

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



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UNIVERSITI MALAYSIA SABAH

PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

**FACULTY OF ENGINEERING
UNIVERSITI MALAYSIA SABAH
2018**

**BIOALCOHOL PRODUCTION FROM
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*SASAD***

HELVIE MANSUIT



**THIS THESIS SUBMITTED IN FULLFILMENT FOR
THE DEGREE OF
MASTER OF ENGINEERING**

PERPUSTAKAAN
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**FACULTY OF ENGINEERING
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2018**

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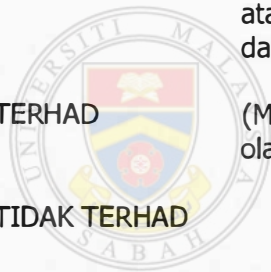
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DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, equations, summaries, and references, which have been duly acknowledged.

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HELVIE MANSUIT
07th September 2018

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 PENAPAIAAN 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 diperolehi daripada *Gracilaria* sp. tanpa hidrolisis selepas 28 hari penapaian iaitu 0.486×10^{-4} g/g diikuti oleh *Gracilaria* sp. yang dimasak serta dihidrolisis menggunakan asid masing-masing menunjukkan hasil sebanyak 0.121×10^{-4} g/g dan 0.030×10^{-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.

TABLE OF CONTENTS

	Page
TITLE	i
DECLARATION	ii
CERTIFICATION	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	v
<i>ABSTRAK</i>	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	xi
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xvii
LIST OF APPENDICES	xviii
CHAPTER 1: INTRODUCTION	19
1.1 Research Background	19
1.2 Biofuels	251
1.3 Macroalgae Fermentation for Biofuels	252
1.4 <i>Sasad</i> – A Mixed Culture Fermentation Agent for Macroalgae	25
1.5 Research Problem	27
1.6 Main Objective	28
1.7 Research Objectives	28
1.8 Scope of Work	29
CHAPTER 2: LITERATURE STUDY	30

2.1	Overview	30
2.2	Biofuels Types and Classification	30
2.3	Macroalgae as Bioalcohol Feedstock	35
2.4	Red Algae (Macroalgae)	41
2.5	<i>Gracilaria</i> sp.	42
2.6	Principal of Bioalcohol Production from Macroalgae	44
2.7	Pretreatment and Hydrolysis of Macroalgae	47
	2.7.1 Physical Pretreatment	48
	2.7.2 Chemical Hydrolysis	49
	2.7.3 Enzymatic Hydrolysis	57
2.8	Macroalgae Fermentation Theory	59
	2.8.1 Macroalgae fermentation using pure culture	62
	2.8.2 Macroalgae fermentation method via traditional and mixed cultures fermentation method	65
2.9	Traditional Fermentation Method	73
	2.9.1 Types of starter culture in traditional fermentation method	73
	2.9.2 Microorganisms associates with traditional fermentation method	75
2.10	Summary	76
	CHAPTER 3: METHODOLOGY	77
3.1	Introduction	77
3.2	Research Venue and Time Introduction	77
3.3	Raw Materials	77
3.4	Chemicals	77
3.5	Equipment and Apparatus	78
3.6	Procedures for <i>Gracilaria</i> sp. Preparation	80
3.7	Procedures for Acid Hydrolysis	80

