

**EFFECT OF VARIOUS PRE-TREATMENTS
ONTO OIL PALM FROND LEAVES FOR
BIOETHANOL PRODUCTION AS RENEWABLE
BIOENERGY SOURCE IN FUEL CELL
APPLICATION**

NUR FATIN SILMI BINTI MOHD AZANI

UNIVERSITI SAINS MALAYSIA

2025

**EFFECT OF VARIOUS PRE-TREATMENTS
ONTO OIL PALM FROND LEAVES FOR
BIOETHANOL PRODUCTION AS RENEWABLE
BIOENERGY SOURCE IN FUEL CELL
APPLICATION**

by

NUR FATIN SILMI BINTI MOHD AZANI

**Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy**

March 2025

ACKNOWLEDGEMENT

First and foremost, I would like to express my uttermost gratitude to my supervisor, Associate Professor Dr. Mohd Hazwan Hussin, for his endless support and advice throughout the years of my research at the School of Chemical Sciences, Universiti Sains Malaysia (USM). Also, the greatest appreciation to my supervisors, Professor Nicolas Brosse and Dr. Isabelle Ziegler-Devin from Université de Lorraine (UL), France for assisting and supporting me throughout my research attachment at the Laboratoire d'études et de recherche sur le matériau bois (LERMAB).

A profound appreciation to the Ministry of Higher Education for providing financial support through the Prototype Research Grant Scheme, PRGS (PRGS/1/2022/STG05/USM/02/1). In addition, an extended gratitude to the RMN platform, Institut Jean Barriol, CRM2, Université de Lorraine – CNRS for the support given to this research. On top of that, I sincerely thank the Embassy of France for providing me with the PhD co-tutelle scholarship *via* Boursier Etranger du Gouvernement Français (French Government Scholarship) throughout my PhD attachment period in France.

I would like to thank all administrative, science officers, and laboratory assistants at School of Chemical Sciences, LERMAB and École nationale supérieure des technologies et industries du bois (ENSTIB). A special appreciation to Dr. Arnaud Besserer for his guidance in assisting me with the enzymatic hydrolysis and preliminary fermentation studies at ENSTIB. Also, thanks to Dr. Laurent Chrusciel for helping me with the steam explosion process.

Last but not least, I would like to express my sincere gratitude to all my research colleagues at USM; Najhan, Hazwani, Liyana, Dr. Ayo and UL; Karina, Victor and

Kyle for their utmost help. Also, thanks to my other friends: Dr. Rabita, Dr. Qiao, Dyia, Resa, Mengxi, Rayan, Alia, Dr. Seddik, Izzati, Hanim and Atikah for their support also. Special thanks to my beloved parents Mr. Mohd Azani Awang and Mrs. Salina Nawi for their highest support and encouragement to me. Thank you, *merci beaucoup à tous*.

TABLE OF CONTENTS

ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iv
LIST OF TABLES.....	ix
LIST OF FIGURES.....	xi
LIST OF SYMBOLS.....	xv
LIST OF ABBREVIATIONS.....	xvi
LIST OF APPENDICES.....	xviii
ABSTRAK.....	xix
ABSTRACT	xxii
ABSTRAIT.....	xxiv
CHAPTER 1 INTRODUCTION.....	1
1.1 Background study.....	1
1.2 Problem statements.....	3
1.3 Research objectives	6
1.4 Scope of study	7
CHAPTER 2 LITERATURE REVIEW.....	10
2.1 Renewable energy.....	10
2.1.1 Biofuels.....	11
2.1.2 Bioethanol from the conversion of lignocellulosic biomass	15
2.1.3 Bioethanol from oil palm biomass	20
2.1.4 Analysis of bioethanol <i>via</i> Gas Chromatography-Flame Ionization Detector (GC-FID)	22
2.2 Fuel cells.....	23
2.2.1 Direct ethanol fuel cell (DEFC) system	25

2.2.2	The exploitation of bioethanol in fuel cell applications.....	30
2.2.3	Evaluation of fuel cell performance by using electrochemical analyses	31
2.3	Lignocellulosic biomass	36
2.3.1	Oil palm frond leaves	40
2.3.2	Valorization of OPFLs into valuable bioproducts.....	44
2.4	Pre-treatment of lignocellulosic biomass	46
2.4.1	Physical pretreatments.....	50
2.4.2	Chemical pre-treatments.....	51
	2.4.2.(a) Acid pre-treatment.....	52
	2.4.2.(b) Alkaline pre-treatment.....	53
2.4.3	Physicochemical pre-treatments	57
	2.4.3.(a) Autohydrolysis pre-treatment.....	57
	2.4.3.(b) Steam explosion pre-treatment	62
2.4.4	Biological pre-treatment.....	65
2.5	Production of sugars from hydrolysis process.....	66
2.5.1	Monosaccharides in lignocellulosic biomass	66
2.5.2	Glucose production from acid hydrolysis.....	68
2.5.3	Glucose production from enzymatic hydrolysis.....	71
2.6	Sugars analysis by chromatographic techniques	77
2.6.1	HPAEC-PAD.....	78
2.6.2	HPLC-UV.....	80
	CHAPTER 3 RESEARCH METHODOLOGY	84
3.1	Chemicals	84
3.2	Raw material.....	86
3.3	Pre-treatments.....	87
3.3.1	Autohydrolysis pre-treatment.....	89

3.3.2	Steam explosion pre-treatment	92
3.3.3	Dilute NaOH pre-treatment	94
3.4	Characterization of raw OPFLs and pre-treated OPFLs.....	96
3.4.1	Chemical composition analysis	96
3.4.1.(a)	Determination of Klason lignin	96
3.4.1.(b)	Determination of Cellulose and Hemicellulose.....	96
3.4.2	Post hydrolysis.....	97
3.4.3	High-performance anionic exchange chromatography (HPAEC-PAD).....	98
3.4.4	Inhibitor analysis	99
3.4.5	Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy	99
3.4.6	Size-exclusion chromatography (SEC) analysis	100
3.4.7	Scanning Electron Microscopy - Energy Dispersive X-ray spectroscopy (SEM-EDX) analysis.....	100
3.4.8	N ₂ Brunauer-Emmett-Teller (BET) analysis	101
3.4.9	X-ray Diffraction (XRD) analysis	101
3.4.10	Extraction of lignin precipitate and Heteronuclear Single Quantum Coherence-Nuclear Magnetic Resonance (HSQC-NMR) analysis	102
3.5	Enzymatic hydrolysis	104
3.6	High Performance Liquid Chromatography (HPLC-UV) analysis	105
3.7	Fermentation.....	107
3.8	Gas Chromatography- Flame Ionization Detector (GC-FID) analysis.....	108
3.9	Electrochemical analysis	110
3.9.1	Open circuit potential (OCP).....	110
3.9.2	Electrochemical impedance spectroscopy (EIS)	113
CHAPTER 4 DISCUSSION		115
4.1	Chemical composition and monosaccharide profile of OPFLs biomass...	115

4.2	Autohydrolysis pre-treatment	116
4.2.1	Chemical composition and monosaccharides profile analysis	116
4.3	Steam explosion pre-treatment	123
4.3.1	Chemical composition and monosaccharides profile	123
4.4	Dilute NaOH pre-treatment	130
4.4.1	Chemical composition and monosaccharides profile	130
4.5	Characterization of raw OPFLs and pre-treated OPFLs.....	135
4.5.1	ATR analysis	135
4.5.2	SEC analysis.....	140
4.5.3	SEM-EDX analysis	142
4.5.4	BET analysis.....	149
4.5.5	XRD analysis.....	151
4.5.6	HSQC-NMR analysis	155
4.6	Enzymatic hydrolysis	163
4.6.1	Effect of substrate (OPFLs) loading and enzyme loading	166
4.6.2	Effects of incubation periods for glucose production from various pre-treated OPFLs pulp.....	168
4.7	Quantification of ethanol from fermentation broth <i>via</i> GC-FID	175
4.8	Energy efficiency of bioethanol <i>via</i> electrochemical analysis	186
4.8.1	Open circuit potential (OCP).....	187
4.8.2	Electrochemical impedance spectroscopy (EIS)	190
4.8.3	Fuel cell performance analysis	197
	CHAPTER 5 CONCLUSION AND FUTURE RECOMMENDATIONS.....	202
5.1	Conclusion	202
5.2	Future recommendations	209
	REFERENCES	210

