty *et al.*, (2012)
Scenedesmus sp.	2L	300	30		Lipid, nr	300^{o}C, 30min	45	~73	8.9	6-8.1	35-35.3	Vardon *et al*., (2012)

nr: not reported

In the same context Vardon *et al*., (2012) after extracting lipid (a high value compound) from *Scenedesmus* sp., processed the residue to biocrude. The obtained result was compared with that from unpretreated *Scenedesmus* sp. In contrast to Miao *et al*., (2012) and Chakraborty *et al.,* (2012) who reported higher biocrude yields from pretreated microalgae, the biorude yield from lipid extracted *Scenedesmus* sp. was 10wt% lower compared to that achieved with untreated *Scenedesmus* sp following liquefaction at 300°C, 30min. This is to be expected as they are different techniques, one removes polysaccharide and the other lipids. It is obvious it will have lower yields. Comparing with untreated *Scenedesmus* sp., there was no substantial difference in HHVs, C, H and O content, but with reduced sulphur and a slight increase in the N content. Nevertheless the pretreatment of microalgae to extract compound prior to HTL is an attractive approach as the co-product has high value and can also be processed to biodiesel.

Another study that examined the pretreatment of microalgae prior to HTL was reported by Biller *et al*., (2013). The main aim of this study was pre-treating the microalga to extract high value compound (lipid) and reducing the nitrogen content in the final biocrude produced. The microalgae *N. occulata, Chlorogloepsis fritchii* and *Pseudochoricystis ellipsoidea* biomass were pretreated in a 45mL capacity microwave between 80°C to 140°C at 12min. This was then followed by the liquefaction of the residue at 300°C and at a reaction time of 15min. There reported data showed no clear trend in the amount of extract relative to the pretreatment temperature; *N. occulata* (10.6wt% to 10wt%), and 1.4wt% to 0.7wt% for *C. fritchii.* On the other hand there was increased in lipid extract 30.6wt% to 37.5wt% with increased in temperature for *P. ellipsoidea*. There was no substantial reduction in nitrogen content of the biocrude from pretreated *Nannochloropsis* and *C. fritchii.* There was significant reduction of nitrogen in the biocrude obtained from HTL of pretreated *P. ellipsoidea*. The biocrude nitrogen content decreased from 1.7%w/w to 0.6%w/w at 140°C. In addition, the yields in biocrude increase from 33.4wt% to 49.5wt%, the maximum obtained from HTL of pretreated microalgae at 120°C. The report also shows a significant increase in biocrude quality following the microalgae pretreatment at mild temperature. The HHV increased with about 10MJ/kg, mostly due to reduced quantities of oxygenated compounds in the final biocrude product.

2.6. Energy Analysis

The amount of input energy required to convert microalgae biomass to biofuels is one of the important factors to consider for a process, including hydrothermal liquefaction. Most research studies on HTL are still on a laboratory scale, and for the feasibility of this process a complete energy balance is needed. Despite the number of research studies there is limited data on the energy requirements through downstream to finished products. Therefore this research area needs further investigation, and is experiencing setbacks due to limited information on continuous operating systems. Review of the scientific literature show: no clear consensus about the profitability of HTL microalgae biofuels in terms of energy balance; and no distinct agreement in how to estimate the yield in energy. In estimating the energy balance, two major parameters are mostly applied, the percentage energy recovery (ER) and the energy conversion ratio (ECR) while the less commonly used method is the process thermal efficiency.

The energy recovery is expressed as the percentage amount of chemical energy in the HTL products that is recovered from the original microalgae. It is estimated by:

$$ER\,(\%) = (HHV_i \times M_i) / (HHV_f \times M_f) \times 100\% \qquad (1)$$

where *i* is product, *f* feedstock, *M* mass (kg), *HHV* is higher heating value (MJ/kg).

The ER of the primary product biocrude is mostly reported and it is always observed to be less than unity because some of the energy is recovered in the solid residue, aqueous and gas phases. A summary of the reported ER data of various published research using different microalgae obtained under optimum operating conditions is presented in Table 6. The ER presented is the quantity of chemical energy recovered from microalgae feed into biocrude, excluding that of solid residue, aqueous and gas phases. Typically, the energy recoveries of the excluded products are low. The amount of energy recoveries in the biocrude oil is in the range 50% to 90%. ER values exceeding 90% are mostly attributable to systematic errors such as in the moisture content of the feed, yields and energy density of the biocrude (Duan and Savage, 2011a). As presented in Eq. (1) it is generally believed that the ER data are strongly dependent on the yields and higher heating values of the biocrude and feedtstock.

In terms of the energy consumption ratio, there is no distinct agreement in estimating as the ratio of energy required for HTL to that in produced biocrude (Jena *et al*., 2012; Sawayama *et* *al*., 1999; Minowa *et al*., 1995). An ECR less than 1 has been largely agreed as a net energy producing system, whereas >1 suggest that the liquefaction system consumes more energy than it produces, and if equal to unity indicates that same amount of heat is used for liquefaction as is produced from the biocrude (Biller and Ross, 2011; Yokoyama *et al*., 1987).

Biller and Ross, (2011) reported energy losses for hydrothermal liquefaction of microalgae, leading to a conclusion that HTL technology would be economically feasible if only a good economic balance is achieved. This is in contrast to other reports, for example, Jena *et al*., (2012); Sawayama *et al*., (1999) and Minowa *et al*., (1995) that achieved positive energy balances. It is noted that all the reported energy balances except Sawayama *et al*., (1999) failed to take into account some paramount heat inputs to the process, e.g. energy inputs for fertilizers, cultivation and harvesting, and pumping of slurry as required in a continuous system. The energy balance carried-out by Sawayama *et al.*, (1999) on HTL of *B. braunii* and *D. tertiolecta* which included energy input for liquefaction, fertilizers, cultivation and harvesting referred to as the energy consumption ratio was found that net renewable energy production was possible using *B. braunii* unlike *D. tertiolecta*. The differing in the ECR data could be due to the higher energy required for cultivation and to a great extent for the supply of nutrients. It was also shown that using recycled wastewater as a source of nutrients decreased the ECR from 0.45 to 0.40. A review of the scientific literature showed that other authors have reported higher ECR values with non-inclusion of downstream energy usage, shown in Table 6.

Table 6: Energy recovery, energy conversion ratio and thermal efficiency as a function of process conditions

Biomass	**Reactor system**	**Temp. ^{o}C**	**Time, min**	**Yield, wt%**	**Energy Recovery, %**	**ECR**	**Thermal efficiency,%**	**Reference**
Nannochloropsis sp.	Batch	350	40	50	71	7.6	NR	Valdez *et al*., (2012)
Scenedesmus sp.	Batch	300	30	41	NR	0.44	NR	Vardon *et al*., (2012)
Spirulina sp.		300	30	31	NR	0.63	NR	
Desmodesmus sp.	Batch	375	5	49.4	75	NR	NR	Alba *et al*., (2011)
Spirulina platensis	Batch	350	60	51.6	91.30	0.56	NR	Jena *et al*., (2012)
Chlorella sor.	Batch	300	20	30	54	NR	35	Miao *et al*., (2012)
Chlorella sp.	Batch	350	60	15-25	54.2	0.8	NR	Biller and Ross, (2011)
Nannochloropsis sp.					66.1	1.2		
Porphyridium sp.					51.6	1.6		
Spirulin sp.					50.7	1.0		
Chlorella pyre.	Batch	280	120	39.4	65.4	NR	NR	Yu *et al*., (2011b)
Spirulina platensis	Batch	350	60	41	67.9	0.70	NR	Jena and Das, (2011)
Nannochloropsis sp.	Batch	350	60	35-57	74-120	NR	NR	Duan and Savage, (2011a)
Nannochloropsis sp.	Batch	350	60	43	90	NR	NR	Brown *et al*., (2010)
B. braunii	Batch	300	60	64	NR	0.4-0.45		Sawayama *et al*., (1999)
D. tertiolecta				42	NR	1.56		
D. tertiolecta	Batch	300	60	43.8	NR	0.75	NR	Minowa *et al*., (1995)
Wood	Continuous	300-360	5-20	NR	NR	NR	72-79	Goudriaan *et al*., (2008)

The solid content of the microalgae feed is also as an important factor to consider during liquefaction. As higher biomass loading leads to more biocrude formation, which could improve HTL energy balance. Also doubling the solid content potentially results to twice as much as biocrude yield for the same amount of water heated. However, high biomass loading could affect the pumping efficiency which could be a challenge. Pumping of slurry into the reactor was an issue during the continuous hydrothermal processing of biomass to biocrude (Ocfemia *et al*., 2006b). Nevertheless, the use of continuous reactor has several advantages over batch system, especially in large-scale. Heat exchange is integrated with continuous mode process, leading to decrease in energy consumption; it avoids long heat up and cooling periods as experienced with batch systems; and high throughputs (Biller and Ross, 2011; Ocfemia *et al*., 2006a).

Importantly continuous system have advantages over batch processes as no solvents are required to extract biocrude as commonly applied in the latter (Elliott *et al*., 2013). However, another study has reported the use of solvent to extract biocrude following liquefaction with a continuous reactor (Jazwari *et al*., 2013). Nevertheless, the development of reacting systems that does not need solvent to extract biocrude from the product mixture in large scale continuous systems, will potentially improve the economics of HTL microalgae fuels.

In conclusion, HTL microalgae-biofuels could be feasible in the nearest future if: availability of a high a biomass productivity microalgae which can produce high yields of biocrude, the use of wastewater and/or natural seawater for cultivation, low-energy harvesting technologies will largely improve the energy recovery and process efficiency, with great reduction in the energy consumption ratio.

2.7. Life cycle assessment and techno-economic analysis

The previous sections have shown that there is increasing research in advanced biofuels production from HTL of algae. However, there is limited scientific literature on the life cycle assessment (LCA) and the techno-economic analysis (TEA) of HTL-algae-biofuels. Since researchers and industrialists continues to embrace biofuels from different feedstocks, the LCA is necessary to ensure that algae feedstocks lead to lower life cycle greenhouse gas (LC-GHG) emissions compared to conventional fuels. Similarly the TEA will provide the economic feasibility of biofuels production.

It is desirable that all fuels have lower life cycle greenhouse gas emissions, and values closer or approximately zero are much more desirable (Liu *et al*., 2013). The few reported studies on HTL-algae-biofuels (Fortier *et al*., 2014; Frank et al., 2013; Liu *et al*., 2013) generally agreed of the potentials of HTL-algae. Liu *et al*., (2013) reported that the GHG emissions for HTL-algae-biofuels are comparable to or better than conventional biofuels such as ethanol and soybean biodiesel. In addition HTL-algae-biofuels have lower GHG emissions when compared to other existing microalgae to energy systems such as transesterification. An important factor for HTL-algae-biofuels is where to site the HTL, as it could affect the GHG emissions. In this context Fortier *et al*., (2014) analysed two pathways of siting HTL at the point (waste treatment plant, (WWTP)) of algae production and siting HTL at a refinery. The results of the two pathways were compared to those of conventional jet fuel. It was reported that siting HTL at the WWTP led to lower life cycle greenhouse gas emissions of 35.2KgCO_{2eq}/GJ than 86.5kgCO_{2eq}/GJ for siting HTL at a refinery. Setting-up of HTL at a WWTP resulted to 76% reduction in LC-GHG emissions (21.2kgCO_2/GJ) compared to 39.3Kg CO_{2eq}/GJ for conventional jet fuel.

On the other hand, most TEA studies have mostly focused on conventional algae to biofuels processes such as transesterification (Delrue *et al*., 2013). Longwen *et al*., (2015) evaluated the techno-economic analysis of HTL–algae-biofuels. The study was performed using a 2000 dry tonne/day biorefinery production facility that produces 112dam^3 and 121dam^3 of gasoline-range fuels and diesel-range fuels, respectively, per year. The total project investment cost was estimated at $US504M, while the annual operating was $US158M. The results showed that the minimum fuel selling price (MFSP) of $US679M/m^3 if the internal rate of return assuming a 30-year plant life is assumed as 10%. It was concluded that the MFSP of the advanced fuels obtained were economically competitive with petroleum-derived transportation fuels.

The LCA and TEA of HTL-algae-biocrude studies were more sensitive to algae production, algae dewatering, energy input for HTL, solid content and biocrude yield, while the uncertainties were on feedstock cost, product yield and capital investment. Despited the uncertainties, the results showed the commercial feasibility of advanced biofuel production from HTL of algae. Though HTL-alga-biofuels are feasible, researchers and industrialists still have important challenges to address. There is need to develop more efficient continuously operated reactors with improved heat integration, enhance biocrude yield, recycling of nutrients, and to consider solar drying systems.

2.8. Effect of operating conditions on yield and properties of biocrude

2.8.1. Temperature

Reaction temperature has direct and indirect effect on the activation energy and solvent properties (Moller *et al.,* 2011). As a result reaction temperature is considered one of the important operating parameters not only because of its high influence, but it can also be directly controlled. Increasing temperature leads to changes in the reaction mechanisms. At low operating temperature, there is prevalence of ionic reactions whereas high temperature promotes formation of radicals. The occurrence of free radicals is more likely to occur in subcritical water than below the boiling point of water, but less in supercritical water (Watanabe *et al.,* 2004). This leads to more yield in biocrude, unlike the severe operating temperature that enhances radical reaction that results to diverse of spectrum of gas formation, thus the low biocrude yield.

Generally temperature effect on liquefaction products, particularly in biocrude yield follows an order of increase and decrease. Biocrude yield is low at low temperature and rises with increases in temperatures. After attaining maximum yield, further increases in temperature reduce biocrude production. The effect of reaction temperature on biocrude yield is presented in Figure 3. Based on previous work (e.g. Jena *et al*., 2011a; Alba *et al*., 2011) the temperature range 200^{o}C to 500^{o}C has been mostly investigated for optimal conditions for maximal biocrude yield. As shown in Figure 3 the maximum biocrude has been reported to be obtained close to the critical point of water (375^{o}C, 22.1MPa) but low yield is obtained with further increase in reaction temperature. The increased in temperature improves the conversion rate, leading to more yields in biocrude, decrease in both solid residue and aqueous phases but increase in gas phase yield, Figure 4.

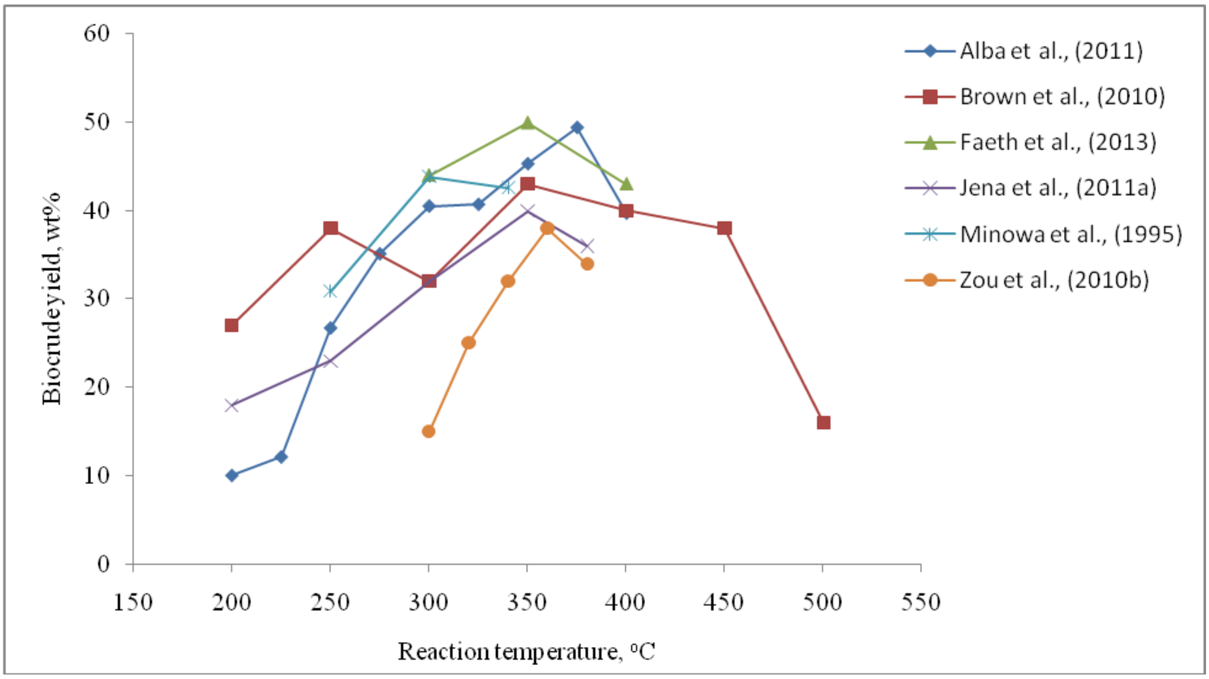

Figure 3: Effect of temperature on biocrude yield

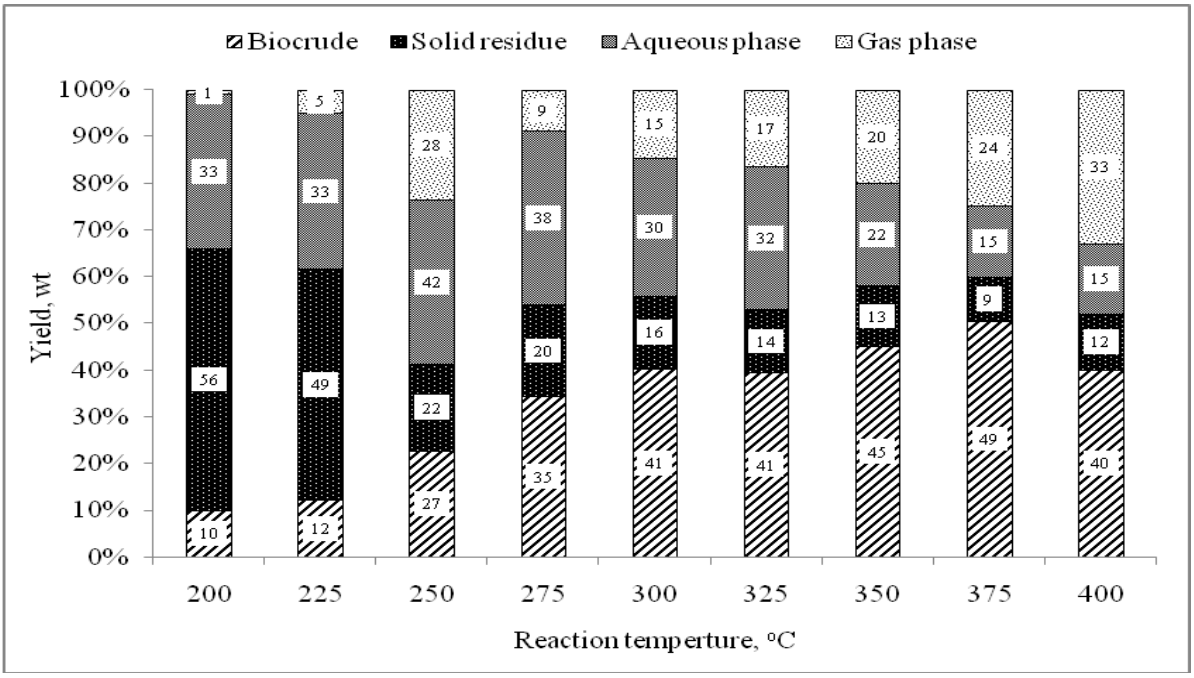

Figure 4: Effect of reaction temperature on HTL biocrude, solid residue, aqueous phase and gas phase products. Source: Alba *et al*., (2011)

2.8.2. Reaction time

The reaction time also known as residence time is the time allowed for reaction after attainment of set point temperature. The reaction time is an important operating parameter that has been proven to have substantial effects on product distribution and quality. Longer reaction times seem to suppress biocrude yield compared to short reaction time. Earlier studies has reported 60min as the optimum reaction time for maximum biocrude yield (Biller and Ross, 2011; Jena *et al.*, 2011a; and Valdez *et al.*, 2012). The trend now is towards achieving maximal biocrude at short reaction time as evidenced by research investigations. Short reaction time is beneficial to reactor capacity in terms of cost and high throughput. The reduction in biocrude yield at longer reaction times could be attributable to the conversion of lighter hydrocarbon fractions in the biocrude into gaseous products. At short reaction time (1min), Faeth *et al.*, (2013) reported 66wt% biocrude yield at a high reaction temperature. High process temperature is uneconomic for biocrude production in terms of operational cost (Moller *et al.*, 2011) and safety of concerns. Alba *et al.*, (2011) reported maximum biocrude of 49wt% at 5min reaction time, but at supercritical condition (375^{o}C). Though high biocrude yields were obtained at short reaction times, more optimization studies are necessary to achieve maximum biocrude yields under subcritical reaction conditions and at short reaction times.

Regarding the effects of reaction time on quality of biocrude, few reports (Jazrawi *et al.*, 2013 and Valdez *et al.*, 2013) reported that it seem challenging to achieve maximum biocrude and best quality at the same operating condition. Irrespective of the type of reactor either batch or continuous, the maximum biocrude yield was only achieved at short reaction times. The reverse was the case for the quality of biocrude, as the best improved properties was obtained at longer reaction times. This phenomenon still remains a challenge to the research community, leading to a situation of trade-off between high yields and optimum qualities. If upgrading is required for produced biocrude, then short reaction times would be suitable otherwise longer reaction times will be necessary if no upgrading is required.

2.8.3. Pressure

Pressure has no major impact on hydrothermal liquefaction compared to reaction temperature and time temperature, and in most cases it is dependent on temperature. As pressure effects on HTL products and properties has received limited studies. Aida *et al.*, (2007) reported that when varying the operation pressure at constant operating temperature had no substantial effect on biocrude yield compared to varying temperature.

2.8.4. Feedstock Concentration

Feedstock concentration which is referred to as solid/substrate concentration, solvent density or mass ratio of feedstock to water is also considered a key parameter in hydrothermal liquefaction. Though the amount of product fraction depends on both the operation temperature and pressure, it also strongly depends on the amount of biomass (solids) in the feed or slurry. The solids to water ratio of feedstock can be altered to specific requirements and capabilities of the process. Higher solid content slurry means the quantity of H_2O per mass of microalgae to be heated will be low, hence a reduction in the energy requirement. However more energy is spent in dewatering the wet microalgae slurry to suitable concentration. High amounts of biomass to water ratio appear to have more influence on biocrude and gas yields unlike on quality (Sato *et al*., 2003). This is probably due to the fact that feedstock dilution minimises cross-reactions of the involved species, which leads to a more defined product spectrum (Moller *et al*., 2011). Due to the varying composition of different microalgae, an optimal water/biomass ratio for biocrude yield is specie dependent. For example, Jena *et al*., (2011a) reported that the yield in biocrude increased from 32.5wt% to 39.9wt% with an increase of 10wt% to 20wt% solid concentration. Further increase in solid content from 20wt% to 50wt% had no substantial effect with increasing biocrude yields. In contrast, Valdez *et al*., (2012) reported steady increased in biocrude yield from 36wt% to 46wt% with 5wt% to 35wt% increase in solid content. An optimum solid content of 15wt% solid content was reported to be more suitable for maximum biuocrude yield. This suggests that different strains of microalgae may behave differently under hydrothermal liquefaction. Valdez *et al*., (2012) reported that the increase in the solid content led to an increase in heavy biocrude yield while light biocrude remained relatively constant.

2.8.5. Catalysts

The application of defined catalyst at suitable reaction temperature, time, and optimum solid concentration has been shown to improve biocrude yield. A wide range of catalyst has been proposed for liquefaction of microalgae mainly towards a specific product and enhanced reaction rate. The tested catalysts mostly include homogeneous and heterogeneous catalyst. The homogeneous catalyst in form of alkali salts; sodium (Na) and potassium (K) has been mostly used, while the heterogeneous catalysts such as nickel (Ni) supported on SiO_2/Al_2O_3 has been less frequently used. Based on previous reports, there has been increased in biocrude yield with the use of catalysts. The increased in yield is dependent on the microalgae composition relative to catalyst type and the percentage loading of catalyst. For example, Biller *et al*., (2011) reported low yield in biocurde during liquefaction of *Nannochloropsis oc.* compared to *C. vulgaris.* The low yield was attributable to the large presence of alkali metals, which could have either promoted alternative reaction pathways. In addition the use of certain catalyst such as Na_2CO_3 has been reported to enhance low biocrude yield if used with biomass of high lipid content (Wang *et al.*, 2013). Furthermore, the catalyst dosage relative to the total amount of feed is an important parameter, as this has effects on the conversion and biocrude yield. Catalysts dosage of 5wt% has been reported to be suitable for increased biocrude yield (Zou *et al*., 2010a, Jena *et al*., 2011b).

2.8.6. Solvent

Solvent acts as both reaction media and reactant in hydrothermal liquefaction. Also suitable solvent are employed to extract biocrude from the HTL product mixture. During HTL, solvent either act as hydrogen donor solvent and a non-hydrogen donor. Wang *et al*., (2007) reported that the presence of a hydrogen donor (HD) solvent enhances the molecular rearrangement and stabilization of the free radicals, resulting in an increase in biocrude yield and decrease in both solid residue and gas yields. The most commonly used solvent as a reaction media is deionised water. This solvent drastically changes in ionic properties ($K_w = [H^+] [OH^-]; [H^+] = [OH^-]$) with an increase in operating temperature close to its critical point (374^oC, 22.1MPa). This allows water to simultaneously act as a solvent and catalyst during liquefaction (Kruse and Dinjus, 2005). At this point hot-compressed water has the ability to hydrolyse biomass components, catalysed through the H^+ and OH^- ions and the hydrothermal cleavage with water molecules (Akiya and Savage, 2002). Thus leads to increase degradation of biomass compounds.