3.8	Procedures for <i>Sasad</i> Preparation	83
3.9	Procedures for <i>Gracilaria</i> sp. Fermentation with <i>Sasad</i>	83
3.10	Procedures for Distillation and Dehydration of Fermentation Product	86
3.11	Determination of Reducing Sugar Concentration , Alcohol and Functional Group Analysis	87
3.11.1	Phenol Sulphuric Method	87
3.11.2	High Performance Liquid Chromatography (HPLC) Method	88
3.11.3	Alcohol Analysis using Gas Chromatography Mass Spectrometry (GCMS) Method	90
3.11.4	Functional Group Analysis using Fourier Transform Infrared Spectroscopy (FTIR) Technique	94
3.12	Experimental Design and Statistical Analysis	94
3.13	Summary	95
	CHAPTER 4: RESULTS AND DISCUSSION	96
4.1	Introduction	96
4.2	Preliminary Study On Acid Pretreatment Conditions Based On Acid Types And Pretreatment Time On <i>Gracilaria</i> sp.	96
4.3	Effect of Varying Acid Concentration and Pretreatment Time on Glucose Concentration (g/L) from <i>Gracilaria</i> sp. Hydrolysis.	98
4.4	Effect of Pretreatment Conditions and Fermentation Days of <i>Gracilaria</i> sp. using <i>Sasad</i> .	104
4.4.1	Effect of Pretreatment Conditions and Fermentation Days of <i>Gracilaria</i> sp. using <i>Sasad</i> .	105
4.4.2	Effect of Pretreatment Conditions and Fermentation Days of <i>Gracilaria</i> sp. on Alcohol Yield.	110
4.5	Analysis of Alcohol Content Presence from the Product of <i>Gracilaria</i> sp. Fermentation.	114
4.5.1	Observation of <i>Gracilaria</i> sp. Fermentation Samples	115
4.5.2	Analysis of Alcohol Presence in <i>Gracilaria</i> sp. Fermentation Product Using GCMS and FTIR	118

4.6	Analysis of <i>Gracilaria</i> sp. Fermentation Using <i>Sasad</i> based on Glucose Uptake and Alcohol Yield	136
4.7	Evaluation on <i>Gracilaria</i> sp. Hydrolysis by Statistical Analysis	140
4.8	Summary	143
	CHAPTER 5: CONCLUSION AND RECOMMENDATION	145
5.1	Conclusion	145
5.2	Future Recommendations	146
	REFERENCES	148
	APPENDICES	158



UMS
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LIST OF TABLES

	Page
Table 2.1:	Table 2.1: Biofuel Types and Classifications 34
Table 2.2:	Table 2.2: Fermentative production of ethanol from macroalgal feedstock 38
Table 2.3:	Table 2.3: Red Seaweed and General Analysis of Total Carbohydrates Red Macroalgae 42
Table 2.4:	Table 2.4: Chemical <i>Composition of Gracilaria sp.</i> 43
Table 2.5:	Table 2.5: Polysaccharides Abundant in Seaweed Biomass. 46
Table 2.6:	Table 2.6: Physical Pretreatment for Macroalgae 49
Table 2.7:	Table 2.7: Comparison of Concentrated and Dilute Acid Hydrolysis Method 50
Table 2.8:	Table 2.8: Summary of Macroalgae Chemical Hydrolysis 54
Table 2.9:	Table 2.9: Summary of Macroalgae Hydrolyzed using Enzymatic Hydrolysis 59
Table 2.10:	Table 2.10: Methods of energy extraction from macroalgal biomass 62
Table 2.11:	Table 2.11: Results on various macroalgae fermentation results with the aid of microbial and enzymes. 70
Table 2.12:	Table 2.12: Starter Culture for Food Fermentation 74
Table 3.1:	Parameters for Acid Hydrolysis Experiment 81
Table 3.2:	Fermentation Parameters 86
Table 3.3:	HPLC Setting for Carbohydrate Analysis 89
Table 3.4:	GCMS Setting for Alcohol Analysis 91
Table 4.1:	Effect of Varying Pretreatment Time and Acid Concentration on Macroalgae. 103
Table 4.2:	Results of Acid Hydrolysis Experiment at Different Acid Molarity and Hydrolysis Time 104
Table 4.3:	Results of glucose yield at different pretreatment method and fermentation days. 106
Table 4.4:	Results of Glucose Loss of <i>Gracilaria sp.</i> Fermentation at Different Pretreatment Method and Fermentation Days 107

Table 4.5:	Results of alcohol concentration produced at different pretreatment method and fermentation days.	112
Table 4.6:	Compound Presence Based on Peaks Detected	132
Table 4.7:	Percentage of Main Compound Detected	133
Table 4.8:	Summarized findings of characteristics peaks of FTIR spectra from various samples crossed checked against GCMS results of this study	136
Table 4.9:	Final model equation for glucose responses resulting from experimental design	140
Table 4.10:	Analysis of Variance (ANOVA) for Glucose Models	141