APPENDICES

LIST OF PUBLICATION AND CONFERENCE

LIST OF TABLES

		Page
Table 2.1	Optimization of conversion process of various lignocellulosic biomass into bioethanol	17
Table 2.2	Composition of various lignocellulosic biomass and its desired application.....	37
Table 2.3	Comparison of different type of pre-treatments with its advantages and disadvantages	48
Table 2.4	Alkaline pre-treatment on different type of lignocellulosic biomass at varying parameters with delignification rate.....	56
Table 2.5	Autohydrolysis and steam explosion pre-treatment for different type of lignocellulosic biomass at varying conditions process	61
Table 3.1	Chemicals used for different pre-treatments, proximate analysis and monosaccharide analysis	84
Table 3.2	Chemicals used for enzymatic hydrolysis, derivation of PMP-sugar, and glucose quantification <i>via</i> HPLC analysis	84
Table 3.3	Chemicals used for fermentation and ethanol extraction for GC-FID analysis.....	85
Table 3.4	Autohydrolysis conditions with the sample code.....	90
Table 3.5	Process condition for steam explosion pre-treatment with the sample code	92
Table 3.6	The process condition of dilute NaOH pre-treatment with the sample code	95
Table 4.1	Experimental condition, chemical composition of autohydrolysis pre-treated OPFLs pulp at varying parameters and hemicellulose removal percentage	119
Table 4.2	Experimental condition, monosaccharides profiles of pre-treated OPFLs pulp and OPFLs hydrolysate fraction after autohydrolysis pre-treatment.....	120
Table 4.3	Experimental condition, chemical composition of steam exploded OPFLs pulp at varying parameters, and hemicellulose removal percentage	124

Table 4.4	Experimental condition, monosaccharides profiles of steam exploded OPFLs pulp and OPFLs hydrolysate fraction after steam explosion pre-treatment	125
Table 4.5	Experimental condition, chemical composition, monosaccharides profile of pre-treated OPFLs pulp at varying parameters, and lignin removal percentage after dilute NaOH pre-treatment.....	132
Table 4.6	Assignment of functional groups based on the ATR-FTIR spectrum of raw OPFLs and pre-treated OPFLs obtained from different pre-treatments	136
Table 4.7	Average molecular weight and degree of polymerization for cellulose OPFLs after various pre-treatments at varying severity	141
Table 4.8	Elemental composition of raw OPFLs pulp and pre-treated OPFLs after various pre-treatments	149
Table 4.9	The specific surface area of OPFLs before and after pre-treatments at varying severity factor	151
Table 4.10	Crystallinity index of raw OPFLs and pre-treated OPFLs after various pre-treatments.....	154
Table 4.11	Assignment of ¹³ C- ¹ H signal of 2D-HSQC-NMR spectrum for lignin samples derived from raw OPFLs and pre-treated OPFLs (after steam explosion and autohydrolysis pre-treatments).....	156
Table 4.12	Electrochemical impedance parameters for direct-ethanol PEM-fuel cell in the presence of analytical ethanol and bioethanol derived from raw OPFLs and pre-treated OPFLs	195
Table 4.13	Consumption of ethanol, fuel utilization factor and power density of the PEM-fuel cell by using analytical ethanol and bioethanol derived from raw OPFLs and pre-treated OPFLs as a fuel source	201

LIST OF FIGURES

		Page
Figure 2.1	Biofuels conversion technologies from different type of feedstock.....	12
Figure 2.2	Conversion of lignocellulosic biomass into 2G bioethanol	15
Figure 2.3	GC-FID chromatogram of the extracted organic layer from the fermentation broth showing three apparent peaks for ethanol (EtOH), ethyl acetate (EtOAc) and butanol (n-BuOH)	23
Figure 2.4	Schematic diagram of A) membrane electrode assembly (MEA) and B) polymer electrolyte membrane-direct ethanol fuel cell (PEM-DEFC)	26
Figure 2.5	A commercial bio-energy direct ethanol fuel cell (DEFC) kit produced by Horizon Fuel Cell Technologies.....	29
Figure 2.6	OCP variation of polymer electrolyte membrane fuel cell as a function of time	32
Figure 2.7	Equivalent circuit model for fuel cell system fitted with generated Nyquist plot	35
Figure 2.8	Nyquist plot for polymer electrolyte membrane direct ethanol fuel cell (PEM-DEFC) system	36
Figure 2.9	The main lignocellulosic biomass derived from oil palm tree	39
Figure 2.10	Oil palm frond leaves biomass cell wall interior with its main components.....	41
Figure 2.11	Impact of pre-treatment process on the biomass cell wall and its fractionation into cellulosic and non-cellulosic (hemicellulose & lignin) components for desirable applications or products	48
Figure 2.12	A) Mechanism of bond breaking in lignocellulose by using NaOH and B) Cleavage of non-phenolic aryl ether linkage during alkaline pre-treatment.	54
Figure 2.13	A proposed mechanism of hydrolysis of hemicellulose during autohydrolysis reaction for hydrothermal pre-treatments (autohydrolysis and steam explosion).....	58

Figure 2.14	SEM micrograph of autohydrolysis pre-treated bamboo chip	60
Figure 2.15	The conversion of cellulose and hemicellulose into simple sugars.....	67
Figure 2.16	Mechanism of glucose conversion <i>via</i> acid hydrolysis.....	69
Figure 2.17	A) Schematic diagram of enzymatic hydrolysis process in lignocellulosic biomass and B) Proposed detail mechanism of enzymatic cellulose degradation to glucose monomer.....	73
Figure 2.18	HPAEC-PAD chromatogram for standard mixed sugars (black line) and hydrolyzed sugars from various agricultural waste.; Fuc: fucose, Rha: rhamnose, Ara: arabinose, Gal: galactose, Glc: glucose, Man: mannose, Xyl: xylose, GalA: galacturonic, and GlcA: glucuronic acid.....	80
Figure 2.19	A) Derivatization reaction of glucose and PMP and B) HPLC chromatogram for PMP derivative of monosaccharide sugar	82
Figure 3.1	Preparation of raw OPFLs biomass for the pre-treatments process.....	87
Figure 3.2	Research flow chart of application of various pre-treatment onto OPFLs biomass and its characterizations along with the production of bioethanol <i>via</i> separate hydrolysis and fermentation and the energy efficiency of bioethanol <i>via</i> electrochemical analysis.....	88
Figure 3.3	Autohydrolysis and dilute NaOH pre-treatments of raw OPFLs biomass at varying parameters.....	91
Figure 3.4	A Parr pressure and reactor components.....	91
Figure 3.5	Steam explosion pre-treatment of raw OPFLs biomass at varying conditions	93
Figure 3.6	A steam explosion reactor components.....	94
Figure 3.7	Extraction of lignin precipitate from steam-exploded pulp and autohydrolysis pre-treated pulp for HSQC-NMR analysis	104
Figure 3.8	Enzymatic hydrolysis process for the production of glucose from the pre-treated OPFLs pulp derived from different pre-treatments	105
Figure 3.9	Fermentation process for production of bioethanol from enzymatic hydrolysate pre-treated OPFLs from different pre-treatments	108

Figure 3.10	A) Electrochemical analysis set-up for fuel cell system and B) A commercial direct-ethanol PEM-fuel cell	112
Figure 4.1	The ATR spectra of raw OPFLs and pre-treated OPFLs after (a) autohydrolysis, (b) water-impregnated steam explosion, (c) acid-impregnated steam explosion, and (d) dilute NaOH pre-treatment	138
Figure 4.2	SEM images of raw OPFLs and pre-treated OPFLs pulp after different pre-treatments at varying severity factor	144
Figure 4.3	XRD diffractogram of cellulose derived from raw OPFLs and pre-treated OPFLs from different pre-treatments.....	152
Figure 4.4	The 2D HSQC-NMR spectrums for the a) OPFL-lignin and the extracted lignin from b) water-impregnated steam exploded OPFLs (SEW ₂₀₀₋₁₀) and c) autohydrolysis pre- treated OPFLs (AH ₁₈₀₋₃₀) (A: aliphatic side chain; B: aromatic region)	160
Figure 4.5	Evolution of β -O-4 linkage amount and S/G value in lignin extracted from raw OPFLs and pre-treated OPFLs pulp after hydrothermal and steam explosion pre-treatment	163
Figure 4.6	The HPLC chromatograms for a) monosaccharides standard, b) control (filter paper), c) raw OPFLs, d) autohydrolysis pre- treated OPFLs, e) water-impregnated steam-exploded OPFLs, f) acid-impregnated steam exploded OPFLs and g) dilute NaOH pre-treated OPFLs.....	166
Figure 4.7	The glucose yield and cellulose conversion of raw OPFLs and pre-treated OPFLs after 9 h of enzymatic hydrolysis at varying cellulase blend dosage and OPFLs pulp dosage.	167
Figure 4.8	The glucose yield and cellulose conversion of raw OPFLs and autohydrolysis pre-treated OPFLs after enzymatic hydrolysis at varying incubation periods	170
Figure 4.9	The glucose yield and cellulose conversion of raw OPFLs , water-impregnated (SEW) and acid-impregnated (SEA) steam-exploded OPFLs after enzymatic hydrolysis at varying incubation periods	172
Figure 4.10	The glucose yield and cellulose conversion of raw OPFLs and dilute NaOH pre-treated OPFLs after enzymatic hydrolysis at varying incubation periods	174
Figure 4.11	GC chromatogram for a) 2.5% v/v ethanol (STD), b) ethanol from 40 g/L glucose (control), c) SEA190-10 bioethanol, and d) N2.5-120-120 bioethanol	178

