

**DILUTE SULFURIC ACID HYDROLYSIS
OF RED MACROALGA *EUCHEUMA
DENTICULATUM* WITH MICROWAVE-
ASSISTED HEATING FOR BIOCHAR AND
BIOETHANOL PRODUCTION**

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UNIVERSITI SAINS MALAYSIA

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EUCHEUMA DENTICULATUM WITH MICROWAVE-ASSISTED HEATING
FOR BIOCHAR AND BIOETHANOL PRODUCTION**

by

TEH YONG YI

**Thesis Submitted in Fulfillment of the
Requirements for the Degree of
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LIST OF ABBREVIATIONS

ACE	ASEAN Centre for Energy
ADF	Acid detergent fiber
ADL	Acid detergent lignin
AIA	Acid insoluble ash
AOAC	Association of Official Agricultural Chemists
ASEAN	Association of Southeast Asian Nations
ASTM	American Society for Testing and Materials
CS	Combined severity
DCM	Dry cell mass
DOF	Department of Fisheries Malaysia
DSC	Differential scanning calorimetry
DTG	Derivative thermogravimetry
EA	Elemental analyzer
EGA	Evolved gas analysis
EEF	Energy enhancement factor
ELSD	Evaporating light scattering detector
GHz	Gigahertz
FAO	Food and Agriculture Organization of the United Nations
FC	Fixed carbon
FID	Flame ionization detector
FT-IR	Fourier transform infrared
GC	Gas chromatography
GHG	Greenhouse gas
GW	Gigawatt

HHV	Higher heating value
HTL	Hydrothermal liquefaction
HPLC	High performance liquid chromatography
IBI	International Biochar Initiative
IEA	International Energy Agency
IRENA	International Renewable Energy Agency
mb	Million barrels
mHz	Megahertz
MSW	Municipal solid waste
mtoe	Million tonnes oil equivalent
MW	Megawatt
NDF	Neutral detergent fiber
NREL	National Renewable Energy Laboratory
PV	Photovoltaic
RFA	Renewable Fuels Association
RO	Reverse osmosis
SEDA	Sustainable Energy Development Authority of Malaysia
SEM	Scanning electron microscope
SIEW	Singapore International Energy Week
TG	Thermogravimetry
TGA	Thermogravimetric analyzer
tmoe	Thousand million oil equivalent
TMA	Thermomechanical analysis
USDA	United States Department of Agriculture
UV	Ultraviolet

VM	Volatile matter
VRE	Variable renewable energy
XRD	X-ray diffractometer
YEPG	Yeast extract peptone galactose
5-HMF	5-(hydroxymethyl)furfural

LIST OF SYMBOLS

Symbol	Description	Unit
ADF_F	Final weight of sample for ADF	g
ADF_I	Initial weight of sample for ADF	g
ADL_F	Final weight of sample for ADL	g
D_E	Energy density of biochar	Dimensionless
$[EtOH]_{max}$	Highest ethanol concentration achieved during fermentation	g/L
$HHV_{biochar}$	HHV of biochar	MJ/kg
HHV_{raw}	HHV of raw biomass	MJ/kg
$m_{biochar}$	Mass of biochar	g
m_{raw}	Mass of raw biomass	g
NDF_F	Final weight of sample for NDF	g
NDF_I	Initial weight of sample for NDF	g
$[Sugar]_{ini}$	Total initial sugar concentration at onset of fermentation	g/L
W_C	Weight of crucible	g
W_{CC}	Weight of crucible and cover	g
W_I	Initial weight	g
W_F	Final weight	g
W_1	Weight of sample and crucible	g
W_2	Weight of ash and crucible	g
$Y_{biochar}$	Solid yield of biochar	%
Y_E	Energy yield of biochar	%
$Y_{P/S}$	Ethanol yield	g/g
$Y_{\%T}$	Percent theoretical yield	%

**HIDROLISIS MAKROALGA MERAH *EUCHEUMA DENTICULATUM*
DENGAN LARUTAN ACID SULFURIK DENGAN MENGGUNAKAN
SISTEM PEMANASAN GELOMBANG MIKRO BAGI PENGHASILAN
BIOARANG DAN BIOETANOL**

ABSTRAK

Eucheuma denticulatum ialah sejenis makroalga merah yang berpotensi sebagai stok suapan untuk penghasilan biotena kerana makroalga ini mengandungi kandungan karbohidrat yang tinggi, mudah diperolehi serta proses penghasilan hanya menggunakan tanah yang sedikit. Oleh sebab kandungan karbohidrat yang tinggi, makroalga boleh dihidrolisis menjadi gula mudah ditapai dan ditukar kepada bioetanol oleh mikroorganisma semulajadi. Walau bagaimanapun, pemanasan haba konvensional yang memindahkan haba dari permukaan ke pusat bahan menjadikan proses pemanasan agak lambat dan tidak cekap. Oleh itu, masa pemanasan yang berpanjangan telah mengurangkan kuantiti gula dan meningkatkan pula penghasilan produk sampingan yang boleh menjejaskan proses penapaian seterusnya. Dalam kajian ini, hidrolisis asid dengan pemanasan gelombang mikro dijalankan dalam ketuhar gelombang mikro pada frekuensi 2.45 GHz dan kuasa 800W. Dengan pemanasan dielektrik gelombang mikro, ia bukan sahaja dapat menangani masalah yang dibangkitkan di atas, malahan ia dapat meningkatkan prestasi proses hidrolisis dan menghasilkan bioarang, gula dan bahan kimia yang bernilai tinggi daripada makroalga. Tujuan utama kajian ini adalah untuk mengkaji sifat-sifat bioarang yang dihasilkan sebagai sumber biotena selepas makroalga melalui proses hidrolisis gelombang mikro. Proses hidrolisis dijalankan pada kepekatan asid sulfurik 0.1M dan 0.2M, suhu pemanasan antara 150-170 °C dan masa pemanasan selama 10 minit.

Kualiti bioarang yang dihasilkan didapati lebih baik dengan peningkatan kandungan karbon unsur dan kandungan abu dan lembapan yang lebih rendah. Nilai kalori bioarang dapat ditingkatkan sehingga 45%, dan 39% hasil tenaga dapat dipulih kembali. Selain daripada bioarang, kepekatan gula dan produk sampingan juga ditentukan dan seterusnya ditukarkan kepada bioetanol. Hasil gula sebanyak 74.84% (51.47 g/L) dan produk 5-HMF yang berjumlah 0.20 g/L telah dicapai apabila biomassa dihidrolisiskan pada suhu 160 °C dengan kepekatan asid sulfurik 0.1M. Hasil bioetanol yang diperolehi adalah sebanyak 0.33 g/g (64.21%). Sebagai kesimpulan, hidrolisis dengan larutan acid sulfurik dalam sistem pemanasan gelombang mikro berpotensi untuk menghasilkan biotenaga, bioarang yang kaya dengan nutrien sebagai penambahbaik tanah dan bahan kimia yang bernilai tinggi.

