BIOALCOHOL PRODUCTION FROM *GRACILARIA* SP. FERMENTATION USING *SASAD*

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FACULTY OF ENGINEERING
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ABSTRACT

Marine biomass or macroalgae has been denoted as a potential feedstock due to its high carbohydrate content which can be a source for biofuel production through fermentation process. In this research, *Gracilaria* sp. from red macroalgae group was used as raw material to produce biofuel via fermentation process. The carbohydrate content in *Gracilaria* sp. can be breakdown into simple sugar through hydrolysis process such as acid hydrolysis which was reported to be the most affordable and available method to be done. This process, however, may release inhibitors such as furfural and 5 HMF (hydroxymethyl furfural). Nonetheless, common ethanol producing agent such as *Saccharomyces cerevisiae* was reported unable to undergo a succesfully alcohol metabolic pathway in any substrate containing inhibitors. Thus, in this research *Gracilaria* sp.. was fermented using a local Borneo bioagent, *Sasad*. The effect of acid concentration variation from 0.2 M to 0.8 M in acid hydrolysis on glucose content and alcohol yield of *Gracilaria* sp. was described. Acid concentration of more than 0.8 M was not explored as it higher acid concentration of more than 1 M was believed to yield higher inhibitors such as levulinic acid that was formed from HMF. Then, the effect of acid hydrolysis duration ranging from 15 to 60 minutes on glucose content and alcohol yield was also measured. The alcohol content of *Gracilaria* sp. fermentation product was also analysed based on the effect of fermentation duration and pretreatment types. The highest glucose content before fermentation process was recorded from *Gracilaria* sp. hydrolyzed using 0.8 M sulphuric acid for 30 minutes yielded 18.25 g/L compared to unhydrolyzed and hot water treated *Gracilaria* sp., 13.34 g/L and 14.22 g/L respectively. After fermentation, lowest glucose content showed by untreated *Gracilaria* sp. after 7 days fermentation; with only 7.57 g/L glucose left. Alcohol content analysis using GCMS showed that 1 – butanol – 3 – methyl or isoamyl can be detected in all fermentation product. Highest alcohol yield was obtained from untreated *Gracilaria* sp. after 28 days fermentation which was 0.486×10^{-4} g/g followed by hot water treated and acid hydrolyzed samples of 0.121×10^{-4} g/g and 0.030×10^{-4} g/g respectively. This study has showed that *Sasad* was able to ferment *Gracilaria* sp. under different hydrolysis type and it maybe possible to eliminate the pretreatment stage since highest alcohol yield was reported from the untreated sample.

ABSTRAK

PENGHASILAN BIOALKOHOL DARIPADA PENAPAIAN GRACILARIA SP. *MENGGUNAKAN SASAD*

Sumber biojisim daripada laut atau makroalga merupakan bahan mentah yang berpotensi kerana kandungan karbohidrat yang tinggi yang boleh menjadi sumber untuk pengeluaran biofuel melalui proses penapaian. Dalam kajian ini, *Gracilaria* sp. daripada kumpulan makroalga merah telah digunakan sebagai bahan mentah untuk menghasilkan biofuel melalui proses penapaian. Kandungan karbohidrat dalam *Gracilaria* sp. boleh diturunkan menjadi gula ringkas melalui proses hidrolisis seperti hidrolisis asid yang dilaporkan menjadi kaedah yang paling berpatutan dan mudah dijalankan. Proses ini, bagaimanapun, menghasilkan penghalang penapaian seperti furfural dan 5 HMF (hydroxymethyl furfural). Namun, ejen penapaian biasa seperti *Saccharomyces cerevisiae* dilaporkan tidak dapat menjalani proses metabolik alkohol dengan jaya dalam keadaan substrat yang mengandungi inhibitor. Oleh itu, dalam kajian ini penapaian *Gracilaria* sp. menggunakan ejen penapaian dari Borneo, *Sasad* telah dijalankan. Kepekatan asid lebih daripada 0.8 M tidak dieksp..lorasi kerana kepekatan asid yang lebih tinggi lebih daripada 1 M dipercayai menghasilkan inhibitor yang lebih tinggi seperti asid levulinic yang terbentuk daripada HMF. Kesan perubahan kepekatan asid daripada 0.2 M ke 0.8 M dalam hidrolisis asid terhadap kandungan glukosa dan hasil alkohol daripada hasil *Gracilaria* sp. telah diterangkan. Kemudian, kesan jangka masa hidrolisis asid antara 15 hingga 60 minit terhadap kandungan glukosa dan hasil alkohol juga diukur. Kandungan alkohol daripada produk penapaian *Gracilaria* sp. juga dianalisis berdasarkan kesan tempoh penapaian dan jenis proses hidrolisis. Kandungan glukosa tertinggi sebelum proses penapaian direkodkan daripada *Gracilaria* sp. yang dihidrolisiskan menggunakan 0.8 M asid sulfurik selama 30 minit menghasilkan 18.25 g/L berbanding *Gracilaria* sp. yang dimasak dan tidak dihidrolisis, 13.34 g/L dan 14.22 g/L masing-masing. Selepas penapaian, kandungan glukosa paling rendah ditunjukkan oleh *Gracilaria* sp. tanpa hidrolisis selepas 7 hari penapaian; dengan berbaki hanya 7.57 g/L. Kandungan analisis alkohol menggunakan GCMS menunjukkan bahawa 3 - metil - 1 - butanol boleh dikesan dalam semua produk penapaian. Hasil alkohol tertinggi diperoleh daripada *Gracilaria* sp. tanpa hidrolisis selepas 28 hari penapaian iaitu 0.486×10^{-4} g/g diikuti oleh *Gracilaria* sp. yang dimasak serta dihidrolisis menggunakakn asid masing-masing menunjukkan hasil sebanyak 0.121×10^{-4} g/g dan 0.030^{-4} g/g. Kajian ini menunjukkan bahawa *Sasad* dapat menapai *Gracilaria* sp. dalam jenis hidrolisis berbeza dan ia mungkin mungkin dapat meringkaskan proses penapaian *Gracilaria* sp. dengan mengetepikan peringkat hidrolisis kerana hasil alkohol tertinggi dilaporkan daripada sampel tanpa hidrolisis.