Figure 4.12	The ethanol yield, ethanol concentration and fermentation efficiency of control (40 g/L glucose), raw OPFLs and pre-treated OPFLs from various pre-treatments under fermentation process at varying incubation periods.....	181
Figure 4.13	The pH of fermentation broth at varying fermentation periods	183
Figure 4.14	OCP variations at 1 h running time for analytical ethanol and bioethanol derived from raw OPFLs and pre-treated OPFLs from different pre-treatments	188
Figure 4.15	Equivalent circuit model (ECM) for Warburg impedance.....	191
Figure 4.16	Nyquist plot for analytical ethanol and bioethanol derived from raw OPFLs and pre-treated OPFLs from different pre-treatments	192
Figure 4.17	Proposed schematic diagram of electrochemical reaction in direct ethanol fuel cell system equipped with proton-exchange membrane (PEM) based on the Nyquist plot and equivalent circuit model (ECM).....	193

LIST OF SYMBOLS

μ	micro
μm	micrometer
Ω	ohm
cm	centimeter
F	Farad
$^{\circ}\text{C}$	celcius
g	gram
h	hour
L	litre
mL	mililitre
min	minutes
m	meter
s	seconds
v/v %	percentage by volume
V	Volt
W	watt
wt.%	percentage by weight (w/w)

LIST OF ABBREVIATIONS

ATR	Attenuated Total Reflection
AH	Autohydrolysis pre-treated OPFLs
CS	Combined severity
CPE	Constant phase element
CrI	Crystallinity index
DP	Degree polymerization
DEFC	Direct ethanol fuel cell
ECM	Electrical circuit model
EH	Enzymatic hydrolysis
EIS	Electrochemical impedance spectroscopy
FPU	Filter paper unit
GC-FID	Gas chromatography flame ionization
HMF	5-hydroxymethylfurfural
HPAEC	High-performance anion-exchange chromatography
HPLC	High-performance liquid chromatography
HSQC-NMR	Heteronuclear Single Quantum Coherence- Nuclear Magnetic Resonance
M_w	Average molecular weight
N	NaOH pretreated pulp
OCP	Open circuit potential
OPFLs	Oil palm frond leaves
PD	Power density
PEM	Proton exchange membrane
R_{ct}	Charge transfer resistance
R_{sol}	Solution/electrolyte resistance
S	Severity
SEA	Acid-impregnated steam-exploded OPFLs
SEC	Size-exclusion chromatography
SEM	Scanning electron microscopy
SEW	Water-impregnated steam-exploded OPFLs

T	Temperature
t	Residence time/incubation time
U_F	Fuel utilization factor
V_{cell}	Fuel cell voltage
XRD	X-ray diffraction

LIST OF APPENDICES

Appendice A1	Water-impregnated steam-exploded (SEW) samples
Appendice A2	Acid-impregnated steam-exploded (SEW) samples
Appendice A3	Glucose standard calibration curve (for HPLC-UV analysis)
Appendice A4	Ethanol standard calibration curve (for GC-FID analysis)
Appendice A5	Preliminary study of fermentation by using glucose solution

**KESAN PELBAGAI PRA RAWATAN TERHADAP DAUN PELEPAH
KELAPA SAWIT UNTUK PENGHASILAN BIOETANOL SEBAGAI SUMBER
BIOTENAGA BOLEH DIPERBAHARU DALAM APLIKASI SEL BAHAN API**

ABSTRAK

Kajian terkini mencadangkan kaedah alternatif menggunakan OPFLs biojisim sebagai sumber biotena. Kajian ini membincangkan kesan pra-rawatan yang berbeza terhadap sifat fizikokimia, keberkesanan hidrolisis berenzim, dan kecekapan penapaian OPFLs biojisim selepas proses pra-rawatan. Setiap kaedah pra-rawatan mendedahkan ciri-ciri tersendiri yang boleh mempengaruhi process penukaran selulosa kepada bioetanol. Daripada pra-rawatan fizikokimia, pra-rawatan letupan wap lebih berkesan untuk mengurangkan kepadatan struktur dinding sel OPFL biojisim berbanding dengan pra-rawatan autohidrolisis, berdasarkan penghapusan kandungan hemiselulosa dan penyahpolimeran lignin. Pra-impregnasi dengan asid sulfurik sebelum pra-rawatan letupan wap berkesan untuk meningkatkan penyingkiran hemiselulosa sehingga 89.94%. Kandungan selulosa bagi pulpa OPFLs biojisom selepas pra-rawatan autohidrolisis dan pra-rawatan letupan wap telah meningkat masing-masing iaitu 31.03 wt.% dan 39.18 wt.%. Sementara itu, pra-rawatan NaOH meningkatkan delignifikasi sebanyak 87.14 %, menyebabkan peningkatan kandungan selulosa sehingga 51.21 wt.%. Penyingkiran kandungan hemiselulosa dan lignin telah disahkan dengan analisis spektrum pantulan total dilemahkan (ATR) berdasarkan pengurangan puncak xylan C=O pada 1730 cm⁻¹ dan pengurangan puncak unit struktur lignin C=O pada 1600 cm⁻¹. Analisis kromatografi penyisihan saiz (SEC) mendedahkan tahap pempolimeran (DP) bagi semua pulpa selulosa OPFLs biojisim berkurangan apabila tahap pra-rawatan dipertingkatkan. Selain itu, analisis pembelauan sinar-X (XRD) mendapati indeks

kehabluran (% CrI) pulpa OPFL biojisim telah dipertingkatkan sehingga 40.85 %, 45.20 %, dan 47.59 % masing -masing selepas pra-rawatan menggunakan NaOH, autohidrolisis dan letupan wap. Analisis permukaan mendedahkan struktur pori OPFLs biojisim yang telah dirawat mempunyai pecahan selulosa yang lebih tinggi dengan nilai ukuran keluasan yang lebih tinggi iaitu $4.875 \text{ m}^2 \text{ g}^{-1}$ hingga $9.701 \text{ m}^2 \text{ g}^{-1}$. Analisis 2D-koheren kuantum tunggal- resonans magnet nuklear (HSQC-NMR) mengesahkan pengurangan kaitan β -O-4 untuk lignin yang diekstrak daripada OPFLs biojisim selepas pra-rawatan autohidrolisis dan letupan wap. Dapatan ini mengesahkan belahan kaitan lignin-karbohidrat (LCC) OPFLs biojisim. Analisis keberkesanan hidrolisis berenzim mendedahkan bahawa OPFLs biojisim selepas pra-rawatan menghasilkan hasil glukosa yang lebih tinggi sebanyak 29.81 wt.% hingga 49.98 wt.%. Hasil glukosa yang tertinggi dicapai oleh OPFLs biojism selepas pra-rawatan NaOH, iaitu nilainya 3 kali ganda lebih tinggi daripada OPFLs biojisim tanpa rawatan (i.e.;14.31 wt.%). Sementara itu, penukaran selulosa yang maksimum (100%) dicapai oleh pulpa OPFLs biojisim yang dirawat menggunakan pra-rawatan letupan wap, selepas 72 jam process hidrolisis berenzim. Hasil bioetanol tertinggi dicapai oleh OPFL biojisim yang dirawat oleh pra-rawatan NaOH iaitu 24.28 wt.% diikuti oleh OPFLs biojisim yang dirawat oleh pra-rawatan asid letupan wap (i.e.: 22.47 wt.%), bersamaan dengan peratusan kecekapan penapaian masing-masing iaitu 94.19 % dan 81.63 %. Akhir sekali, analisis elektrokimia mendedahkan bahawa bioetanol yang diperolehi mempunyai keupayaan tenaga yang memuaskan dengan nilai V_{cell} dan R_{ct} masing-masing iaitu 0.447 V dan $7.105 \Omega \text{ cm}^2$ yang setanding dengan etanol komersial. Anggaran kecekapan penggunaan faktor bahan api menggunakan bioetanol ialah 34.90 % dengan ketumpatan kuasa sel bahan api sebanyak 16.40 W cm^{-2} . Secara ringkasnya, kajian ini menyimpulkan bahawa penggunaan pra-rawatan yang berbeza telah terbukti berkesan dalam meningkatkan

penukaran OPFLs biojisim kepada bioetanol. Selain itu, kajian ini mengesahkan bahawa bioetanol yang dihasilkan mempunyai keupayaan tenaga yang setanding dengan etanol komersial, yang boleh digunakan sebagai bahan api alternatif untuk aplikasi sel bahan api etanol secara langsung (DEFC).