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FOR BIOCHAR AND BIOETHANOL PRODUCTION**

ABSTRACT

Eucheuma denticulatum is a type of red macroalgae which are considered as potential feedstock for biofuel production due to high carbohydrate content, ease of availability and low arable land usage. Being such abundant in carbohydrates, macroalgae can be hydrolysed into fermentable sugars and converted to bioethanol by natural microorganisms. However, the conventional thermal heating that delivers heat from the surface to center of a material is comparatively slow and inefficient. As a result, the longer heating time gives rise to a dramatic reduction of reducing sugars but higher content of by-products, consequently inhibiting the subsequent fermentation. In this study, acid hydrolysis with microwave-assisted heating of macroalga was conducted in a 2.45 GHz microwave oven with 800W of power. The rapid and efficient of dielectric heating effect of microwave-assisted heating could address aforementioned problem, enhance the hydrolysis process and co-produce biochar, reducing sugars and valuable chemicals. This study was set out to study the properties of biochar as a source of biofuel after macroalga was subjected to microwave-assisted hydrolysis. The hydrolysis reactions were operated at sulfuric acid concentrations of 0.1M and 0.2M, reaction temperatures of 150–170 °C and a heating time of 10 min. The produced biochars were characterized and it was found that biochar qualities were improved with increased elemental carbon content and lower ash and moisture contents. The calorific value of the biochar was intensified up to 45%, and 39% of energy yield was recovered. Apart from biochar, the

concentrations of reducing sugars and by-product of hydrolysate were also determined and subsequently converted to bioethanol. The highest total reducing sugars were 51.47 g/L (74.84% yield) along with a low by-product 5-HMF of 0.20 g/L, when the biomass was treated under the conditions at 160 °C with 0.1M H₂SO₄. The ethanol yield obtained was 0.33 g/g, which corresponded to a 64.21% of yield. As a conclusion, acid hydrolysis with microwave-assisted acid hydrolysis has its great potential applications for the production of bioenergy, nutrient-rich biochar for soil amelioration and value-added chemicals.

CHAPTER ONE

INTRODUCTION

1.1 Energy sector landscape

Energy is an important sector which interplays between power generation, transportation, heating and cooling. The dynamics of energy consumption in the industry, transport, power and buildings sectors are depicted in Figure 1.1. Global energy consumption rose 53% from 8589 million tonnes oil equivalent (mtoe) in 1995 to 13147 mtoe in 2015 and it is expected to increase, reaching 17158 mtoe in 2035 (BP, 2017). Remarkably, energy consumption in Asia Pacific nearly increased 2.5 times over the two decades, contributing 5499 mtoe in 2015. This is due to Asia Pacific's economy shifted from an agricultural to an energy-intensive manufacturing industrial base (IRENA, 2018). Coal is mainly used in industry sector, contributing 44% of the total global consumption by energy source (BP, 2017).

Driven by road infrastructure development and rising vehicle ownership, the energy use in transport sector grew modestly from 1995 to 2015. Oil is used primarily for road transport (95% of the total global consumption by energy source) (BP, 2017). The global car fleet will be doubled up and reached 2 billion by 2040. The energy use in the transport sector remains dominated by oil next decades, which fuel demand has grown rapidly amid growing economic, greater freight activities and rising consumption for trucks, cars, aviation and shipping (IEA, 2017c).

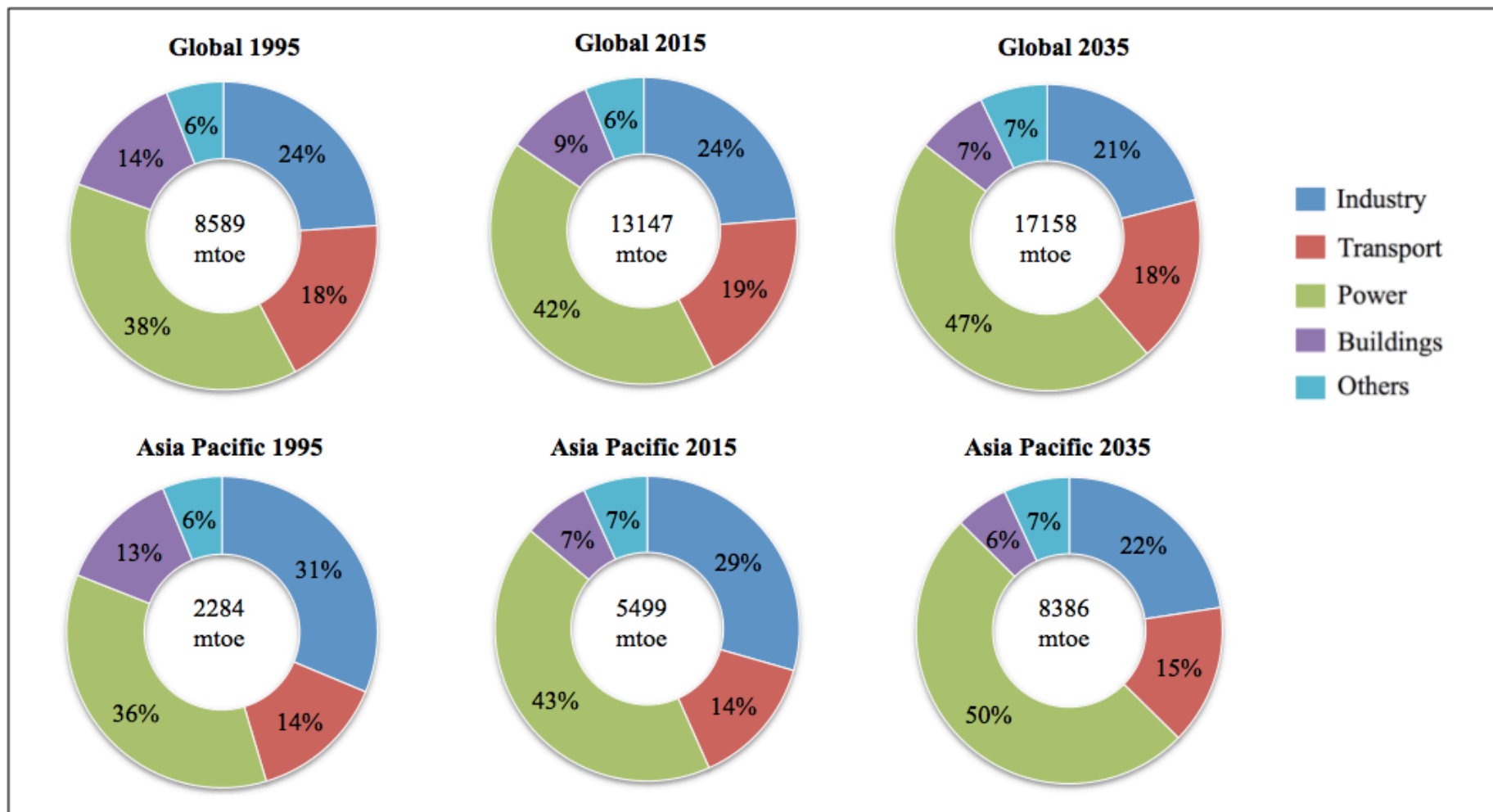


Figure 1.1 Total final energy consumption by sector for the year 1995, 2015 and 2035 (BP, 2017)

