OPTIMIZATION OF BIOETHANOL PRODUCTION FROM SEAWEEDS (*EUCHEUMA* spp.)

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ABSTRACT

The depletion of fossil fuels and increase in energy demand give rise to the urgent need to find a new alternative energy resource. The production of bioethanol using land-based and food crops have brought much controversy and debate on their sustainability. Respective to this, the use of marine biomass such as seaweeds in bioethanol production provides a possible solution for this energy issue. In this study, the potential for bioethanol production of the two species that abundantly found in Sabah, Malaysia: *Eucheuma cottonii* (*Kappaphycus alvarezii*) and *Eucheuma denticulatum* was studied by determining the amount of reducing sugars after acid hydrolysis as higher reducing sugars content will increase the potential of higher bioethanol yield. *E. cottonii* was selected due to the higher reducing sugars content and expected to produce higher bioethanol yield during fermentation. *E. cottonii* samples were hydrolysed by autoclave using sulphuric acid as catalyst with different acid concentrations (0.05-0.5 M), hydrolysis temperatures (110-130 °C) and hydrolysis time (5-25 minutes). The optimum conditions for hydrolysis were achieved at 0.1 M, 130 °C and 15 minutes. Before fermentation, the microscopic view and growth of yeast *Saccharomyces cerevisiae* were studied in the preparation of yeast inoculum. The yeast cells were harvested at the earliest exponential phase that is after nine hours of incubation. Subsequently, *E. cottonii* hydrolysates were fermented using different concentration of inoculum (10-30 v/v %) and fermentation time (12-72 hours). Bioethanol content was quantified by using Gas Chromatography- Mass Spectrometry (GC-MS). The maximum bioethanol concentration (9.331 g/L) and bioethanol yield (0.164 g/g) were achieved at 20 v/v % yeast inoculum and 24 hours of fermentation duration. These results may provide useful information for development of more efficient methods for bioethanol production from seaweeds.

ABSTRAK

OPTIMASI PENGHASILAN BIOETANOL DARIPADA RUMPAI LAUT (EUCHEUMA spp.*)*

Pengurangan bahan api fosil dan peningkatan permintaan tenaga menimbulkan keperluan mendesak untuk mencari sumber tenaga alternatif baru. Penghasilan bioetanol menggunakan tanaman yang tumbuh di darat dan sumber makanan telah menimbulkan banyak kontroversi dan perdebatan mengenai kemampanan mereka. Oleh itu, penggunaan biomas dari laut seperti rumpai laut dalam penghasilan bioetanol adalah penyelesaian yang berpotensi untuk menangani isu tenaga ini. Dalam kajian ini, potensi untuk penghasilan bioetanol daripada dua spesies yang banyak dijumpai di Sabah, Malaysia: Eucheuma cottonii (Kappaphycus alvarezii) dan Eucheuma denticulatum telah dikaji dengan menentukan jumlah gula penurun selepas asid hidrolisis oleh sebab kandungan gula penurun yang lebih tinggi akan meningkatkan potensi untuk menghasilkan kandungan bioetanol yang lebih tinggi. E. cottonii dipilih kerana ia mempunyai kandungan gula penurun yang lebih tinggi dan dijangkakan boleh mengeluarkan hasil bioetanol yang lebih tinggi semasa fermentasi. Seterusnya, sampel E. cottonii telah dihidrolisiskan dengan menggunakan autoklaf dan menggunakan asid sulfurik sebagai pemangkin dengan kepekatan yang berbeza asid (0.05-0.5 M), suhu hidrolisis (110-130 °C) dan masa hidrolisis (5-25 minit). Keadaan optimum bagi hidrolisis telah dicapai pada 0.1 M, 130 °C dan 15 minit. Sebelum fermentasi, gambaran mikroskopik dan pertumbuhan yis Saccharomyces cerevisiae telah dikaji dalam penyediaan inokulum yis. Sel-sel yis telah dituai pada fasa awal eksponen iaitu selepas sembilan jam pengeraman. Selepas itu, hidrolisat E. cottonii telah ditapai menggunakan kepekatan inoculum yang berbeza (10-30 v / v %) dan masa penapaian (12-72 jam). Kandungan bioetanol telah diukur dengan menggunakan Kromatografi Gas-Spektrometri Jisim (GC-MS). Maksimum konsentrasi bioetanol (9.331 g/L) dan hasil bioetanol (0.164 g/g) telah dicapai pada 20 v/v % inokulum yis dan 24 jam tempoh penapaian. Keputusan ini boleh memberikan maklumat yang berguna untuk perkembangan kaedah yang lebih berkesan bagi penghasilan bioetanol daripada rumpai laut.