**EFFECT OF VARIOUS PRE-TREATMENTS ONTO OIL PALM FROND
LEAVES FOR BIOETHANOL PRODUCTION AS RENEWABLE BIOENERGY
SOURCE IN FUEL CELL APPLICATION**

ABSTRACT

Oil palm frond leaves (OPFLs) are one of the biomass by-products originating from oil palm plantations. Currently, OPFLs are under-valued since they can be classified as a high recalcitrance feedstock due to the high lignin and hemicellulose content. This current study proposed an alternative of using OPFLs as a bioenergy source. This study highlighted the impact of different pre-treatments on the physicochemical properties, enzymatic digestibility, and fermentation efficiency of pre-treated OPFLs. Each of the pre-treatments revealed the distinctive traits that influence the efficiency of the conversion of cellulose into bioethanol. From physicochemical pre-treatments, steam explosion much more effective in disrupting the compact cell wall structure of OPFLs in contrast to autohydrolysis pre-treatment, by a vast dissolution of hemicellulose and depolymerization of lignin. The pre-impregnation with H₂SO₄ prior to steam explosion was beneficial to enhance the hemicellulose removal up to 89.94 %. The cellulose content of the autohydrolysis pre-treated and steam-exploded OPFLs pulp were enhanced up to 31.03 wt.% and 39.18 wt.%, respectively. Meanwhile, the dilute NaOH pre-treatment enhanced the delignification by 87.14 %, led to the enrichment of cellulosic fraction up to 51.21 wt.%. The removal of hemicellulose and lignin fraction was confirmed by the reduction of xylan C=O peak at 1730 cm⁻¹ and lignin structural units C=O peak at 1600 cm⁻¹, observed in attenuated total reflection (ATR) spectra. The size exclusion analysis divulged a decreasing pattern of degree of polymerization for all pre-treated cellulosic OPFLs as the severity factor was intensified. Besides, the X-ray

diffraction analysis revealed that the crystallinity index of the OPFLs biomass was enhanced up to 40.85 %, 45.20 %, and 47.59 % after the autohydrolysis, steam-explosion and dilute NaOH pre-treatment, respectively. The surface analyses revealed a well-disrupted structure of the pre-treated OPFLs exposing a higher cellulosic fraction with higher specific surface ranging from 4.875 m² g⁻¹ to 9.701 m² g⁻¹. The 2D-Heteronuclear Single Quantum Coherence- Nuclear Magnetic Resonance (HSQC-NMR) analysis confirmed the reduction of β-O-4 linkage for the extracted lignin from the autohydrolysis pre-treated and steam-exploded OPFLs, verifying the cleavage of lignin-carbohydrate linkage of OPFLs biomass. The enzymatic analysis revealed that pre-treated OPFLs yielded higher glucose yield ranging from 29.81 wt.% to 49.98 wt.%, which the highest was attained by dilute NaOH pre-treated OPFLs, which was ~3-fold higher than raw OPFLs (14.31 wt.%). Meanwhile, a maximum cellulose conversion (100%) was achieved by steam-exploded OPFLs pulp after 72 h of enzymatic hydrolysis. The highest bioethanol yield was attained by dilute NaOH pre-treated OPFLs which was 24.28 wt.% followed by acid-impregnated steam exploded OPFLs (i.e:22.47 wt.%), amounting to 94.19 % and 81.63 % fermentation efficiency, respectively. Finally, the electrochemical analyses divulged that the produced bioethanol exhibited promising energy capability with V_{cell} and R_{ct} value of 0.447 V and 7.105 Ω cm², respectively which was comparable with an analytical ethanol. The estimated fuel utilization factor for the produced bioethanol was 34.90 % with fuel cell power density of 12.60 mW cm⁻². In summary, this current study concluded that the application of different pre-treatments has proven to be effective in enhancing the conversion of OPFLs into bioethanol. Also, this study confirmed that the produced bioethanol possessed a comparable energy capability as analytical ethanol, which can be utilized as an alternative biofuel for direct ethanol fuel cell application (DEFC).

**EFFET DU PRÉ-TRAITEMENT SUR LA PRODUCTION DE BIOÉTHANOL
À PARTIR DE FEUILLES DE PALMIER À HUILE ET APPLICATION DE
BIOÉTHANOL COMME ÉNERGIE PROPRE DANS LES PILES À
COMBUSTIBLE**

ABSTRAIT

Les feuilles de palmier à huile (OPFLs) sont l'un des sous-produits de la biomasse provenant des plantations de palmiers à huile. Actuellement, ces feuilles sont sous-évaluées, car elles peuvent être classées comme une matière première très récalcitrante en raison de leur teneur élevée en lignine et en hémicellulose. La présente étude propose une alternative à l'utilisation des OPFL comme source de bioénergie. Cette étude a mis en évidence l'impact de différents prétraitements sur les propriétés physicochimiques, la digestibilité enzymatique et l'efficacité de la fermentation des OPFL prétraitées. Chacun des prétraitements a révélé les caractéristiques distinctives qui influencent l'efficacité de la conversion de la cellulose en bioéthanol. Parmi les prétraitements physicochimiques, l'explosion à la vapeur est beaucoup plus efficace pour perturber la structure compacte de la paroi cellulaire des OPFLs que le prétraitement par autohydrolyse, par une vaste dissolution de l'hémicellulose et une dépolymérisation de la lignine. La pré-imprégnation avec H₂SO₄ avant l'explosion à la vapeur a permis d'améliorer l'élimination de l'hémicellulose jusqu'à 89.94 %. La teneur en cellulose de la pâte d'OPFLs prétraitée par autohydrolyse et explosée à la vapeur a été augmentée jusqu'à 31.03 wt.% et 39.18 wt.%, respectivement. Parallèlement, le prétraitement au NaOH dilué a amélioré la délignification de 87.14 %, ce qui a conduit à l'enrichissement de la fraction cellulosique jusqu'à 51.21 wt.%. L'élimination de l'hémicellulose et de la lignine a été confirmée par la réduction du pic C=O du xylane à

1730 cm^{-1} et du pic C=O des unités structurales de la lignine à 1600 cm^{-1} , observée dans la réflectivité totale atténuée (ATR). L'analyse d'exclusion de taille (SEC) a révélé un schéma décroissant du degré de polymérisation pour tous les OPFLs cellulosiques prétraités au fur et à mesure que le facteur de sévérité s'intensifiait. En outre, l'analyse par diffraction des rayons X (XRD) a révélé que l'indice de cristallinité de la biomasse des OPFLs était augmenté de 40.85 %, 45.20 % et 47.59 % après l'autohydrolyse, l'explosion à la vapeur et le prétraitement au NaOH dilué. Les analyses de surface ont révélé une structure bien perturbée des OPFLs prétraitées, exposant une fraction cellulose plus importante avec une surface spécifique plus élevée allant de 4.875 $\text{m}^2 \text{g}^{-1}$ à 9.701 $\text{m}^2 \text{g}^{-1}$. L'analyse 2D-résonance magnétique nucléaire à cohérence quantique unique hétéronucléaire (HSQC-NMR) a confirmé la réduction de la liaison β -O-4 pour la lignine extraite des OPFL prétraitées par autohydrolyse et explosées à la vapeur, vérifiant le clivage de la liaison lignine-glucides de la biomasse des OPFLs. L'analyse enzymatique a révélé que les OPFL prétraitées donnaient un rendement en glucose plus élevé allant de 29.81 wt.% à 49.98 wt.%, le plus élevé étant atteint par les OPFL prétraitées au NaOH dilué, qui était ~ 3 fois plus élevé que les OPFLs brutes (i.e.:14.31 wt.%). Par ailleurs, une conversion maximale de la cellulose (i.e.:100 %) a été atteinte par la pulpe de OPFL expulsée à la vapeur après 72 heures d'hydrolyse enzymatique. Le rendement en bioéthanol le plus élevé a été atteint par les OPFL prétraitées au NaOH dilué, soit 24.28 wt.% en poids, suivi par les OPFL imprégnées d'acide et explosées à la vapeur (22.47 wt.%), soit 94.19 wt.% et 81.63 wt.% d'efficacité de fermentation. Enfin, les analyses électrochimiques ont révélé que le bioéthanol produit présentait une capacité énergétique prometteuse avec des valeurs V_{cell} et R_{ct} de 0.447 V et 7.105 Ωcm^2 , respectivement, qui étaient comparables à celles d'un éthanol analytique. Le rendement énergétique estimé du bioéthanol produit était de 34.90 % avec une densité

de puissance de la pile à combustible de 16.40 W cm^{-2} . En résumé, cette étude a conclu que l'application de différents prétraitements s'est avérée efficace pour améliorer la conversion des OPFL en bioéthanol. En outre, cette étude a confirmé que le bioéthanol produit possédait une capacité énergétique comparable à celle de l'éthanol analytique, qui peut être utilisé comme biocarburant alternatif pour l'application directe des piles à combustible à l'éthanol.