On the contrary, the buildings sector accounted for 9% of total final energy consumption in 2015, down from 14% in 1995 (BP, 2017). This trend was accompanied by rapid urbanization coupled with the improved energy access. Electric cooking, heating and cooling system have gained increasing popularity. Subsequently, electricity has replaced less efficient oil and coal for lighting and heating and thus electricity become a common alternative for a variety of energy demands (IRENA, 2018; SIEW, 2017). With the rising electricity demand, this trend is expected to continue over the next two decades. The growth in energy demand will be highest in power sector (45%), followed by transport (23%), industry (16%) and buildings (6%) (BP, 2017).

Interconnectedness, the hallmark of the information age, is spurring the development of information technology and artificial intelligence. This will greatly improve productivity of system and reinforce a more consumer-centric approach in the Industrial Revolution 4.0. Undoubtedly, growing automation and digitalization could boost electricity consumption exponentially (SIEW, 2017).

Moreover, transportation is a key area for electricity replacement. Beyond personal cars, transportation electrification is taking hold in public transit, commercial delivery vehicles and ride-sharing applications. Additionally, the electric vehicle market is now kicking off, with electric and plug-in vehicles amounting to 280 million by 2040 (IEA, 2017c; SIEW, 2017).

However, strong growth in fossil-fuel consumption increased roughly 75% in energy-related carbon dioxide (CO₂) emissions (IEA, 2017a). This alarming rise of carbon emissions has increased ambient temperature and heat wave events, consequently aggravating climate change (SEDA, 2017c). About 80% of natural disasters are “hydro-meteorological” events and these are expected to grow in

number and severity with the effects of climate change. With extreme weather events, high temperatures and extreme precipitation patterns, the climatic impacts can cause extensive damage to infrastructure and disrupt energy supply (IEA, 2017a).

Therefore, rising domestic energy demand and environmental impacts exacerbate energy security concerns. Against this backdrop, there is a need for energy players to stay nimble and forge a progressive energy landscape.

1.2 Overall renewable energy

Energy resources that are naturally replenishing such as biomass, geothermal, hydro, solar and wind could be harnessed as renewable energy. Renewable energy could address climate agenda by reducing harmful greenhouse gas (GHG) emissions during power generation, while offering as alternative energy which is available domestically and never-ending in supply.

Over the two decades, renewable energy increased dramatically from 599 mtoe in 1995 to 1258 mtoe in 2015, accounting 10% of total final energy generation (BP, 2017). The hydropower, bioenergy and geothermal are the main renewable energy resources. Despite the cost competition from onshore wind and solar photovoltaic (PV) technologies, bioenergy remains an important contributor to renewable energy. This is because bioenergy plants generally have higher capacity factor than variable renewable energy (VRE). Capacity factor is the ratio of electricity generation over an extended period (e.g. a year) compared to maximum theoretical generation possible given by the rated capacity of the technology. The capacity factors of renewable energy vary greatly depending on the availability of

energy source. In 2015, the average capacity factor of bioenergy was relatively higher (50%) when compared with 13% for solar PV and 26% for onshore wind (IEA, 2017b).

Moreover, countries with the hot and humid climate encountered certain limitations with their traditional solar power plants such as difficulties in installation and maintenance as well as frequent failures of consumables like external fans and fuses (SEDA, 2017a). As solar and wind are variable in nature, thus some intelligence in the network is required to balance the grid in terms of the dynamic electricity supply and demand (SEDA, 2017c). During January 2017, there was very prolonged cold weather throughout Europe, which coincided with very little wind and low hydro-electric reserves (Agora Energiewende and Sandbag, 2018). Hence, bioenergy is an excellent complement to renewables as a sustainable alternative fuel, providing grid stability and supply security.

1.3 Development of bioenergy in Southeast Asia

According to IEA (2017a), the energy demand in the Southeast Asia region has grown by 60% over the past 16 years, largely due to robust economic and demographic growth. Being a new heavyweight in global energy, a wide range of policy developments has been made in this region. As depicted in Figure 1.2, the share of renewable energy increased from 21.05 gigawatt (GW) (19% of the region's total electricity generation) in 2006 to 51.42 GW (27% of the region's total electricity generation) in 2015 (ACE, 2018).

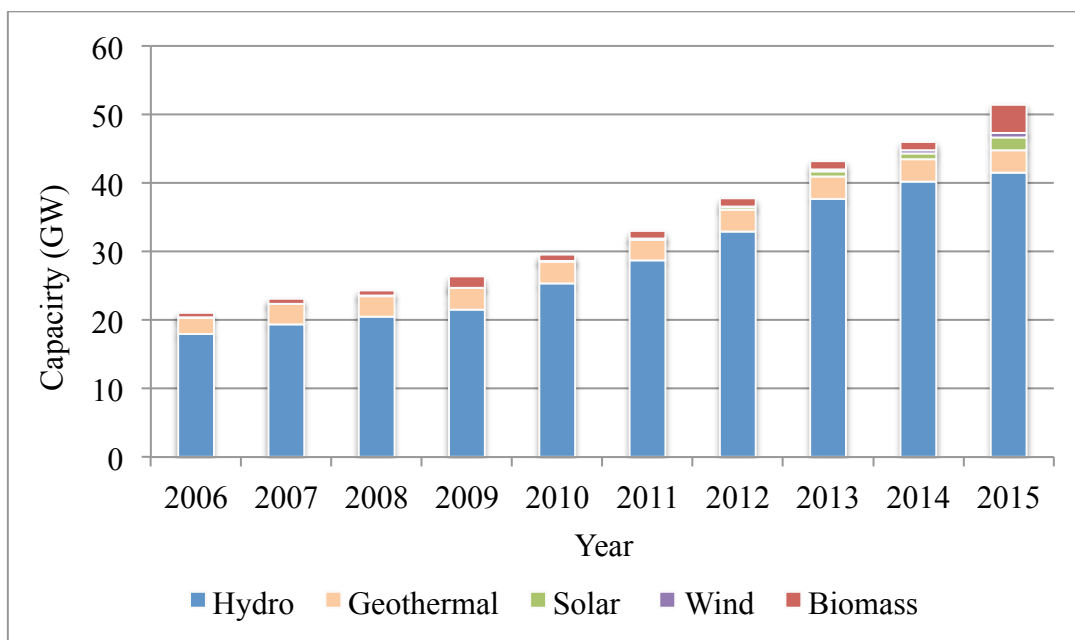


Figure 1.2 Renewable energy capacity by source in Southeast Asia, 2006-2015 (ACE, 2018)