Other solvent in place of deionised water are the acidic, alkaline and organic solvents. This alternative solvent is employed to modify the ionic environment, improving the conversion rates, yields and properties of biocrudes. Liu and Zhang, (2008) investigated the influence of three solvents (acetone, water and ethanol) during the HTL of biomass. It was reported that acetone had more influence on conversion rate (75.9%) compared to both ethanol (74.2%)

and water (69.4%) at high temperature. Water recorded the highest conversion rate at low temperature (250^{o}C). There were increases in biocrude yield with an increase in temperature with all employed solvents. However, a decrease in yield was noticed with further increase of temperature to: 330^{o}C (water) and 400^{o}C (acetone and ethanol). Moreover, Yuan *et al*., (2011) investigated the influence of three organic solvents (methanol, ethanol and 1, 4-dioxane) on hydrothermal liquefaction of *Spirulina* sp. Higher conversion rate and biocrude yield of higher HHV was obtained with the use of methanol and ethanol in comparison to 1, 4-dioxane, although methanol was reported to be more efficient amongst the used solvents. In a related study, Liu and Zhang, (2008) investigated the effects of acetone, water and ethanol on product distribution and property during hydrothermal liquefaction. It was reported that acetone had the maximum conversion rate and higher energy value biocrude, while the maximum biocrude yield was achieved with ethanol. Recently, Zhang and Zhang, (2014) examined the effects of ethanol-to-water ratio as co-solvent on yield and properties (hydrogen-to-carbon ratio, oxygen content and HHV) of biocrude during hydrothermal liquefaction of *C. vulgaris*. It was found that the co-solvent, a mixture of ethanol and water showed better performance in terms of biocrude yields, deoxygenation, and improved HHV compared with the mono-solvents. An ethanol-to-water-ratio 5:2 was reported more suitable for improving yields and properties of the biocrudes. Conclusively, it was suggested that further research in selection of effective solvent exhibiting catalyst characteristics for desired increased in biocrude production is necessary.

For the choice of solvent in extraction of biocrude from the reaction mixture depends on the properties of microalgal lipids. The algal lipids are classified into two groups. Group one consist of open-chain compounds with polar head groups and long non-polar tails, while the second group consists of fused-ring compounds. Microalgal lipids are in group one, which includes fatty acids, triacylglycerols, phosphoacylglycerols and glycolipids. Lipids algal cells consist of glycerine and fatty acids. In addition, the three ester groups of the triacylglycerol are polar whereas the tails of the fatty acids are nonpolar. Ralston and Hoerr, (1942) reported that the occurrence of the polar group brings about a solubilising effect on a paraffin-chain compound, especially in a polar solvent. It could therefore be inferred that the polar solvents enhances the solubility of post liquefaction reaction mixture, improving the yield/recovery of biocrude than the non-polar solvents.

In 2011, Valdez *et al*. investigated the effects of solvents on biocrude recovery and its effects on compositions of product fractions. This study involved use of non-polar (e.g. hexadecane, decane, hexane, cyclohexane) and polar (e.g. methoxycyclopentane, dichloromethane, chloroform) solvents. The maximum yields of biocrude of about (39wt%) were obtained with the use of the non-polar solvents (hexadecane, decane) compared with 30wt% with dichloromethane. However, the recovered biocrude has lower carbon content (69%w/w for decane*)* compared with biocrude obtained with polar solvent (74%w/w for chloroform and for 76wt% for dichloromethane). Also the yields in aqueous phase were higher with the use of non-polar solvents compared to the polar solvents.

Although employing organic solvent in liquefaction could lead to an increase in conversion rate, and biocrude product with better higher heating value. Their use in commercial scale could be uneconomic mostly due to cost and difficulties in the recycling of nutrients (Zhang and Zhang, 2014). Regarding cost, the application of renewable solvent that is less expensive could be used. Importantly, calls for biocrude recovery protocols without the use of organic solvent is seem more necessary. Following liquefaction with a continuous reactor, Elliott *et al*., (2013) recovered biocrude from the product mixture without the use of organic solvent. Another study (Jazrawi *et al*., 2013) with a continuous reactor employed organic solvent to extract biocrude from the product mixture. The aforementioned reports showed differences in biocrude recovery due to the employed method adopted for recovery. Though the recovery of biocrude without employing solvent to extract biocrude from the product mixture might be specie dependent, but it also suggests that biocrude can be obtained without using organic solvent, which calls for further research. Moreover, in addition to cost of organic solvent, during the extraction of biocrude from the product mixture some light fractions of biocrude are lost when evaporating solvents from mixture of solvent and organic phase. This also affects or reduces the amounts of final biocrude yield. Therefore the developments of high separation efficiency for product recovery procedures that will give accurate measurements of product fractions might be needful.

2.8.7. Reducing agent

The use of reducing gas/or hydrogen donor agents functions in a similar way as solvents. A reducing gas atmosphere has the ability to break down microalgae biomass by abstraction. As previously mentioned, one of the main aims of liquefaction is reducing the content of oxygen in the resultant biocrude. Preferably, the oxygen could be removed as CO_2 or H_2O. However it is more advantageous to remove the oxygen as CO_2, since this increases the H:C ratio for a more energetic fuel product (Peterson *et al.*, 2008). The use of reducing gas has limited effects on biomass liquefaction. Among the most commonly used reducing gases are CO and H_2. Reducing gases such as CO helps in the removal of oxygen in the organic compounds, improving biocrude yield.

The removal of elemental oxygen is possible in two ways. Firstly, CO produces hydrogen via the water-gas shift reaction. The hydrogen reacts with the carbonyl groups to form H_2O, thereby removing elemental oxygen. Secondly, CO directly reacts with elemental oxygen to produce CO_2. Inert gases have also been used as substitute for reducing gas but reducing gas is still of better option due to inert gases low reactivity. However, using reducing gas has some negative effects. CO as a reducing gas is safety implicative due its hazardous nature, while H_2 could also be expensive. The use of air as a reducing gas leads to combustion of the biomass, hence not encouraged (Aktar and Amin, 2011). The reducing gas effect on yield of biocrude is presented in Figure 5. As shown in Figure 5, the biocrude yield was higher at 310°C, but the yields vary with different reducing gas at same conditions.

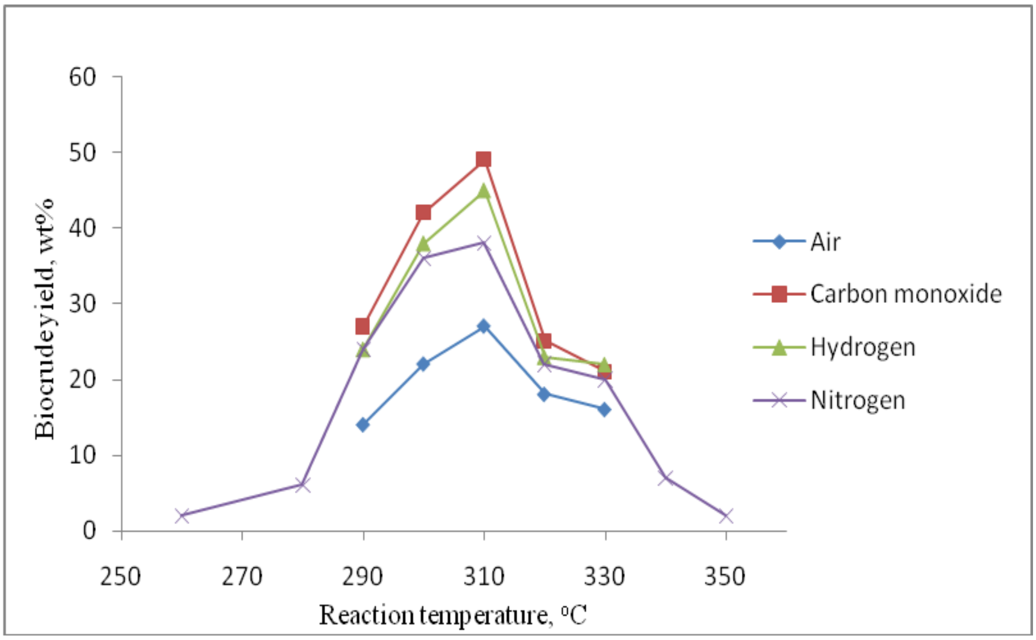

Figure 5: The influence of reducing gas in biocrude yield as a function of temperature. Source: Yin *et al.*, (2010).

This finding suggests that the fragmented radicals are more stabilized with higher reactivity of carbon monoxide and hydrogen during liquefaction. Also the affinity of biomass is more towards CO or H_2, thus easily get stabilized. As a result, carbon monoxide and hydrogen as reducing gas seem to have more positive effect on product yield. Moreover, hydrogen shuttling property of hydrogen donor (HD) solvents which contains uniform supply of hydrogen could improve biocrude yield. Tetralin seem to be the most commonly applied hydrogen donor solvent for HTL process (Akhtar and Amin, 2011), however, their use in addition other reducing gases is unnecessary and could be uneconomical on large scale production due to cost.

2.8.8. Particle size

The influence of particle size on hydrothermal liquefaction is usually not discussed in literatures, as it is less important than other process parameters. Particle size relates to the surface area for mass and heat transfer during liquefaction. Fine particle size leads to increased surface area and invariably increased reaction rate. Feedstocks of smaller particle sizes enhance the degree of fragmentation and an increase in reaction rate due to the larger surface area. An optimum biomass particle size increases biocrude yield, hence the necessity to determine an optimum size to avoid excessive cost. However, it is expensive in reducing the biomass particle size. Microalgae biomass particle size is insignificant and warrants no need for excessive particle size reduction, since wet harvest microalgae slurry is up to 90% water content. Also the operating conditions in sub/supercritical water condition facilitate faster defragmentation and act as a medium for heat transfer and extraction. Zhang *et al.*, (2009) investigated the influence of three particle sizes (0.5mm, 2mm and 1 in length) on yield of biocrude at sub/supercritical conditions. It was reported that the particle size reduction did not enhanced biocrude yield at 350°C, 20MPa for 10min. However, at 374°C and 22.1MPa, the effect of size reduction was observed with decrease in the solid residue yield (14wt% to 7wt%). Based on previous report feedstock particle size in the range of 4mm to 10mm is preferred for hydrothermal liquefaction.

2.8.9. Heating rate

The reactor heating rate effect in product distribution and properties is rarely mentioned in HTL literature. Heating rate is believed to enhance the fragmentation of biomass during liquefaction, particularly at higher temperature. However, it has no substantial effects on biocrude properties. Only few reports including Faeth *et al*., (2013); Biller and Ross, (2011); Biller *et al*., (2011) and Brown *et al*., (2010) has given information on the heating rate, but the impact of heating rate on biocrude yield is still unclear. There were no substantial effects on biocrude yield from *Nannochloropsis oc*. and *C. vulgaris* when the heating rates was increase from 10-25°C (Biller *et al*., 2011). A maximum biocrude yield of 35.8wt% and 38.9wt% was obtained for *Nannochloropsis oc*. and *C vulgaris*, respectively. This data is relatively close to the 43wt% biocrude yield obtained at a heating rate of 20°C/min reported by Brown *et al*., (2010). The effects of heating rates on biocrude yield could be due to the solvent property during liquefaction. The fragmentation of biomass happens so fast that the decomposed by-products are quickly stabilized by the reaction media. As a result further variation in heating rates becomes insignificant. However, Zhang *et al*., (2009) reported an increased in biocrude yield from 63wt% to 76wt% when operating at higher heating rate interval of 5°C/min to 40°C/min. Their findings let to the proposed equation (Eq. 2) to estimate biocrude yield, but the equation is not applicable to other biomass species, as it could not accurately estimate biocrude yield

$$\text{Biocrude yield} = [0.0042 \times \ln(\text{heating rate}) + 0.5514] \times 100\%. \tag{2}$$

Furthermore, operating with a high heating rate of 230°C/min, Faeth *et al*., (2013) reported maximum biocrude yield of 66wt%. This report suggests that the high heating rates could influence an increased in biocrude yield. Low heating rates leads formation of solid residue due to secondary reactions while the high heating rates seem to enhance dissolution and stabilization of fragmented biomass. However, high heating rates could also lead to high gas yields due to secondary reactions. Nevertheless, more investigation is needed in this research area.

2.9. Nutrient recycling

Microalgae biomass production is expensive mostly due to the associated cost of nutrients. As a result nutrients are perceived as a perpetual challenge to the development of large-scale microalgae-bioenergy. In addition the production of nutrient which occurs upstream is one of the life cycle issues associated with the microalgae biofuel facilities. Fortunately, following hydrothermal liquefaction, majority of the microalgae biomass is converted into biocrude. The aqueous phase co-product has been shown to mostly contain significant quantity of the feed nutrients such as nitrogen, potassium, magnesium and phosphates (Valdez *et al*., 2013;

Yu *et al*., 2011b). The severity of reaction leads to more decomposition of nitrogen in initial biomass to ammonia, whereby the dissolved ammonia increases the bio-availability of nitrogen for microorganisms. This facilitates recycling of nutrients to cultivation ponds which can potentially offset the fertilizer requirements (Frank *et al*., 2013; Pham *et al*., 2013). This is an advantage in HTL over other process routes such as in biodiesel production, and one of the important long-term considerations for environmental sustainability.

Nevertheless, several studies have experience difficulties using the recycled aqueous phase for the cultivation of additional microalgae biomass. Several challenges on microalgae growth rates has been reported due to toxicity or nutrient limited (Alba *et al*., 2013; Biller *et al*., 2012). The aqueous phase contains some organic compounds, thus recycling it to an open for microalgae growth could lead to substantial increase in toxicity to cells (Pham *et al*., 2013) and the risk of invasion of heterotrophs contaminant in the culture. Fortunately it has been demonstrated that the minimal dilution of the aqueous phase and nutrient supplements could be utilized for cultivating *Escherichia coli and Pseudomonas putida* monocultures (Nelson *et al*., 2013).

The potential of nutrient recycling from the aqueous phase to cultivate additional microalgae biomass has been demonstrated in two conditions. First is growing microalgae specie in recovered process water following HTL of different microalgae (Jena *et al*., 2011b; Tsukahara *et al*., 2001). Secondly trials involving cultivating microalgae in the same process water obtained after its liquefaction (Biller *et al*., 2012). Other studies have been successful to grow microalgae in industrial wastewater. The main challenges of previous reports are the toxicity of ammonia, metallic ions, organic compounds and fear of invasion of contaminants. This research area has received limited studies, this is summarized below.

Based on the reviewed scientific literature, nutrient recycling for cultivating additional microalgae biomass was initially investigated at the National Institute for Resources and Environment (NIRE) in Japan by Minowa and Sawayama, (1999). The recovered process water obtained from catalytic hydrothermal gasification of *Chorella vulgaris* was employed in the study. It was reported that most of the nitrogen were fractionated into the aqueous phase in form of ammonia. The microalgae growth rate conducted in diluted aqueous phase was compared with that of a standard media. Of which the growth rate in undiluted recycled process water was much lower compared to that of the standard media. However, very good growth rates were observed when the wastewater was blended with standard media. Tsukahara *et al.*, (2001) conducted further investigations to obtain the optimum culture condition(s). Also investigated was the effect of nickel and ammonia ion concentrations with necessary nutrients supplement in the process water on microalgae growth rates. It was reported that 0.22g/l to 1.11g/l of ammonium concentrations was more suitable to grow *C. vulgaris* but above 16.6g/l was observed to be toxic.

It was therefore concluded that a 30 fold dilution of the recovered aqueous phase would be necessary to avoid ammonium toxicity. Similar growth rates were observed with standard growth media and that of diluted process water: up to 75 to 300 dilution times with the addition of magnesium and phosphorus as nutrient supplements. In addition, nickel concentrations were high and the same 30 fold dilution times was reported to be suitable to avoid inhibitory growth. Other studies such as Biller *et al.*, (2012); Haiduc *et al.*, (2009) and Bordons and Jofre (1987) have reported nickel to be inhibitory to microalgae growth rate. Based on this report the nutrient uptake by cells in the medium is affected by the accumulation of nickel on the cell surface which inhibit microalgal growth. Nickel is believed to be leached from reactor wall and its growth inhibitory effect is also similar to that of copper, cadmium and zinc. Also several microalgae strains were able to grow well up to 5g/l nickel. The use of 10g/l led to reduced growth rate while 25g/l highly affected the growth rate. It was therefore concluded that the concentration of nickel in the recycled process water should be well monitored. If necessary the process water should be diluted severally to ensure <10g/l, however, dependent on microalgae strain (Haiduc *et al.*, 2009; Bordons and Jofre, 1987).

Jena *et al.*, (2011b) investigated the growth of *Chorella minutissima* in different dilutions; 10, 100, 300, 400 and 500 times of the wastewater recovered from liquefaction of *S. platensis.* The reported data show that growth was possible using the diluted water of which the higher growth was observed in 100, 300 and 500 times dilution. No growth was observed for the 10 fold dilution which was attributed to the presence of growth inhibitors e.g. nickel. A maximum growth rate of ~80% was observed with the 500 fold dilution compared to that of standard BG11 growth medium. Jena *et al.*, (2011b) and Tsukahara *et al.*,'s (2001) investigations were conducted using different microalgae strains in their trials than for the

HTL experiments. In contrast Biller *et al*., (2012) performed growth trials with *C. vulgaris*, *Scenedesmus dimorph*, *S. platensis* and *C. fritschii* using their respective recycled aqueous phase following liquefaction.

Biller *et al*.,'s (2012) study focussed on the feasibility of nutrient recycling, and to obtain the optimum dilution for all microalgae species compared to standard growth media. To avoid growth inhibition and maintaining similar nitrogen level to that of standard growth medium, the respective process water was diluted in the folds of 50, 100, 200, 400 and 600 times. It was reported that all the strains reproduced in the recovered wastewater but each strain growth rates was a function of the different dilutions. The optimum dilution for all microalgae strains were in the range 200 to 400 times dilution. All the tested microalgae effectively utilized acetate in the recycle aqueous phase as a substrate for mixotrophic growth. Of which *C. vulgaris* and *C. fritschii* recorded the highest biomass productivity compared to the standard media. Based on analyses of the aqueous phase prior to and after cultivation, it was concluded that recycling of nitrogen, potassium and phosphorus was possible without nutrient supplement. This is in contrast to Alba *et al*., (2013) who reported that the recycled aqueous phase need additional nutrients, that growth inhibitory is not only because of undiluted aqueous phase media.

Inserting microbial growth into the recycled aqueous phase might reduce the concentrations of toxic compounds and dilution rates before its further utilization for cultivating microalgae biomass. During growth, a microbial culture utilizes some organic compounds as their carbon source, resulting in reduced toxicity and provision of supplementary biomass. Nelson *et al*., (2013) used recycled aqueous phase from liquefaction of *N. oculata* as a growth media for model heterotrophic microorganisms *E. coli*, *P. putida*, and *S cerevisiae.* It was reported that *E. coli* and *P. putida* were able to grow in the process water as the only source of carbon, nitrogen and phosphorus in the media containing 10vol% to 40vol%. The best growth was observed at 20vol%; however *S. cerevisiae* could only grow under these conditions with glucose supplement. It was concluded that recycling the aqueous phase via the microbial cultures significantly reduces dilution and lower nutrient supplement. In the same context, Pham *et al*., (2013) reported 30% removal of toxicity following treatment with microbial cultures; however they suggested further processing of the aqueous phase before application.

If the aqueous phase organic components proves too toxic, other methods like as anaerobic digestion (AD) or catalytic hydrothermal gasification (CHG) has been proposed as a means to remove the organic and recover the energy as fuel (Barreiro *et al*., 2013a; Elliot *et al*., 2013). Though these processes still lead to recovery of nutrient in the final aqueous product, CHG is an energy intensive process. The use of AD might not be successful as the N/C ratio of the aqueous phase is unsuitable for efficient anaerobic fermentation (Frank *et al*., 2013). Another option is using the aqueous phase as a HTL reaction media could improve the biocrude yield.

2.10. Summary of reviewed literature

In the research area of hydrothermal liquefaction, a range of organic biomass materials, including microalgae biomass has been tested as potential feedstocks. The effect of operating condition such as reaction temperature, reaction time, solid content, catalyst (homogeneous and heterogeneous), and reaction atmosphere has been investigated. The results of these studies mostly agreed that maximum biocrude yield can be produced at 280^{o}C to 375^{o}C, a reaction time ~60min, 15wt% to 30wt% solid content with or without catalyst. It was found that using initial elevated headspace is a normal practice as it ensures that the reaction media remains liquid during liquefaction.

The reviewed scientific literature shows numerous gaps that provide opportunities for further research investigation. It was found that microalgae have great potential as a third generation feedstock for biofuels production due to their faster growth rate and renewability. However, microalgae biomass production is still expensive, representing the largest energy input. Since the yield and quality of the resultant biocrude is directly influenced by the composition of microalgae (Ross *et al*., 2010), has prompted individuals and several research groups to continued search and develop microalgae species. Other conditions considered in selecting particular species are the biomass productivity, resistance to external factor (e.g. bacteria), the ease of culturing in either sea or brackish water, and their possibility for high biocrude yields. For instance, high lipid content microalgae have been reported for maximum biocrude yield (Biller and Ross, 2011). However, such species, particularly marine strains have much lower biomass productivity and are usually related to stress condition compared to low or moderate lipid content species (Rodolfi *et al*., 2009). The feasibility of producing high biocrude yield from low or moderate lipid content microalgal as obtained from high-lipid content strains seems necessary.

Furthermore, it was found that the operating conditions, yields and qualities of biocrude are highly dependent on specific microalgae strains. This suggests the optimum condition for yield and quality of biocrude will be different. Also, the maximum biocrude yields from liquefaction of microalgae have been mostly obtained at long reaction times of 60min. Biocrude yield significantly impacts life-cycle analysis (LCA) data. Most LCA sensitivity analysis has indicated many vital areas to improve the energy efficiency of microalgae-to-biocrude systems. It has been suggested that improving the biocrude yield and recovery would substantially improve the energy-return on energy-invested (EROI) (Liu *et al.*, 2013 and Frank *et al.*, 2013). However, the data employed in computing the EROI analysis in the previous HTL studies were obtained at longer reaction times i.e. about 60min. The few studies that have reported high biocrude yield at short reaction times in order of few minute were obtained at high reaction temperatures (e.g. Alba *et al.*, 2011; Barreiro *et al.*, 2013b). Decreasing the HTL reaction temperatures to subcritical conditions has been suggested to reduce the energy consumption (Biller and Ross, 2011). It is therefore envisaged that the EROI values would be attractive if higher yields of biocrude can be achieved at relatively shorter short reaction times and at subcritical condition.

The resultant biocrude from microalgae has been widely reported to contain undesired metals and heteroatoms, particularly nitrogen and oxygen. Biocrude with high nitrogen content leads to refining issues (Anastasakis and Ross, 2011; Biller and Ross, 2011; Jazrawi *et al.*, 2013), whereas the biocrude with high oxygen content reduces its higher heating value. As a result several techniques has been employed: the use of catalyst (homogeneous and heterogeneous) during liquefaction (Duan and savage, 2011a; Jena *et al.*, 2012; Ross *et al.*, 2010; Zou *et al.*, 2010a & 2009); and in catalytic hydrotreating (Biller *et al.*, 2011; Duan and Savage, 2011b-d; Li and Savage, 2013). The results of some of these studies showed improved yield, HHV and lower sulfur content of the biocrude. However, the nitrogen content is still considerably higher compared to that of conventional crude oil. This is an area that needs further research studies.

One of the important factors in HTL is the present of metals in the resultant biocrude, and since microalgae, particularly marine strains are high in alkali metals, the fate of metals during HTL is a long-term concern. Metals in biocrude seemed unavoidable due to adsorption and leaching, especially if operating at high temperature, and with feedstocks rich in metals. This can also lead to an increase in the metallic constituent in the biocrude. Metals in biocrude could also cause problems, such as in poisoning the catalysts employed for desulfurization and denitrogenation, an increase in gas and coke formation and subsequent decrease in gasoline yields (Speight, 1999). The review of the scientific literature showed limited information on the amounts of metals fractionated in biocrude including their removal. Therefore further studies are required to develop novel technologies to reduce metallic content in the biocrude in order to avoid issues during the downstream processing operations.

Several catalyst and organic solvents (e.g. ethanol, methanol) have been employed as reaction media purposely aimed to enhance the yield and quality of biocrude. The use of catalyst and organic solvent could be acceptable in laboratory but may not be at commercial scale because of the additional cost. The source of natural occurring solvent that could simultaneously act as a reaction media and catalyst to improve the yield and quality of biocrude will be an important contribution in HTL studies.