CHAPTER 1

INTRODUCTION

1.1 Background study

An increase in energy demand poses significant challenges due to concerns about sustainability, environmental impact, and the finite nature of fossil fuels. A transition towards renewable energy such utilization of bioenergy from biomass will bring great benefits to the environment. Biofuel such as bioethanol is one of the available bioenergy that can be derived from biomass. As a biofuel, bioethanol possesses intrinsic properties such as renewability, excellent fuel stability due to its high-octane number and, and emits much lower greenhouse gasses (GHG) than petroleum fuels, thus promoting cleaner combustion[1]. This indicates bioethanol's credibility as a promising biofuel to replace petroleum, which is prominent in reducing vast reliance on petroleum-based fuels. As a liquid biofuel, bioethanol has been utilized as an alternative fuel for vehicles mainly in Brazil and the United States in the form of blended gasoline-bioethanol[2]. In addition, the utilization of bioethanol as a direct fuel source in the fuel cell system to generate electricity offers a new alternative to green technologies[3]. For instance, bioethanol can be directly utilized as a biofuel in a direct ethanol fuel cell (DEFC) system in supplying electricity to the fuel cell system. Among the available fuel cell systems, the DEFC is preferable due to its easy application and high efficiency than the indirect method[4]. The utilization of bioethanol as a bioenergy source has been implemented in the fuel cell-powered vehicle system by a group of researchers from Nissan Motor Corporation in 2016[5]. The fuel cell-powered vehicle system is known as e-Bio-fuel-cell. This e-Bio-fuel-cell prototype vehicle utilized 100 % bioethanol or blended ethanol with water, which is capable of charging the car battery

and supplying electrical power to the vehicle. It was reported that the e-Bio-fuel-cell prototype vehicle fuelled by bioethanol cruised over 600 km[5]. This suggested the potential of bioethanol as an alternative electrical energy source in the prospect of renewable power generation systems particularly for transportation and industrial sectors[5, 6].

As energy needs to grow, there is a pressing need to explore alternative sources of energy that are renewable, environmentally friendly, and economically viable. One such alternative is the utilization of abundant and neglected non-woody lignocellulosic biomass such as leaves. In Malaysia, oil palm plantation activities have generated over 94 million tonnes (Mt) of biomass[7]. Annually, half of that amount about 59 Mt was contributed by oil palm frond (OPF). OPF is composed of two main parts which are the petiole and leaflets. Usually, leaflets, known as oil palm frond leaves (OPFLs) will be discarded and left over without further utilization. It is worth highlighting that as a valuable dry matter, the cellulosic fraction in OPFLs is useful for obtaining bioethanol, whose potential has not yet been explored and studied. Bioethanol derived from lignocellulosic biomass highlights the renewability property of second-generation (2G) bioethanol. The conversion of OPFLs into bioethanol involves multiple processes such as pre-treatments, enzymatic hydrolysis and fermentation processes. The application of pre-treatment is vital to reduce the recalcitrance of leafy biomass by the fractionation of biomass components. In plants, the presence of lignin and hemicellulose influences the cell wall recalcitrance of biomass. This is because lignin is a complex phenolic compound that is covalently bonded to hemicellulose with a strong lignin-carbohydrate complex (LCC) linkage, creating a highly condensed structure of biomass[8]. Thus, a high amount of lignin and hemicellulose will hinder the cellulose that is embedded within the lignocellulose matrix from being susceptible to subsequent saccharification

and fermentation processes. This issue can be overcome by using different approaches of pre-treatments to optimize the digestibility of biomass, thus enhancing the cellulosic fraction by the removal of lignin and hemicellulose fraction[9]. Physicochemical pre-treatments such as autohydrolysis and steam explosion that utilize water as a pre-treatment medium are reported to be highly effective in removing hemicellulose and disrupting the LCC linkage[10, 11]. Meanwhile, chemical pre-treatment such as dilute NaOH pre-treatment is efficient for the delignification of biomass, resulted in higher cellulose content due to the removal of lignin and slight removal of hemicellulose[12]. These pre-treatments were reported to increase the cellulose content of lignocellulosic biomass. The enhancement of the cellulosic fraction of OPFLs biomass is vital to produce a high yield of glucose during enzymatic saccharification. Moreover, the application of pre-treatment also improved the physicochemical properties of biomass in terms of biomass surface area, crystallinity index, and degree of polymerization[13, 14]. Thus, a high availability of cellulose with a high surface area is beneficial to facilitate the reaction between enzyme-substrate complexes, thus increasing the saccharification process and producing higher glucose yield[15]. Eventually, a higher glucose yield can maximize the production of bioethanol during the fermentation process.

1.2 Problem statements

Petroleum-based fuels significantly contribute to total GHG emissions, which navigate the uprising global warming. One alternative to tackle this issue is shifting towards the utilization of renewable energy sources. Notably, the current uprising of electrical-powered vehicles implies the need of variety power sources that are competent to supply electrical power. One such power source is a fuel cell application

such as a direct ethanol fuel cell (DEFC) system. By using bioethanol produced from OPFLs biomass as a fuel source to generate electricity, this particular approach develops a carbon-neutral cycle that has a low impact on the environment. This is because the carbon emission from bioethanol originates from biomass. Thus, it is vital to assess the energy capability of bioethanol produced from OPFLs biomass to supply electrical power, particularly in the DEFC system.

The expansion of oil palm plantations in Malaysia has shown an increasing trend over the years. This is indeed a great advantage for the growth of the economy, however, it also creates a serious environmental issue as only 10 % of oil palm trees contribute to the production of palm oil while 90 % are unconsumed residues. An inefficient disposal of high amounts of oil palm wastes mainly derived from oil palm plantation activities such as milling, pruning and replanting caused environmental issues such as air pollution, and soil contamination[16]. For instance, after the pruning activities, most leaf waste from oil palm trees will be leftover on the ground to naturally degrade over an extended period (3- 5 months). The exposure of the leaf wastes to rainfall will promote microbial growth on the leaf's surface, which later may affect the soil nutrients when they naturally decompose. In addition, the traditional method of disposing of these leaf wastes, such as the burning method was also used. This disposal approach resulted in the release of hazardous smoke that contains a condensed particulate matter that can pollute the environment and also endanger public health. Therefore, a more sustainable approach such as the valorization of oil palm frond leaves wastes (OPFLs) to produce biofuels or bioproducts can be implemented. To the best of our knowledge, a minimal number of studies have been carried out to explore the potential of leaf waste as an alternative feedstock for biofuel production, whereas most published literature focuses on the potential of woody biomass. Moreover, in comparison with other oil palm

biomass, the OPFLs biomass can be classified as underutilized. In view of this, the utilization of OPFLs biomass is the best approach to explore its potential as an alternative cellulosic source for bioethanol production, which has not yet been discovered.

As a lignocellulosic biomass, OPFLs are composed of three valuable components such as cellulose, hemicellulose, and lignin that are present in a compact biomass matrix. In the context of bioethanol production, the cellulose fraction in OPFLs is valuable as it can be converted to bioethanol. However, without pre-treatment the cellulosic fraction in OPFLs cannot easily be assessed as it is guarded by lignin component *via* strong LCC linkage. In other words, a high amount of lignin and hemicellulose are the prominent cause of the high recalcitrance of OPFLs structure that hinders the availability of cellulose for the saccharification and fermentation process. This issue highlights the necessity to disrupt the compact structure of OPFLs biomass by using pre-treatment as a prerequisite step. This step is vital to reduce the cell wall recalcitrance of OPFLs by the removal of non-cellulosic constituents (lignin and hemicellulose) to extract a cellulose-rich OPFLs pre-treated pulp. To date, among the oil palm biomass, much literature has discussed the impact of single pre-treatment on the physicochemical properties of the pre-treated oil palm frond, pre-treated oil palm trunk, and pre-treated oil palm empty fruit bunches. Yet, many types of pre-treatments were available and each of them improved the physicochemical properties of lignocellulosic biomass in unique ways. In other words, the study that focused on various kinds of pre-treatments will have extensive findings to elucidate the traits that were representative of pre-treatments on the different approaches. The traits such as the composition, and physical and chemical properties of the biomass, after each of the pre-treatments will be beneficial to assess the extent of digestibility of OPFLs biomass.

Moreover, the application of various pre-treatments will distinctly reveal the effectiveness of each pre-treatments in improving the subsequent saccharification and fermentation process to maximize the bioethanol yield.

It is reported that the subsequent separate hydrolysis and fermentation processes (SHF) accounted for about 25 % of the total industrial bioprocess cost[17]. At the industrial scale, bioprocessing needs to be operated under controlled condition using optimal hydrolysis and fermentation conditions. This aspect highlights the significance of the optimization of enzymatic hydrolysis and fermentation process to ensure a high yield of the final product, which in this study is bioethanol yield. Enzymatic hydrolysis is the subsequent saccharification process to hydrolyze cellulose-rich pre-treated pulp to obtain glucose solution. The main challenge in enzymatic hydrolysis is its significantly slower process, which usually requires a longer incubation period, ranging from 8 h to 96 h, to achieve complete glucose conversion[18]. To mitigate this issue, a detailed study focusing on the effect of cellulase dosage, substrate dosage and incubation period is prominent to optimize the enzymatic hydrolysis process, to produce a high glucose yield. Similarly for the fermentation process, due to its slower conversion process, the optimization of the fermentation period will be beneficial to maximize the production of bioethanol.