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°C	degree Celsius
%	percentage
g	gram
kg	kilogram
mg	milligram
L	litre
ml	millilitre
µl	microliter
M	molar
m	meter
mm	millimetre
µm	micrometre
nm	nanometre
m^3	cubic meters
min	minute
EJ	exajoule
m/z	mass-to-charge ratio
v/v	volume/volume
rpm	revolutions per minute
CO_2	carbon dioxide
H_2O	water
O_2	oxygen
H_2SO_4	sulphuric acid
NaOH	sodium hydroxide
HCl	hydrochloric acid
$KNaC_4H_4O_6 \cdot 4H_2O$	potassium sodium tartrate
DNS	3,5-Dinitrosalicyclic acid
$NaSO_4 \cdot H_2O$	sodium sulphate anhydrous
C_2H_5OH, EtOH	ethanol
$C_6H_{12}O_6$	glucose
$CH_3COCOOH$	pyruvic acid
CH_3CHO	acetyladehyde

5-HMF	5-hydromethylfurfural
GC-MS	gas chromatography – Mass spectrometry
ICE	internal combustion engine
FGB	First-generation bioethanol
SGB	Second-generation bioethanol
TGB	Third-generation bioethanol
YDP	Yeast Dextrose Peptone
OD	optical density
CFU	colony forming unit
&	and
λ	lambda
ι	iota
k	kappa

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Overview

In this 21st century, the dwindling fossil fuels energy sources is one of the major crisis that threatens the sustainability of human kind and civilization (Goh *et al.*, 2010). According to the analysis of global oil depletion by UK Energy Research Centre, it is forecasted that the conventional reserves will be substantially used up between 2020 and 2030 (Sorrell *et al.*, 2009). The depletion of fossil fuels, which are non-renewable, is attributed to the excessive dependency of the world on fossil fuel as the predominant energy source. Besides, the exploitation of non-renewable fossil fuels is unsustainable and leads to the emission of greenhouse gases into the atmosphere which may increase the risk of global warming and climatic change (Mutripah *et al.*, 2014). Therefore, in order to tackle these problems of energy scarcity, global warming and climatic change, there is an urgent need to develop and increase the usage of renewable or alternative energy to meet the demands in the present as well as the needs of future generations.

'Biofuel' or 'biorenewable' fuel is defined as fuel in solid, liquid, or gaseous state which is derived from biomass. It is one of the most significant renewable energies that attract public and scientific interest. In Malaysia, biomass has become the most appealing alternative for energy generation on account of large amount of agriculture waste produced annually (Goh *et al.*, 2010). The European Union had targeted that the share of biofuels of all transport fuels to increase to 10 % by 2020, replacing the older 5.75 % goal for 2010 (Saikkonen *et al.*, 2014). Besides, the International Energy Agency estimated that an average annual growth rate of 7 %, which means that by 2030, biofuels would account for about 5 % of the total road transport demand, compared to approximately 2 % today (Gadonneix, 2010). There are various types of biofuels, such as bioethanol, biogas and biodiesel, which are commonly used nowadays. Biofuels are free of carbon dioxide because when they are burned, they emit the same amount of carbon dioxide as they absorbed during photosynthesis (Clark *et al.*, 2012). Therefore, the use of biofuel for generation of energy is environmental friendly.