It was found that some researchers have applied freshly harvested or wet microalgae slurry as feedstock, while some has used diluted pulverised dried/freeze-fried microalgae biomass. Apparently the drying/freeze-drying of microalgae biomass is employed to maintain/store feedstock prior to liquefaction, but such practice will be unnecessary at commercial scale. Moreover, it is not clear if either freshly harvested microalgae biomass slurry or diluted pulverised biomass has any effect on HTL product yields and properties. This is a research area that might require further investigation.

The review literature showed that few studies have been performed in downstream processing of the biocrude. The studies in this area have applied catalytic hydrotreating and thermal treatment of resultant biorude in improving its properties such as increased higher heating values and lower heteroatoms content. However, there is limited information on the distillation of the resultant biocrudes. The distillation of the biocrude will provide further knowledge on the yields and properties of distilled biocrude. It is envisaged that the properties of the biocrude after distillation will be improved and comparable as obtained from catalytic hydrotreating, thus saving cost, and benefitting shipping and transportation.

Finally, combining hydrothermal liquefaction with other organic waste treatment facilities has been strongly suggested to improve the sustainability of renewable energy production and reduction in environmental pollution (Cantrel *et al*., 2007; Hoffmann *et al*., 2013). The sludge also known as digestate from waste treatment plants are high in bacteria and viruses. Importantly the digestate, biosolid contains about 50wt% of unconverted organic matter of initial feedstock. HTL of this digestates, biosolids is a potential means to recover residual carbon. This will produce additional and valuable energy source biocrude that is upgradable to transportation fuels. Also the combined processes will reduce environmental pollution and risk disease epidemics.

Chapter 3

Effect of operating conditions on yield and quality of biocrude during hydrothermal liquefaction of halophytic microalga *Tetraselmis* sp.

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Bioresource Technology, 2014, 170, 20-29.

Statement of Authorship

Title of Paper	Effect of operating conditions on yield and quality of biocrude during hydrothermal liquefaction of halophytic microalga *Tetraslemis* sp.
Publication Status	○ Published ○ Accepted for Publication ○ Submitted for Publication ○ Publication Style
Publication Details	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 microalga *Tetraslemis* sp. Bioresource Technology, 170, 20-29.

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Contribution to the Paper	Designed and performed all experiments, analysed and processed data, and wrote manuscript.		
Overall percentage (%)			
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i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate to include the publication in the thesis; and
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Bioresource Technology, v. 170, pp. 20-29

Chapter 4

Hydrothermal liquefaction of microalgae for biocrude production: Improving the biocrude properties with vacuum distillation

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Bioresource Technology, 2014, 174, 212-221.

Statement of Authorship

Title of Paper	Hydrothermal liquefaction of microalgae for biocrude production: Improving the biocrude properties with vacuum distillation.
Publication Status	○ Published ○ Accepted for Publication ○ Submitted for Publication ○ Publication Style
Publication Details	Eboibi, B.E., Lewis, D.M., Ashman, P.J., Chinnasamy, S. (2014). Hydrothermal liquefaction of microalgae for biocrude production: Improving the biocrude properties with vacuum distillation. Bioresource Technology, 174, 212-221.

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ii. permission is granted for the candidate to include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Eboibi, B.E., Lewis, D.M., Ashman, P.J. & Chinnasamy, S. (2014) Hydrothermal liquefaction of microalgae for biocrude production: Improving the biocrude properties with vacuum distillation
Bioresource Technology, v. 174, pp. 212-221

Chapter 5

Influence of process conditions on pretreatment of microalgae for protein extraction and the production of biocrude during hydrothermal liquefaction of pretreated *Tetraselmis* sp.

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RSC Advances, 2015, 5, 20193-20207.

Statement of Authorship

Title of Paper	Influence of process conditions on pretreatment of microalgae for protein extraction and production of biocrude during hydrothermal liquefaction of pretreated *Tetraselmis* sp.
Publication Status	○ Published ○ Accepted for Publication ○ Submitted for Publication ○ Publication Style
Publication Details	Eboibi, B.E., Lewis, D.M., Ashman, P.J., Chinnasamy, S. (2015). Influence of process conditions on pretreatment of microalgae for protein extraction and production of biocrude during hydrothermal liquefaction of pretreated *Tetraselmis* sp. RSC Advances, 5, 20193–20207.

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Overall percentage (%)			
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iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Cite this: *RSC Adv.*, 2015, **5**, 20193

Influence of process conditions on pretreatment of microalgae for protein extraction and production of biocrude during hydrothermal liquefaction of pretreated *Tetraselmis* sp.

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Received 2nd October 2014
Accepted 13th January 2015

DOI: 10.1039/c4ra11662c

www.rsc.org/advances

Direct conversion of microalgae to advanced biofuels with hydrothermal liquefaction (HTL) is an attractive option which has drawn attention in recent years. The presence of heteroatoms in the resultant biocrude, energy input and the process water has been a long-term concern. In this study, the pretreatment of microalgae biomass for protein extraction was conducted prior to HTL for biocrude production. The impact of operating conditions on both the pretreatment and hydrothermal liquefaction steps was investigated. Following HTL using the pretreated algae with an initial solid content of 16% w/w for 30 min at 310 °C, the biocrude yield was 65 wt%, which was more than a 50% improvement in yield as compared to HTL of untreated algae under the same reaction conditions. To achieve a similar biocrude yield using the untreated algae required a much higher reaction temperature of 350 °C. Using recycled process water as reaction media led to a 25 wt% higher biocrude yield. HTL of pretreated algae led to 32–46% nitrogen reduction in resultant biocrude. The biocrude had a higher heating value (HHV) of 28 MJ kg^{-1} to 34 MJ kg^{-1}. A maximum of 15 wt% protein extract was obtained during pretreatment at 150 °C, 20 min. A similar energy input was required in biocrude production from the untreated route and the combined pretreatment and HTL.

1. Introduction

Hydrothermal liquefaction is a promising technology for the production of biofuel as it provides a direct pathway in the conversion of biomass to biocrude.[1] Hydrothermal liquefaction (HTL) is typically operated at subcritical temperature and pressure, and with hot compressed water as both reaction media and catalyst. This process is suitable for the conversion of low energy-density biomass feedstocks with higher moisture content, such as microalgae. Importantly, HTL avoids the high energy cost associated with drying feedstocks that is normally required in other conversion processes such as pyrolysis and transesterification. In addition, the resultant biocrude could potentially be used directly in existing crude oil refineries.

Recently, there has been increased study of the application of HTL for biocrude production from microalgae. These previous reports have investigated effects of temperature, residence time, solid concentration, catalyst, solvent, and microalgae strain on the yield and composition of HTL products.[2–9] It is clear from the above work that biocrude production is feasible irrespective of differing biochemical composition of individual species and almost complete conversion can be achieved with reaction times in the order of minutes. However, the resultant biocrude typically contains undesirable heteroatoms, especially nitrogen. According to previous work, the nitrogen content of biocrude typically ranges between 5% w/w to 8% w/w, as compared to 0% w/w to 1.1% w/w for petroleum crude.[10] Also the sulphur and oxygen contents are in the range 0.3% w/w to 3% w/w and 5% w/w to 28% w/w, respectively. The heteroatoms in the biocrude prevent its use directly in conventional petroleum refineries.[1] Thus, further research to reduce the nitrogen content of biocrude produce from microalgae is necessary.

Most of these previous reports agreed that high nitrogen content in biocrude was due to decomposition of the microalgae protein fractions into simpler nitrogenous compounds (*e.g.* amides, amino acids) through series of decarboxylation and deamination reactions during HTL. Based on previous research investigations, three methods have been proposed to deal with the issue of high nitrogen content in biocrude: the use of heterogeneous catalysts during liquefaction;[11,12] downstream catalytic upgrading; and pre-treatment of microalgae prior to liquefaction.[2,4]

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The catalytic liquefaction of microalgae *Chlorella vulgaris* and *Nannochloropsis* sp. with Pt/Al_2O_3, Ni/Al_2O_3, Ce/HZSM-5, and $Co/Mo/Al_2O_3$ was shown to improve the yield and energy density of the resultant biocrude and also lower its sulphur content.[11–15] However, the nitrogen content of the biocrude was 6% w/w compared to that of petroleum crude.

The subsequent catalytic hydrotreating of HTL biocrude could in principle be useful to reduce the nitrogen content of biocrude. A review of the scientific literature found that the catalytic hydrotreating step is a simple process, essentially aimed to improve biocrude quality. However, the nitrogen content of biocrude produced in this manner is still high (4.5% w/w to 6% w/w).[16–18]

Thus, while these previous reports have so far demonstrated that catalytic liquefaction and catalytic hydrotreating leads to increased biocrude yield and enhanced fuel properties, neither of these processes appear to have been successful in achieving substantial reductions in nitrogen content. Besides, the use of catalysts in HTL would be expensive due to the high cost of catalyst in addition to problems such as sintering, dissolution and instability within the hydrothermal environment.[19,20] Additionally, there could be increased energy consumption and loss of valuable products *e.g.* protein for pharmaceutical use.[2] Thus, the hydrotreating could be uneconomical.

Another proposed route to address the challenge of biocrude with high nitrogen content is the pretreatment of microalgae for the extraction of protein prior to liquefaction. Extraction of protein from biomass before HTL would not only reduce the nitrogen content in biocrude but also improve the economics of HTL since the extracted protein is a value co-product. Limited information is available on the extraction of protein from microalgae prior to liquefaction. However, some previous studies have investigated the extraction of protein from other types of biomass.[21–23] These reports made use of mild to harsh extraction techniques to recover highly soluble proteins with good techno-functional properties. As a result, protein de-naturation was not a problem since the methods used were for analytical purposes and primarily aimed to achieve the production of enzymatic protein hydrolysates. It is clear from this previous work that the protein and the properties of the by-products strongly depend on reaction conditions during pre-treatment, and that no information is available on biocrude production. It is envisaged that HTL of the solid residue (pre-treated microalgae) obtained after extraction could be valuable for the production of biocrude with low nitrogen content since much of the nitrogen associated with the protein fraction is extracted during the pre-treatment step.

A review of the scientific literature shows that few studies have been conducted in the extraction of pharmaceutical grade products (*e.g.* protein, polysaccharides, and lipids) prior to biocrude production from microalgae. Biller *et al.*[24] investigated the extraction of lipids from *Nannochloropsis oculata* using microwave before processing of the microalgae biomass to biocrude. Chakraborty *et al.*[25] investigated hydrothermal liquefaction of *Chlorella sorokiniana* by extraction of polysaccharides. They concluded that biocrude production is feasible after microalgae pre-treatment. Also the production system does not need additional energy input as compared with conventional liquefaction. However, it was found that a quantity (about 40%) of residual organic carbon is lost in the process water following pretreatment. Recovering the organic carbon from the process water is necessary, as it will improve biocrude yield and lower carbon footprint of HTL-microalgae biofuels systems. Production of co-products simultaneously with biocrude *via* hydrothermal liquefaction of microalgae biomass could improve the viability of HTL microalgae biofuels.

The aim of this paper is to investigate the influence of thermal pretreatment of algae on HTL, product yields and biocrude characteristics. The effect of the pretreatment process water as a reaction media on the HTL product yield and properties was examined.

2. Materials

The hypersaline microalga *Tetraselmis* sp. was used in the present study. *Tetraselmis* sp. was grown and cultivated in an outdoor open raceway pond owned and operated by Muradel Pty Ltd in Karratha, Australia. Commercial grade carbon dioxide, nitrate and phosphate were employed as nutrients. The other essential mineral elements for cultivation were source from natural seawater. Biomass was harvested by centrifugation after electroflocculation. The detail of cultivation and harvesting has been reported elsewhere.[26] This present study (pretreatment and HTL) was conducted at the Aban Infrastructure Pvt Ltd in Chennai, India. To convey the harvested *Tetraselmis* sp. biomass to Chennai, the biomass was freeze-dried (Labcono FreeZone) at a temperature of −48 °C and pressure 0.133 mBar (absolute). The biochemical and elemental compositions of the microalgae are shown in Table 1.

2.1. Methods

2.1.1. Pre-treatment. Microalgae biomass pretreatment and liquefaction steps were performed batch-wise in a custom made 1 L high-pressure Inconel reactor (100 mm × 160 mm). Pre-treatment was performed isothermally at temperatures in the range 130 °C to 200 °C for a fixed reaction time of 20 min with 16% w/w solid content. A slurry was prepared by adding 60 g of freeze-dried *Tetraselmis* sp. (7 wt%) to 300 mL water. Then the reactor was sealed and heated with an electrical band ceramic heater to the desired set-point temperature while stirring at 300 rpm using an inbuilt specialized two bladed magnetic drive impeller type device made of Inconel. At the end of desired reaction time, the reactor was cooled to room temperature. It took about 30 min to attain set-point temperature and 90 min to reach room temperature. After cooling, the reaction mixture was transferred to a separating funnel followed by filtration to recover the pretreated algae as solid phase. The solid phase was oven dried at 40 °C, the resultant mass, labelled as "pretreated algae", was determined using eqn (1).

The filtrate was mixed with ethanol in the ratio 1 : 4 and vigorously agitated for about 5 min. The mixture was allowed to sit for 10–12 hours then decanted to separate the upper and lower

Table 1 Elemental and biochemical composition of *Tetraselmis* sp.

Tetraselmis sp. composition (wt%, afdw[a])	
Carbon (C)	42
Hydrogen (H)	6.8
Nitrogen (N)	8.0
Sulfur (S)	3.0
Oxygen[b] (O)	40.2
H/C	1.94
HHV[c] (MJ kg^{-1})	19.2
Protein	58
Lipids	14
Carbohydrate	22

[a] afdw: ash free dry weight. [b] Determined by difference. [c] Higher heating value.

phases. Soluble protein extract was recovered from the lower phase using ethanol extraction following previous method.[27] The lower phase was again mixed with ethanol (0.5 v/v) and vigorously agitated for about 5 min. The remixed mixture was allowed to settle and the lower phase was decanted. This step was then repeated up to four times, or until the lower phase became clear. The lower phase from the final ethanol extraction step was then oven dried at 50–70 °C. The mass yield of the resultant solid, labelled as "Protein Extract", was determined also using eqn (1). The upper phases (ethanol mixture) remaining after each ethanol extraction stage (above) were combined. Ethanol was recovered from this mixture using vacuum distillation at ~80 °C. A fraction of the process water was oven dried at 100 °C. The mass yield of the wastewater was estimated by relating the resultant dried fraction to the mass of feed.

$$Y_i = \frac{M_\mathrm{i}}{M_\mathrm{f}} \times 100\% \tag{1}$$

where Y_i is the yield of the *i*th fraction, i is the pretreated algae, protein extract or process water, *M* is mass, and f is original feed.

2.1.2. Hydrothermal liquefaction.

For hydrothermal liquefaction, the recovered pretreated algae were diluted to ~16% w/w solid concentration with deionised water or the recycled process water. HTL experiments were performed at 310 and 350 °C, for either 5 or 30 min. We followed the HTL and separation procedures as described in previous work.[6] In each HTL experiment, duplicate runs were performed and average yield reported. The list of separate treatments with experimental variables used is presented in Table 2.

The gravimetric yields (wt%) of biocrude, residual char and aqueous phase (precipitate) were determined by relating the weight of the product to the initial mass of microalgae loaded to the reactor. The biocrude yield was estimated on ash free dry basis. The gas phase yield was determined by difference using the calculated yields of the remaining fractions.

2.2. Analysis

Dried fractions of pretreated algae, process water, residual char, aqueous phase and the liquid biocrude were analysed for elemental composition (carbon, hydrogen, nitrogen, and sulphur) according to ASTM D-5291 method using a VarioEL III Elemental Analyser System GmbH. The oxygen content was determined by difference. The elemental composition data was used to calculate the higher heating value (HHV) using the unified correlation (eqn (2)) proposed by Channiwala and Parikh.[28]

$$\begin{aligned}\mathrm{HHV}\ (\mathrm{MJ\ kg^{-1}}) = {} & 0.3491\mathrm{C} + 1.1783\mathrm{H} + 0.1005\mathrm{S} \\ & - 0.1034\mathrm{O} - 0.0151\mathrm{N} - 0.0211\mathrm{A}\end{aligned} \tag{2}$$

where C, H, N, S, O and A represent the mass of carbon, hydrogen, nitrogen, oxygen, sulphur and ash, on a dry weight basis.

The dried fractions were also analysed for their functional groups and structure with Fourier Transform Infrared Spectroscopy (FT-IR) (Nicolet 6700, Thermo scientific) according to

Table 2 List of separate treatments with experimental variables

Tag	HTL temperature (H/L)	Microalgae pretreatment (Y/N)	Pretreatment temp. (°C)	Process water recycling (Y/N)
350NP[a]	H	N	N/A	N/A
310NP[a]	L	N	N/A	N/A
130PWH	H	Y	130	Y
150PWH	H	Y	150	Y
150PWL	L	Y	150	Y
170PWH	H	Y	170	Y
200PWH	H	Y	200	Y
200DWH	H	Y	200	N

[a] NP = no pretreatment, HTL = hydrothermal liquefaction, H = high (350 °C), L = low (310 °C), Y = yes, N = no, N/A = not applicable. *e.g.* 130PWH = microalgae pretreated at 130 °C followed with liquefaction of pretreated algae with recycled process water at high temperature (H) (350 °C). 350NPH: HTL of untreated algae (no pretreatment (NP)) at high temperature (H) (350 °C). 310NPL: HTL of untreated algae (no pretreatment (NP)) at low temperature (L) (310 °C). 130PWH: algae pretreated at 130 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 150PWH: algae pretreated at 150 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 150PWL: algae pretreated at 150 °C followed by HTL of pretreated algae with process water (PW) at low temperature (L) (310 °C). 170PWH: algae pretreated at 170 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 200DWH: algae pretreated at 200 °C, followed with HTL of pretreated algae with deionised water (DW) at high temperature (H) (350 °C).

the method by Duan and Savage.[12] The FT-IR spectra were assigned.[16,29–31]

The micrographs of initial microalgae biomass and the product fractions (in powder form) were inspected with Philips XL 30 FEG scanning electron microscopy (SEM). This allows a high-resolution of samples, providing more details that are undetected using a conventional optical microscopy. A fraction of the powder samples were coated with platinum after being placed in a sticky and aluminium stab, before being placed in the SEM. The metallic compositions of dried fraction were analyzed by Inductively Couple Plasma-Mass Spectrometry (ICP-MS). About 5 g of dried sample were prepared by wet digestion in nitric acid.

The chemical composition of the biocrude was determined using an Agilent 6890N series GC-MS coupled with a HP5-MS column (length: 30 m, internal diameter: 0.25 mm, film thickness: 0.25 μm). GC-MS analyses were performed according to the method described by Jena *et al.*[7] The biocrude sample size was 1 μL, prepared by diluting to 2.5% v/v with acetone. The injector temperature was 200 °C, oven temperature 50 °C to 250 °C at 10 °C min^{-1}, carrier gas HP helium, flow rate 1 mL min^{-1}, GC interface temperature 250 °C, mass spectrometer temperature 250 °C, scan range: 50 to 600 amu, detector photon multiplier, ionisation method electron impact ionisation. Chemical composition of the biocrude was identified with the mass spectral library of National Institute of Standards and Technology's 1998 version (NIST 98).

The carbon and nitrogen balance in the pretreated algae, biocrude, residual char and aqueous phase was calculated by using the mass balances across the product. The carbon and nitrogen balance in the gas phase plus losses was obtained by mass difference using the estimated recovery of remaining elements.[32]

The percentage energy recovery (ER%) in individual product fractions from initial feedstock were calculated using eqn (3). The energy recovery does not include the external energy input for heating the reactor.

$$\text{ER} = \frac{M_\text{i} \times \text{HHV}_\text{i}}{M_\text{f} \times \text{HHV}_\text{f}} \times 100\% \tag{3}$$

where M is mass (g), HHV is the higher heating value (MJ kg^{-1}), i represent biocrude, residual char or aqueous phase and f the initial algae feed. Assuming the initial microalgae biomass slurry loaded into the reactor was 80% w/w with 20% w/w dried microalgae. The reactor being insulated, the heat capacity of dry microalgae biomass was assumed to be half of that of water.[4,33] The heat input (eqn (4)) required to produce a unit of biocrude from untreated algae, by heating from room temperature to predefined temperature with no vaporization was estimated using the enthalpies of saturated liquids (h_f). Similarly, the heat input for HTL of pretreated algae (E_LP) was determined, but after substituting M_dw and M_ua with M_pw and M_pa, respectively.

$$E_\text{L}\left(\text{MJ kg}^{-1}\right) = \frac{\Delta h_\text{f} \times M_\text{dw} + 0.5 \times \Delta h_\text{f} \times M_\text{ua}}{M_\text{b}} \tag{4}$$

where Δh_f is the change in enthalpies of water at T_1 base temperature, assumed to be ~28 °C and T_2 predefined temperature. M_dw, M_ua, M_pw, M_pa, and M_b are the mass (kg) of deionised water, untreated algae, process water, pretreated algae and biocrude, respectively.

Assuming heat loss to environment and container is negligible, the heat input for vacuum distillation (E_VD) of ethanol from the mixture of ethanol and water was determined using eqn (5).

$$E_\text{VD} = \frac{\left(\left(M_\text{e} \times C_\text{pe} \times \Delta T\right) + \left(M_\text{e} \times \text{HVAP}_\text{e} \times \Delta T\right) + \left(M_\text{w} \times C_\text{pw} \times \Delta T\right) + \left(M_\text{wd} \times \text{HVAP}_\text{w} \times \Delta T\right)\right)}{M_\text{m}} \tag{5}$$

where M_e, M_w is the mass (kg) of ethanol and water respectively, M_wd is the mass (kg) of water in the distilled liquid (mass of water distilled alongside the ethanol), M_m the mass (kg) of ethanol–water mixture, ΔT is the temperature difference, C_pe and C_pw is the specific heat capacity of ethanol (2.3 kJ kg^{-1} K^{-1}) and water (4.2 kJ kg^{-1} K^{-1}), respectively, HVAP_e the latent heat of vaporization for ethanol (841 kJ kg^{-1}), and HVAP_w for water (2260 MJ kg^{-1}). In principle, an ethanol–water solution forms an azeotrope at 78.2 °C at standard atmospheric pressure.[34] The boiling point of ethanol is 78.4 °C, and 100 °C for water but the azeotrope boils at 78.2 °C, which is lower than that of either ethanol or water.[35] Also the distilled liquid from a mixture of ethanol–water contains 95.6% ethanol and 4.4% water. For example, assuming the total amount of ethanol–water mixture for vacuum distillation was 5 kg containing 80% ethanol (4 kg) and 20% water (1 kg). Then T_1 = 28 °C (room temperature), T_2 = 78.2 °C (azeotrope boiling point), ΔT = 50.2 °C. 4.4% water of 4 kg ethanol = 0.176 kg = M_wd. The amount of water remaining = 1 kg − 0.176 kg = 0.824 kg, then the total mixture evaporated = 4 kg ethanol + 0.824 kg water.

For ethanol:

$$\begin{aligned}\text{Heat input for temperature rise} &= M_\text{e} \times C_\text{pe} \times \Delta T \\ &= 4\ \text{kg} \times 2.3\ \text{kJ kg}^{-1}\ \text{K}^{-1} \times 50.2\ ^\circ\text{C} \\ &= 461.84\ \text{kJ}\end{aligned}$$

$$\begin{aligned}\text{Heat input for vaporization} &= M_\text{e} \times \text{HVAP}_\text{e} \times \Delta T \\ &= 4\ \text{kg} \times 841\ \text{kJ kg}^{-1}\ \text{K}^{-1} \\ &= 3364\ \text{kJ}\end{aligned}$$

Similarly for water:

$$\begin{aligned}\text{Heat input for temperature rise} &= M_\text{w} \times C_\text{pw} \times \Delta T \\ &= 1\ \text{kg} \times 4.2\ \text{kJ kg}^{-1}\ \text{K}^{-1} \times 50.2\ ^\circ\text{C} = 210.84\ \text{kJ}\end{aligned}$$

$$\begin{aligned}\text{Heat input for vaporization} &= M_{\text{wd}} \times \text{HVAP}_{\text{w}} \times \Delta T\\ &= 0.176\ \text{kg} \times 2260\ \text{kJ kg}^{-1}\ \text{K}^{-1}\\ &= 397.76\ \text{kJ}\end{aligned}$$

$$\begin{aligned}\text{Total amount of heat input} &= 461.84\ \text{kJ} + 3364\ \text{kJ} + 210.84\ \text{kJ}\\ &\quad + 397.76\ \text{kJ} = 4434.44\ \text{kJ kg}^{-1}\end{aligned}$$

Therefore, the E_{VD} required to evaporate 5 kg of ethanol–water solution $= 4434.44$ kJ/5 kg $= 0.8868$ MJ kg^{-1}.