1.3 Research objectives

This current study highlights the impact of various pre-treatments on the physicochemical properties of OPFLs to optimize the saccharification and fermentation processes to maximize bioethanol yield. Also, the utilization of the produced bioethanol as an alternative biofuels for fuel cell application. Thus, the objectives of this current work are as follows:

1. To study effect of various pre-treatments namely autohydrolysis, steam explosion and dilute NaOH pre-treatment at varying parameters and to characterize the obtained pre-treated OPFLs pulp and hydrolysate by using complementary analyses
2. To optimize enzymatic hydrolysis of pre-treated OPFLs pulp at varying parameters and to quantify glucose content by using high-performance liquid chromatography ultraviolet detector (HPLC-UV)
3. To quantify bioethanol content derived from different pre-treated OPFLs pulp after fermentation process by using gas chromatography flame ionization detector (GC-FID)
4. To evaluate energy capability of the produced bioethanol as a bioenergy sources in a commercial direct ethanol fuel cell (DEFC) system *via* electrochemical analyses

1.4 Scope of study

Among the oil palm biomass, the valorization of OPFLs as an alternative cellulosic source for bioethanol feedstock has not yet been explored. To the best of the author's knowledge, there is no present literature that has extensively discussed the impact of pre-treatments by using different approaches on the digestibility of OPFLs to maximize the bioethanol yield. Later, the produced bioethanol was used *via* direct utilization method in fuel cell application as a renewable bioenergy source, which makes this current work relatively new.

In this current work, OPFLs biomass was subjected to different pre-treatment approaches which were environmental-friendly pre-treatments (autohydrolysis and steam explosion) and chemical pre-treatment (dilute NaOH pre-treatment) at varying

parameters. The extent of digestibility of OPFLs biomass after different pre-treatment approaches is evaluated *via* characterizations of the pre-treated OPFLs to elucidate the change in physicochemical properties of the pre-treated OPFLs. The evaluation was based on the change in the chemical composition of the pre-treated OPFLs pulp and the recovered hydrolysate OPFLs, mainly highlighting the enhancement of cellulose content by the removal of lignin and hemicellulose fractions. The improvement in chemical properties of the pre-treated OPFLs pulp was also highlighted by analyzing the change in chemical structure, degree of polymerization, and crystallinity index of the pre-treated OPFLs pulp. In addition, the occurrence of lignin condensation and formation of pseudo-lignin due to the impact of autohydrolysis and steam explosion pre-treatments on the OPFLs biomass is also explained by qualitative analysis *via* 2D-HSQC-NMR analysis. For the improvement in physical properties of the pre-treated OPFLs pulp, surface analyses such as analyzing the change of OPFLs cell wall structure and OPFLs surface area after application of pre-treatments were performed. The characterizations of the pre-treated OPFLs pulp are imperative to determine the key properties of OPFLs as a promising cellulosic source to produce bioethanol.

Moreover, the saccharification of the pre-treated OPFLs pulp under enzymatic hydrolysis at varying parameters was compared with raw OPFLs pulp, by highlighting the effect of substrate dosages (low and high solid loading), cellulase dosages, and incubation periods on the glucose yield and cellulose conversion rate. Then, the effect of fermentation periods was assessed to determine the optimum condition to obtain a high yield of bioethanol from the pre-treated OPFLs. Finally, as a renewable bioenergy source, the produced bioethanol from raw OPFLs and the pre-treated OPFLs was analyzed to determine its energy capability in supplying electricity by using a direct ethanol fuel cell (DEFC) application. The energy capability of the DEFC in the presence

of the produced bioethanol was evaluated by using electrochemical analyses such as open circuit potential (OCP) and electrochemical impedance spectroscopy (EIS).

CHAPTER 2

LITERATURE REVIEW

2.1 Renewable energy

Over the past years, the uprising of global carbon footprint has shown serious implications for our planet, leading to accelerating global warming. Thus, many researchers have been involved in creating a better future for our planet by encouraging the world to shift towards renewable energy. Renewable energy is a form of energy that can be obtained from infinite natural resources. The utilization of this type of resources will greatly reduce our dependency on the exhausted fossil resources which are deleterious to our environment. In 2021, the combustion of fossil fuels caused an increase in greenhouse gasses (GHG) emission by up to 71 % globally[19]. This implies the urgency to replace fossil fuels with renewable energy such as solar, wind and tidal power, geothermal energy, and bioenergy from biomass[20]. Focusing on the utilization of biomass as a bioenergy source, the high availability of different types of biomass offers a sustainable production of bioenergy. For instance, Akbari et al.[21] divulged the potential of biomass derived from agriculture waste (i.e.: corn stover and wheat straw) and forest residues (i.e.: branches, barks and leaves from logging of pine trees) as a raw material for the production of renewable natural gas (RNG) *via* gasification process. The gasification process involves the transformation of organic compounds in biomass into renewable natural gases (i.e.: hydrogen, methane and carbon dioxide) by reacting the biomass with oxygen or steam. The study divulged that the biomass derived from agriculture and forest residues yielded an average of 39 % RNG from the gasification process[21]. Besides, biomass also can be transformed into biofuels, for instance, bioethanol as an alternative fuel to replace petroleum-based fuels. In addition,

bioethanol also can be utilized as a fuel source to supply electricity for power sources such as fuel cell applications[22]. Electricity production by using biomass as a raw material is a key to navigating the world's transition to decarbonization and sustainable bioenergy development that may offer great benefits to society, economic and environmental aspects[20].

2.1.1 Biofuels

Yearly, global energy production and consumption have tremendously increased commensurate with the growth in world population, and growth in economic and industrial sectors. For instance, biofuel is a one of the bioenergy that has been used as an alternative fuel to replace the exhaustible fossil fuels. According to the latest report by the World Bioenergy Association (WBA), about 160 billion tons of biofuels were produced in 2018[23]. From the total production, 62 % was bioethanol followed by biodiesel and cellulosic ethanol which accounted for 26 % and 12 %, respectively. Biofuels are known as versatile fuels that can be derived from food crops, algae, and biomass including agricultural waste, wood residue, animal waste, and food waste. Figure 2.1 illustrates the classification of biofuels and its conversion technologies for the production of different bioenergy[24]. Assorted kind of biofuels can be derived from several types of processes such as thermochemical conversion, biochemical conversion, and transesterification.

Thermochemical approaches such as pyrolysis, gasification, and hydrothermal carbonization are the common methods used to produce bio-coal from renewable sources[25, 26]. Among the methods proposed, pyrolysis and hydrothermal carbonization are the most reported for biomass conversion due to their low environmental impact and ease of operation[26]. For instance, the pyrolysis process involves the heating process of dried lignocellulosic biomass at an elevated temperature

of 450°C-850°C with a heating rate of 1.6 °C/min-20 °C/min[27]. The final products of pyrolysis are biochar/bio-coal (solid form) and bio-oil (liquid form). A past study by Nudri et al.[28] reported the conversion of oil palm trunk (OPT) biomass into bio-coal was attained by using a top-lit updraft reactor at a peak temperature of 550 °C with an airflow rate of 4.36 L/min. The study revealed that the volatile matter and fixed carbon yield of OPT bio-coal decreased from 77.1 % to 41.3 % and increased from 4.2 % to 30.9 %, respectively as compared to raw OPT biomass. The increment of fixed carbon yield of OPT bio-coal suggested that the energy density of OPT bio-coal was also improved which can produce more combustion heat with an extended burning period.

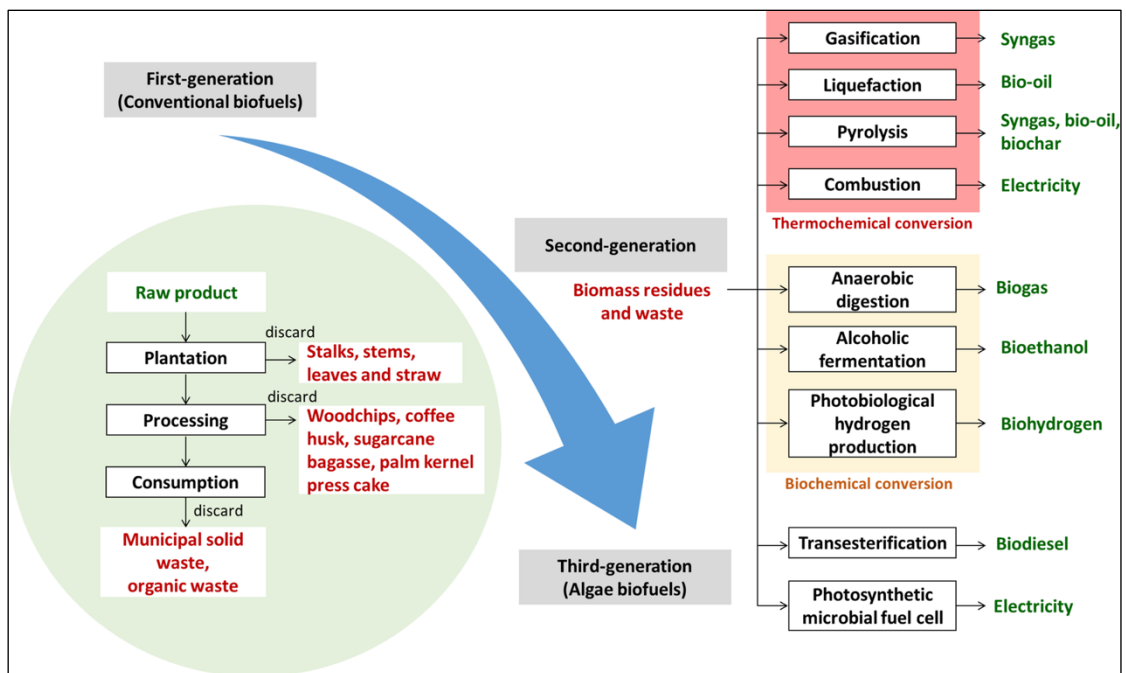


Figure 2.1: Biofuels conversion technologies from different type of feedstock [24]