UMS
UNIVERSITI MALAYSIA SABAH

LIST OF FIGURES

	Page
Figure 1.1: World Temperature Modern Record began in 1880 up to 2017	20
Figure 1.2: Growth in Renewable Energy Generation from 2012 – 2016	21
Figure 1.3: Block Flow of Macroalgae to Biofuel Production via Fermentation	25
Figure 2.1: First and Second Generation Biofuel Processing Steps	32
Figure 2.2: Macroalgae Division Chart	42
Figure 2.3: Agarobiose Structure	44
Figure 2.4: Major Steps for bioethanol and/or biogas production from seaweed biomass.	45
Figure 2.5: Structural Information about Polysaccharides Abundant in Seaweed Biomass	47
Figure 2.6: The Formation of Levulinic and Formic Acids during Dilute Acid Hydrolysis.	53
Figure 2.7: Formation of Inhibitors.	53
Figure 2.8: Hydrolysis of <i>Gracilaria verrucosa</i> using enzymatic hydrolysis and acid hydrolysis.	58
Figure 2.9: Bacterial fermentation products of pyruvate. Pyruvate formed by the catabolism of glucose is further metabolized by pathways which are characteristic of particular organisms and which serve as a biochemical aid to identification. End products of fermentations are italicized.	60
Figure 2.10: a) Effect of temperature on the hydrolysis of <i>Gracilaria salicornia</i> . b) Ethanol production from hydrolysate derived from <i>Gracilaria salicornia</i> feedstock using the ethanologenic <i>E. coli</i> strain KO11.	63
Figure 2.11: Ethanol fermentation using hydrolysate of <i>Gracilaria</i> sp. by PVA-Ca-alginate immobilized <i>S. cerevisiae</i> Wu-Y2 in repeated batch culture.	64
Figure 2.12: Growth of moulds and yeasts (●) Growth of lactic acid bacteria (■) during fermentation of tapai for 20 days.	66
Figure 2.13: Alcohol content in <i>Tapai</i> during fermentation	67

Figure 2.14:	Fuyukama vinegar fermentation using Koji mould (A) and AAB biofilm (B)	68
Figure 2.15:	Schematic of Biofilm	68
Figure 2.16:	Production of ethanol from <i>L. japonica</i> used to isolate microorganisms from Nuruk; both aerobic and anaerobic conditions were used. The results shown are averages of triplicate tests. (a) Anaerobic: the bottles were capped with butyl rubber covers. (b) Aerobic: the bottles were capped by wrapping with aluminum to prevent contamination.	72
Figure 3.1:	Flowchart of the Experimental Process	79
Figure 3.2:	Procedures for Raw <i>Gracilaria</i> sp. Preparation	82
Figure 3.3:	Procedures for Acid Hydrolysis	82
Figure 3.4:	Procedures for <i>Sasad</i> Preparation	83
Figure 3.5:	Procedures for <i>Gracilaria</i> sp. Fermentation with <i>Sasad</i>	85
Figure 3.6:	Distillation Unit Setup for Sample Distillation	87
Figure 3.7:	Calibration curve of Glucose Standard using UV-VIS	88
Figure 3.8:	Calibration curve of Glucose Standard using HPLC	90
Figure 3.9:	Calibration curve of Isoamyl Standard using GCMS 104	93
Figure 3.10:	GCMS standard curve for isoamyl. Retention time was found at 3.85 minutes	93
Figure 3.11:	GCMS standard curve for ethanol. Retention time was found at 1.87 minutes	94
Figure 4.1:	Reducing sugar yield of <i>Gracilaria</i> sp. for different pretreatment time and types of acid.	98
Figure 4.2:	Percentage Of Glucose Yield From <i>Gracilaria</i> sp. Hydrolysis At Different Time And Acid Molarity.	101
Figure 4.3:	Glucose Concentration of <i>Gracilaria</i> sp. Hydrolysis at Different Acid Molarity and Hydrolysis Time	102
Figure 4.4:	Glucose Concentration at Different Pretreatment Method and Fermentation Days	110
Figure 4.5:	Alcohol yield of NT, HT and AT against fermentation days.	114
Figure 4.6:	Observations on <i>Gracilaria</i> sp. and Rice Fermentation Using <i>Sasao</i>	118