The energy consumption ratio (ECR) for processing untreated algae was calculated with eqn (6a), while that for the combined process including pretreatment (E_{P}), vacuum distillation of ethanol (E_{VD}) and liquefaction of pretreated algae (E_{LP}) was calculated with the eqn (6b).

$$\text{ECR} = \frac{E_{\text{L}}}{E_{\text{OUT}}} \tag{6a}$$

$$\text{ECR} = \frac{E_{\text{LP}} + E_{\text{P}} + E_{\text{VD}}}{E_{\text{OUT}}} \tag{6b}$$

where E_{L}, E_{LP}, E_{P}, E_{VD} is the amount of heat input for liquefaction of untreated algae, liquefaction of pretreated algae, pretreatment and vacuum distillation, respectively, E_{OUT} energy produced from the biocrude (MJ kg^{-1} biocrude). An ECR greater than 1 suggest that the process consumes more energy than it produces while a ratio <1 means that a net energy producer.[36] If equal to unity indicates that same amount of heat is used for liquefaction as is produced from the biocrude.[4]

3. Result and discussion

3.1. Pre-treatment

The mass yields of pretreated algae, protein extract and process water following pretreatment at 130, 150, 170 and 200 °C are shown in Fig. 1. The yield of pretreated algae varies in the range 54–65 wt% and decreases with increasing pretreatment temperature. The combined yield of the remaining phases (protein extract and process water) varies in the range 28 wt% to 39 wt%. The yield of protein extract varies in the range 4 wt% to 15 wt%, with the maximum yield observed a pretreatment temperature of 150 °C. The decrease in the pretreated algae is mostly due to increased breakdown of the algae cells into water soluble products, which also led to an increase in process water. The lower protein extract at 130 °C could be that the pretreatment temperature was not high enough to break down the algae cell, while the decreased extract after 150 °C could be due to formation of new products. The slight increase in the process water with an increase in pretreatment temperature could be mostly due to the increase in the algae cell metabolites such as lipids that could not precipitate along protein extract phase. It should be noted that a portion of the initial mass of microalgae remains unaccounted following pretreatment. This mass loss could be due to the production of gases (such as CO_2) as a result of decarboxylation reactions during pretreatment and is labelled as the gas phase in Fig. 1. This is in the range 6 wt% to 10 wt% and is independent of the pretreatment temperature. This could relate to the original moisture content of the feed or loss of volatiles, which was more pronounced at pretreatment temperature of 200 °C.

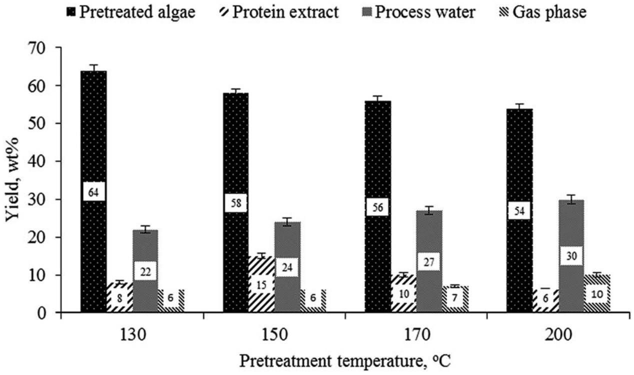

Fig. 1 Mass yield of pretreated algae, protein extract, process water and gas phase following pretreatment of *Tetraselmis* sp. microalgae.

The carbon and nitrogen recovery in the pretreated algae is presented in Fig. 2. It was found that there was general reduction in each of the element with an increase in the pretreatment temperature. The amount of carbon and nitrogen obtained after pretreatment were 59–95% and 54–88%, respectively. This suggests that up to 41% carbon and 46% nitrogen is fractionated in other product fractions such as protein extract and process water. At the optimum pretreatment temperature (based on maximum yield of protein extract and recovered pretreated algae) about 85% of the carbon was recovered while the nitrogen content in the pretreated algae was reduced by 33%. The pretreatment of microalgae resulted in structural changes to the microalgae biomass, as shown in Fig. 3. These modifications led to the fractionation of carbon and nitrogen in protein extracts, process water and gas phase. These structural changes could improve the quality of the pretreated algae as a feedstock for biocrude production *via* HTL. Based on the data in Fig. 2 these changes were higher at the pretreatment temperature of 200 °C, but led to low carbon recovery in the pretreated algae. This suggests that operating at 200 °C is unsuitable.

The micrographs of untreated and pretreated algae are presented in Fig. 3. The micrographs of the pretreated algae for different pretreatment conditions appear similar; hence, only one is presented and compared with the untreated algae. As shown in Fig. 3 the untreated algae cells appear to be highly clustered, however, the cells seem disrupted after pre-treatment. This cell disruption reaffirmed the fractionation of components during pretreatment, thus a substantial impact on recovered products. It is also noted that the microalgae is not a monoculture as Fig. 3b revealed the presence of diatoms.

The FT-IR spectra of the untreated and pretreated algae is illustrated in Fig. 4. As shown in Fig. 4, there were no substantial changes in the peak and wave-numbers of the untreated and pretreated algae. One of the revelations is the wider transmittance in the peak 1200 cm^{-1} to 800 cm^{-1}, which represent the C–O bonds, O–H phenoxy structures, aliphatic ester, sulphonic acid and aromatic substituted benzenes. Heterocyclic compounds lead to higher nitrogen content in

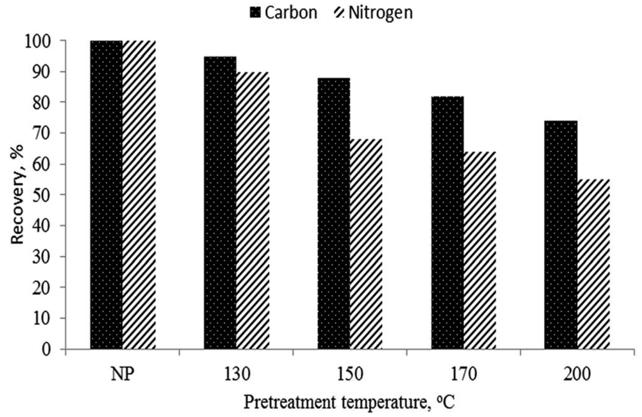

Fig. 2 Comparison of carbon and nitrogen recovery in pretreated algae and no pretreatment.

biocrude following liquefaction. This finding suggests that the undesired N-compounds could have been reduced during pretreatment. Also the peak and wave-numbers suggests that the pretreated algae still contain similar functional groups to that of the untreated algae. It could be inferred that the functional group of the pretreated algae is unaffected following pretreatment. For the process water, there were changes in peak 2900 cm^{-1} to 2800 cm^{-1}, 1550 cm^{-1} to 550 cm^{-1} compared to either untreated or pretreated algae. These changes could be due to new products formed, as a result of the interactions among the algae components during pretreatment. Moreover, the similarity in the process water wavenumbers to that of the algae (untreated or pretreated) suggests that the process water could still contain important dissolved organic compounds such as hydrocarbons. Therefore employing the process water as HTL reaction media could enhance biocrude yield.

The data from the elemental and ICP-MS analysis of the process water is presented in Table 3. The ICP-MS data revealed presence of dissolved trace elements of vary concentration. The elemental analysis of the process water show the presence of residual organic carbon up to 32%, which is believed to be from the initial algae biomass during pretreatment. It is therefore envisaged that recycling the process water as HTL reaction media will influence product yields and distribution, particularly biocrude yield. The reuse of the process water to mix/dilute the pretreated algae is to take advantage of the dissolved organics to improve the carbon efficiency of biocrude. Also it will avoid employing freshwater for liquefaction of pretreated algae, thus water conservation. Recycling the process water to algae cultivation pond will be useful.[24] But it will be of more benefit to be initially employed as hydrothermal media recovering residual organic carbon. Essential nutrient for cultivation will be eventually recovered in the final aqueous phase. In addition, employing the process water is a potential means to reduce the organic and toxicity that has been identified to adversely inhibit microalgae growth during cultivation.[37,38]

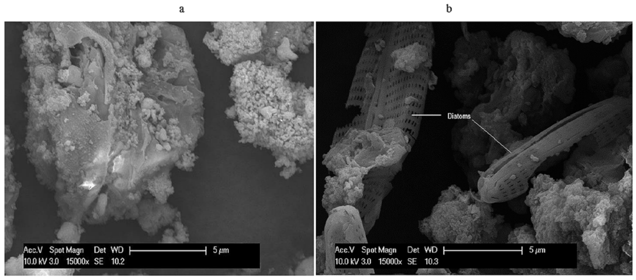

Fig. 3 SEM micrographs of (a) untreated algae and (b) pretreated algae following pretreatment at 150 °C.

The colour of the protein extract was whitish. An increase in pretreatment temperature led to changes in colour for the pretreated algae and the process water. The pretreated algae slightly changed from green to dark green with an increase in the pretreatment temperature. At 200 °C, a darker green colour was observed with pretreated algae, which could be mostly due to hydrophobic peptides or Maillard reaction. The filtrate colour was light green, which was similar to that observed when untreated algae was mixed deionised water and stirred for 20 min at room temperature. This suggests that the temperature below 130 °C has no substantial effect on disruption of the algae. The filtrate colour became light yellow, light brown and ember at 150 °C, 170 °C and 200 °C, respectively.

After complete cycle, about 85% of the employed ethanol was recovered following distillation, which can be reused after purification. The remaining fraction could have been lost as vapour during distillation. In order to avoid error, fresh ethanol was used for each experiment.

The previous section has clearly demonstrated the feasibility to extract protein from microalgae prior to liquefaction; effects of the pre-treatment conditions in the composition and structural changes of product yields and properties. The effect of reaction conditions on HTL products yield and properties, particularly biocrude during liquefaction of the pretreated algae is discussed in the next section.

3.2. HTL product yields

The yield in biocrude, residual char, aqueous and gas phases during processing of pretreated algae is presented in Fig. 5. Due to the recycled process water having residual carbon led to positive effect on biocrude yields. As shown in Fig. 5a biocrude yield from pretreated algae with the process water were generally higher than that from pretreated algae with deionised water. The HTL of pretreated algae with recycled process water led to additional 25 wt% biocrude yield at 350 °C. Also at this condition, the residual char, aqueous and gas phase yields were lower with 10 wt%, and 2.5 wt% and 38 wt%, respectively, than that obtained without recycled process water (*i.e.* using deionised water). It is therefore imperative to recycle the process water for liquefaction as it favours biocrude yields.

However, employing the recycled process water with pretreated algae led to lower biocrude yields compared to that from HTL of untreated microalgae biomass at 350 °C, shown in Fig. 5b. The biocrude yields from the untreated algae were generally higher 10–16 wt% with about 6 wt% in gas phase. Similar yields in residual char and aqueous phases were obtained at same condition. The slight variations in product yields from pretreated algae (shown in Fig. 5b) were simply the

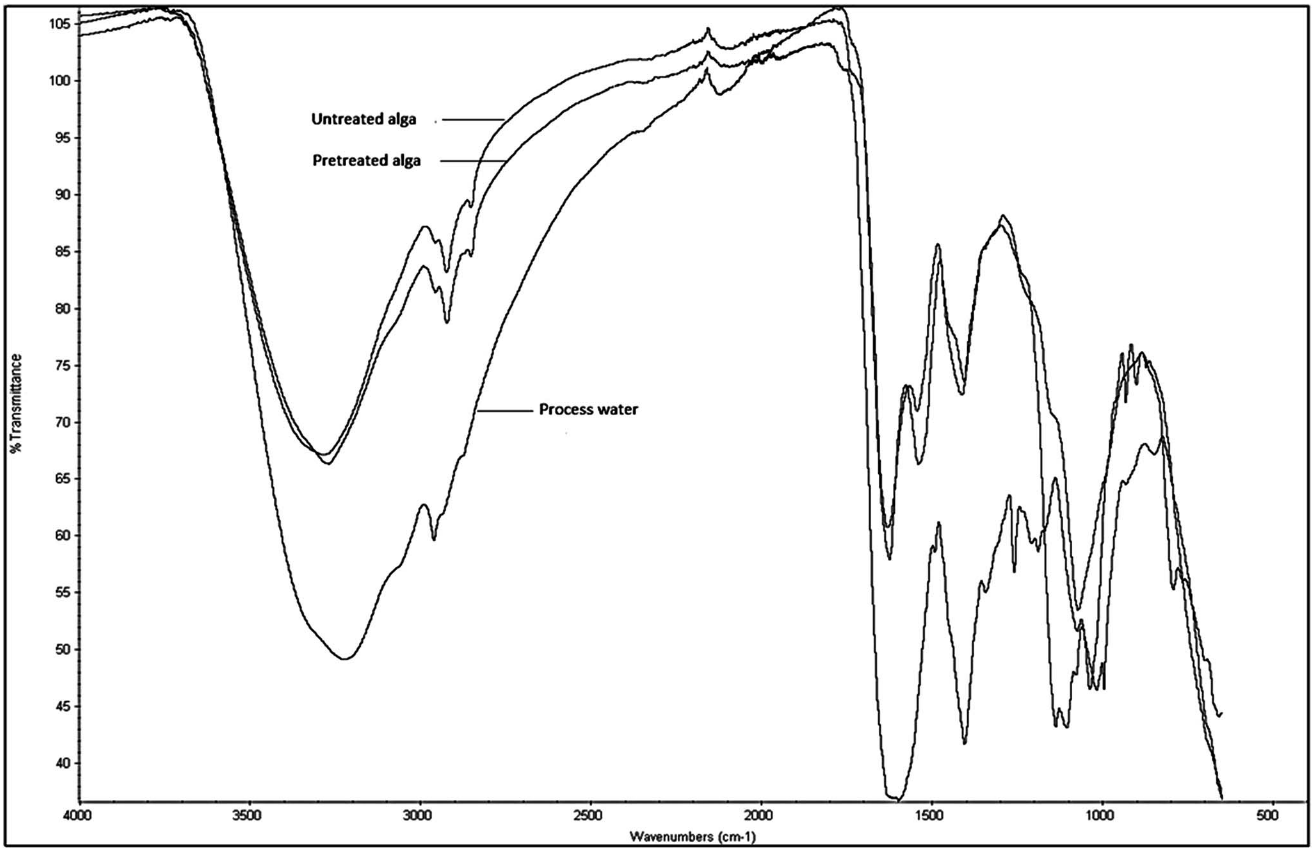

Fig. 4 Comparison of FT-IR of untreated and pretreated algae.

Table 3 Elemental and trace metals in process water following pretreatment

Elemental composition %w/w		Trace elements	Concentration	
			mg kg^{-1}	mmol kg^{-1}
C	15.02	Na	9129.30	396.90
H	3.15	Mg	389.49	16.20
N	1.23	Al	13.30	0.50
S	0.70	K	569.90	14.60
HHV (MJ kg^{-1})	9.00	Ca	150.30	3.50
		Mn	1.00	0.02
		Fe	14.40	0.30
		Ni	3.20	0.10
		Cu	16.80	0.30
		Zn	38.70	0.60

manifestation of amount of algae during pre-treatment (Fig. 1). This finding suggests that: processing of pretreated algae at high temperatures (350 °C) simply favours gasification; and intact cell may require more energy than hydrolysed cell. The difference in biocrude yields obtained from the pretreated and untreated algae at 350 °C was in agreement with previous reports. For example, a 10 wt% less biocrude yield was reported by Zou *et al.*[39] after HTL of pretreated *Dunaliella tertiolecta* compared to processing of untreated *D. tertiolecta*.[40] Vardon *et al.*[41] reported 31 wt% biocrude yield from defatted *Scenedesmus* sp. compared to 45 wt% obtained from non-lipid extracted *Scenedesmus* sp. These studies were mostly conducted at high reaction temperature (360 °C), hence the lower yields in biocrude from pretreated microalgae compared to untreated algae.

At low reaction temperature (310 °C), there was no substantial difference in biocrude yield from liquefaction of pretreated algae compared with that from untreated algae processed at much higher temperature. Processing of pretreated algae at 310 °C, led to ~65 wt% biocrude yield similar to 68 wt% obtained from untreated microalgae at 350 °C. The difference in biocrude yield was only about 3 wt%, which reaffirms that higher reaction temperature may not be suitable for HTL of pretreated algae. Moreover, the biocrude yield from pretreated algae at 310 °C (150WL) was 23.6 wt% higher than that obtained from untreated algae at same condition (310NPL).

In comparison with previous research investigation, the biocrude yield obtained in this study was within range compared to yields from catalysed, un-catalysed HTL and microalgae pretreatment prior to liquefaction. At optimum condition, the biocrude yields obtained in this study was relatively higher than the 50 wt% biocrude yield from *Chlorella pyrenoidosa* at 300 °C, 20 min using HZSM-5 catalyst,[14] 46 wt% from *Chlorella pyrenoidosa* at 280 °C, 120 min using heterogeneous catalyst (Pd/Al_2O_3),[15] 57 wt% from *Nannochloropsis* sp. at 350 °C, 60 min with heterogeneous catalyst (Pd/C, Pt/C, $NiSiO_2$, Co/Mo/γ-Al_2O_3, Zeolite)[12] but lower than 82 wt% from *Chlorella* sp. at 220 °C, 90 min.[42] The biocrude yield was found higher

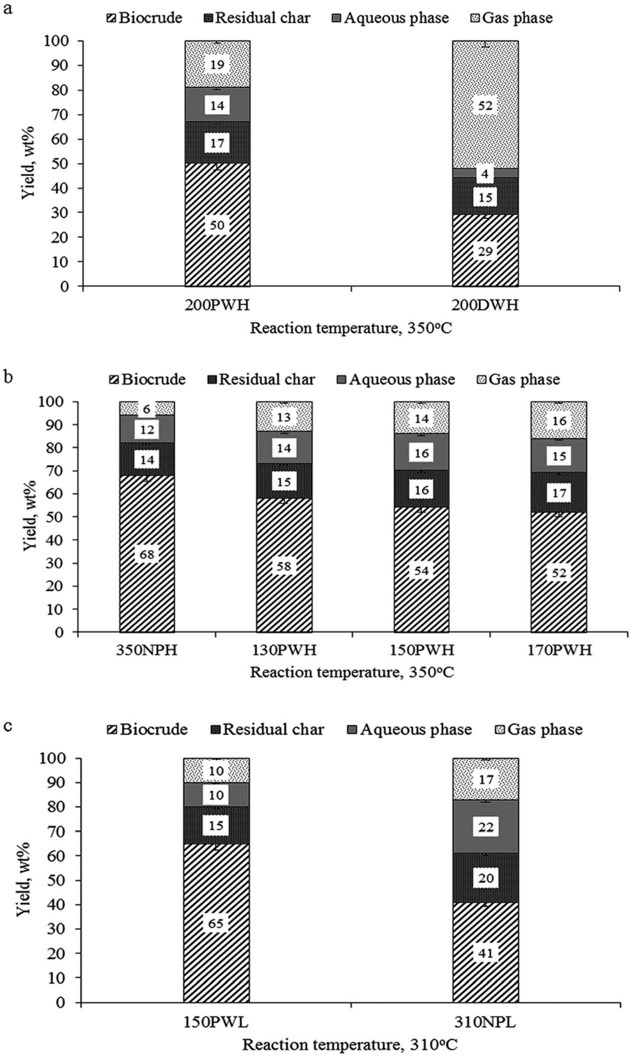

Fig. 5 Mass yield of biocrude, residual char, aqueous and gas phases following hydrothermal liquefaction pretreated and untreated microalgae (a) HTL of pretreated algae with and without recycled process water. (b) Comparison of HTL product from untreated algae with and algae at higher temperature (350 °C) (c) comparison of HTL of pretreated and untreated algae at lower temperature (310 °C). 200PWH: algae pretreated at 200 °C, followed with HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 200DWH: algae pretreated at 200 °C, followed with HTL of pretreated algae with deionised water (DW) at high temperature (H) (350 °C). 350NPH: HTL of untreated algae (no pretreatment (NP)) at high temperature (H) (350 °C). 130PWH: algae pretreated at 130 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 150PWH: algae pretreated at 150 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 170PWH: algae pretreated at 170 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 150PWL: algae pretreated at 150 °C followed by HTL of pretreated algae with process water (PW) at low temperature (L) (310 °C). 310NPL: HTL of untreated algae (no pretreatment (NP)) at low temperature (L) (310 °C).

than ∼24 wt% to 49.5 wt% from *Nannochloropsis oc.*, *Chlorogloeopsis fritschii* and *Pseudochoricystis ellipsoidea* at 300 °C, 15 min (ref. 24) and 30 wt% to 45 wt% from *Chlorella sorokiniana* and *Scenedesmus* sp. at 240 °C to 300 °C in previous reports[12,43] following pretreatment of microalgae.

Furthermore, the residual char obtained from HTL of pretreated and untreated algae were fairly constant. Lowest residual char (3.3 wt%) was obtained from pretreated algae without recycled process water at 350 °C (Fig. 5a) which contain some inorganics such as salt (Table 3). At 310 °C, the aqueous phases from liquefaction of pretreated algae were 3.3 wt% to 13.7 wt% lower compared to 16 wt% to 22.7 wt% for untreated algae at same reaction condition. At higher reaction temperature (350 °C), the yields in gas phase (10 wt% to 22 wt%) from HTL of pretreated algae was generally higher than those from untreated algae. However, at lower process temperature, the gas phase yield from pretreated algae was ∼12 wt% lower than the 22 wt% from untreated algae. This finding again reaffirms that liquefaction of pretreated algae at high temperature appear to favour gasification, thus much lower reaction temperature is required for the processing of pretreated algae.

This present study has shown that high yields in biocrude could still be produced from pretreated algae at a lower temperature. Application of the recycled process water as reaction media increases biocrude yields, suggesting that higher concentration of organic carbon in the process enhances biocrude yields. If the pretreated algae are processed at high temperature as normally required for conventional liquefaction, would lead to ∼10 wt% to 16 wt% reduced biocrude yields. It is therefore concluded that biocrude yields similar to that obtained from untreated algae at high operating temperature (350 °C) can be produced at much lower temperature (310 °C) from pretreated algae. This is encouraging as producing maximum biocrude at lower reaction temperature will economically favour the envisaged HTL commercialization. Decreasing liquefaction reaction temperature from 350 °C to 300 °C will reduce the energy consumption by 22% and only by 3 wt% the yield of biocrude.[11] Akhtar and Amin[44] reported that high operating temperature is usually unsuitable for the production of biocrude in terms of operational cost and biocrude yield. Moreover, the cultivation and harvesting of microalgae biomass for biofuels production is still too expensive.[3,45,46] Therefore producing valuable co-products alongside the primary product biocrude at lower temperature and the use of waste products could improve microalgae biofuels.

3.3. Biocrude analysis

A dark biocrude was produced from both pretreated and untreated algae, but a lighter, more volatile and less viscous biocrude was obtained from the former. This could suggest the elimination of some fatty acids and nitrogenous compounds or the formation of new compounds in the biocrude. There was no much difference in the biocrude HHV produced from both routes. The higher heating values were 32.1 MJ kg^{-1} to 34 MJ kg^{-1} and 28 MJ kg^{-1} to 32.3 MJ kg^{-1} with H/C ratio of 1.03–1.41 and 1.3–1.57 for biocrude obtained from untreated algae and pretreated algae, respectively.