Besides, biodiesel is another type of biofuel that has been widely used to replace petroleum diesel due to its renewability, low greenhouse gas (GHG) emission and its capability to reduce 78 % of CO₂ emissions in contrast to petroleum diesel[29]. In Asia, Indonesia is the leading producer of biodiesel, amounting to 5600 million L of biodiesel per year, followed by Malaysia, Thailand, and China[30]. In Indonesia, Malaysia and

Thailand, biodiesel is produced from palm oil, whereas China utilize the used cooking oil as a biodiesel feedstock[29]. The most common conversion technology used for biodiesel is the transesterification process which is the chemical reaction between the alkyl group of an ester from feedstock oil (sources: vegetable oil/plant oil/used oil) with methyl group of alcohol such as methanol or ethanol[29, 31]. The transesterification of oil is preferably to be conducted by the presence catalyst to facilitate the esterification reaction. Degfie et al.[32] optimized transesterification process of waste cooking oil to obtain a high yield of biodiesel by using calcium oxide nanocatalyst at varying dosages. The study observed that the incorporation of 0.5 to 1.0 % w/w of nanocatalyst increased the biodiesel yield up to 96 % and further increment in catalyst loading (i.e.;2 to 5 % w/w) showed a decrement pattern in the yield of biodiesel.

Biochemical conversion such as alcoholic fermentation and dark fermentation were employed to produce bioethanol and biohydrogen, respectively. For instance, dark fermentation is a promising option for biohydrogen production which involves the degradation of carbohydrate-rich substrate by anaerobic bacteria in the absence of light[33, 34]. It was reported that acid pre-treated empty fruit bunch oil palm which was composed of 28.30 g/L of total sugars (TS) was successfully fermented to biohydrogen, producing 282.17 mL H₂ g⁻¹ TS of biohydrogen[35]. Biohydrogen production has shown promising outcomes at the laboratory and pilot scale levels. However, some concerns such as complex processes, difficulties in transportation and storage of biohydrogen may cause its production at the industrial scale to be very challenging[34].

In contrast, the production of bioethanol is less complex and its production has steadily emerged over the years. Bioethanol sales accounted for 75.2 % of biofuels sales, accounting for a \$2 billion market value. Globally, the main producer of bioethanol is the United States followed by Brazil. To date, US and Brazil ethanol

production was estimated at 15,620 million gallons and 8,260 million gallons in 2023, respectively[36]. In the context of sources, different types of renewable sources can be utilized to produce bioethanol, thus bioethanol can be categorized into first-generation (1G) bioethanol, second-generation (2G) bioethanol, and third-generation (3G) bioethanol based on the type of feedstock used.

For 1G bioethanol, food crops such as corn, sugarcane, and wheat are the common feedstock used [37]. In the US, corn is a major crop that has been cultivated with a yearly production estimated at approximately 381 billion kg, of which 28 % of total production was used for bioethanol production[37]. Meanwhile, Brazil utilizes about 633.3 Mt of sugarcane to produce 8 million gallons of ethanol[2]. However, 1G bioethanol has been facing a controversial issue of “food-to-fuel” for past years. This was due to the problems with food insecurities and high-cost feedstock of 1G bioethanol because of the utilization of food crops as a main source for the production of 1G bioethanol. Thus, an alternative feedstock which is lignocellulosic biomass namely sugarcane bagasse[38], corn stover[39], oil palm wastes[40], banana leaf waste[41], grasses [42], and wood residues[43] can be used to produce 2G bioethanol. For instance, Brazil has implemented a new approach to boost up its bioethanol production by the inclusion of sugarcane residues as the alternative feedstock for the production of 2G bioethanol[2]. Currently, 100 % of the produced bioethanol in Brazil is a 1G bioethanol obtained from the conversion process of sugarcane juice. However, the extraction process of the juice from the sugarcane stalk produced a vast amount of residues including sugarcane bagasse, which can be used as a substrate to produce 2G bioethanol *via* enzymatic hydrolysis and fermentation process[2].

Besides, algae from the macroalgae[44] and microalgae[45] species have been proposed as promising substrates for the production of 3G bioethanol. However, more

studies must be conducted to optimize the production of 3G bioethanol from algae species. While 2G bioethanol is extensively reaching full-scale commercialization, 3G bioethanol is primarily a forthcoming technology of the future[46]. Among the available feedstocks, lignocellulosic biomass is the most preferable feedstock to produce bioethanol due to its high availability, renewability, and low cost.

2.1.2 Bioethanol from the conversion of lignocellulosic biomass

The conversion technologies are widely available for different types of lignocellulosic biomass to obtain bioethanol[1]. Figure 2.2 illustrates the conversion of biomass into 2G bioethanol. Due to the variation in the recalcitrance of lignocellulosic biomass, the optimizations of the conversion of biomass into bioethanol which include the processes namely pre-treatment, enzymatic hydrolysis and fermentation are varied based on the type of biomass used[13, 15, 47]. Pre-treatment is a pre-requisite step to disrupt the complex structure of biomass by removing the non-cellulosic component (i.e.; lignin and hemicellulose). Then, the glucose solution can be obtained from the enzymatic saccharification of the pre-treated biomass pulp, which glucose can be fermented into ethanol *via* a fermentation process.

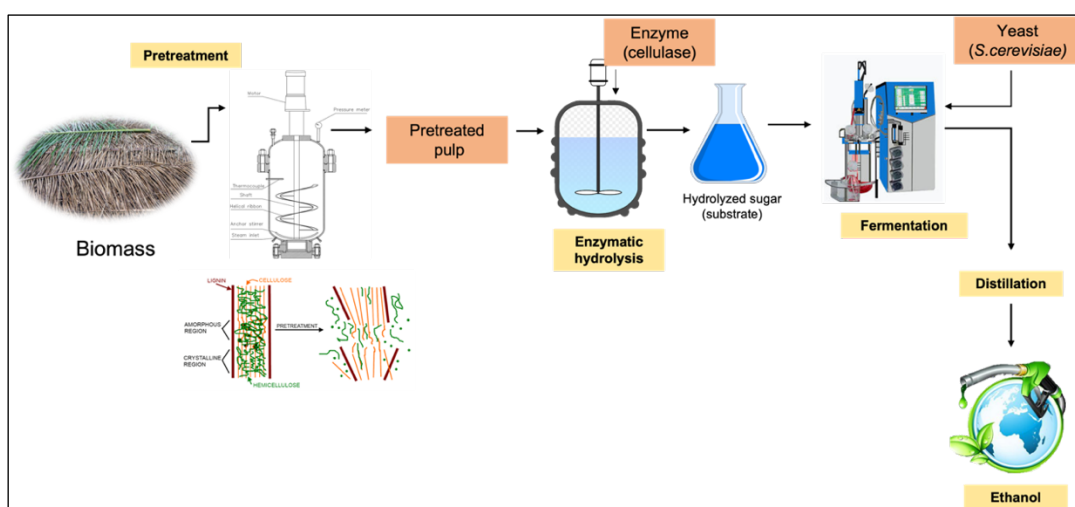


Figure 2.2: Conversion of lignocellulosic biomass into 2G bioethanol

Generally, fermentation is an anaerobic process to break down monomers mainly glucose by the action of microorganisms into ethanol and carbon dioxide as by-products. It can be carried out by a variety of yeast under mild conditions of 30-37 °C for 24h-96 h[48, 49]. The most common yeast is *Saccharomyces cerevisiae* which efficiently convert glucose (C₆ sugar) to ethanol. However, this type of yeast is unable to metabolize pentose sugar (e.g.; xylose). The conversion of xylose to ethanol is possible by another type of yeast namely *Pichia stipites* [50]. Fermentation of C₅ sugar is also possible by using engineered *Saccharomyces cerevisiae*. The modification of commercial *Saccharomyces cerevisiae* is carried out by introducing xylose-utilizing yeast, bacteria, and fungi to construct a xylose-fermenting *S. cerevisiae* strain[51]. This type of strain has shown promising findings that exhibited a high yield of ethanol (i.e.;0.44 g/g) from a mixture of glucose, xylose, and cellobiose hydrolysate[52].

Fermentation process can be performed either simultaneously or sequentially with enzymatic hydrolysis which is known as simultaneous saccharification and fermentation (SSF) or separate hydrolysis and fermentation (SHF), respectively[1]. In view of that, the optimization of fermentation parameters such as substrate loading, concentration of yeast, fermentation period, and temperature by using different types of biomass have been reported by many studies as tabulated in Table 2.1. The optimization of fermentation parameters is vital to maximize the production of bioethanol.