Certainly, investments in renewable energy coupled with improvements in energy-efficient technologies could scale up renewable energy in the electricity mix in order to ensure supply security. In 2016, USD 2.6 billion was invested in the renewable energy and bioenergy received most of the investment (USD 920 million), followed by solar PV (USD 662 million) and wind (USD 589 million) (IRENA, 2018). As shown in Figure 1.2, the share of bioenergy increased substantially from 0.69 GW in 2006 to 4.12 GW in 2015 (ACE, 2018). Bioenergy is more prominent in the recent renewable generation mix for Southeast Asia region.

In Thailand, bioenergy fuel is used in co-generation plants to produce heat and electricity for industry sector (IRENA, 2018). Thailand is one of the major markets using liquid biofuel blending mandates. Thailand introduced B2 (2% biodiesel, 98% petroleum diesel) mandate in 2008, followed by B5 mandate in 2012, B7 mandate in 2014 and is planning to raise the current B7 mandate to B10. Tax

incentives are given for the manufacturing of vehicles compatible with ethanol blending. Thailand also supported E20 and E85 blends at the pump (IRENA, 2018; USDA, 2016).

In Malaysia, an option for B5 mandate was introduced in 2011, rising to a B7 mandate in 2014 and going to roll out B10 mandate (IRENA, 2018). Up to June 30, 2017, renewable energy projects in Malaysia have been approved with total capacity of 1455 megawatt (MW) and a capacity of 376 MW was recorded for biomass plants (SEDA, 2017b).

The mix of fuels and technologies varies country-by-country, but a common challenge is shared by policy-makers to meet the rising electricity demand. By 2040, installed power generation capacity in Southeast Asia increases to more than 565 GW from 240 GW in 2017 and 70% of the new coal-fired capacity uses high efficiency supercritical or ultra-supercritical technologies. Coal leads the charge for new power gen by taking the most prominent role in the energy mix (40%). The two-thirds of the increase in the electricity demand comes from the residential and service sectors. This mainly is due to the rising urban middle class and high demand for electrical applications. However, in transport sector, less progress has been taken for the electrification and energy use remains dominated by oil products. With growing economies and rising demand for mobility, the oil demand expands from 4.7 million barrels per day (mb/d) in 2017 to 6.6 mb/d in 2040. Ultimately, it reflects an emerging preference for increased deployment of renewables and greater diversification of energy mix focusing on bioenergy (IEA, 2017a).

1.4 Potential of bioenergy as alternative renewable energy

A wide range of feedstock (biomass) can be used as sources of bioenergy. These include: lignocellulosic plants (such as eucalyptus), agricultural residues (such as straw and bagasse), oil-bearing plants (such as jatropha and camelina), food crops (such as sugar cane and corn), microalgae, macroalgae and organic fraction from municipal solid waste (MSW).

A number of processes are available to turn these feedstocks into a product that can be used for power generation and electricity and transportation. For instance, biomass can be converted into biochar, bio-oil or syngas via thermochemical conversion (torrefaction, liquefaction, pyrolysis and gasification), bioethanol by fermentation or biogases through anaerobic digestion, as illustrated in Figure 1.3 (Chen et al., 2015a; IEA, 2017b; Rowbotham et al., 2012).

Thermochemical treatment of biomass could improve the fuel qualities of the resulting products such as biochar with higher calorific value close to that of coal, as a consequence of increased energy density. Undeniably, the properties of biochar with increased carbon content, higher hydrophobicity and lower ash content could be a viable option for direct combustion or co-combustion with coal for heat and power generation (Liu et al., 2013).

Furthermore, the large surface area of biochar provides another value-added function, say, for soil applications. This feature promotes the capability of nutrients and water adsorption in the soil and can be further improved by chemical and physical activation to enhance the biomass utilization efficiency (Rajapaksha et al., 2016).