3.3.1. Elemental composition of biocrude. The elemental composition of biocrude produced at different operating condition is shown in Fig. 6. There were some slight variation in carbon and nitrogen content of the biocrudes. The carbon content was 66% w/w to 70% w/w and 68% w/w to 72% w/w for biocrude from pretreated and untreated algae, respectively. It was found that the biocrude carbon and nitrogen content was lower in experiments involving non application of recycled process water (Fig. 6a) at 350 °C. At high process temperature (350 °C) (Fig. 6b), the nitrogen content was generally lower in biocrudes obtained from pretreated algae than untreated algae. But there were no substantial difference in nitrogen content at lower reaction temperature (310 °C). This was expected and thus signified the importance of the pretreatment step prior to liquefaction. During pretreatment, hydrolysis of the microalgae led to reduction in protein fraction which was recovered as protein extract (Fig. 1). At the initial hydrolysis of microalgae, it is inferred that some protein fraction can decompose to N-compounds such as amines, amino acid[4,24,27] *via* decarboxylation and deamination reactions,[47] leading to lower nitrogen content in biocrude (Fig. 6). This finding confirms with our previous report[6] that low nitrogen content biocrude could be produced from liquefaction of pretreated algae. However, the level of nitrogen in resultant biocrude is unsuitable to be directly applied as transportation fuel. For transportation fuel, further upgrading of the biocrude is required or blended with petroleum crude. Also, it was found that the nitrogen content in biocrude obtained at lower reaction temperature were relatively lower compared to those obtained at high process temperature The increase in nitrogen content at higher temperature could be due to increase decomposition of protein fractions in the biocrude, which also led to higher biocrude yields. This finding is

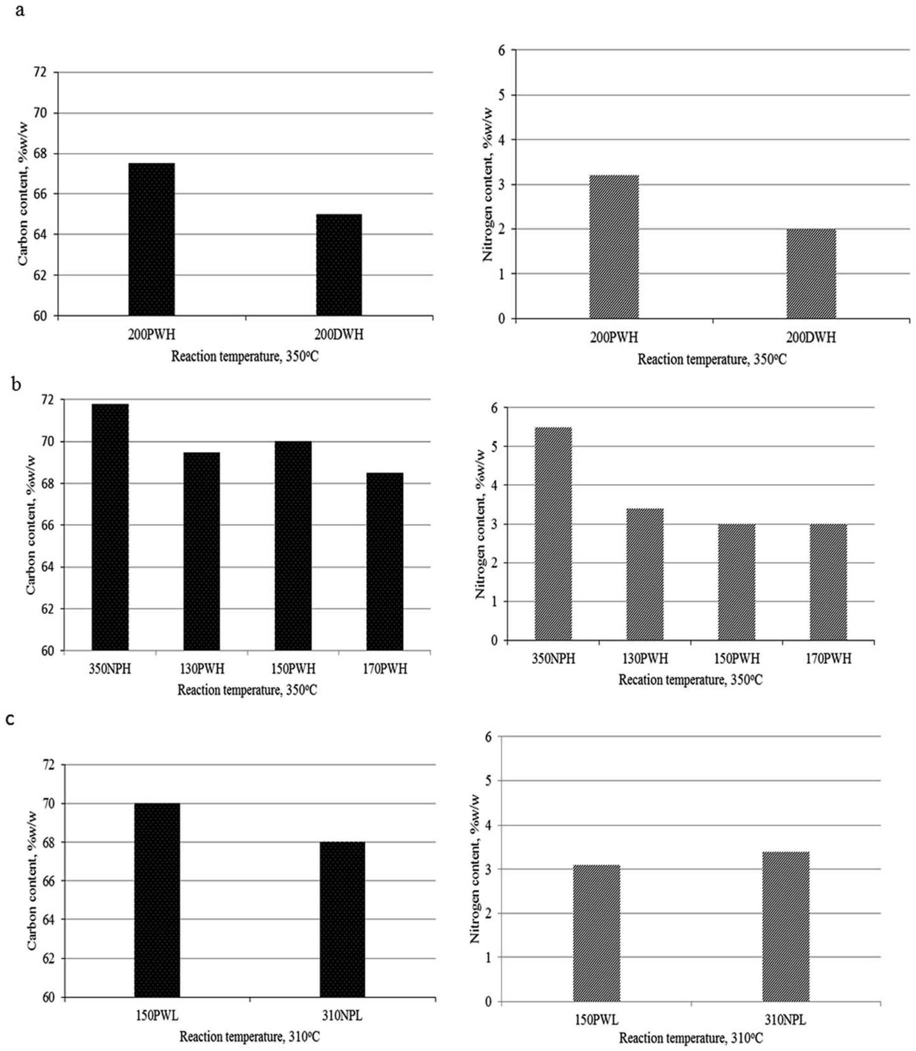

Fig. 6 Elemental composition of biocrude from pretreated and untreated algae at different reaction conditions. 200PWH: algae pretreated at 200 °C, followed with HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 200DWH: algae pretreated at 200 °C, followed with HTL of pretreated algae with deionised water (DW) at high temperature (H) (350 °C). 350NPH: HTL of untreated algae (no pretreatment) (NP)) at high temperature (H) (350 °C). 130PWH: algae pretreated at 130 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 150PWH: algae pretreated at 150 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 170PWH: algae pretreated at 170 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 150PWL: algae pretreated at 150 °C followed by HTL of pretreated algae with process water (PW) at low temperature (L) (310 °C). 310NPL: HTL of untreated algae (no pretreatment (NP)) at low temperature (L) (310 °C).

in agreement with previous reports reporting increase in nitrogen content. For example, Alba *et al.*[2] reported an increase in nitrogen content of 0.4% w/w to 6.5% w/w at 175 °C to 450 °C during the liquefaction of *Desmodesmus* sp. Jazrawi *et al.*[48] reported increase in nitrogen content from 2.6% w/w to 7.7% w/w with an increase in process temperature of 250 °C to 350 °C.

3.3.2. FT-IR characterization of biocrude. The FT-IR spectra for biocrude from pretreated and untreated algae is presented in Fig. 7. As presented in Fig. 7, the FT-IR spectra of both biocrude obtained from pretreated and untreated algae appear similar. This suggests biocrude from pretreated algae has similar functional groups to that of untreated algae. However, the composition of the biocrude from pretreated and untreated algae may differ due to the difference in transmittance of the biocrudes. The use of GC-MS provides more detail, which will be discussed later. One of the main features in the FT-IR spectra of both biocrudes is the absence of major peaks in 3500 cm^{-1} to 3000 cm^{-1} wavenumbers, initially exhibited by the spectra of algae (Fig. 3). This major peak absence shows scission of protein derivatives such as amine.[29] Both the FT-IR spectra of biocrude from pre-treated and untreated algae display sharp peaks between 3000 cm^{-1} to 2800 cm^{-1}, suggesting higher content of C–H stretching vibration of methylene group.[49] The band initially present at 2926.25 cm^{-1} in the biocrude from initial microalgae shifted towards a lower wavenumber of 2920.32 cm^{-1} in biocrude produced from pretreated algae, which suggests the formation of new compound.[50] Similarly, the shift in peak 1700 cm^{-1} to 1600 cm^{-1} represents C═O group stretching vibration in carboxylic acids. The peak originally present at 1701.32 cm^{-1} in untreated algae shifted to 1666.49 cm^{-1} with much lower intensity. The biocrude produced from pretreated algae show a proportionately lower transmittance in this wavenumbers. The peak located at 1451.19 cm^{-1} to 1258.05 cm^{-1} shows the presence of CH_2 and CH_3C–O bending vibrations, and possibly with some aromatic rings.[30,31] The weak peaks between 1163.06 cm^{-1} to 966.75 cm^{-1} strongly suggests C–O bonds, O–H in phenoxy structures, aliphatic esters and sulphonic acid. The peaks detected at 887.60 cm^{-1} to 694 cm^{-1} wavenumbers show the presence of aromatic substituted benzenes.[31] Since both biocrude show relatively similar peaks, it reaffirms that the biocrude from both route still had similar functional groups as determined by the FT-IR. Therefore, pretreatment of microalgae lead to no negative influence, but enhance the quality of resultant biocrude.

In comparison, previous reports[16,47,51] reported differences in intensities of FT-IR spectra among the produced and catalytically upgraded biocrudes. This was attributed to improved quality/properties such as reduced carboxylic acids, nitrogen content in upgraded biocrude. Similar phenomenon was observed in the present study, thus, it reaffirmed that microalga pretreatment led to improved quality biocrude. Nevertheless, the resultant biocrude still contains some heteroatoms, suggesting that pretreatment route does not totally remove

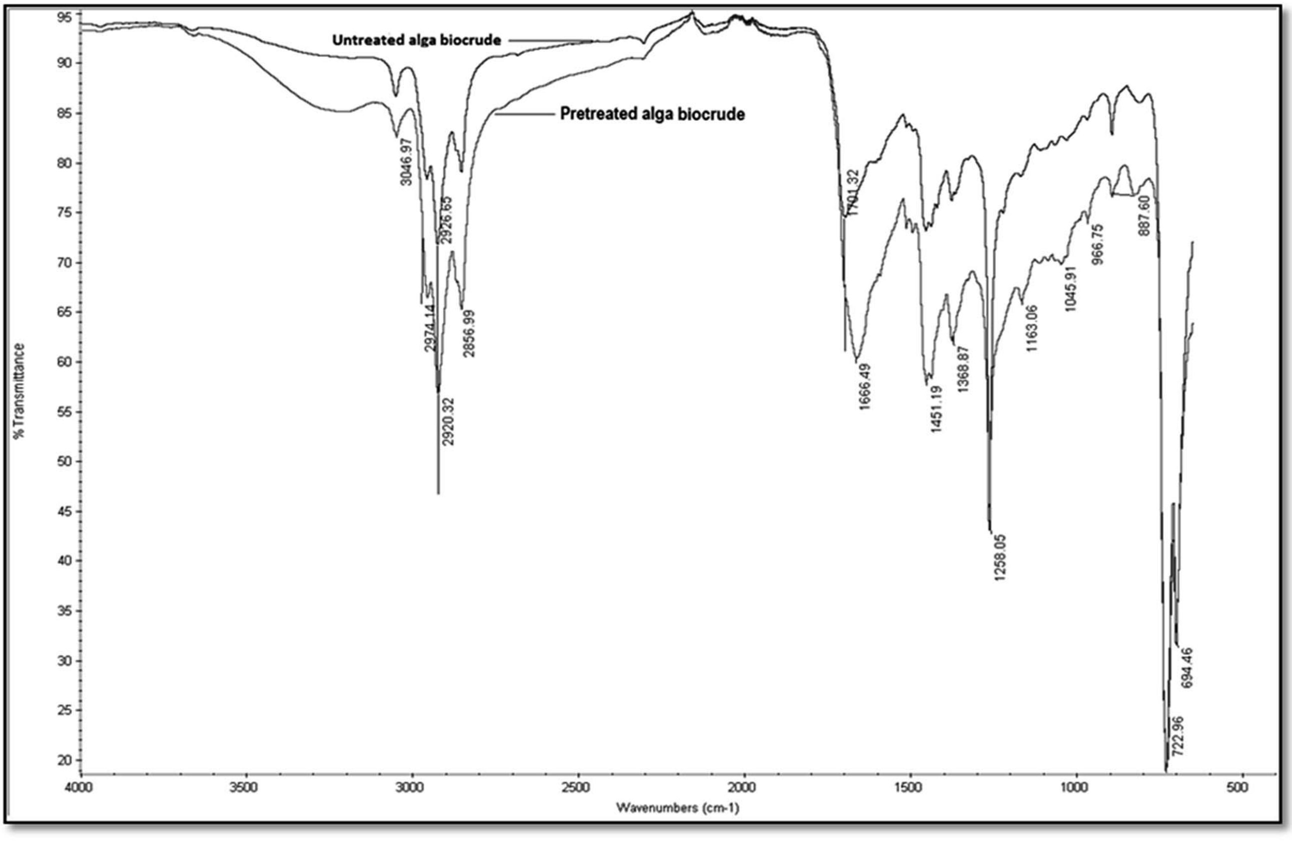

Fig. 7 FT-IR spectra of biocrudes produced from hydrothermal liquefaction of untreated and pretreated algae.

heteroatoms such as nitrogen in the biocrude. Hence, upgrading of the biocrude is required.

3.3.3. GC-MS analysis of biocrude. The data obtained from the GC-MS analysis of the biocrudes are presented in Table 4. As shown in Table 4, the GC-MS results revealed presence of complex compounds, mainly containing long-chain fatty acids, alcohols, nitrogenated compounds and some alkane hydrocarbons. The N-containing compounds such as pyrrole, indole, phenol, egtazic, are typical protein derivatives obtained through series of decarboxylation and deamination reactions[4,47] during liquefaction. However, these N-compounds were below detection level in the biocrude derived from pretreated algae. This suggests that the protein that leads to the formation of these compounds could have been removed during the pretreatment step. The biocrude produced from untreated algae contained heavier molecular weight compounds unlike that from pretreated algae. The constituents of the biocrude derived from pretreated algae were simpler compared to untreated algae but still high in long-chain fatty acids. The presence of long-chain fatty acids could due to the reactions of fatty acids and ammonia released from the pretreated algae. Nevertheless, the long-chain fatty acids can be catalytically converted to hydrocarbons. In summary, it could be inferred that the biocrude obtained from pretreated algae was of better quality due to the reduced amount of the N-compounds.

3.4. Nitrogen recovery in biocrude, solid residue, aqueous and gas phases

The fate of nitrogen and its recovery in HTL product fractions is important as it significantly affects product quality.[32,52,53] For example, it is desirable for biocrude to be low in nitrogen in order to reduce NO_x emission during combustion.[46,54] The nitrogen recovery (NR) in biocrude, residual char, aqueous and gas phases from liquefaction and untreated microalgae is presented in Fig. 8. As shown in Fig. 8, there was generally lower nitrogen distribution in biocrude obtained from pretreated algae than untreated algae. The NR were 15.4% to 33.7% and 28.6% to 49.6% in biocrude derived from pretreated and untreated algae, respectively. This suggests that 32% to 46% of nitrogen was reduced in biocrudes derived from pretreated algae.

The nitrogen recovery (NR) in the residual char was 3.6% to 7.2% and 3.8% to 6.2% from HTL of pretreated algae and untreated algae, respectively. The NR in aqueous phase varies in 3.8% to 22.4% for pretreated algae and 12.2% to 21.2% for untreated algae. This finding confirmed earlier suggestion of recovering nutrient such as nitrogen in the aqueous phase if the recycled process water is employed as a reaction media. The recovery of nitrogen in the aqueous phase is important as it facilitate nutrient recycling[2,8] for cultivation of microalgae.[37,38] The NR in the gas phases from HTL of pretreated and untreated algae was 40.3% to 76.7% and ~53%, respectively, similar to 20% to 70% at 200 °C to 450 °C, 60 min reported by Alba *et al.*[2]

3.5. Energy analysis

The heat input and output applied for the pre-treatment and liquefaction of pretreated and untreated algae is presented in Table 5. It was found that the pretreatment step required input of 2.95 MJ kg^{-1} to 3.7 MJ kg^{-1}. At optimum conditions, the heat-

Table 4 Chemical composition of biocrude from untreated and pretreated algae at 310 °C[a]

S/N	Compounds	Chemical formula	Molecular weight,[b] g mol^{-1}	Retention time, min	Area, % Untreated algae biocrude	Area, % Pretreated algae biocrude
1	Pyrrole	C_4H_5N	137	10.28	1.80	bdl
2	Heptadecene, 17-chloro	$C_{17}H_{33}Cl$	272	12.27	bdl	4.0
3	Egtazic acid	$C_{14}H_{24}N_2$	380	12.61	3.3	bdl
4	Indole	C_8H_7N	117	14.25	2.4	bdl
5	2-Heptacosanone	$C_{27}H_{54}O$	394	14.22	3.8	bdl
6	Tetradecanoic acid, 2-hydroxy	$C_{14}H_{28}O_3$	244	15.1	1.5	bdl
7	Phenol, 2-cyclohexyl-4,6-dinitro	$C_{12}H_{14}O_5$	234	16.14	2.9	bdl
8	9-Hexadcenoic acid, methyl ester	$C_{17}H_{34}O_2$	270	16.25	15.6	bdl
9	8-Octadecenal	$C_{18}H_{34}O$	266	16.82	1.7	bdl
10	Hexadecane	$C_{16}H_{30}$	226	16.28	bdl	4.9
11	Pentadecanoic acid, 14-methyl	$C_{15}H_{30}O_2$	242	17.18	—	21.8
				17.27	13.6	—
12	Hexadecanedioc acid, 3-methyl, dimethyl ester	$C_{17}H_{32}O_4$	361	18.41	4	bdl
13	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	18.92	38.5	—
14	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	18.97	—	65.5
15	Oleic acid	$C_{18}H_{32}O_3$	296	19.17	5.0	bdl
16	8-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	19.27	0.6	bdl
	Glycidol sterate	$C_{21}H_{40}O_3$	340	19.63	2	bdl
17	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	19.82	1.3	bdl
18	Oxiraneoctanoic acid	$C_{19}H_{36}O_3$	312	20.63	bdl	3.8
19	Eicosanoic acid	$C_{20}H_{40}O_2$	312	23.28	2.0	bdl

[a] bdl: below detection level. [b] Calculated using atomic mass of respective elements (C: 12, H: 1, N: 14, Cl: 35, O: 16).

input for HTL of untreated algae was 10.28 MJ kg^{-1} at 350 °C and 13.92 MJ kg^{-1} for pretreated algae at 310 °C. The heat input was found to be similar to 14 MJ kg^{-1} obtained for liquefaction of *Spirulina* sp.[54] The differences in energy values were predominantly due to the variation in biocrude yield and higher heating value. It was found that the combined heat load of 13.81 MJ kg^{-1} for microalgae pre-treatment at 150 °C and liquefaction at 310 °C was similar (13.92 MJ kg^{-1}) to that required for only HTL of untreated algae at same reaction condition.

Total heat-input for production of microalgae biomass and HTL of biomass shows the possibility of net energy production. As shown in Table 5, the energy consumption ratios of 0.3 to 0.66 suggests that processing pretreated and untreated algae could produce renewable fuels. The ECR values were within the range of those reported by Sawayama *et al.*,[9] but were considerably lower than those in some previous reports.[4,7] The ECR difference is predominantly due to varying biocrude yields, heating values and process temperatures. It also suggests that a favourable ECR at lower temperature is recommended based on energy consumption. In conclusion, pretreatment at 150 °C with subsequent liquefaction of pretreated algae at 310 °C with recycled process water appear more suitable for a biorefinery concept. Operating at 350 °C, 5 min was found as the optimum condition based on biocrude yield and HHV for conventional liquefaction of untreated algae.

Furthermore, the ER in biocrude from pretreated algae at 310 °C (150WRL) was 54.9% and higher than 37.3% derived from untreated algae at the same reaction condition. However, it was $\sim$10% less compared to that obtained from untreated algae at 350 °C (350NP). The ER difference was mainly due to the <3 wt% biocrude yield. However, the ECR could still be improved if higher biocrude yields are obtained at lower liquefaction temperature. The chemical energy recovered in the biocrude was found to be similar to that of previous reports.[2,4,5,55] Alba *et al.*[2] reported 11% to 75% ER during HTL of *Desmodesmus* sp. at 175 °C to 450 °C, 5 and 60 min. Biller and Ross[4] reported an ER of 50.7% for *Spirulina* sp., 51.6% for *Porphyridium* sp., 54.2% for *Chlorella* sp. and 66% for *Nannochloropsis* sp. Brown *et al.*[5] obtained 55–90% ER from *Nannochloropsis* sp. at 200 °C to 500 °C, 60 min while 56–90% ER was reported by Jena *et al.*[55] for *Spirulina* sp. at 350 °C, 60 min. This suggests that the pretreatment of microalgae had no negative effect on the energy recovery in biocrude rather a potential to improve product yields, properties and the viability of HTL microalgae biofuels.

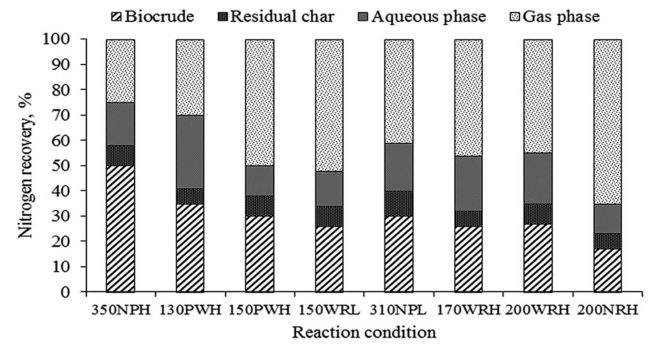

Fig. 8 Nitrogen recovery in biocrude, residual char, aqueous and gas phases from liquefaction of pretreated and untreated algae. 200PWH: algae pretreated at 200 °C, followed with HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 200DWH: algae pretreated at 200 °C, followed with HTL of pretreated algae with deionised water (DW) at high temperature (H) (350 °C). 350NPH: HTL of untreated algae (no pretreatment) (NP)) at high temperature (H) (350 °C). 130PWH: algae pretreated at 130 °C then HTL of pretreated algae at 130 °C with process water (PW) at high temperature (H) (350 °C). 150PWH: algae pretreated at 150 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 170PWH: algae pretreated at 170 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 150PWL: algae pretreated at 150 °C followed by HTL of pretreated algae with process water (PW) at low temperature (L) (310 °C). 310NPL: HTL of untreated algae (no pretreatment) (NP)) at low temperature (L) (310 °C).

The ER and HHV distributed to the residual char and aqueous phases following HTL of pretreated and untreated algae is presented in Fig. 9. As shown in Fig. 9, the ER and HHV of residual char and aqueous phases obtained from liquefaction of pretreated algae were generally lower compared to that from untreated algae. The lower ER and HHV data could be mostly due to the energy loss during the initial pretreatment process. In summary, the total amount of energy recovered in biocrude, residual char and aqueous phase was less than 100%, the remaining is believed to have been fractionated to the gas phase. It was found that the combined ER for biocrude, residual char and aqueous phases obtained from pretreated algae was lower than that from untreated algae. The trade-off here is the improved quality biocrude that will require less amount of catalyst (and hence cost) during refining to transportation fuels and the revenue from the extracts. The energy balance can still be improved with a continuous reactor. Though no study has compared biocrude yields from continuous and batch reactors, it is generally believed that the former is more efficient mostly due to heat exchange systems, far less heating and cooling periods leading to reduce energy consumption and higher biocrude yields,[48,56] although the pumping of feedstock still remains a challenge.

Although, an improved quality biocrude was obtained from pretreated algae, an important long-term concern is the high heat input for algae cultivation and harvesting, which in most cases were more than that required for the combined process route (Table 5). For a viable and sustainable production of biofuels and chemicals from algae, developing advanced technology that requires less amount of heat input for cultivation and harvesting of algae is necessary. In addition, the extractability of valuable pharmaceutical grade chemicals from the biocrude prior to refining to transportation fuels may be interesting.

4. Conclusion

This study has demonstrated the feasibility of producing biocrude low in nitrogen content from liquefaction of pre-treated algae. The pre-treatment of algae led to the lowest nitrogen content in resultant biocrude. The pretreated algae do not require high reaction temperature to achieve yield in biocrude

Table 5 Comparison of energy analyses for biocrude derived from untreated and pretreated algae at different reaction conditions

	Heat input (E_{in})					Heat output (E_{out})				
Condition	Cultivation[a] (MJ kg^{-1} biocrude)	Harvesting[b] (MJ kg^{-1} biocrude)	Pre-treatment (MJ kg^{-1} biocrude)	HTL (MJ kg^{-1} biocrude)	Total E_{in} MJ kg^{-1}	E_{out} = HHV oil MJ kg^{-1}	$\Delta E = E_{in} - E_{out}$ MJ kg^{-1} biocrude	H/C atomic ratio	Energy recovery, %	ECR
350NPH	9.78	6.15	NP	10.28	26.21	34	7.79	1.41	64.9	0.30
130WRH	9.78	6.15	3.33	14.1	33.36	32.1	−1.26	1.55	52.4	0.57
150WRH	9.78	6.15	3.7	16.36	35.99	31.8	−4.19	1.33	46.7	0.60
170WRH	9.78	6.15	3.5	15.48	34.91	32.3	−2.61	1.57	49.9	0.61
150WRL	9.78	6.15	2.95	10.86	29.74	30	0.26	1.3	54.9	0.49
310NPL	9.78	6.15	NP	13.92	29.85	32.1	2.25	1.03	37.3	0.43
200WRH	9.78	6.15	3.53	15.65	35.11	30.3	−4.81	1.42	46.6	0.66
200NRH	9.78	6.15	3.53	29.25	48.71	28	−20.71	1.32	23.8	1.20

HHV: higher heating value; HTL: hydrothermal liquefaction; NP: no pre-treatment. [a] Sawayama *et al.*[9]; [b] estimated in accordance to Lee *et al.*[57] and Shelef *et al.*[58] Total heat input [E_{in} (MJ kg^{-1})] = energy for cultivation + harvesting (electroflocculation and centrifugation) + pre-treatment + liquefaction. Heat output [E_{out} = HHV MJ kg^{-1}] = energy produced from biocrude estimated based on the CHNS data. Net energy balance (ΔH) = $E_{in} - E_{out}$ (MJ kg^{-1}). ECR: energy consumption ratio.

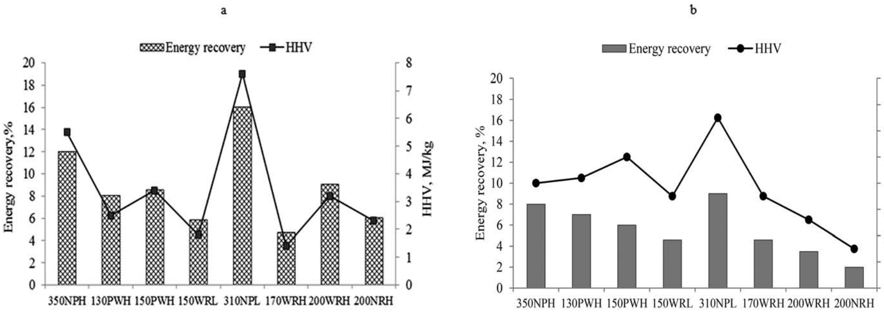

Fig. 9 Energy recovery and HHV for residual char and aqueous phase following HTL of pretreated and untreated algae at different reaction conditions. 200PWH: algae pretreated at 200 °C, followed with HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 200DWH: algae pretreated at 200 °C, followed with HTL of pretreated algae with deionised water (DW) at high temperature (H) (350 °C). 350NPH: HTL of untreated algae (no pretreatment (NP)) at high temperature (H) (350 °C). 130PWH: algae pretreated at 130 °C then HTL of pretreated algae at 130 °C with process water (PW) at high temperature (H). 150PWH: algae pretreated at 150 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 170PWH: algae pretreated at 170 °C then HTL of pretreated algae with process water (PW) at high temperature (H) (350 °C). 150PWL: algae pretreated at 150 °C followed by HTL of pretreated algae with process water (PW) at low temperature (L) (310 °C). 310NPL: HTL of untreated algae (no pretreatment (NP)) at low temperature (L) (310 °C).

as normally applied in liquefaction of untreated algae. Recycling of process water from the pre-treatment step generally improved the biocrude yield. Though, the pre-treatment process did not totally eliminate the nitrogen content in the resultant biocrude, it significantly reduced by 32% to 46%. This suggests that further reduction of protein fraction during pretreatment could reduce nitrogen content in the biocrude. Therefore further optimization studies might be necessary, but caution needs to be adhered as this could have negative effect.