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LIST OF ABBREVIATIONS

AT	-	Acid Treatment
Conc	-	Concentration
FTIR	-	Fourier Transform Infrared Spectroscopy
GCMS	-	Gas Chromatography Mass Spectrometry
HPLC	-	High Performance Liquid Chromatography
HT	-	Hot Water Treatment
Min	-	Minutes
NT	-	Untreated
RSM	-	Response Surfaces Methodology
RT	-	Retention Time

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Climate change and global warming has become a new challenge in our world as well as to our generation recently. An increment of 1°C of our world average temperature since the late 19th century had immense adversely effects on the world climate (Nasa, 2017). Based on Figure 1.1, the graph illustrates the change in global surface temperature relative to 1951-1980 average temperatures. Seventeen of the 18 warmest years in the 136-year record all have occurred since 2001, with the exception of 1998. The year 2016 was ranked the warmest on record by 0.99 °C (NASA/GISS, 2017). This research is significant with similar constructions prepared by the Climatic Research Unit and the National Oceanic and Atmospheric Administration (NOAA, 2017).

The temperature rise was due to the increase of carbon dioxide and other pollutant emissions to the atmosphere. One of the main reason of rising carbon dioxide levels in the atmosphere was due to the burning of fossil fuels. Fossil fuels such as coal, oil and gas were also derived from biological material where carbon has been captured by photosynthesis million of years ago. Energy was released from these sources by burning of these materials releases carbon dioxides, carbon monoxide and vapour. This process returns the previously trapped carbon into the atmosphere, resulting in an increase of carbon concentration. Besides that, carbon monoxide CO that produces from natural resources such as volcanoes and forest burning as well as vehicle emissions are readily reacts with hydroxyl radical (OH) and forming carbon dioxide (ESSEA, 2010).

The concern over climate change and global warming has brought on the search for a new and sustainable alternative energy. Figure 1.2 showed the statistics of renewable electricity generation in TWh, Terawatt/hour from 2012 to 2016. Over the years, the renewable electricity generation had increased which was the highest recovery in hydropower generation and rapidly continued in solar and wind generation. This has showed that our world is demanding for alternative energy and to have less dependancy on fossil fuel energy. Biomass energy can be a potential option to replace the energy from fossil fuel. It manages to reduce the greenhouse gas emissions since it can utilize the carbon dioxide that was released from biomass combustion during photosynthesis process for its growth. Besides that, statistical analysis shows that global consumption of our natural gas had increased by 1.5% (63 billion/m^3), but the global gas production was flat of 0.3%, 21 63 billion/m^3 showed the weakest growth in gas output for 34 years, other than in the immediate effect due to financial crisis (BP, 2017). Hence, it was crucial to search for another renewable and sustainable energy for our future generation.

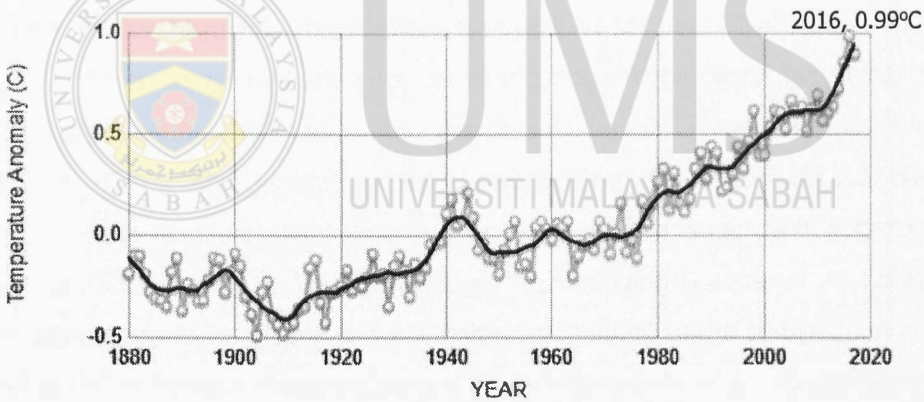

Figure 1.1 : World Temperature Modern Record 1880 up to 2017

Source : NASA's Goddard Institute for Space Studies (GISS)