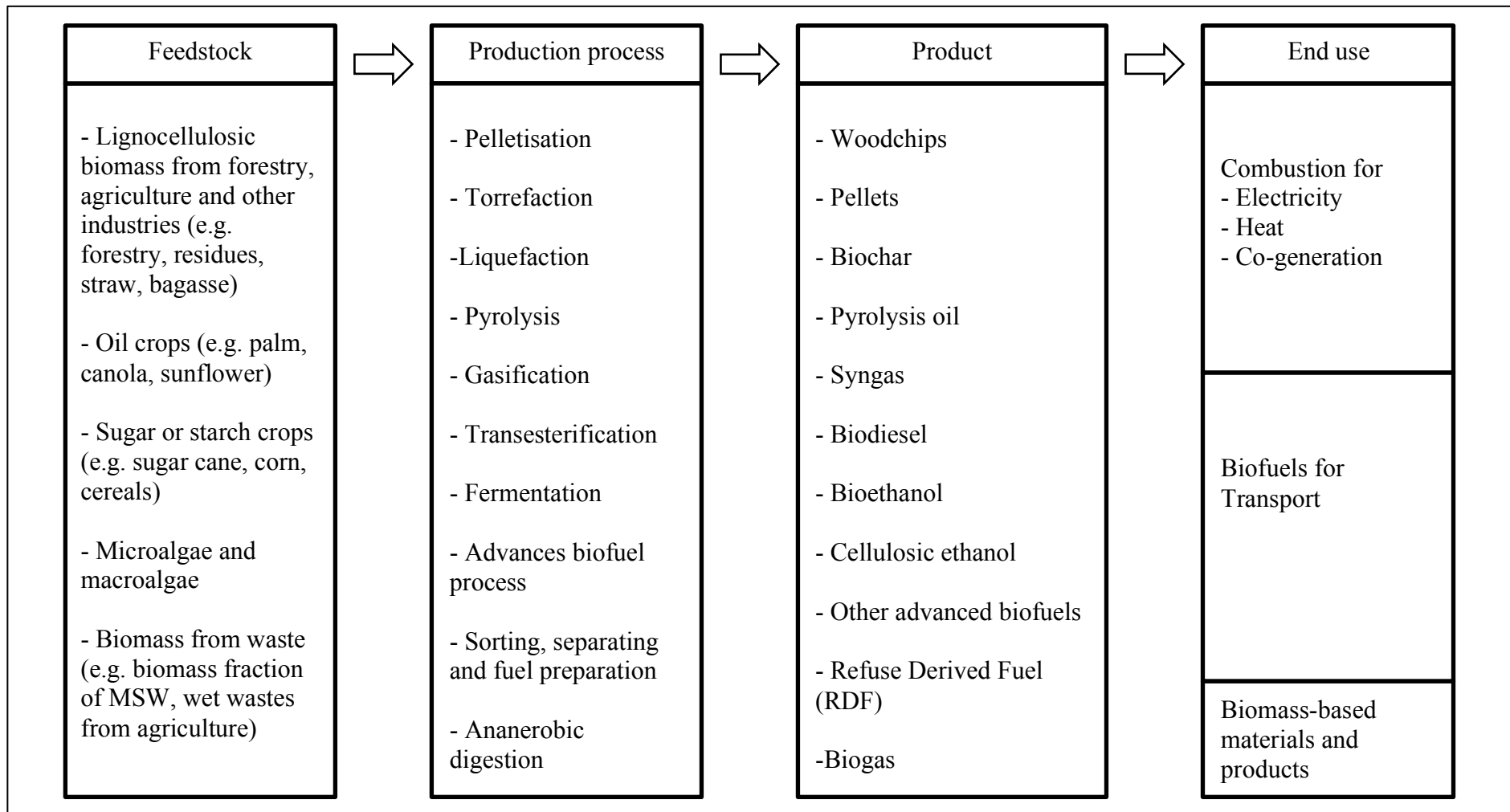


Figure 1.3 Potential bioenergy pathways: From feedstock to final energy use (Chen et al., 2015a; IEA, 2017b; Rowbotham et al., 2012)

Global production of biofuels increased dramatically from 9861 thousand million oil equivalent (tmoe) in 1995 to 80024 tmoe in 2015 (BP, 2017). The two most abundant and feasible liquid biofuels are bioethanol from carbohydrate based crops and biodiesel from oil crops (Wei et al., 2013). The biomass would utilize sunlight and carbon dioxide that released during a vehicle's fuel combustion in order to synthesis organic molecules, particularly carbohydrate and lipids, for the production of biofuels and value-added chemicals (RFA, 2017). Bioethanol has been recognized as one of the most affordable and safest option available to replace gasoline because bioethanol is an oxygenated fuel which can improve combustion and reduce hydrocarbon, carbon monoxide and particulate emissions (Balat et al., 2008).

Till date, four generations of biofuel have been developed based on different feedstocks. Bioethanol produced from sugar or starch edible crops is termed as first generation bioethanol. Corn and sugarcane are the main feedstocks for bioethanol production in USA and Brazil, respectively. As the conversion process is relatively simple, first generation bioethanol has been extensively consumed. However, first generation bioethanol has raised the "food versus fuel" feud. Thus, biofuel refined from non-edible lignocellulosic biomass, namely second generation biofuel is an excellent option to displace fossil fuels. However, scaling-up second generation biofuel requires vast areas of arable land and advance technologies to unravel the recalcitrant structure of lignocellulosic biomass (Goh and Lee, 2010; Jambo et al., 2016). In this respect, third generation biofuel derived from microalgae and macroalgae offers great promise to be renewable energy source because algae-derived biofuel overcomes the major drawbacks associated with the first and second generations of biofuels. For the latest fourth generation biofuel, advanced technology

with genetic modification is adopted but the technical performance is still in development stage (Liew et al., 2014).

Therefore, in terms of technical and economic performance, algae-derived biofuel is preferred as sustainable solution to address climate change and energy security. Algae-derived feedstocks could offer great potential as solid biochar for industrial heat generation and cogeneration with coal and as liquid biofuels for transport sector.

1.5 Problem Statement

A number of mature technologies are available to turn biomass feedstocks into biochar, liquid biofuel and biogas that can be used for transport, electricity and heat. The rich marine biodiversity in the east coast of Malaysia comes with an abundance of marine algae, namely seaweed or macroalgae. Being such abundant in carbohydrates, macroalgae can be hydrolysed into fermentable sugars and converted to bioethanol by natural microorganisms. In order to render the carbohydrates in macroalgae accessible for ethanol fermentation, different physical and chemical treatment methods have been applied. However, conventional thermal heating transfers heat from the surface towards the center of the material by convection, conduction and radiation (Brodie, 2015). Therefore, reaction vessel normally requires a higher temperature than the required reaction temperature, which is relatively slow and inefficient (Yin, 2012). As a result, the longer heating time gives rise to a dramatic reduction of reducing sugars but higher content of by-products, consequently inhibiting the subsequent fermentation (Rosatella et al., 2011).

As an alternative, microwave-assisted heating could be adopted to addressing the aforementioned problems, coproducing biochar, sugars and value-added chemicals. The microwave-assisted heating is a direct conversion of electromagnetic energy into thermal energy within the volume of the material, providing a rapid and energy efficient heating mode (Brodie, 2015; Chen et al., 2012a). Therefore, biomass can be heated in a shorter time and with a more even temperature distribution. These advantages often yield an increased sugar production and reduced by-products or inhibitor of fermentation.

Till date, only brown seaweed *Ascophyllum nodosum* has been studied for biochar and bioethanol production from microwave-assisted acid treatment (Yuan and Macquarrie, 2015). In fact, red macroalgae contain higher carbohydrate composition than brown macroalgae (Jambo et al., 2016; Park et al., 2012). Thus, acid hydrolysis with microwave-assisted heating of red macroalga *Eucheuma denticulatum* (*E. denticulatum*) has the potential to produce high yield of reducing sugars for bioethanol production in addition to increasing biochar densification. The upgraded solid residues, biochar can be utilized to partially replace coal for power generation (Chen et al., 2015a). With the high mineral and nutrient contents, algal biochar could provide another value-added function as soil amendment for agriculture application (Yu et al., 2017). Hence, it is beneficial to conduct a process study on the conversion of red macroalga to biochar and bioethanol via acid hydrolysis with microwave-assisted heating.

1.6 Objectives

The main aim for this research is to produce biochar and bioethanol from red macroalga *E. denticulatum* by using dilute sulfuric acid hydrolysis with microwave-assisted heating. Specific objectives can be divided into 3 parts as listed below:

1. To perform biochar characterization and investigate the effect of microwave-assisted heating process parameters (heating temperature and concentration of dilute sulfuric acid) towards hydrolysis process.
2. To determine the concentrations of reducing sugars and by-product produced from the hydrolysis process.
3. To produce hydrous bioethanol from *E. denticulatum* by fermentation.

1.7 Scope of study

Eucheuma denticulatum (*E. denticulatum*) used in this study is a red macroalga that is widely cultivated along the east coast of Sabah (Malaysia). Apart from avoiding significant land-use and threats to food security, red macroalga with high carbohydrate content and fast growing ability are heralded as the principal advantages as the biomass feedstock of this study.