Acknowledgements

The authors greatly acknowledge the financial support of the Australian Research Council's Linkage Grant (100200616), the industry partner SQC Pty Limited, and the Australian Renewable Energy Agency (ARENA) advanced biofuels investment readiness program funding (Q00150). Eboibi acknowledge the support in form of Postgraduate Research Award provided by the Education Trust Fund (ETF) of the Federal Republic of Nigeria. The authors greatly appreciate the support and technical assistance provided by Aban Infrastructures Pvt and the R&D Division of Chennai Petroleum Corporation Limited (CPCL), Chennai, India.

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Chapter 6

Effect of seawater and wet feedstock on product distribution and properties during hydrothermal liquefaction of microalgae

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Fuel Processing Technology, Under Review.

Statement of Authorship

Title of Paper	Effect of seawater and wet feedstock on product distribution and properties during hydrothermal liquefaction of microalgae.		
Publication Status	○ Published ○ Submitted for Publication	○ Accepted for Publication ○ Publication Style	
Publication Details	Eboibi, B.E., Lewis, D.M., Ashman, P.J., Chinnasamy, S. Effect of seawater and wet feedstock on product distribution and properties during hydrothermal liquefaction of microalgae. Fuel Processing Technology, Under review.		

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Overall percentage (%)			
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate to include the publication in the thesis; and
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Abstract

Improving biocrude yield and energy efficiency of hydrothermal conversion processes are considered important to ensure the commercial viability of microalgae biofuels. The microalgae feedstock composition affects these parameters and was the focus of the reported work, which investigated the effects of seawater as a reaction media during hydrothermal liquefaction. The microalgae *Amphora* sp., *Spirulina* sp., and *Tetraselmis* sp., were employed in the study. Comparison of product distribution and properties using fresh microalgae slurry and diluted pulverised microalgae biomass were considered. HTL experiments were performed in a high-pressure batch reactor at reaction temperatures of 310, 350 and 370°C, reaction times of 5min, and using a microalgae slurry feedstock with a 16%w/w dry solid content. The maximum biocrude yields were obtained at reaction temperature of 350°C. The yields of biocrude were higher with seawater than deionised water. However, there was no substantial effect on properties of biocrudes. The use of fresh unprocessed microalgae slurry also led to higher biocrude yields and a lower solid phase compared with diluted pulverised microalgae biomass. In all cases an energy dense biocrude with similar functional properties to petroleum crude was obtained.

Keywords: Biocrude; Hydrothermal Liquefaction; Microalgae; Seawater; Solvent.

1. Introduction

Hydrothermal liquefaction (HTL) is one of the promising technologies for the direct conversion of renewable biomass to biofuel precursors like bio-oil or biocrude[1]. Biocrude derived from biomass can be subsequently refined to wide range of drop-in transportation fuels that include gasoline/petrol, diesel and aviation turbine fuels[2]. HTL converts the biomass to biocrude in an aqueous medium that is maintained at subcritical temperature[3]. In comparison to conventional biofuel production processes, HTL can directly process high moisture content feedstocks to biocrude, thus, HTL significantly reduces energy consumption otherwise used for dewatering and drying of biomass slurry[4,5]. Recently, much attention has been on the utilization of microalgae as a suitable feedstock for biocrude production via HTL[6-9]. Microalgae are considered potential renewable feedstocks for the production of biofuels due to their faster growth rates than terrestrial plants and ability to grow in non-arable lands and poor quality water. Microalgae are also capable of removing pollutants from wastewater streams[10,11].

Commercial viability of biocrude production process from microalgae using HTL process currently faces some challenges. HTL reaction mechanisms are not yet fully understood and the influence of biochemical composition of feedstock on biocrude yield and quality has not been studied in great detail. As reported in earlier studies, achieving maximum yield of biocrude with desired fuel quality using the same reaction conditions may not be possible as these are directly influenced by various parameters[12,13]. Improved biocrude yield with ideal fuel properties will improve the economic viability of HTL processes, however; it still remains as a challenge to the burgeoning algae biofuels industry.

The major focus of previously reported research on HTL of microalgae was on the effect of various operating conditions including temperature, reaction time, solids concentration, type of strain, application of catalysts and solvents on the yield and quality of biocrude[3,12-19]. These studies were mainly conducted using batch reactors, at varying temperatures (200-500^{o}C), reaction times (5-60min) and feedstock solids concentration (10-35%w/w) . These studies concluded that feedstocks containing ~20%w/w dry solids resulted in the maximum yield of biocrude while operating close to the critical point of water (374^{o}C and 22.1MPa). Different types of catalysts were reported to increase biocrude yield with improved “quality”. These included alkali catalysts such as carbonates and hydroxides of sodium and potassium, $Ni/SiO_2/Al_2O_3$, $CoMo/Al_2O_3$, Pt/ Al_2O_3, Pd/C, Pt/C, Fe sulphides, and Ce/HZSM-5[8,16,18,21]. The presence of catalysts reduced activation energy, improved conversion rate and increased biocrude yield. However, most catalysts are expensive and can lead to the fouling and clogging of processing equipment and importantly, their use in commercial scale production of biofuels (a commodity product) could incur additional production costs.

A review of the scientific literature highlighted that the yield and properties of biocrude can be enhanced by replacing the normally used deionised water with organic solvents as a HTL reaction media[9,22-24]. Employing different solvents could strongly affect the distribution of HTL products and their properties[22]. HTL of microalgae with defined solvents could be developed with optimal process conditions for the production of specific fuels and valuable chemicals. The variation in yield and properties of biocrude could be mostly attributable to the different reaction pathways created with various solvents during liquefaction.

The use of polar and non-polar solvents in biocrude recovery has been reported to have significant impact on the biocrude yield and its qualities[25]. Though, the biocrude yield varied with different solvents used in biocrude extraction step, it is still not clear if the extraction protocol involved any chemical reaction which could lead to either an increase or decrease in the resultant biocrude yield.

Therefore, it could be inferred that the biocrude yield and properties are primarily determined by the HTL reaction steps, where a defined solvent is normally used[26].

Previous reports[9,22,23] confirmed that the use of acidic, neutral, alkaline and organic solvents as HTL reaction media could improve the yield and quality of biocrude. Moreover, these studies proposed further research in selection of an effective solvent exhibiting catalytic properties for enhancing the yield and properties of biocrude. Though, these results show increases in biocrude yield with improved properties, the potential use of organic solvents with commercial scale HTL processes may be uneconomical. In addition, the application of organic solvents as a reaction media could make it more challenging to recycle the nutrients for cultivating new microalgae.

The use of natural seawater as HTL media is predicted to enhance the biocrude yield and improve their fuel properties. Seawater is alkaline and its application as a reaction media will lead to the modification of the HTL ionic environment. This will improve base-catalysed reaction pathways, improving biocrude oil formation than the acid-catalysed reaction that normally leads to more solid products. In general, seawater contains varying concentrations of trace elements such as sodium, calcium, magnesium, iron, and magnesium. These elements are essential components in catalysts, which were previously reported to increase biocrude yield with improved properties[27,28]. Therefore seawater could exhibit catalytic properties if employed in HTL. Seawater is naturally occurring, abundant and an environmentally benign source. Importantly, its use as a HTL solvent would add minimal cost to laboratory or commercial scale operations compared to organic solvents or conventional catalysts.

Furthermore, a review of the scientific literature also highlighted that previous studies used microalgae slurries with different solids concentration as feedstocks for HTL. In most of the studies conducted earlier, dried or freeze-dried microalgae biomass mixed with deionised water was subjected to HTL process[3,19-21]. HTL studies were also carried out using wet or freshly harvested intact microalgae cells with water containing residual nutrients from the culture medium as HTL feedstocks[13,15]. Data from these previous reports was inconsistent and it was difficult to accurately compare results from various studies conducted earlier, even though the same microalgae feedstocks were utilised in the experiments. For example, using the slurry prepared with freeze dried microalgal biomass, Biller and Ross[3] obtained 5wt% to 25wt% biocrude yield with HHV of 34.5MJ/kg to 36.8MJ/kg at 350°C and 60min reaction time. Barreiro *et al.*[15] employed wet microalgae and reported 18wt% to 58wt% biocrude yield with HHV in the range 29.3MJ/kg to

37.2MJ/kg at 250°C to 375°C and 5min reaction time. Similarly, Alba *et al.*[14] obtained 8.6wt% to 49.4wt% biocrude yield, HHV of 30.1MJ/kg to 36.6MJ/kg at 175°C to 450°C, and reaction times ranging from 5min to 60min. It is not clear how HTL of wet/freshly harvested microalgal biomass or slurry prepared with dried/freeze-dried microalgal biomass could affect product yields and properties. Freeze-drying and/or thermal drying of microalgae biomass are perceived as preservation techniques, but could lead to a reduction in the quantity and quality of final target products[29,30]. Drying may either partially or totally affect the quality of a product, which may involve various changes in physical, chemical and biological nature during processing, storage and distribution. This change could lead to significant influence on product yield and distribution when used as feedstocks for HTL. Thus a comparative analysis of the products yield, distribution and properties from HTL feedstock prepared using freshly harvested wet algal biomass and slurry prepared with dried/freeze-dried algae was carried out and reported in this study.

The main objectives of this reported work was to (1) elucidate the effects of different concentrations of seawater on HTL biocrude yield and quality, and (2) examine the trends in HTL product yields particularly biocrude, distribution and properties using wet algae with whole cells and slurry prepared with dried microalgae biomass as feedstocks for hydrothermal liquefaction.

2. Materials and Methods

2.1 Feedstocks

Three microalgae strains *Amphora* sp., *Spirulina* sp. and *Tetraselmis* sp. were used in this present work. The microalgae strains were grown and harvested in outdoor open raceway ponds owned and operated by Biotechnology Division, Aban Infrastructure Pvt India. Only a brief summary of culturing and harvesting procedures is provided here, the details can be found elsewhere[31]. Carbon dioxide and commercial grade chemicals were used as nutrients during growth period. The microalgae strains *Amphora* sp. and *Tetraselmis* sp. were harvested using a novel electro-flocculation approach; the slurry obtained was further dewatered and concentrated using a filter press. S*pirulina* sp. was harvested and concentrated using a filter press. A portion of the wet algal biomass harvested was stored in a refrigerator (~4°C) till its further use. The remaining portion was dried and the dry algal biomass was used for preparing slurries containing 16%w/w solids as feedstocks for HTL studies.

2.2 Feedstock characterization

The microalgae slurry obtained were analysed for biochemical and elemental composition prior to HTL. Total carbohydrate, lipid and protein concentrations were determined according to the methods of Dubois *et al.*[32], Folch *et al.*[33] and Lowry *et al.*[34], respectively. The elemental carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) contents of the microalgae were analysed using a VarioEL III elemental analyser system GmbH. The oxygen content was determined by difference. The higher heating value (HHV) was estimated using the consolidated Eq. (1) proposed by Channiwala and Parikh[35]:

$$\text{HHV (MJ/kg)} = 0.3491\text{C} + 1.1783\text{H} + 0.1005\text{S} - 0.10340 - 0.0151\text{N} - 0.0211\text{A} \qquad (1)$$

Seawater was vacuum filtered through Whatman micro filters (27 mm diameter, 25µ pore size, 98% efficiency particle retention). The filtrate was analysed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

2.3 Hydrothermal liquefaction

HTL experiments were performed in a 1L high-pressure batch reactor at 310, 350 and 370^{o}C and 5min reaction time using the feedstock containing 16%w/w dry solids dissolved in seawater or deionised water. Two different concentrations of seawater were used in the experiments viz. 24ppt (SW1) and 36ppt (SW2).

In each HTL run, the reactor was loaded with the required feedstock (360g of slurry) and tightly sealed. The reactor was then heated to a predefined set-point temperature using an in-built electrical heating jacket. All the HTL runs were maintained at the desired reaction temperature for the fixed reaction time of 5min. The reaction time commenced when the reactor reached the desired reaction temperature and stopped after the completion of the reaction time. During the HTL reaction, the reactor was continuously stirred with an in-built specialised magnetic drive impeller operated at 300rpm to maintain homogeneity in the reaction vessel. After the HTL run, the reactor was cooled (~30min) to ambient temperature. HTL runs were performed in triplicates, and the average values were reported.

2.4. HTL product separation and analysis

HTL products were obtained through series of extraction and filtration methods shown in Fig. 1. The details of the separation procedure have been previously reported[12]. The gravimetric yields of HTL products viz. biocrude (on ash free dry weight), solid residue, and dissolved aqueous solids were determined using Eq. (2). The gas phase yield was estimated by mass difference using the calculated yields of the remaining fractions.

$$Y_i = M_i/M_f \times 100\% \qquad (2)$$

where Y_i is yield (wt%) of the *ith* fraction, *i* is biocrude, solid residue, aqueous phase or gas phase, *f* is feedstock, and *m* represents mass (g). Each product sample except the gas phase was analysed for CHNSO according to the method mentioned previously. The amount of chemical energy recovered (ER) in each individual product from the initial feedstock was estimated with Eq. (3):

$$ER = (HHV_i \times M_i) / (HHV_f \times M_f) \times 100\% \qquad (3)$$

where *i* and *f* represent product and feedstock, respectively. *HHV* is higher heating value (MJ/kg) and *M* is mass (g). The elemental carbon and nitrogen recovery in the HTL products were calculated using the elemental mass balances across the reactor.

The metallic analysis of the ash fractions from biocrude, solid residue, aqueous phase including the initial microalgae were performed using Inductively Coupled Plasma–Mass Spectroscopy (ICP-MS). The ash fractions of biocrude solid residue and aqueous phase were obtained by incinerating each dried fraction in a muffle furnace at 450^{o}C for 4h. Then about 300 mg of the resultant ash was wet digested in 10ml nitric acid in a closed vessel followed by the addition of reverse osmosis water for dilution. The diluted solution was agitated for about 5min and left to stand overnight. The solid particles from this solution were removed by vacuum filtration using Whatman microfilters. The filtrate obtained was analysed for metallic contents and the elemental balance/distribution in the individual HTL products was estimated.

3. Results and discussion

3.1. Feedstock Analysis

The elemental and biochemical composition of the microalgae strains used in this reported study is presented in Table 1, which shows compositional variation of various microalgal species. There were differences in CHNSO content amongst all strains. All the strains exhibited very high oxygen contents. The oxygen content of *Tetraselmis* sp. was higher in comparison with that of *Spirulina* sp. and *Amphora* sp., due to its lower carbon content. The HHV of the strains were 19MJ/kg to 24.3MJ/kg. The lowest HHV was reported for *Tetraselmis* sp., however its H/C ratio was higher compared to the other species. Feedstocks exhibiting high H/C ratio are believed to be highly beneficial for HTL as they lead to more energy release upon combustion[12]. *Amphora* sp. had the highest carbohydrate and lowest protein content, but with the highest lipid content compared to

Spirulina sp. and *Tetraselmis* sp. The protein and lipid composition of *Spirulina* sp. and *Tetraselmis* sp. were similar. The typical trace elemental composition of SW1 and SW2 is presented in Table 2.

3.2. HTL products yield

3.2.1. Effects of reaction media

The yields of various HTL products of *Tetraselmis* sp. viz. biocrude, solid residues and aqueous and gas phases obtained from the treatments with DW, SW1 and SW2 are presented in Fig. 2. As shown in Fig. 2, at 310°C there was no significant difference in product yields, which was mostly attributable to the low operating temperature. The biocrude yields were 36wt% for DW, 42wt% for SW1 and ~40wt% for SW2. Irrespective of HTL reaction media, the yield in solid residues was 20wt%, aqueous phase was ~23wt% and 13wt% to 23wt% for the gas phase. The high yield in both solid residue and aqueous phase was expected because at this reaction temperature, hydrolysis reaction is dominant.

An increase in the reaction temperature to 350°C led to substantial increase in biocrude yields to 58wt% for DW, 68wt% for SW1 and 62wt% for SW2. The general increase in biocrude yields could be attributed to subsequent cracking and polymerisation reactions. Mostly, an increase in biocrude yields reduces the yields in both solid residue and aqueous phases. The increase in biocrude yield corresponded to a decrease in the solid residue (~14wt% to 10wt%) and aqueous phase (18.3wt% to 12.2wt%), whilst the gas phase yield was ~7wt% to 12wt%. However, further increases in the reaction temperature to 370°C improved gasification, resulting to a decrease biocrude yields. The yield in biocrude at 370°C with DW, SW1 and SW2 was ~37wt% to 48wt%. The yields in solid residues were between 14wt% and 17wt% for DW, SW and SW2. The aqueous phase yield varies between 20wt% and 23wt%, while the gas phase yields increased up to 30wt%. It could be inferred that higher reaction temperatures, especially close to the critical point of water lead to an increase fractionation of low molecular weight compounds to gaseous products. Based on these results, it seems needless to go up to 370°C to maximise the biocrude yield. It can therefore be concluded that 350°C was the optimum reaction temperature based on biocrude yield in this reported work. Most previously reported studies generally agreed that maximum biocrude yields were achieved at 350°C[3,12,19]. In summary, about 4wt% to 11wt% higher biocrude yield was obtained with SW1 and SW2 than DW. Approximately 6wt% increase in yield of aqueous phase was obtained with SW1 and SW2 compared to DW. This could be due to an increase in precipitation of the soluble inorganic salts. Generally, there was no substantial difference in yields of solid residues.

This study has shown that seawater of suitable concentration could improve biocrude yields compared with the use of DW alone during hydrothermal liquefaction. However, the chemistry behind the increase in biocrude yields among the different reaction media is not yet clear, but there are multiple potential reasons. HTL follows decomposition and repolymerization reaction mechanism leading to the production of unstable free radicals. These free radicals can be stabilized or recombined into larger ones by the hydrogen atoms made available by the water-gas shift reaction[5]. The stability of the free radicals could have significantly reduced repolymerisation reactions, thus enhanced the yields in biocrude. Moreover, the alkalinity of seawater buffered the acidic component, thus improving biocrude yield. Furthermore, the high operating temperatures significantly affected the biocrude yield.

3.2.2. Yield of HTL products from wet algae and slurry prepared using dry algae

The HTL product yields from the freshly harvested microalgae (wet) and slurry prepared with dry microalgae diluted with deionised water (DW) are presented in Fig. 3. The biocrude yields of wet and dry biomass samples of *Amphora* sp. were 55wt% (12g dry basis) and 46wt% (8 g dry basis), respectively. HTL of wet microalga *Spirulina* sp., led to biocrude yield of 50wt% (24g dry basis), whereas it was 42wt% (19g dry basis) for the slurry prepared with *Spirulina* dry biomass. HTL of freshly harvested wet biomass of *Tetraselmis* sp. recorded 66wt% (24g dry basis) yield in biocrude, while it was 57wt% (18g dry basis) for the slurry prepared using *Tetraselmis* sp. dry biomass. The solid residues were 20wt% (wet) and 27wt% (dry) for *Amphora* sp., 9wt% (wet) and 14wt% (dry) for *Spirulina* sp. and 11wt% (wet) and 15wt% (dry) for *Tetraselmis* sp. Generally, the gas phase yield was between 12wt% and 30wt%, the maximum was obtained from *Spirulina* sp. Importantly, the data presented in Fig. 3 shows that wet microalgae recorded ~9wt% to 12wt% higher yields in biocrude and lower solid residues and aqueous phases, but similar gas yields compared to dry microalgae. One of the reasons behind this phenomenon was attributed to the rapid decomposition of wet microalgae biomass. The cell wall of freshly harvested microalgae is readily broken down compared with freeze dried microalgae; hence there could have been faster release of cell contents into HTL environment. Thus, leads to higher conversion rates and an increase in biocrude yield. The quick decomposition of wet microalgae biomass led to lower yields in solid residue compared to higher solid residues obtained from dry biomass. Also the natural constituents present in the freshly harvested biomass media could have played an important role in improving the biocrude yields.

Another possible explanation for lower yields in biocrude obtained from dried algae could be due to further decomposition and repolymerisation of the biocrude and induced formation of DAS and solid residue. Thus the higher degree of DAS and solid residue found following HTL of freeze-dried microlgae. These findings are relevant for the future commercialization of HTL for the production of biofuels, as biocrude yields impact life cycle greenhouse gas emissions and the overall economic viability of HTL-algae-biofuels[36,37,38].

It was also noted in Fig. 3 that there were variations in product fractions amongst individual species. The differences observed in the products yield were mostly due to variations in the biochemical composition of the microalgae strains used in the study which are shown in Table 1. It was found that the biocrude yields were always higher than the total lipid content, as biocrude formation is derived from all the fractions. The interaction of protein, carbohydrate and lipid is believed to significantly influence biocrude yields[5].

Though it is difficult to relate biocrude yield to individual feedstock biochemical composition, it can be seen from the data shown in Fig. 3 that lower yields in biocrude with corresponding high yields in solid residues were obtained for *Amphora* sp. The lower yields in biocrude from *Amphora* sp. could be due to the higher carbohydrate content. During depolymerisation carbohydrates mostly result in the formation of polar organic compounds, which may end up in other product fractions (aqueous phase and solid residue) but not in the non-polar hydrocarbon fractions. It has been shown previously that the carbohydrate fraction is a minor contributor to biocrude yields[3,39] in comparison to proteins or lipids. The use of sodium carbonate catalyst with microalgae species rich in carbohydrates could enhance biocrude yields[3], which is supported from the reported data.

3.3. Biocrude elemental analysis

The elemental composition of biocrudes derived from HTL of *Tetraselmis* sp. with DW, SW1, and SW2 are presented in Table 3. Elemental carbon and hydrogen content of the biocrude were substantially higher and the oxygen content was lower than that of the initial algal biomass (*Tetraselmis* sp) (Table 1). There was no wide variation in carbon content, which was between 67.6%w/w and 74.2%w/w under all treatment conditions. The hydrogen content increased from 5.8w/w to 8.6%w/w with an increased in reaction temperature. At lower reaction temperature (310^{o}C), the hydrogen content was between 5.8%w/w and7.1%w/w, and then increased up to 8.6%w/w with increase in reaction temperature. Similarly, the nitrogen content increased from 3.5%w/w to 3.8%w/w to ~5%w/w. This suggests that at higher reaction temperature, biocrude formation is facilitated by enhanced protein deploymerization reactions. The sulphur content was relatively constant at 0.6% w/w while the oxygen content decreased from 22.9%w/w to 18.5%w/w to 18.6%w/w to 14.3%w/w. The oxygen content of the biocrude was always lower in the treatments SW1 and SW2 compared to DW. This could be due to the presence of chemical compounds/metals in the seawater, which could have enhanced deoxygenation reactions. Due to the decrease in oxygen content, the higher heating value (HHV) increased from 27.8MJ/kg to 34.8MJ/kg at 350^{o}C. However, further increase in reaction temperature to 370^{o}C led to minor decrease in HHV (31.3MJ/kg to 33.3MJ/kg).

Also presented in Table 3 are the atomic ratios and energy recovery for biocrude obtained at different experimental conditions. As shown in Table 3, the hydrogen-to-carbon ratio increased from 1.2 to 1.5 with an increase in reaction temperature. The N/C and O/C ratios showed no variation which were within 0.1 and 0.2, respectively. The energy recoveries (ER) in biocrudes corresponded with the differences in the biocrude yields. The ER was in the range 40% to 72%, and in most cases higher values were obtained with SW1 and SW2.

The elemental analysis of biocrude from wet and dry microalgae is presented in Table 4. Despite the variations in biocrude yield, there were no distinct differences in the elemental data amongst the species, although *Amphora* sp. and *Spirulina* sp. showed higher carbon and nitrogen contents respectively. The overall C H N and S contents were between 68.9%w/w and 76.7%w/w, 7.8%w/w and 9%w/w, 3.5%w/w and 6.3%w/w and 0.5%w/w and 1.4%w/w, respectively. There were substantial reductions in oxygen content from 40%w/w to 28%w/w to 13.6%w/w to 9.7%w/w, respectively. These observations clearly show that HTL treatment facilitates decarboxylation and deoxygenation reactions. However, the levels of the oxygen and sulphur content and particularly nitrogen content are still considered high compared to that of petroleum crude[40]. So, further upgrading of the biocrude may be required before its utitization as a feedstock in the refineries for the production of drop-in transportation fuels. Upgrading of the biocrude is paramount in order to reduce NO_x and SO_x emissions upon combustion of fuels produced from biocrude. The ER (48.5% to 70%) was relatively higher with wet algal feedstock due to the higher biocrude yields.

3.4. Elemental analysis of solid residue and aqueous phases

The elemental composition of the solid residue and aqueous phases obtained from the liquefaction of *Tetraselmis sp*. are presented in Fig. 5. The solid residue and aqueous phase elemental carbon, hydrogen and nitrogen, and higher heating value (HHV) obtained at all operating conditions were generally lower in comparison to that of the initial *Tetraselmis* sp. microalgae feed (Table 1). There was no substantial difference in the elemental C, H, N, S and HHVs of solid residue and aqueous phases obtained with DW, SW1 and SW2. The trend showed a slight decrease in carbon content and HHV with an increase in reaction temperature. This confirmed with the earlier suggestion that gasification reaction is dominant at high temperature (370^{o}C). The nitrogen content seems higher in the aqueous phase than in solid residue, while the solid residue had higher carbon content and resulted in higher HHVs. This suggests that the nitrogen compounds are not easily fractionated in the solid residue compared to the aqueous phase. Recycling of the elements from the solid residues and aqueous phases back to the cultivation pond is an essential step to reduce the cost of production of algal biomass. The lower elemental C, H, N in both solid residue and aqueous phase reaffirmed that most of the important organic elements such as carbon and hydrogen, and energy content of the initial microalgal biomass are fractionated in the primary biocrude product (Fig. 4).