Currently, among different types of biofuels, 'bioethanol' or 'fuel alcohol' is the most widely utilized liquid biofuel. The production of bioethanol accounts for about 80 % of the global biofuel market and is dominated by United States and Brazil (Clark *et al.*, 2012). The complete combustion of bioethanol will result in carbon dioxide and water only, hence the uses of bioethanol in transportation sector can help to safeguard the next generation by reducing the risk of global warming (Goh *et al.*, 2010). Bioethanol produced from different types of renewable feedstocks is considered clean and renewable energy, which is an ideal alternative to gasoline (Wu *et al.*, 2014). In addition, ethanol is a more environmentally-friendly substitute to methyl tertiary butyl ether which is a toxic chemical compound but most common additive to gasoline (Demirbas, 2007).

Bioethanol can be produced by fermentation of sugars derived from hydrolysis of starch from various feedstocks, for example, sorghum, sugarcane and corn (Zhu *et al.*, 2014). However, bioethanol production using starch-based feedstocks, which generally known as first-generation biomass may result in competition with human food production and subsequently lead to rise in the price of crops as well as cause negative impacts to the economy (Kim *et al.*, 2014). Inedible lignocellulosic biomass (second-generation biomass) such as grasses, crop residues and sawdust, are alternative low-priced and abundant feedstocks but the use of these materials has disadvantages such as low output and high cost processing (John *et al.*, 2011). In addition to the first- and second-generation of biomass, algae, which are named as "third-generation biomass", is gaining immense attention as a promising and renewable alternative biomass source for bioethanol production (John *et al.*, 2011).

Seaweeds are potential bioethanol feedstock because they can be easily transformed to ethanol, since they contain low amount of lignin or no lignin at all (Yanagisawa *et al.*, 2011). Besides, production of bioethanol by utilizing seaweed does not impact food supply as seaweed is not a main food for human (Mutripah *et al.*, 2014). According to John *et al.* (2011), seaweeds also exhibit the ability to grow at a high rate and produce large amounts of biomass because they require lower energy for the production of supporting tissues compared to land plants, as well as their capability to absorb nutrients over their whole surface area. Moreover, the use of seaweeds is energy-conserving as they have no demand for internal transport of nutrients or water (Wi *et al.*, 2009). Most importantly, seaweeds can be widely found in Sabah, Malaysia. The high availability of seaweeds enhances their potential as the feedstock of bioethanol production.

Seedweeds or macroalgae are categorised into three major groups based on their photosynthetic pigments: Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae). *Eucheuma* spp. is red alga and also one of the most widely found species in Sabah, Malaysia. Carrageenan, a sulphated linear polysaccharide that consists of alternating α(1-3)-D-galactose-4-sulphate and β(1,4)-3,6-anhydro-D-galactose residues, is largely found in *Eucheuma* spp as cell wall component (Fakhrudin *et al.*, 2014). According to Goh & Lee (2010), *Eucheuma* spp. is a prospective candidate for the production of macroalgae-based bioethanol (third-generation bioethanol). *Eucheuma cottonii,* which commercially known as *Kappaphycus alvarezii* is the major source of κ-carrageenan while *E. denticulatim* is also a type of red alga, which is found to contain mainly ι-carrageenan.

Generally, bioethanol production involves three steps: hydrolysis of polysaccharides into monosaccharides, bioethanol production from monosaccharides, and recovery and purification of bioethanol. Carrageenan, which is a form of polysaccharide in cell wall of *Eucheuma* spp. can be hydrolysed to monosaccharides using acidic and enzymatic hydrolysis. Acidic hydrolysis is more favourable because of its low cost. *Saccharomyces cerevisiae* is the most commonly used microorganism in ethanol fermentation and has several advantages including its efficiency to produce ethanol from hexoses without oxygen, low pH and high tolerance to ethanol and inhibitors (Meinita *et al.*, 2012).