The acid hydrolysis with microwave-assisted heating was conducted in the temperature range of 150 to 170 °C and concentration of dilute sulfuric acid of 0.1M to 0.2M but at a constant heating time (10 min). The hydrolysis temperature was limited to 170 °C because that would result to a pressure of 10 bar in the reactor. Since the reactor was conducted in a household microwave oven, the operating pressure was limited to 10 bar to ensure safe operation.

Detailed compositional characterizations of biochar were performed with bomb calorimeter, elemental analyzer (EA), scanning electron microscope (SEM), thermogravimetric analyzer (TGA), X-ray diffractometer (XRD) and fourier transform infrared (FT-IR) spectrometer. Contents of sugars and by-product were also determined with high performance liquid chromatography (HPLC) for subsequent fermentation experiment.

The cultivation activities of macroalgae were excluded from this study. The bioethanol produced in this research is the hydrous ethanol (as part of fermentation broth), thus the purification stage (distillation and dehydration) is not within the scope of this study.

1.8 Thesis organization

This research study is presented in five chapters: Introduction, Literature Review, Materials and Methods, Results and Discussion and Conclusions and Recommendations.

Chapter 1 covers the current global energy sector landscape, focusing on the final energy consumption and the factors of the rising energy demands. This chapter gives a brief introduction on the renewable energy and a snapshot of the recent progress and developments of bioenergy in Southeast Asia. It further discusses the potential of bioenergy as alternative renewable energy, including the bioenergy pathways and biofuel feedstocks. The problem statement, research objectives and scope of the study can be found here in detailed.

Chapter 2 initially provides basic backgrounds of macroalgae including their taxonomical classification, carbohydrate composition and seaweed cultivation. This

is followed by an overview on the current biofuel production technologies, covering thermochemical conversion, enzymatic hydrolysis and chemical hydrolysis. The principles of microwave-assisted heating, together with pyrolysis and hydrolysis, are presented. Effects of process parameters on the properties of biochar and yield of reducing sugars during biomass hydrolysis in microwave-assisted heating are also discussed thoroughly. Biochemical pathways for ethanol synthesis from glucose and galactose as well as fermentation process by various researchers are included.

Chapter 3 covers the research methodology and analysis applied in this research work. The details of materials and chemicals used throughout the study are listed. It also includes the description of experimental setup, acid hydrolysis with microwave-assisted heating and fermentation process, product analysis and characterization methods.

Chapter 4 presents the results and discussion of the research study. The first part consists of fuel characterization based on chemical properties, physical properties and thermal analysis of biochar. Detailed compositional characterizations are carried out and compared with various biomass in order to elucidate the scalability of microwave-assisted heating. The effects of acid concentration and heating temperature in the acid hydrolysis with microwave-assisted heating process are also discussed in detail. Next, the potential applications of biochar are explored with respect to their composition and characterization. The investigation of the effect of process parameters towards the yield of reducing sugars and performance of fermentation are discussed as well.

Chapter 5 provides a summary of the results obtained from the research study and suggestions about works in dilute acid hydrolysis with microwave-assisted heating for future biofuel developments.

CHAPTER TWO

LITERATURE REVIEW

2.1 Macroalgae as biofuel feedstock

As discussed in the previous chapter, energy demand is increasing fast, driven by rapid economic growth, increasing industrial activities and growing populations. Therefore, there is an urgent need to find alternative renewable energy in order to reduce heavy reliance of fossil fuels for energy supply and subsequently reduce the greenhouse gas (GHG) emission from fossil fuel use. Biomass, one of the world's most abundant materials, has a huge potential to replace the existing conventional fossil fuels as a renewable energy source. The potential and advantages of macroalgae as third generation biofuel feedstock is briefly discussed in this subsection.

Macroalgae/seaweed are multicellular algae and classified based on pigments and chlorophylls in their thallus. Macroalgae are categorized into three groups which are brown macroalgae (Phaeophyta), green macroalgae (Chlorophyta) and red macroalgae (Rhodophyta) (Figure 2.1) (Bharathiraja et al., 2015; Jung et al., 2013; Lobban et al., 1985; Sze, 1993).

It is noteworthy that carbohydrate composition of macroalgae is greatly different from microalgae and lignocellulosic biomass. Due to living in marine environment, macroalgae almost do not have lignin and hemicellulose, which are structural support in terrestrial plants (Jung et al., 2013; Wargacki et al., 2012; Wegeberg and Felby, 2010; Wei et al., 2013). Furthermore, in contrast to microalgae and lignocellulosic biomass, macroalgae have a variety of carbohydrates depending on their species, as listed in Table 2.1 (Jung et al., 2013; Lobban and Wynne, 1981).

The carbohydrate contents of brown, green and red macroalgae are 30-50%, 25-50% and 30-60% dry wt., respectively (Becker, 1994; Jensen, 1993; Jung et al., 2013; Ross et al., 2008). Being such abundant of carbohydrate, macroalgae can be depolymerized relatively easily and converted efficiently to fermentable sugars.

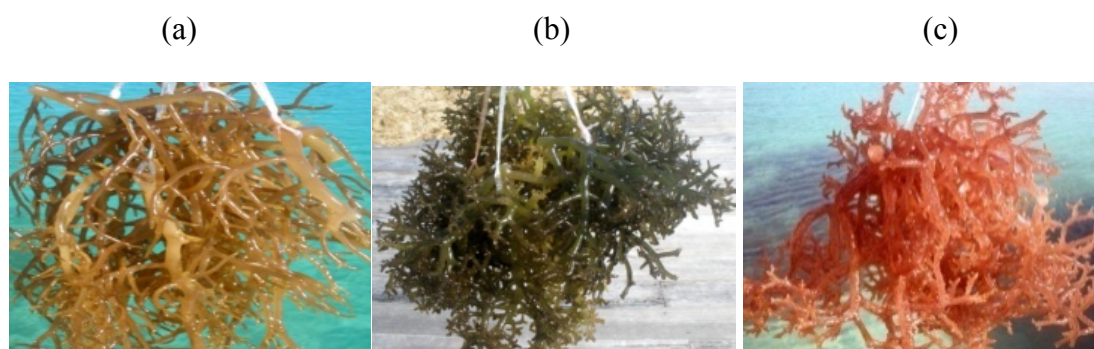


Figure 2.1 (a) Brown macroalgae (b) Green macroalgae (c) Red macroalgae (Photo by DOF, 2016)

Table 2.1 Carbohydrate composition of macroalgae, microalgae and lignocellulosic biomass (Jung et al., 2013; Lobban and Wynne, 1981)