In comparison with previous work[19], the elemental composition and HHV of solid residue and aqueous phase obtained with DW, SW1 and SW2 were very similar. For example, the HHVs of the solid residue (5.4MJ/kg to 7.5MJ/kg) and aqueous phase (2.6MJ/kg to 6.4MJ/kg) reported in this study were similar to 3.8MJ/kg to 9.3MJ/kg and 3.6MJ/kg to 5.2MJ/kg obtained at 300^{o}C to 380^{o}C for solid residues and aqueous phase, respectively[19]. This suggests that the use of seawater have insignificant effect on the solid residue and aqueous phase elemental composition and HHVs. Also similar properties of solid residue and aqueous phase as obtained with deionised water can also be obtained with seawater.

3.5. Elemental mass balance in HTL product streams

3.5.1. Carbon and nitrogen recovery

The fate of carbon and nitrogen in HTL product fractions is important as it significantly affects the quality of the products. For example, it is important for the biocrude to be high in carbon and low in nitrogen in order to reduce NO_x formation during refining. The carbon recovery (CR) and nitrogen recovery (NR) in various fractions of products are illustrated in Fig.6. It was found that there were some substantial differences in the CR and NR in product streams among SW1, SW2 and DW. The differences could be mostly due attributed to the variation in biovrude yields. About 40% to 70% of carbon was fractionated in the biocrude, 7% to 25% in solid residues, 18% to 40% in aqueous phase and 7% to 34% in gas phase. The nitrogen distribution in biocrude was 16.8% to 42%, whereas it was 8% to 25%, 18% to 45%, and ~12% to 38% for solids residues, aqueous and gas phases, respectively. Previous studies have shown that up to 70% carbon and 22% to 48% of nitrogen is recovered in the biocrude[3,14], 3.1% to 30% in solid residues[20,41], and up to 24% to 70% in the gas and aqueous phases, respectively[41,42].

Irrespective of the types of reaction media used in this study, an increase in the reaction temperature from 310°C to 350°C resulted in higher CR and NR in the biocrude. A further increase to 370°C led to lower CR and NR in the biocrude, but increased the fractionation of carbon and nitrogen in the aqueous and gas phases. Previously reported works demonstrated that an increase in reaction temperature leads to the rapid hydrolysis of proteins in the feedstocks to nitrogen compounds such as amines and amides via series of decarboxylation and deamination reactions[3,5]. The significant concentration of the nitrogen compounds in the resultant biocrude is undesired, and as a result denitrogenation would be necessary before the biocrude can be fractionated directly for biofuels.

3.5.2. Metallic distribution in product streams

One of the important long-term operational concerns is the fate of metals in feedstock and their distribution along product streams during hydrothermal liquefaction. With the fact that microalgae particularly marine strains are high in alkali and earth metals, the distribution of metals along various fractions of HTL products, particularly biocrude is of major concern as high metal content in biocrude will lead to refining issues. The amount of metals distributed in different product fractions is presented in Fig. 7. As shown in Fig. 7, the detected metals include potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), aluminium (Al), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu) and zinc (Zn). Based on the data shown in Fig. 7, there were no substantial differences in recovered metals in the biocrudes obtained with DW, SW1 and SW2. The biocrude obtained from the above three treatments had approximately 1% to 1.5% K, 2.8% to 4% Ca, 2% to 7.5% Mg, 1.8% to 2.2% Na, 2% to 4% Al, 1.5% to 3% Mn, ~2% Fe, 2% to 3% Ni, 2% to 3% Cu, and ~2% to 3% Zn. The observed percentage compositions of metals (Ca, Mg, Na and K) were in accordance with Anastasakis *et al.*[41] investigation who reported about 2% Ca, 8% Mg, 1% Na and ~0% K in biocrude following HTL of *Laminaria saccharina.* The metallic constituents in the biocrude in the present study may not contribute significantly to the issues associated with slagging or fouling during refining. Otherwise thermal upgrading of the biocrude to remove/reduce the metallic content is necessary[6,43].

Though there were no differences in the metallic composition in product streams obtained with deionised water (DW) and seawater (SW1 or SW2), apparently seawater contains chlorides which is an important concern. Similar challenge is notable being experienced with feedstocks particularly marine microalgae strains that contain salts. Since marine algae strains are mostly preferred to freshwater strains for HTL-algae biofuels. It is inferred that reactor systems that could handle marine algae would be able to handle feedstocks mixed with seawater of suitable concentration.

As presented in Fig. 7, most of the metallic elements were mainly distributed in the aqueous phase and solid residues. Interestingly, with all employed solvents (DW, SW1, and SW2), the Na, K, Al, Mn, Fe, Ni, Cu and Zn metals were mostly distributed in the aqueous phase, while most of the calcium and magnesium were present in the solid residues. In agreement with previous studies[42,44] reporting distribution of metals in HTL product streams, these findings suggest that the monovalent metals such as Na^+ and K^+ ions were mostly dissolved in the aqueous phase, whereas the divalent Ca^{2+} and Mg^{2+} ions were precipitated and recovered in the solid residues. Of course there was no closure with metals recovered in product streams. The overall closure or mass balance following product separation and analysis was 85% to 95%. The lack of closure could be due to being unable to estimate the amount of metals distributed in the gas phase.

The elemental balances show that the aqueous phase is rich in metals such as K, Na and nitrogen, and the solid residues were higher in Ca and Mg. Meaning that the solid residue and aqueous phases could be a potential source for the recovery of essential nutrients for crop and microalgae cultivation. Thus recycling of these nutrients back to the cultivation pond for growing microalgae could significantly reduce the amount of fresh nutrients used and associated costs. Cultivating new microalgae in post-HTL aqueous phase has been investigated with success, but inhibition on growth rate with undiluted aqueous phase has been mostly observed. In this context, Biller *et al*[44] and Jena *et al*.[45] suggested that the inhibitory effects are due to high concentrations of nutrients and poisonous organics such as phenols and fatty acids. Another study by Alba *et al*.[46] reported that the lower growth rate is not only because of the insufficient aqueous phase dilution, but largely due to the unavailability of some essential nutrients such as K, Ca, Mg, and Na required for microalgae growth. In their study, the addition of nutrients such as potassium and magnesium to the culture media led to similar growth as obtained with standard growth media. It is inferred that application of seawater as HTL media could provide more nutrients which could be recycled from the aqueous and solid residue fractions. In addition to providing some catalytic effect similar to conventional alkali catalyst, seawater could also provide economic and environmental benefits to replace freshwater as cultivation medium for microalgae.

4. Conclusion

This work has demonstrated that seawater as a HTL reaction medium significantly impacts product yield and distribution. Using seawater led to improve biocrude yield, however, with no substantial effect on quality relative to the use of deionised water. The increase biocrude yield is dependent on microalgae composition and seawater concentration. Freshly harvested wet algal biomass recorded maximum biocrude yield compared to the slurry prepared with dried biomass. Energy-intensive pre drying/freeze-drying processes for storage and handling of microalgae biomass prior to HTL may negatively impact HTL product distribution. Integrating freshly concentrated microalgae biomass with continuous HTL reactor would provide increased energy efficiencies and biocrude yields with better quality.

5. Acknowledgement

This research was supported under the Australian Research Council's Linkage Projects funding scheme (Project LP100200616) with industry partner SQC Pty Ltd, and the Australian Renewable Energy Agency (ARENA) advanced biofuels investment readiness program funding agreement number Q00150. Eboibi acknowledge the financial support in form of the postgraduate research scholarship received from the Education Trust Fund (ETF) through the Delta State University Abraka, Nigeria. Eboibi appreciate the scholarship received from the Study Abroad Scholarship Scheme from The University of Adelaide, Australia. The Authors also thank Dr. R. Rengasamy, Professor Emeritus, University of Madras and Mr. M. G. Rajaram, Ph.D Scholar, Centre for Advanced Study in Botany, University of Madras for providing algal biomass of *Amphora* sp. for HTL studies. The authors express their profound gratitude to Mr. Sailendra Bhaskar, Advisor (R&D), J. Nallasivam, Assistant Research Officer and other researchers in the Biotechnology Division of Aban Infrastructure Pvt Ltd for providing necessary research facilities and support. The authors thank Dr. Meenakshisundaram, Chief Manager (R&D) and Ms. Lavanya, Senior Manager (R&D) and the R&D Division of Chennai Petroleum Corporation Limited, India for their technical assistance.

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7. Figure captions

Figure 1: Schematic of biomass production and hydrothermal liquefaction of biomass products and separation.

Figure 2: Mass yield in biocrude, solid residue, dissolved aqueous solid (DAS) and gas phase following hydrothermal liquefaction of *Tetraselmis* sp. with DW, SW1 and SW2 at 310°C, 350°C and 370°C.

Figure 3: Products distribution from wet and freeze dried microalgae at 350°C and 5 min reaction time.

Figure 4: Elemental composition and HHV of solid residue and aqueous phases following HTL of *Tetraselmis* sp. at different reaction conditions.

Figure 5: Carbon and Nitrogen recovery in biocrude, solid residue, aqueous and gas phases from liquefaction of *Tetraselmis* sp. with DW, SW1 and SW2 at 310°C, 350°C, and 370°C.

Figure 6: Distribution of metals in biocrude, solid residue, and aqueous phase following hydrothermal liquefaction of *Tetraselmis* sp. at 350°C, 5min.

8. Table captions

Table 1: Elemental and biochemical composition of microalgae.

Table 2: Typical trace elemental composition of SW1 and SW2.

Table 3: Table 3: Elemental composition, atomic ratios and energy recovery for biocrude obtained under different reaction conditions.

Table 4: Elemental composition of biocrude produced from different microalgal strains from wet and dry biomass.

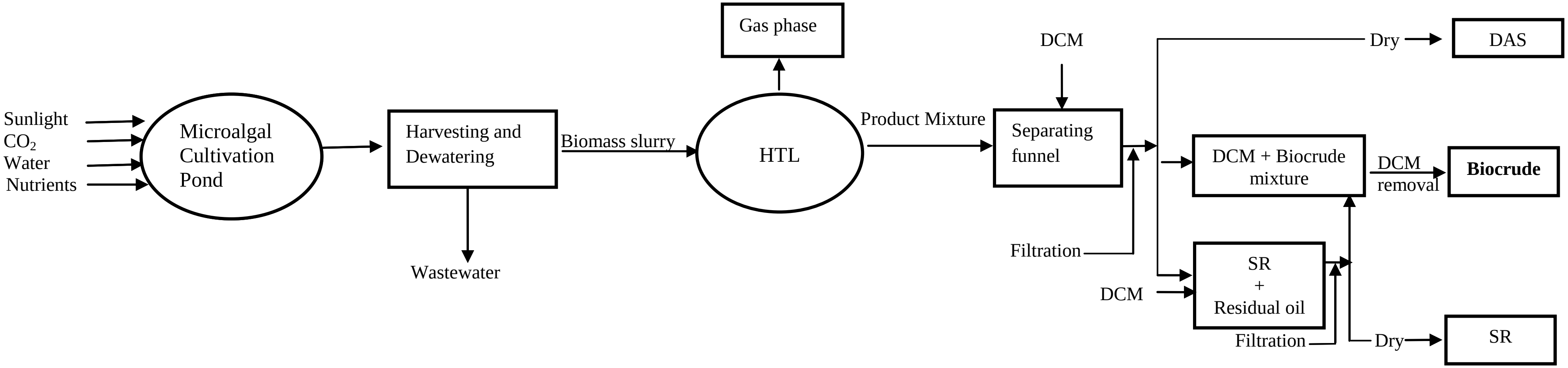

FIG.1: Schematic of algal biomass conversion to biocrude through hydrothermal liquefaction (HTL) process
CO_2: carbon dioxide; DAS: dissolved aqueous solid; DCM: dichloromethane; HTL: hydrothermal liquefaction; SR: solid residue.

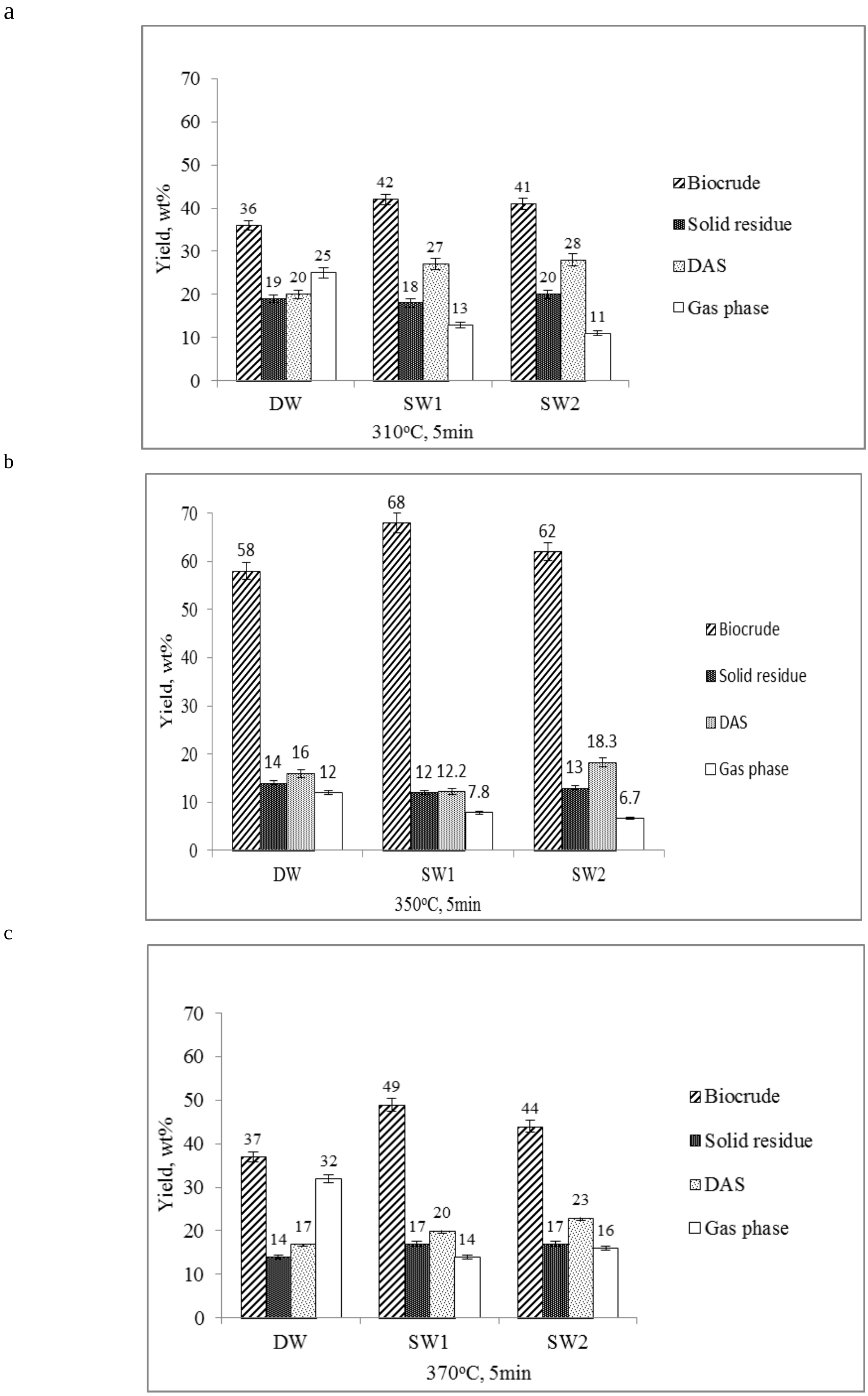

FIG. 2: Mass yield in biocrude, solid residue, dissolved aqueous solid (DAS) and gas phase following hydrothermal liquefaction of *Tetraselmis* sp. with DW, SW1 and SW2 at (a) 310°C, (b) 350°C and (c) 370°C.

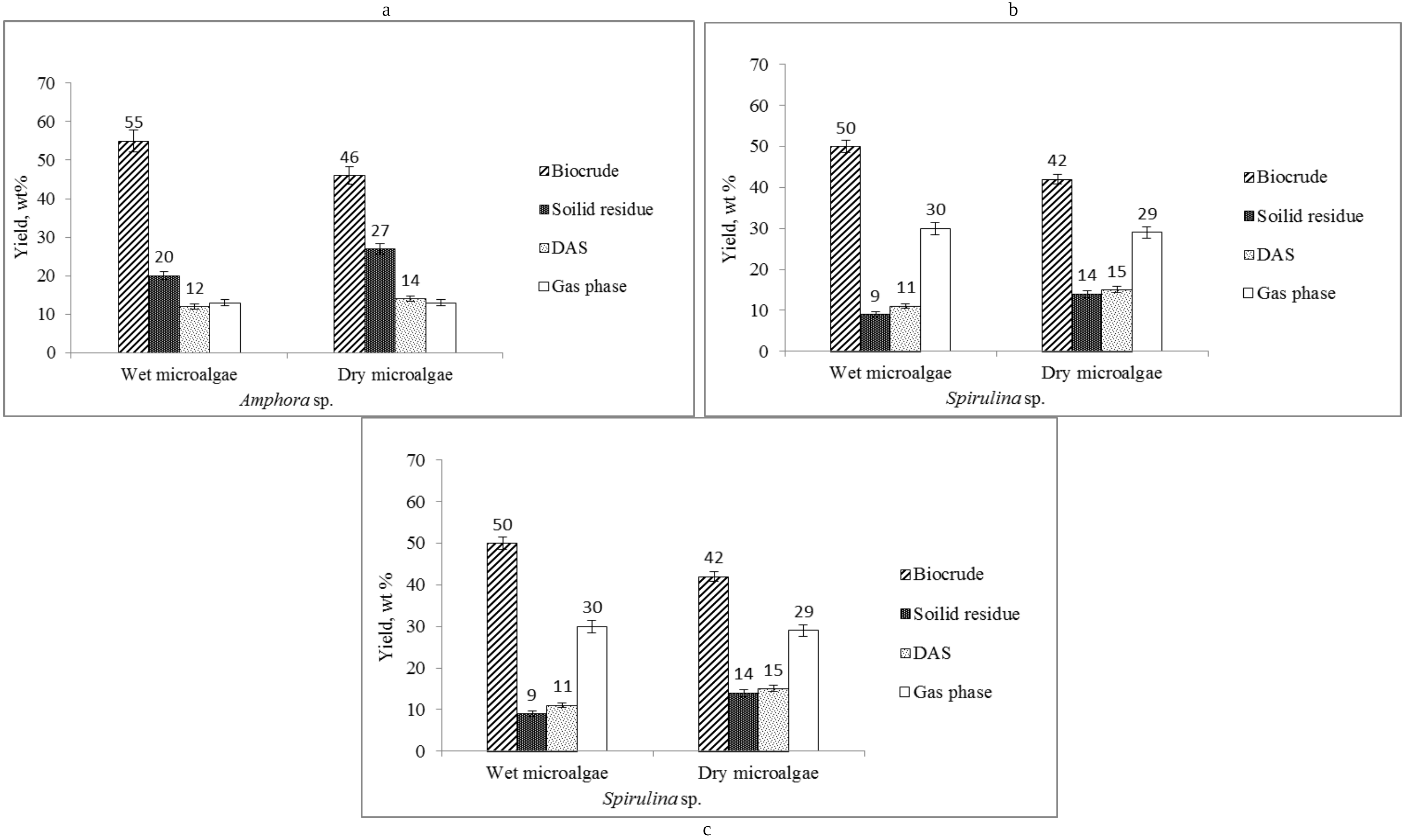

FIG 3: Products distribution from wet and dry microalgae at 350°C and 5 min reaction time. (a) *Amphora* sp., (b) *Spirulina* sp., (c) *Tetraselmis* sp. DAS: Dissolved aqueous solid

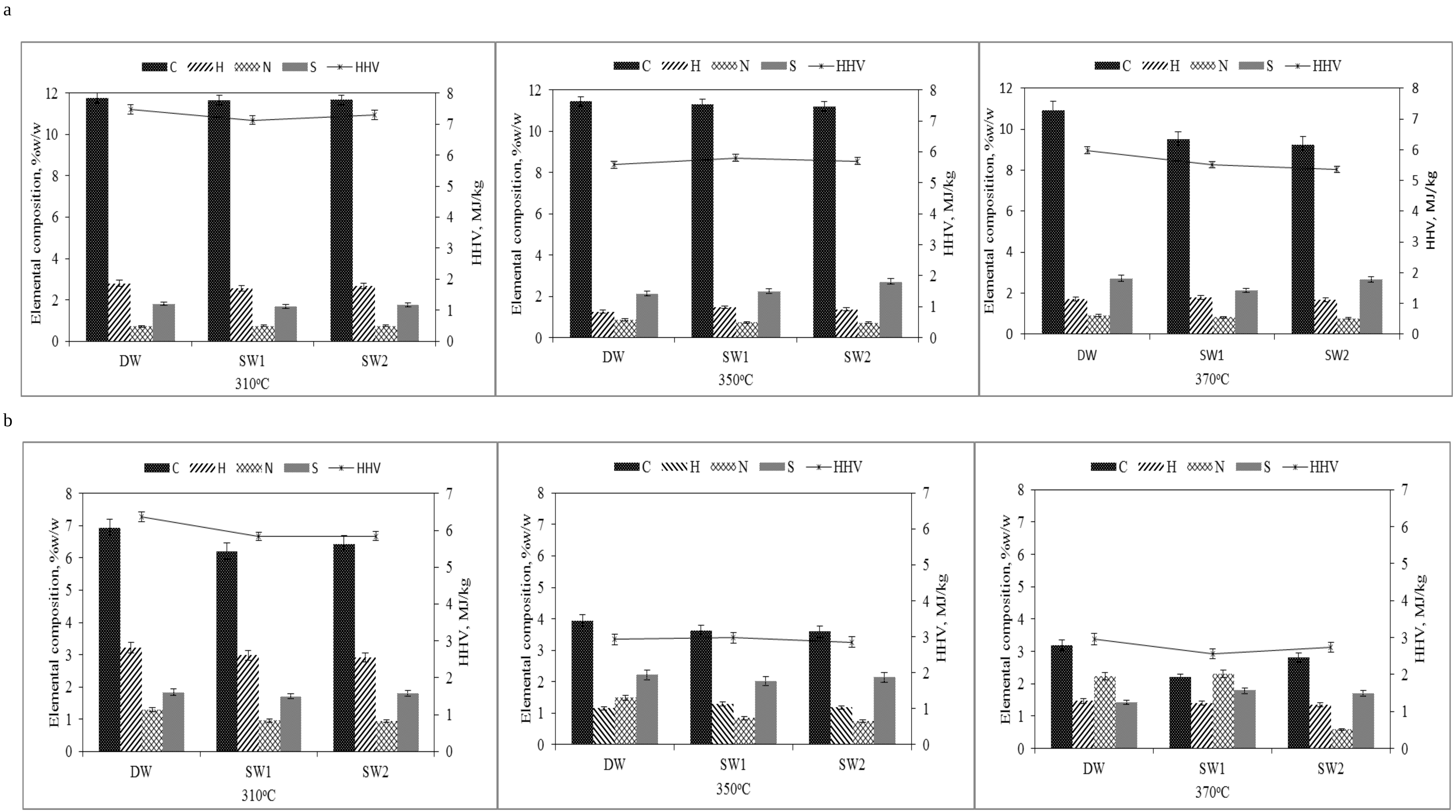

FIG. 4: Elemental composition and HHV of solid residue and aqueous phases following HTL of *Tetraselmis* sp at different operating conditions. (a) Solid residue (b) Aqueous phase.