Figure 4.7:	Chromatography Graph of <i>Gracilaria</i> sp. Fermentation Product after 3 Days Fermentation at Different Pretreatment Method; a) Acid Treated <i>Gracilaria</i> sp., b) Hot Water Treated <i>Gracilaria</i> sp. c) Untreated <i>Gracilaria</i> sp.	120
Figure 4.8:	Mass spectrum of isoamyl in 3 days <i>Gracilaria</i> sp. fermentation samples using <i>Sasad</i> ; Acid Treated <i>Gracilaria</i> sp., b) Hot Water Treated <i>Gracilaria</i> sp. c) Untreated <i>Gracilaria</i> sp.	120
Figure 4.9:	Chromatography Graph of <i>Gracilaria</i> sp. Fermentation Product after 7 Days Fermentation at Different Pretreatment Method; a) Acid Treated <i>Gracilaria</i> sp., b) Hot Water Treated <i>Gracilaria</i> sp. c) Untreated <i>Gracilaria</i> sp.	121
Figure 4.10:	Mass spectrum of isoamyl in 7 days <i>Gracilaria</i> sp. fermentation samples using <i>Sasad</i> ; Acid Treated <i>Gracilaria</i> sp., b) Hot Water Treated <i>Gracilaria</i> sp. c) Untreated <i>Gracilaria</i> sp.	122
Figure 4.11:	Chromatography Graph of <i>Gracilaria</i> sp. Fermentation Product after 14 Days Fermentation at Different Pretreatment Method; a) Acid Treated <i>Gracilaria</i> sp., b) Hot Water Treated <i>Gracilaria</i> sp. c) Untreated <i>Gracilaria</i> sp.	123
Figure 4.12:	Mass spectrum of isoamyl in 14 days <i>Gracilaria</i> sp. fermentation samples using <i>Sasad</i> ; Acid Treated <i>Gracilaria</i> sp., b) Hot Water Treated <i>Gracilaria</i> sp. c) Untreated <i>Gracilaria</i> sp.	124
Figure 4.13:	Chromatography Graph of <i>Gracilaria</i> sp. Fermentation Product after 21 Days Fermentation at Different Pretreatment Method; a) Acid Treated <i>Gracilaria</i> sp., b) Hot Water Treated <i>Gracilaria</i> sp. c) Untreated <i>Gracilaria</i> sp.	125
Figure 4.14:	Mass spectrum of isoamyl in 21 days <i>Gracilaria</i> sp. fermentation samples using <i>Sasad</i> ; Acid Treated <i>Gracilaria</i> sp., b) Hot Water Treated <i>Gracilaria</i> sp. c) Untreated <i>Gracilaria</i> sp.	126
Figure 4.15:	Chromatography Graph of <i>Gracilaria</i> sp. Fermentation Product after 28 Days Fermentation at Different Pretreatment Method; a) Acid Treated <i>Gracilaria</i> sp., b) Hot Water Treated <i>Gracilaria</i> sp. c) Untreated <i>Gracilaria</i> sp.	127
Figure 4.16:	Mass spectrum of isoamyl in 28 days <i>Gracilaria</i> sp. fermentation samples using <i>Sasad</i> ; Acid Treated <i>Gracilaria</i> sp., b) Hot Water Treated <i>Gracilaria</i> sp. c) Untreated <i>Gracilaria</i> sp.	128
Figure 4.17:	Chromatography Graph of Isoamyl Standard at Retention Times of 3.85 minutes	130
Figure 4.18:	Mass Spectrum Data at 3.44 minutes for Isoamyl Standard	130
Figure 4.19:	FTIR spectra of untreated sample (NT)	134