1.2 Problem Statement

The depletion of fossil fuels is one of the key challenges in this century. Thus, the production of bioethanol which can be used in the energy generation is one of the ways to face this challenge. However, the utilization of food-based feedstock (first generation) and non-food feedstock (second generation) has led to many problems. So, the research on the production of bioethanol using algae feedstock (third generation) should be given attention. Besides, various studies have been performed on the biodiesel production by using microalgae but there are few reports on production of bioethanol from seaweeds. Their hydrolysis and fermentation are also less commonly studied. Thus, special efforts are required to understand these processes with a view to identify the potential of such seaweeds for bioethanol production.

1.3 Objectives

1. Screening of seaweeds (*Eucheuma denticulatum* and *Eucheuma cottonii* (*Kappaphycus alvarezii*)) with greater potential for bioethanol production.
2. Optimization of the acid hydrolysis parameters to produce fermentable sugars from seaweed.
3. Preparation of yeast inoculum for ethanol fermentation.
4. Determination of optimum parameters for ethanol fermentation.

1.4 Scope of the study

The scope of study for this research is to optimize the production of bioethanol from the third-generation feedstock: seaweeds (*Eucheuma* spp.). In the first part of research, two types of seaweeds: *Eucheuma denticulatum* and *Eucheuma cottonii (Kappaphycus alvarezii)* were screened for the best yield of bioethanol by performing acid hydrolysis. The type of seaweed which produced the highest amount of reducing sugars was chosen to be optimized for acid hydrolysis and bioethanol production. In the next part of this research, the parameters of acid hydrolysis, including concentration of sulphuric acid, temperature and duration of acid hydrolysis were optimized to determine the best conditions that produce highest yield of reducing sugars. Lastly, the parameters in ethanol fermentation were optimized to determine the fermentation conditions that convert reducing sugars to ethanol most efficiently. The optimized parameters in ethanol fermentation include inoculum concentration, pH and duration of fermentation.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter covers the review of published research literature related to the bioethanol production using seaweeds as feedstock. The importance of the seaweeds in production of bioethanol as the key solution of energy crisis is discussed in detail. Besides, the important past research's findings and the theories related to the methodology used in this research are focussed to achieve the objectives.

2.2 Energy crisis and global warming

Energy crisis is one of the most threatening issues in the sustainable humanity and civilization (Goh *et al.*, 2010). In the 19th century, the discovery of crude oil had produced a source of inexpensive liquid fuel that helped in industrialization and improving living standards (Huber *et al.*, 2006). However, the expansion in human population has resulted in the accelerated worldwide energy consumption and this has raised prices in the competitive energy market. Besides, the use of fossil fuels is related to several issues such as limited reserves, unstable supply due to regional bias and global warming (Hong *et al.*, 2013).

Generally, fossil fuels include natural gas, petroleum and gas (Ahmed *et al.*, 2014). The world is greatly dependent on fossil fuels as the primary source of energy (Goh *et al.*, 2010). In fact, according to Pandey *et al.* (2014), "57.7% of the primary energy consumed has been used in the transportation sector, where the consumption rate of fossil diesel fuel was estimated to be 934 million tonnes per year". In Malaysia, the energy usage also has soared to a new height of 44, 268 ktoe in 2007 (Goh *et al.*, 2010). In addition, the world's energy demand is expected to increase exponentially by one-third from 2010 to 2035 (Ahmed *et al.*, 2014). However, fossil fuels are non-renewable resources that cannot be supplied limitlessly and will be completely used up one day.