Macroalgae			Microalgae	Lignocellulosic biomass
Brown macroalgae	Green macroalgae	Red macroalgae		
Alginate	Cellulose	Agar	Starch	Cellulose
Cellulose	Mannan	Carrageenan		Hemicellulose
Fucoidan	Starch	Cellulose		
Laminarin	Ulvan	Lignin		
Mannitol				
Galactose	Glucose	Agarose	Arabinose	
Glucose	Mannose	Galactose	Fucose	
Fucose	Rhamnose	Glucose	Galactose	
Xylose	Xylose		Glucose	
			Mannose	
Glucuronic acid	Glucuronic acid		Rhamnose	
Guluronic acid	Uronic acid		Ribose	
Mannuronic acid			Xylose	
Uronic acid				

The cell wall of red macroalgae is constructed with long-chain structural polysaccharides (agar or carrageenan) and inner rigid component cellulose fibrils, as depicted in Figure 2.2. Agar is a linear polymer consisting of *D*-galactose, *L*-galactose and anhydro-*L*-galactose units whereas carrageenan is a sulfated polysaccharide of alternating *D*-galactose and anhydro-*D*-galactose units (Gómez-Ordóñez and Rupérez, 2011; Jung et al., 2013; Lobban and Wyne, 1981). Cellulose is a skeletal polysaccharide consisting of glucose monomer units with β -1,4 glycoside linkages. The basic repeating unit of cellulose is termed as a cellobiose unit comprising of two glucose anhydride units (Mohan et al., 2006; Murphy and McCarthy, 2005; Wei et al., 2013; Yu et al., 2008).

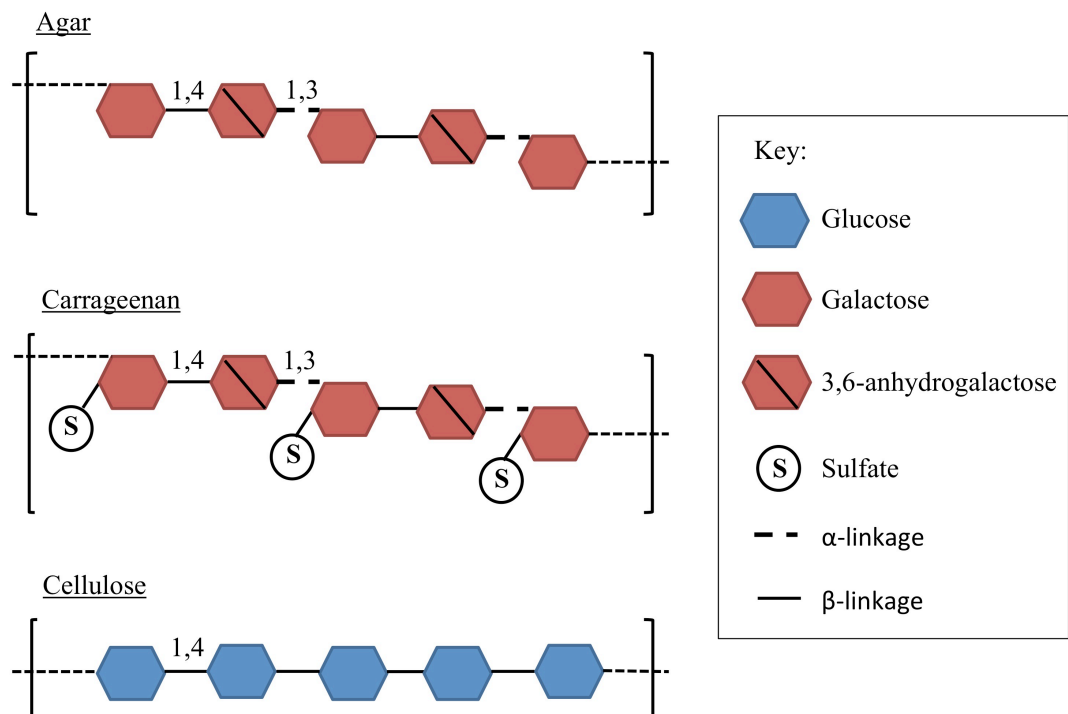


Figure 2.2 Structure of polysaccharides in red macroalgae (Wei et al., 2013)

Gelidium spp. and *Gracilaria* spp. are the type of red macroalgae that used for commercial agar extraction whereas *Kappaphycus alvarezii* (*Eucheuma cottonii*) and *Eucheuma denticulatum* (*Eucheuma spinosum*) are carrageenan-containing macroalgae (McHugh, 2003). Carrageenan is commercially extracted as stabilizing and thickening agents and used as additives in food, cosmetics and pharmaceutical industries (FAO, 2016; Jung et al., 2013; McHugh, 2003). Due to the economic potentials for seaweed aquaculture, the Department of Fisheries Malaysia (DOF) aims to expand the farming area by creating the Aquaculture Industry Zone to develop around 22500 hectares for seaweed cultivated and increase the seaweed production from 24 thousand tonnes to 730 thousand tonnes by 2020 (DOF, 2016). Undeniably, the continuous supply of seaweed throughout the year could ascertain the biomass availability for biofuel production.

Generally, one cycle of the seaweed farming takes roughly 2 months for planting preparation (4-5 days), growing (45 days), harvesting (2 days) and drying (3 days). Thus, annually, tonnes of seaweed can be produced with 4 to 6 cycles (Alin, 2009). Hence, the higher productivity rate of macroalgae has served as added advantages for biofuel and biomaterial production (Brinkhuis et al., 1987; Gao and McKinley, 1994; Wei et al., 2013).

Moreover, macroalgae are found mostly in marine environment and thus, arable land is not required for cultivation (Guiry, 2012; Jung et al., 2013; Lobban and Wynne, 1981; Sze, 1993; Wei et al., 2013). Above all, macroalgae are considered as the potential feedstock for biofuel production due to its high carbohydrate content, ease of availability and abundance, high production yield and low arable land usage.

2.2 Thermochemical conversion

In the section 1.4, it is mentioned that a number of technologies can be used to convert feedstock into useable fuels in term of solid, liquid and gaseous forms. The technologies for algae conversion can be classified into four categories: direct combustion, thermochemical (torrefaction, liquefaction, pyrolysis and gasification), chemical (transesterification) and biological (fermentation and anaerobic digestion) processes (Chen et al., 2015a; Rowbotham et al., 2012).

The significant levels of alkali earth metals in macroalgae makes them not suitable for direct combustion because fouling in the reactors could be caused (Budarin et al., 2011; Ross et al., 2008). Therefore, in order to overcome this challenge, researchers are investigating alternative thermochemical conversion of macroalge to biofuels, and promising findings have been obtained and reported. In his comprehensive review, Rowbotham and his research team concluded that thermochemical conversion could be a promising approach for biofuel production because thermochemical processing methods represent a simpler and direct route of conversion as well as very little pretreatment or extraction is required prior to biomass processing (Rowbotham et al., 2012). As listed in Table 2.2, a wide range of products can be attained from thermochemical conversion based on their operating conditions (temperature, pressure and duration).