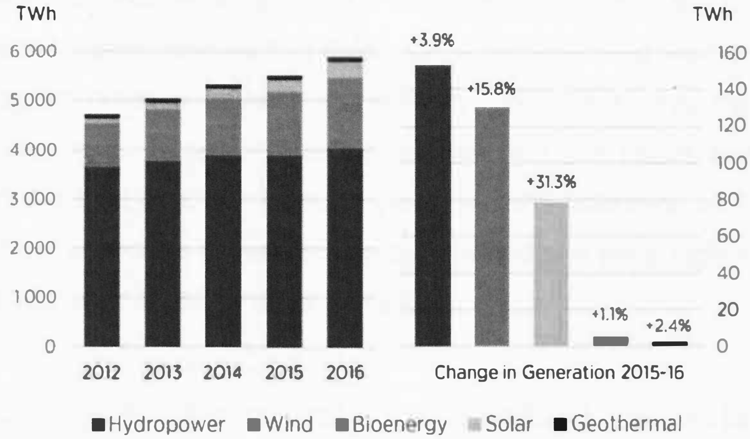

Figure 1.2 : Growth in Renewable Energy Generation from 2012 – 2016

Source : IRENA, 2018

1.2 Biofuels

Biofuels was produced from fossil fuels thus it is consider as carbon neutral supply since carbon dioxide from the atmostphere was utilized for photosynthesis. (Ogum *et al.,* 2012). Thus, liquid biofuels such as bioalcohol and biodiesel may offer an environmental friendly alternative to petroleum based transportation fuels (Demirbas, 2010). The first generation biofuels were referred to as biofuels that were manufactured from readily available energy crops such as corn, sugarcane, sugarbeet and rice (edible feedstock) using conventional technologies (Luque *et al.,* 2010). However, biofuel production using these substances resulted in the competition with food production that leads to increase in food price (Khambhaty *et al.,* 2012). Besides that, first generation biofuel based on edible crops has raised morality and ethical issues as there are millions of people around the world suffering from malnutrition and hunger (Goh and Lee, 2010).

The second generation biofuel feedstock are biofuel refined from lignocellulose biomass. Although, this biofuel feedstock mostly come from non-edible feedstock, however, the separation of lignin in lignocelluloses was found to be a highly expensive process (Goh and Lee, 2010). Thus, for long term and commercialization reasons, a cheaper process would be preferred for the development and production of biofuel. In order to overcome the issues from the first and second generation biofuel feedstock, a sufficient and inexpensive biomass is necessary to produce ethanol economically. Macroalgae has the potential to overcome the issue. It was interesting to note that the issues arising from the increase in the land used for biomass crops and the utilization of food crops for biofuel are not applicable to macroalgae or seaweed and have high photosynthetic efficiency (Adams *et al.*,2009; Su *et al.*, 2017).

Macroalgae, is a third generation biofuel feedstock that can be cultivated in no requirement of fresh water under free sunlight, pesticides and fertilizers for growth (Buck & Buchholz, 2004; Gaurav *et al.*, 2017). Macroalgae has the ability to grow at a fast rate and yield huge amounts of biomass (John *et al.,* 2011; Wei *et al.,* 2013). It has higher annual primary production rates of 30 to 83 dry metric ton per hectar (Roesijadi *et al.*, 2010) It also contains low concentration of lignin making it a suitable biomass materials that can easily be converted into ethanol without the expensive lignin removal process (Jung *et al.,* 2013; Park *et al.,* 2012). A review by Kim *et al.*, (2012) showed that marine biomas has little or no recalcitrant lignin and crystalline cellulose so that it can be depolymerized relatively easily as compared to land crops.

1.3 Macroalgae Fermentation for Biofuels

As a plant, macroalgae are also comprised of cellulose based cell walls and complex polysaccharides in it and these compositions can be hydrolyzed into sugars and further fermented to produce biofuel (Goh & Lee, 2010; Adams *et al.,* 2011). It comprises of three different groups: red, brown and green macroalgae. Among these three groups of macroalgae, red macroalgae contain the highest amount of carbohydrate (57.79% to 74.11% of dry weight) followed by green seaweeds (53.08% to 67.40% of dry weight) and brown seaweeds (26.86% to 41.03% of dry weight) (Ahmad *et al.,* 2012).

Brown algae group such as *Laminaria* sp. contained laminarin and mannitol as their main reserved food that is applicable to be converted into bioalcohol (Ge *et*