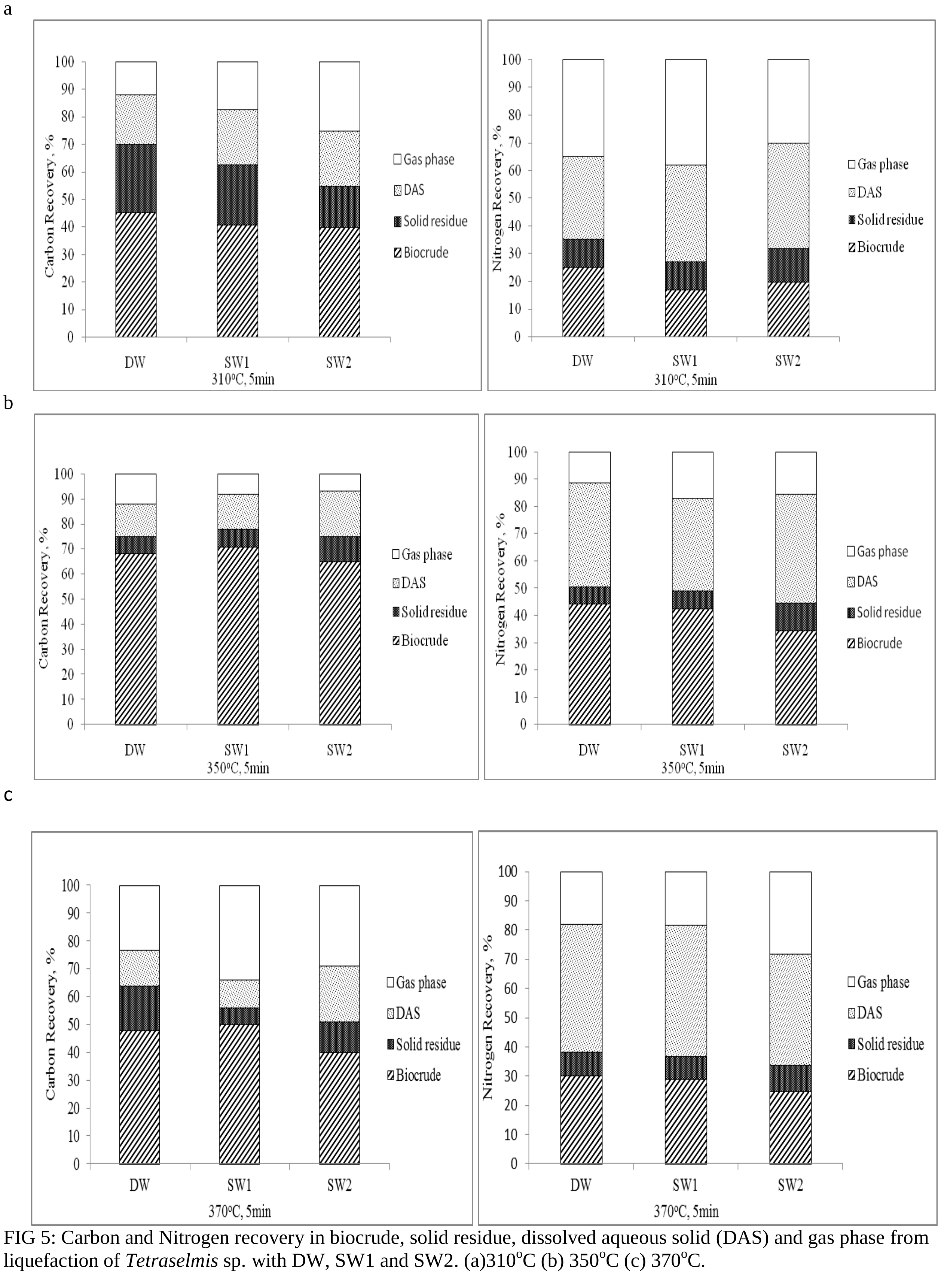

FIG 5: Carbon and Nitrogen recovery in biocrude, solid residue, dissolved aqueous solid (DAS) and gas phase from liquefaction of *Tetraselmis* sp. with DW, SW1 and SW2. (a)310^{o}C (b) 350^{o}C (c) 370^{o}C.

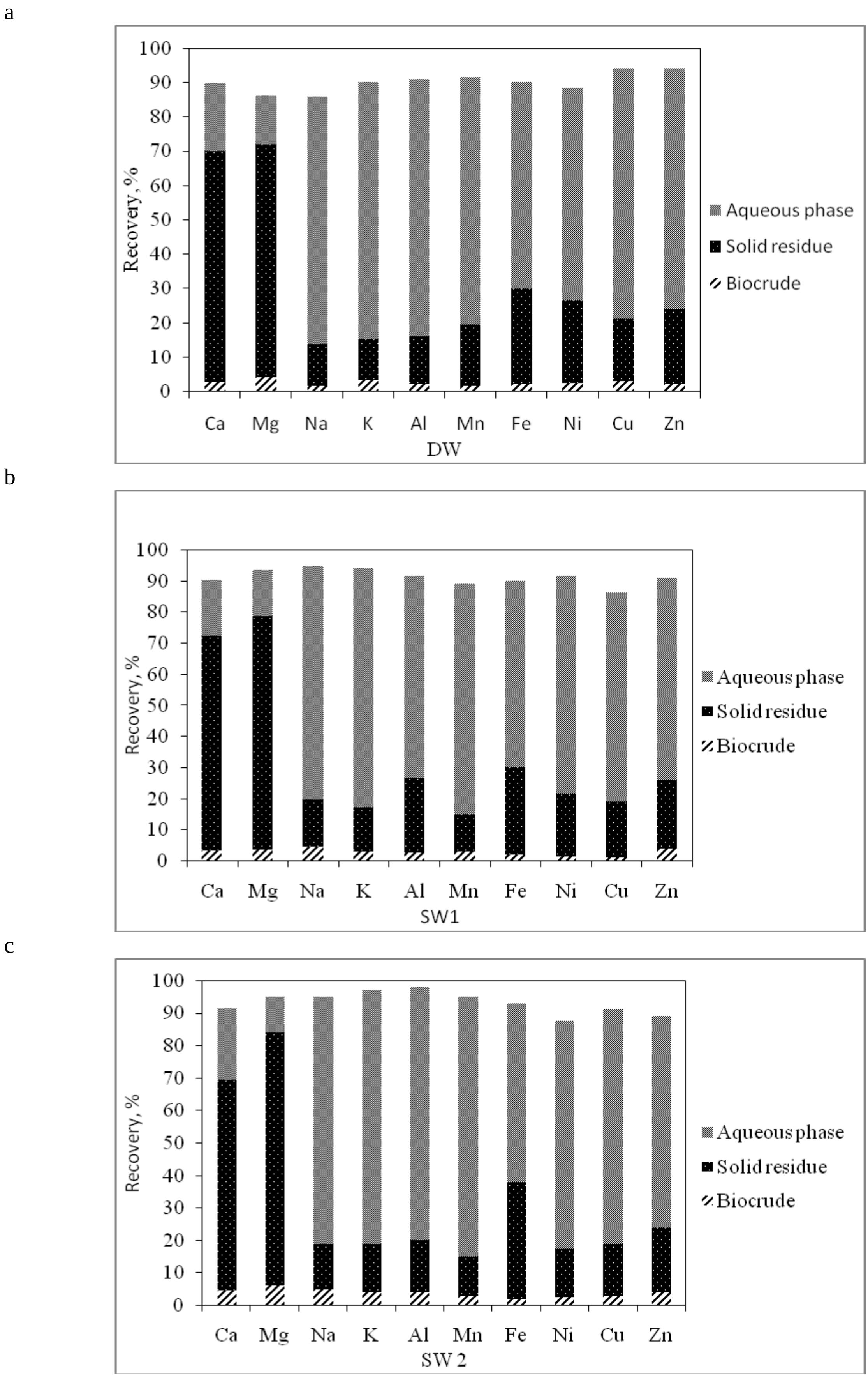

FIG. 6. Distribution of metals in biocrude, solid residue, and aqueous phase following hydrothermal liquefaction of *Tetraselmis* sp. at 350°C, 5min (a) DW: deionised water (b) SW1: seawater, 24ppt. and (c) SW2: seawater, 36ppt.

Table 1: Elemental and biochemical composition of microalgae

Microalgae strain	**Elemental composition (%w/w)**						**HHV**	**Biochemical composition %wt**			**Reference**
	C	**H**	**N**	**S**	**O**^{a}	**H/C**	**(MJ/kg**	**Carbohydrate**	**Protein**	**Lipids**	
Desmodesmus sp.	51.9	7.3	6.9	nr	30.3	1.6	23.2	13-20	38-44	10-14	Alba *et al.*[14]
Nannochloropsis. oc.	57.8	8.0	8.6	nr	25.7	1.7	26.8	8	57	32	Biller and Ross[3]
Spirulina sp.	46.6	7.1	10.6	0.7	35.4	1.8	20.7	30	48	13	Jena *et al.*[42]
Chlorella sp.	60.5	9.1	1.9	nr	28.5	1.8	28.7	13.2	9.3	59.9	Li *et al.*[7]
Amphora sp.	46.0	9.0	6.0	3.0	36.0	2.3	23.2	43	33	24	Present work
Spirulina sp.	51.0	8.0	12.0	1.0	28.0	1.9	24.3	11	53	18	Present work
Tetraselmis sp.	42.0	7.0	8.0	3.0	40.0	2.0	19.0	22	58	14	Present work

^{a}: obtained by difference, HHV: higher heating value.

Table 2: Typical trace elemental composition of SW1 and SW2

Trace Element	**Molecular weight, g/mol**	**SW1**		**SW2**	
		Concentration, mg/kg	**mmol/kg**	**Concentration, mg/kg**	**mmol/kg**
Calcium (Ca)	40.10	273.70	6.83	473.50	11.80
Sodium (Na)	22.99	7120.52	309.73	12239.04	532.40
Potassium (K)	39.10	263.51	6.74	443.70	11.30
Magnesium (Mg)	24.30	863.30	35.33	1474.10	60.70
Aluminium (Al)	27.00	200.80	7.43	281.10	10.41
Manganese (Mn)	54.90	22.00	0.40	28.60	0.52
Iron (Fe)	55.80	160.00	10.83	261.00	4.67
Nickel (Ni)	58.70	48.70	0.83	95.60	1.62
Copper (Cu)	63.50	115.00	1.81	162.00	2.55
Zinc (Zn)	65.40	225.00	3.44	375	5.73

Table 3: Elemental composition, atomic ratios and energy recovery for biocrude obtained under different reaction conditions.

Reaction conditions		Elemental composition, w/w%					[a]HHV, MJ/kg	Atomic ratio, mol/mol			Energy recovery, %
Temperature, ^oC	Media	C	H	N	S	O		H/C	N/C	O/C	
310	DW	66.0	5.8	3.5	0.9	22.9	27.8	1.0	0.1	0.2	40
	SW1	69.4	6.8	3.5	0.6	19.6	30.0	1.2	0.1	0.2	58
	SW2	70.3	7.1	3.8	0.6	18.5	30.0	1.2	0.1	0.2	52
350	DW	71.1	8.5	5.2	0.6	16.7	33.3	1.4	0.1	0.1	64
	SW1	74.2	8.6	4.7	0.6	11.9	34.8	1.4	0.1	0.1	72
	SW2	72.0	8.2	4.5	0.6	14.2	34.4	1.4	0.1	0.2	60
370	DW	67.6	8.2	5.0	0.6	18.6	31.3	1.5	0.1	0.2	50
	SW1	72.3	8.2	4.1	0.6	14.3	33.3	1.4	0.1	0.2	57
	SW2	72.0	8.1	5.0	0.6	14.3	33.3	1.4	0.1	0.1	55

[a]: obtained by difference, HHV: Higher heating value.

Table 4: Elemental composition of biocrude obtained from different microalgae strain under wet and dry conditions

Microalgae	**Condition**	**Elemental composition (%w/w)**					**HHV**	**Atomic ratios (mol/mol)**			**Energy recovery, %**
		C	**H**	**N**	**S**	**O**[a]	**(MJ/kg)**	**H/C**	**N/C**	**O/C**	
Amphora sp.	Dry	76.7	9.0	4.6	1.0	8.7	36.5	1.4	0.1	0.1	48.5
	Wet	74.2	8.6	4.2	0.5	12.5	34.7	1.4	0.1	0.1	60.7
Spirulina sp.	Dry	72.6	8.9	6.3	0.7	11.5	34.6	1.5	0.1	0.1	56.3
	Wet	73.1	8.9	5.9	1.4	9.7	35.4	1.4	0.1	0.1	67.7
Tetraselmis sp.	Dry	68.9	7.8	5.9	0.4	17.0	31.4	1.4	0.1	0.2	66.9
	Wet	73.2	8.4	3.5	1.4	13.5	34.1	1.4	0.1	0.1	72.1
	[b]Petroleum crude [A,E, S,US]	83-87	10-14	0.1-2	0.05-6	0.05-1.5	40-44	1.4-1-9	-	-	-

[a]: obtained by difference, [b]: Speight[39], [A]: Asia, [E]: Europe, [S]: South America, [US]: United States, HHV: higher heating value. HTL performed at 350°C, 5min.

Chapter 7

Integrating anaerobic digestion and hydrothermal liquefaction for renewable energy production: An experimental investigation

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Environmental Progress & Sustainable Energy, Under Review.

Statement of Authorship

Title of Paper	Integrating anaerobic digestion and hydrothermal liquefaction for renewable energy production: An experimental investigation.
Publication Status	○ Published ○ Accepted for Publication ○ Submitted for Publication ○ Publication Style
Publication Details	Eboibi, B.E., Lewis, D.M., Ashman, P.J., Chinnasamy, S. Integrating anaerobic digestion and hydrothermal liquefaction for renewable energy production: An experimental investigation. Environmental Progress & Sustainable Energy, Under review.

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Contribution to the Paper	Designed and performed all experiments, analysed and processed data, and wrote manuscript.		
Overall percentage (%)			
Signature		Date	

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate to include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Eboibi, B.E., Lewis, D.M., Ashman, P.J. & Chinnasamy, S. (2015) Integrating anaerobic digestion and hydrothermal liquefaction for renewable energy production: An experimental investigation.
Environmental Progress & Sustainable Energy, v. 34 (6), pp. 1662-1673

Chapter 8

Conclusion and Recommendations

8.1. Conclusions

This study contributes to the understanding of direct conversion of microalgae biomass to biocrude using hydrothermal liquefaction. Knowledge of the composition of the HTL products, and the process mass and energy balances is important in the design of future HTL systems. The composition of the resultant biocrude is paramount as it impacts significantly on its suitability for either direct utilization or upgrade to better quality fuels. The amount of nitrogen, oxygen, and metallic impurities in the biocrude are among the main factors to be considered for biocrude applications. The amount of undesired content has significant effects on the cost of upgrading, process equipment and environmental issues. The experimental data obtained in this study is important as it will enable other researchers to improve the life-cycle or techno-economic analysis of an envisaged HTL-microalgae-biofuel system. The following section outlines some of the specific conclusions related to the objectives of this study.

8.1.1. Effect of operating conditions on yield and quality of biocrude during hydrothermal liquefaction of halophytic microalga *Tetraselmis* sp.

The optimum conditions based on maximum yield and most improved quality biocrude in terms of reaction temperature and reaction time was determined during hydrothermal liquefaction of halophytic microalga *Tetraselmis* sp. From this investigation, the following conclusions were drawn:

1. It was shown that high biocrude yields can also be obtained from HTL of halophytic microalga *Tetraselmis* sp. This conclusion addressed the downstream objective of selecting suitable microalgae for high biocrude yield.
2. The resultant biocrude yields were in the range 35wt% to 65wt%, the maximum of 65wt% was obtained at 350^{o}C with 5min short reaction time. The yield of biocrude decreased with a further increase in reaction temperature.
3. The biocrude has a higher heating value of 28MJ/kg to 39MJ/kg, which is similar to 40MJ/kg to 44MJ/kg of petroleum crude.
4. The quality of the biocrude at each process condition was assessed using the elemental analysis. The analytical results showed that the maximum yield and best quality of the biocrude are independent variables. A better quality biocrude was achieved at a longer reaction time (45min) than at short reaction time of 5min. This finding suggests that obtaining maximum yield and quality of biocrude at the same reaction temperature and time is challenging.

5. It was found that the resultant biocrude contains undesired nitrogen and oxygen content up to 6%w/w to 20%w/w, respectively. It also contains metals which is one of the main long-term concerns in HTL-microalgae-biocrudes. As a result, the biocrude cannot be used directly as transportation fuel, even if blended with petroleum crude still requires refining like petroleum crude. For direct utilization, upgrading of the biocrude to remove the undesired contents is necessary.
6. The energy recovery in the biocrude from the initial microalgae feedstock was 38% to 87%, while the carbon and nitrogen recovery were 63% to 88% and 24% to 53%, respectively. Nevertheless, using continuous systems will improve the yield and energy recovery of biocrude.

8.1.2. Hydrothermal liquefaction of microalga for biocrude production: Improving the biocrude properties with vacuum distillation

Due to some undesirable elements in the composition of the resultant biocrude, an experimental method for: reducing the oxygen, nitrogen, and metallic content, and enhancing the molecular weight biocrude fractions without employing catalyst or hydrogen was investigated. Based on the results of this study, the following conclusions were drawn:

1. Biocrude yield of 36wt% and 42wt% was obtained from HTL of *Spirulina* sp. at 300°C and 350°C, respectively. The biocrude yield for *Tetraselmis* sp. was 34wt% at 300°C, and 58wt% at 350°C.
2. The vacuum distillation of biocrude obtained from *Spirulina* sp. at 300°C and 350°C led to 62wt% and 67wt% distilled biocrudes yield, respectively. Vacuum distillation of *Tetraselmis* sp. biocrude obtained at 300°C resulted in a yield of 70wt%, and 73wt% from 350°C.
3. The distillation was shown to highly deoxygenate the resultant biocrudes from 12.5%w/w to 17.3%w/w to 0.4%w/w to 1.1%w/w, resulting in an oxygen removal of 87% to 96.8%.
4. Importantly, the carbon contents in the distilled biocrudes generally increased from 68.5%w/w to 71.4%w/w to 83.8%w/w to 86.9%w/w, which were very similar to 83%w/w to 87%w/w for petroleum crude.
5. The improve carbon and oxygen content led to a substantial increase in higher heating value of the biocrudes from 32.1MJ/kg to 40MJ/kg following distillation.
6. Approximately 78% to 83% energy was recovered in the distilled biocrudes.

7. The metallic content of the distilled biocrudes were found to substantially reduce after distillation.
8. The experimental result showed an increase in the low molecular weight biocrude fractions after distillation as evidenced by the simulated distillation data.
9. The nitrogen content was found to be lower in distilled biocrudes than the raw HTL biocrudes and similar to previous studies reporting catalytic hydrotreating of biocrude. However, it was found that the nitrogen content of distilled biocrude was 1.6%w/w to 1.9%w/w higher than of petroleum crude. Nevertheless, it is envisaged that lower amount of catalyst would be required during refining to transportation fuels.

8.1.3. Influence of process conditions in pretreatment of microalgae for protein extraction and in production of biocrude during hydrothermal liquefaction of pretreated *Tetraselmis* sp.

Due to the undesired high nitrogen content in the biocrude, mostly due to high-protein fraction biomass, the pretreatment of microalgae biomass prior to HTL of the resultant residue was investigated. This study demonstrated the possibility of producing biocrude low in nitrogen content from pretreated microalgae biomass. The results of this study advances knowledge on the feasibility of producing by-products alongside the primary product biocrude. The study also for the first time enhances carbon efficiency by using the recycled aqueous phase by-product as HTL reaction media. From the experimental results of this work, the following conclusions were drawn:

1. A maximum biocrude yield of 65wt% was obtained from liquefaction of pretreated microalgae biomass at 310^{o}C, but it was only 3wt% less than that derived from untreated biomass at 350^{o}C.
2. The reuse of recycled aqueous phase from the pretreatment as reaction media was found to significantly improved yields in biocrudes. The use of the recycled aqueous phase led to a 25wt% increase in biocrude yield, compared to employing fresh deionised water. The increased in biocrude yield with the use of recycled aqueous phase could be due to the presence of organic carbon. This is important as the reuse will be a means to improve the HTL carbon footprint. Also it is advantageous to reduce the toxicity and number of dilutions before they are applied in microalgae cultivation.

3. The results showed substantial reduction of about 32% to 46% nitrogen in the resultant biocrude obtained from pretreated microalgae biomass. However, the combined pretreatment and HTL route were found to be insufficient to reduce the nitrogen content in the biocrude to a standard which does not require further upgrading.
4. It was found that the biocrudes from pretreated and untreated biomass had similar functional groups. This suggests that pretreatment prior to liquefaction of the resultant residue has no negative effects rather it is a means to improve the quality of biocrude.
5. The energy analysis showed positive energy balance for both routes. A similar energy input at low reaction temperature (310^{o}C) was required for convectional liquefaction of untreated microalgae and the combined pretreatment step and HTL of pretreated biomass.
6. The concept of integrating microalgae pretreatment with subsequent liquefaction of treated biomass seems to have great potential as valuable co-products that can be produced alongside the primary product biocrude.

8.1.4. Effect of seawater and wet feedstock on product yield and properties during hydrothermal liquefaction of microalgae

In this study the use of seawater as reaction media on HTL yields and properties were examined. In addition, the effects of freshly harvested microalgae biomass and diluted pulverised biomass on product yields during hydrothermal liquefaction were investigated. The experimental data of this study led to the following conclusions:

1. The application of seawater led to up to 11wt% additional biocrude yield compared to deionised water.
2. The use of seawater as HTL reaction media does not have substantial effects on denitrogenation and deoxygenation, hence the biocrude will require further upgrading.
3. It was found that most of the metals in the feed were mostly distributed in the solid residue and aqueous phases. Similar amounts of metallic content in the biocrude were obtained for employed seawater and deionised water following liquefaction. Meaning that the use of seawater does not increase the amount of metallic content in biocrudes.
4. The use of freshly harvested microalgae biomass resulted in an increase biocrude yield ~10wt%, leading to lower yields in solid residue, aqueous and gas phases.
5. Despite variations in biocrude yields, there were no substantial differences in elemental composition and higher heating values.

8.1.5. Integrating anaerobic digestion and hydrothermal liquefaction for renewable energy production: An experimental investigation

An experimental investigation for the concept of integrating hydrothermal liquefaction with waste treatment facility such as anaerobic digestion was performed. From this study the following conclusions were drawn:

1. The result showed an average yield ~$0.5 m^3/kgVS_{added}$ of biogas from anaerobic digestion of manure.
2. The liquefaction of the resultant digestate obtained at day 38 hydraulic retention time (HRT) led to biocrude yield of 42wt%. A decrease in biocurde yields was obtained with digestate > 38 day HRT.
3. The biocrudes exhibited high oxygen contents of 16%w/w to 25%w/w, and higher heating value of 28.4MJ/kg to 31.2MJ/kg that require further upgrading.
4. Importantly, an energy recovery and carbon recovery of ~76% and up to 82% was obtained in the biocrude after liquefaction of the digestates.
5. Similarly, the nitrogen recoveries in the biocrude were as low as 1.9% to 3.6%, lower than 50% mostly reported from other HTL feedstocks in biocrude.
6. A positive energy consumption ratio was obtained for the combined anaerobic digestion and hydrothermal liquefaction of the resultant digestate.

8.2. Recommendations

The results of this thesis advance the knowledge of hydrothermal liquefaction of biomass to biofuel. However further studies are required to ensure future feasibility of the promising HTL-microalgae-renewable liquid fuels system as it can complement fossil fuels. Some of the further studies are presented here:

1. The microalgae *Tetraselmis* sp. biomass used in the study reported in Chapter 3 was harvested during the exponential growth phase. Since the composition of biomass varies with growth phase, it might be necessary to investigate the effect of biomass harvested at different growth phase on yield and properties of biocrude.
2. Investigating the influence of biomass solid content on biocrude yields deserves further studies, since it has been reported that solid loading has substantial effect on biocurde yield (Jena *et al*., 2011a; Valdez *et al*., 2012).
3. One of the issues in biocrude production from *Tetraselmis* sp. is that following liquefaction the product mixture forms an emulsion. Though the mixture is self separating from water after a period of time, but the extraction of biocrude from the product mixture

is challenging. Thus developing novel technology for biocrude or a rector design may be necessary.

4. Another issue to address is if time taken before extracting biocrude from the product mixture after adding organic solvent could affect yields and properties of products. It is envisaged that the timing may be important, because leaving the mixed product mixture over a period may lead to side reactions, which may affect the yield and quality of the products after extraction. Similar study could also be carried out on the product mixture before adding organic solvent, because in a commercial scale, there could be storage of product mixture. Thus the time allowed for storage before adding solvent to extract biocrude may be necessary.
5. A further study to understand the reaction mechanisms and kinetics involved in HTL is necessary in order to achieve desirable yield and quality biocrude.
6. The development of a pilot-scale plant is needful for a detailed economic feasibility study in order to assess the profitability of the envisage commercialisation of HTL.
7. The commercial application of hydrothermal liquefaction of microalgae for biofuels production may require the use continuous reactor integrated with harvesting systems such as centrifugation and flocculation. The use of continuous reactor is advantageous: inbuilt heat recovery system to improve energy efficiency, high throughput, it avoids the normal long heat up and cool down periods associated with batch systems (Ocfemia *et al*., 2006a; Jazwari *et al*., 2013). However, the long-term concern would be the cost of feedstock, feedstock salt content and corrosion.
8. Upgrading of biocrude using vacuum distillation showed the potential for decarboxylation and deoxygenation, leading to improved quality of biocrude. However, more work is needed in understanding the chemistry. This would help to select and design suitable catalyst for hydrotreating biocrude if needed.
9. Though, employing HTL process water as reaction media led to an increase in biocrude yields, an extended series of recycle investigation to know the number of times the process water can be recycled to comparable yield may be interesting.
10. It could be concluded that seawater as reaction media has an effect on product yields. However, the underlying chemistry behind this phenomenon is yet to be fully understood, hence further studies are required. In addition the reason why some metals are distributed in the solid residue and aqueous phases may deserve further investigation.
11. The concept for combining AD and HTL for biogas production and the subsequent liquefaction of resultant digestate to biocrude described in Chapter 7 showed the potential

for recovery of carbon from organic waste in sustainable production of renewable energy and reducing environmental pollution. The operating parameters examined were at fixed reaction temperature, reaction time and solid content. The operating parameters need further studies in order to increase biocrude yields, as it has been shown that biocrude yield varies with respect to differing operating parameters.

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