Moreover, extreme consumption of fossil fuels will lead to global warming due to the emission of great amount of greenhouse gases, particularly carbon dioxide (CO_2). About 98% of carbon is emitted as a result of fossil fuel combustion (Demirbas, 2005). According to Goh *et al.* (2010), more than 19% of the total emission of CO_2 is contributed by transportation sector and the CO_2 emission has increased by 221% from 1994 to 2004. The amount of trapped heat will increase (or the heat emitted from surface of the Earth will decrease) when concentration of greenhouse gases in the atmosphere increases, consequently elevating the surface temperature of the Earth (Kalogirou, 2004). So, the usage of fossil fuels will result in many negative consequences to the environment, such as climate change, subsiding of glaciers, elevating sea level and decline in biodiversity (Nigam & Singh, 2011). Hence, minimizing the consumption of fossil fuels by finding renewable alternative energy sources can help in reducing the degree of pollution as well as conserving our 'Mother Earth'.

2.3 Renewable energy

Renewable energy is generally known as energy generated from resources which are readily available in nature, such as wind, tide and wave, solar, geothermal, wood, waste and biomass (Demirbas, 2005; Sebri & Ben Salha, 2013). Renewable energy sources are safe, primary, clean and limitless, in contrast to the conventional non-renewable energy (Amponsah *et al.*, 2014). Therefore, the use of renewable energies is growing at a fast pace around the world and it is expected to succeed many conventional energies and accounts for most of the share of global energy consumption (Sebri & Ben Salha, 2013). According to Destouni & Frank (2010), renewable energy is forecasted to reach 35% of an estimated global primary energy supply by year 2050, which is higher than 12% in year 2007.

Renewable energy sources are important in diminishing emissions of greenhouse gas, especially CO_2 which constitutes greater than 60% of the total greenhouse gases. Demirbas (2009) also stated that "renewable energy sources that use indigenous resources have the potential to provide energy services with zero or almost zero emissions of both air pollutants and greenhouse gases". Besides, according to International Energy Agency, renewable energy is known as a key component to achieve 50% reduction of CO_2 by 2050, provided that the long-term average increase in global temperature is to be restricted to between 2 and 2.4 °C (Sebri & Ben Salha, 2013).

2.3.1 Biomass energy

Biomass is the general name of all the earth's living matter and also defined as all organic materials that originated from crops, trees, plants and algae (Demirbaş, 2006; Demirbas, 2009). Biomass is one of the abundant resources, which is also the only renewable organic resource (Demirbaş, 2006; Demirbas, 2009). Presently, biomass is the fourth largest energy source worldwide after coal, petroleum and natural gas (Chen & Lee, 2014). It is also one of the renewable energy sources that many countries depend on due to its flexibility to be transformed into electricity, transport liquid fuels and heat by chemical and biological means on demand (Chen & Lee, 2014). According to Kaygusuz (2002) & Bilgen *et al.*, (2004), biomass resources are possibly the world's largest renewable and sustainable energy source, which account for 220 billion even dry tons (about 4,500 EJ) of annual primary production.

Different forms of energy can be generated by biomass. It can generate heat by direct combustion of biomass, or by conversion of biomass into gaseous, liquid and solid fuels using conversion technologies including fermentation to yield alcohols, bacterial digestion to yield biogas and gasification to yield a substitute of natural gas (Kaygusuz & Türker, 2002). Most of the biomass energies are generated from wood and wood wastes (64%), followed by municipal solid waste (24%), agricultural waste (5%) and landfill gases (5%) (Chen & Lee, 2014). Biomass energy is increasingly being related to sustainability of environment and stabilization of climate since many studies have suggested that if biomass is manufactured and utilized sustainably, it can give many environmental advantages (Kaygusuz, 2002; Bilgen *et al.*, 2004). For instance, the production of biomass energy is free of CO_2 as the amount of CO_2 released to atmosphere is equal to the amount used in the process (Demirbas, 2005).

2.4 Biofuel

Biofuel is a fuel which is derived from biomass after being processed physically and chemically (Zhu *et al.*, 2014). The term biofuels can be referred to the fuels used in direct combustion for electricity production, although they are commonly used for liquid fuels in transportation (Balat, 2011). Up to present, the production of liquid biofuels is mainly in United States of America, Brazil and several European nations

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