Figure 4.20: FTIR spectra of hot treated sample (HT)	134
Figure 4.21: FTIR spectra of acid treated sample (AT)	134
Figure 4.22: A typical growth curve for a bacterial population	135
Figure 4.23: Untreated <i>Gracilaria</i> sp. fermentation (NT)	138
Figure 4.24: Hot water treated <i>Gracilaria</i> sp. fermentation (HT)	139
Figure 4.25: Acid treated <i>Gracilaria</i> sp. fermentation (AT)	139
Figure 4.26: 3D Surface Model Graph of <i>Gracilaria</i> sp. Acid Hydrolyzed Samples	142
Figure 4.27: Optimum hydrolysis parameters at 30 min pretreatment time and 0.4 M H ₂ SO ₄ yielded 39.21 g/L glucose.	142
Figure 4.28: Optimum hydrolysis parameters at 30 min pretreatment time and 0.6 M H ₂ SO ₄ yielded 42.34 g/L glucose.	143



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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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LIST OF APPENDICES

	Page	
APPENDIX A	Data for Calibration Curve of Glucose Using UV-Vis	158
APPENDIX B	Data for Calibration Curve of Glucose Using HPLC	159
APPENDIX C	Peaks Area of Glucose of <i>Gracilaria</i> sp. Fermentation with <i>Sasad</i> (HPLC)	161
APPENDIX D	1-Butanol-3-Methyl Concentration Calibration Curve (GCMS)	164
APPENDIX D	1-Butanol-3-Methyl Concentration Yield of <i>Gracilaria</i> sp. Fermentation with <i>Sasad</i> (GCMS)	165
APPENDIX E	List of publication, conferences, seminar and achievement	166



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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/m³), but the global gas production was flat of 0.3%, 21 63 billion/m³ 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.

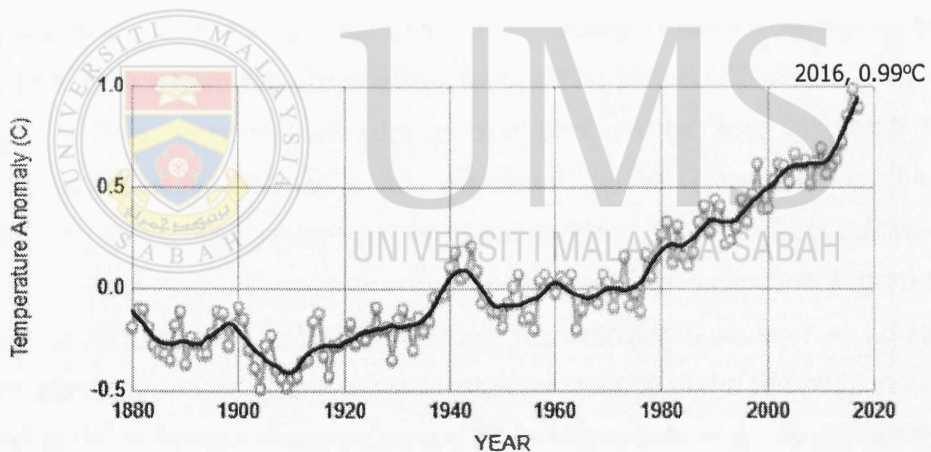


Figure 1.1 : World Temperature Modern Record 1880 up to 2017

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

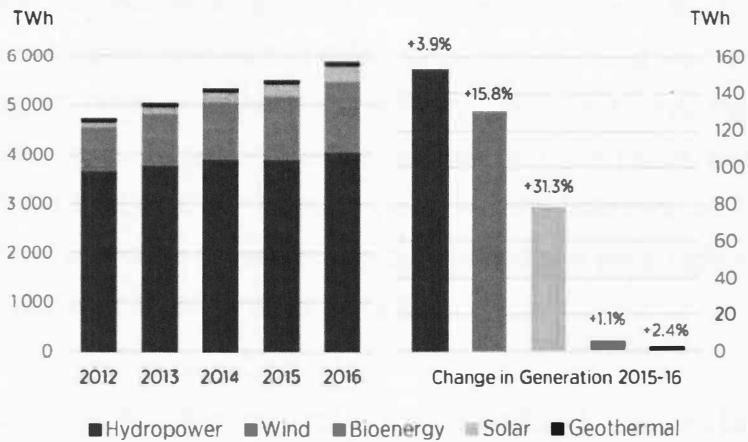


Figure 1.2 : Growth in Renewable Energy Generation from 2012 – 2016

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 atmosphere 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 hectare (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 biomass 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*