Biochar is the solid residue obtained from the thermochemical conversion and can be used as solid fuel in boilers or carbon nanotubes for industrial applications. Meanwhile, syngas is the gaseous fraction consisting of carbon monoxide (CO), carbon dioxide (CO₂), methane (CH₄) and hydrogen (H₂). The liquid fraction produced from thermochemical conversion is termed as bio-oil. The yield of bio-oil depends on biochemical composition of feedstock and thus feedstock

with high lipids content is preferred for bio-oil production. However, the high viscosity of bio-oil contributes to the difficulties associated with utilizing bio-oil as biofuel. In spite of that, bio-oil possess high-value commodity of chemicals. Thus, syngas and bio-oil can further be upgraded as a fuel and chemical source, respectively (Chen et al., 2015a; Liew et al., 2014; Rowbotham et al., 2012).

Table 2.2 Process parameters of thermochemical conversion (Chen et al., 2015a)

	Torrefaction	Liquefaction	Pyrolysis	Gasification
Temperature (°C)	200-300	300-375	Slow: 400-600 (5-10 °C/min)* Fast: 400-600 (10-600 °C/s)* Catalytic: 300-500 Microwave: 500-800	600-1000
Pressure (bar)	1	50-200	1	1
Time (min)	10-90	30-60	30-120	30-90
Main products	Biochar	Bio-oil & syngas	Biochar & bio-oil	Syngas

*Heating rate

The most conventional liquid biofuels produced among the generations are biodiesel from oil crops and bioethanol from carbohydrate based crops. They are primarily produced through transesterification and fermentation, respectively (Liew et al., 2014). In Malaysia, the rising energy demands comes from transport sector which predominantly utilizing liquid fuels (Goh and Lee, 2010). Therefore, breaking down polysaccharide into monosaccharide for subsequent fermentation by using hydrolysis could be a viable option for bioethanol production. Hence, it is necessary to further review the literature and background about hydrolysis and fermentation of red macroalgae.

2.3 Hydrolysis

In red macroalgae, the two most abundant polysaccharides of cell wall, long-chain structural polysaccharides (agar or carrageenan) and cellulose, need to be depolymerized, producing monosaccharides which can be readily fermented to bioethanol and this process is termed hydrolysis or saccharification (Jambo et al., 2016; Wei et al., 2013). This reaction involves the cleaving of a molecule by adding a water molecule (Balat et al., 2008; Vessia Ø, 2005). For instance, the disaccharide sucrose is hydrolyzed into two monosaccharides (glucose and fructose), by the addition of water. Hydrolysis reaction can be catalyzed by enzymes, bases or acids (Balat et al., 2008).

2.3.1 Enzymatic hydrolysis

Enzymatic hydrolysis is a mild approach to convert stored carbohydrate in macroalgae into simple fermentable sugars. Enzymes cellulases are employed mostly and can be categorized into endo-glucanases, exo-glucanases and β -glucosidase. Under enzymatic hydrolysis, the endo-glucanases attack the interior parts and amorphous region of cellulose whereas exo-glucanases cleave the non-reducing end of cellulose into cellobiose units to enable the enzyme attack. Subsequently, β -glucosidases split the cellobiose dimers into two units of glucose (Carere et al., 2008; Demain et al., 2005; Jambo et al., 2016).

As compared to alkaline and acid hydrolysis, enzymatic hydrolysis has many advantages owing to its mild conditions (pH 4.8 and temperature of 50 °C), higher sugar yield and low maintenance costs due to no corrosion problem (Balat et al., 2008; Vessia Ø, 2005). However, a major drawback with this approach is the longer

completion time of the process because the action of the cellulolytic enzyme is hindered by structural and compositional of the biomass (substrate) such as the different varieties of carbohydrate composition, surface area and cellulose crystallinity (Balat et al., 2008; Wei et al., 2013). As a result, additional pretreatment is required prior to enzymatic hydrolysis in order to enhance the physical disruption of biomass to make more accessible to the enzymes, consequently increasing the rate of enzymatic hydrolysis. Pretreatment can be carried out in different ways such as mechanical pretreatment and alkali or acid pretreatment (Balat et al., 2008).

Tan and Lee extracted the polysaccharide contents and studied the sugar production from macroalgae cellulosic residues *Kappaphycus alvarezii* by using commercial cellulase from *Trichoderma reesei* (ATCC 26921) and β -glucosidase from *Aspergillus niger* (Novozyme 188). The highest glucose yield (99.8%) was achieved after 54 h of hydrolysis (Tan and Lee, 2014). Adopting a similar hydrolysis condition, Kumar and his co-workers obtained 88% yield was achieved from *Gracilaria verrucosa* algal pulp (Kumar et al., 2013).

Notwithstanding the higher sugar yield compared to acid hydrolysis, the longer completion time of the process and costly commercial enzyme make the process not economically feasible. Ongoing efforts are being made to provide rapid and efficient of enzymatic hydrolysis on account of the highly specific of enzyme activity to the types of polysaccharides (Balat et al., 2008; Jambo et al., 2016).

2.3.2 Chemical hydrolysis

There are many types of chemical hydrolysis including the use of sodium hydroxide (NaOH), calcium hydroxide (CaOH), sulfuric acid (H₂SO₄), hydrochloric acid (HCl), nitric acid (HNO₃) and phosphoric acid (H₃PO₄). The four key factors for optimal conditions of acid hydrolysis are identified as (i) base/acid concentration, (ii) heating time, (iii) reaction temperature and (iv) biomass loading (Balat et al., 2008; Wei et al., 2013).

2.3.2 (a) Alkaline hydrolysis

Alkaline hydrolysis is one type of chemical hydrolysis and mainly is used to disrupt lignin structure of lignocellulosic biomass and remove acetyl and uronic acid substitutions on hemicellulose that lower the accessibility of the enzyme to the hemicellulose and cellulose surface. However, a limitation occurs due to the saponification of intermolecular ester bonds crosslinking xylan hemicelluloses. Lignin and other hemicelluloses can be converted to irrecoverable salts or incorporated as salts into the biomass by alkaline hydrolysis (Balat et al., 2008; Kuhad et al., 2011; Li et al., 2014).

Eshaq and his co-workers compared the fermentation performance of untreated and treated *Spirogyra* biomass (1% NaOH). They found that treated *Spirogyra* biomass produced lower yield of ethanol compared to untreated biomass. It is because toxic substances were produced when *Spirogyra* biomass was subjected to alkaline hydrolysis, consequently decreasing the fermentation efficiency of yeast (Eshaq et al., 2011). Therefore, alkaline hydrolysis is less effective for algal biomass which almost do not have lignin and hemicellulose.