Table 2.1: Optimization of conversion process of various lignocellulosic biomass into bioethanol

Biomass	Conversion process		Bioethanol yield	Ref
	Pre-treatment	Hydrolysis and Fermentation		
Sugarcane leaves	Hydrothermal and dilute NaOH pre-treatment	SHF: Enzymatic hydrolysis Substrate dosage: 10 %w/v Cellulase dosage: 10 FPU/g T: 50 °C t: 72 h Fermentation S. cerevisiae dosage: 10 %v/v T: 30 °C , t: 2 h-24 h	- 28.97 g L ⁻¹ after 18 h of fermentation - 92.86 % fermentation efficiency	[53]
Pineapple leaves	Hydrothermal	SHF: Enzymatic hydrolysis Substrate dosage: 5 %w/v Cellulase dosage: 10 FPU/g T: 50 °C t: 72 h Fermentation S. cerevisiae dosage: 10 %v/v T: 30 °C , t: 24 h-72 h	- 9.0 g L ⁻¹ after 72 h of fermentation -94.69% fermentation efficiency	[54]
Banana leaves	Dilute H ₂ SO ₄ pre-treatment	SHF: Enzymatic hydrolysis Substrate dosage: 2-6 %w/v Cellulase dosage: 15 FPU/g T: 45 °C t: 70 h Fermentation S. cerevisiae dosage: 5 %v/v T: 30 °C , t: 6 h-30 h	- 15.43 g L ⁻¹ after 12 h of fermentation - ethanol yield decreased after 12 h	[41]
<i>E. grandis</i> sawdust	NaOH impregnation and Steam explosion	SHF: Enzymatic hydrolysis Substrate dosage: 27 %w/w Cellulase dosage: 25 FPU/g T: 50 °C t: 48 h Fermentation (SHF) S. cerevisiae dosage: 5 %v/v T: 30 °C , t: 24 h SSF: Substrate dosage: 27 %w/w Cellulase dosage: 25 FPU/g S. cerevisiae dosage: 5 %v/v T: 30 °C t: 48 h	SHF: - 71.80 g L ⁻¹ after 36 h of fermentation - 78 % fermentation efficiency SSF: - 75.60 g L ⁻¹ after 30 h of fermentation - 83 % fermentation efficiency	[55]

Oil palm trunk (OPT)	Steam explosion and NaOH pre-treatment	SSF: Substrate dosage: 10 %w/v Cellulase dosage: 15 FPU/g S. cerevisiae dosage: 10 %v/v T: 40-45 °C , t: 96 h	- 44.25 g L ⁻¹ [56] after 96 h of fermentation - 90.30 % fermentation efficiency
Oil palm empty fruit bunches (OPEFB)	H ₂ O ₂ pre-treatment	SHF: Acid hydrolysis Substrate dosage: N/A H ₂ SO ₄ dosage: 10 % v/v T: 100 °C t: 60 min Fermentation S. cerevisiae dosage: 4, 6 & 8 g L ⁻¹ T: 30 °C , t: 24 h-120 h	- 41.41 g L ⁻¹ [57] after 96 h of fermentation by using 6 g L ⁻¹ of yeast - ethanol yield decreased after 96 h
Oil palm frond (OPF)	Biological pre-treatment	SHF: Enzymatic hydrolysis Substrate dosage: 6 %w/v Cellulase dosage: 40 U/g T: 50 °C t: 150 min Fermentation S. cerevisiae dosage: N/A T: 30 °C , t: 24 h-60 h	- 7.23 g L ⁻¹ [40] after 24 h of fermentation -79.40 % fermentation efficiency

From the agricultural residues, a few studies have explored the potential of leafy biomass such as banana leaves[41], sugarcane leaves[53] and pineapple leaves[54] as a substrate in the fermentation process to produce bioethanol. Moodley et al.[53] reported that about 28.97 g L⁻¹ of bioethanol is yielded from the pre-treated sugarcane leaves after being subjected to the SHF process. The study conducted the enzymatic saccharification of 10 %w/v of pre-treated sugarcane leaves by using 10 FPU/g cellulase at 50 °C for 72 h, producing 60 g L⁻¹ glucose. Then, the subsequent fermentation process was performed at 30 °C for 24 h by introducing 10 % v/v of *S. cerevisiae* into the fermentation medium. At similar fermentation conditions with a longer fermentation period, Saini et al.[54] divulged that approximately 9.06 g L⁻¹ of bioethanol was attained after 72 h of the fermentation process for pre-treated pineapple leaves, amounting to 94

% of fermentation efficiency. Meanwhile, Suhag et al.[41] studied the optimization of the SHF process for banana leaves waste at varying substrate loading (i.e.; 2 – 6 % w/v), and fermentation periods (i.e.; 6 h – 30 h). The study revealed that the highest ethanol concentration (i.e.; 15.43 g L⁻¹) was attained when 2 % w/v of banana leaves was subjected to a fermentation process for 12 h. The gradual depletion of bioethanol yield was observed when the fermentation period was extended to 30 h. This phenomenon was also observed by Tenkolu et al.[49], who suggested the increase of the fermentation period beyond the limit of the conversion process of glucose into ethanol led to ethanol toxicity. A high concentration of bioethanol produced at an extended fermentation period will become a toxic by-product in the fermentation medium which will initiate a death phase of yeast cells, resulting in the reduction of bioethanol yield[47].

From the forestry residues, *E.grandis* sawdust can be used as an alternative substrate for the production of bioethanol. Rochon et al.[55] discussed the fermentation efficiency of pre-treated sawdust by using SHF and SSF processes. The SHF process is conducted by hydrolysis of 27 % w/w of sawdust with 25 FPU/g cellulase at 50 °C for 48 h, producing 150 g L⁻¹ of glucose, which later was subjected to the fermentation process with 5 %v/v of *S.cerevisiae* (Baker's yeast) at 30 °C for 24 h. Meanwhile, the SSF process is performed by mixing 27 % w/w of sawdust with 25 FPU/g cellulase and 5 %v/v of *S.cerevisiae*, and the saccharification and fermentation process is conducted simultaneously at 30 °C for 48 h. The study concluded that the SSF process has higher fermentation efficiency compared to the SHF process, with bioethanol yield of 71.8 g L⁻¹ and 75.6 g L⁻¹ attained *via* the SHF and the SSF process, respectively.

2.1.3 Bioethanol from oil palm biomass

Among the oil palm biomass, the production of bioethanol from oil palm trunks[58], oil palm empty fruit bunches[59], and oil palm fronds[40, 60] have been studied and reported. For instance, Tareen et al.[56] utilized pre-treated oil palm trunk (OPT) biomass as a substrate in the SSF process *via* saccharification and fermentation process with 15 FPU/g cellulase and 10 %v/v *S. cerevisiae*, respectively by using OPT pulp at a solid loading of 10 % w/v. The SSF process was conducted for 96 h at a temperature range of 40 °C - 50 °C. For the SSF process, determination of optimum incubation temperature is vital as both enzymatic hydrolysis and fermentation processes are conducted at the same time. Commonly, enzymatic hydrolysis and fermentation processes are conducted at 50 °C and 30 °C, respectively, thus an optimum condition must be determined for an enzyme and yeast to be able to function simultaneously during the SSF process. In view of that, Tareen et al.[56] concluded that the optimum temperature for the conversion of OPT into bioethanol *via* the SSF process was 45 °C, producing the highest bioethanol yield of 44.25 g L⁻¹. The study also divulged that the lowest bioethanol yield is attained at 50 °C in contrast to 40 °C and 45 °C. This was due to the depletion of yeast cells at an elevated temperature, at which the best growth of yeast cell was at 30 °C -36 °C. This finding highlights one of the drawbacks of the SSF process compared to the SHF process.

The conversion of oil palm empty fruit bunches (OPEFB) was reported by Siregar et al.[57], who compared the bioethanol yield from OPEFB at varying yeast concentrations and different fermentation periods *via* the SHF process. The pre-treated OPEFB pulp was hydrolyzed to obtain a glucose solution, which was then fermented at 30 °C for 24 h to 120 h by using different concentrations of *S. cerevisiae* at 4 g L⁻¹, 6 g L⁻¹, and 8 g L⁻¹. The study observed the increment of bioethanol yield as the yeast

concentration increased from 4 g L⁻¹ to 6 g L⁻¹, however at a high concentration of 8 g L⁻¹, the bioethanol yield started to decrease gradually. In the context of incubation temperature, the maximum bioethanol (i.e.; 41.41 g L⁻¹) was attained at 96 h, and increasing the fermentation period up to 120 h led to the gradual decrease in bioethanol yield. The study proposed that at an extended fermentation period (i.e.; beyond 96 h), the low availability of glucose in the presence of a high amount of yeast cells from the high concentration (i.e.; 8 g L⁻¹) of *S. cerevisiae*, led to the instability in the conversion process of glucose to ethanol, thus caused bioethanol production to decline.

Besides, past studies have proposed oil palm fronds (OPF) as an alternative substrate for the production of bioethanol. Abdul Halim et al.[40], subjected 6 % w/v of pre-treated OPF pulp (i.e.; petiole part without leaves) to the SHF process, where the saccharification was conducted by using cellulase at 50 °C for 150 min, followed by a fermentation process by *S. cerevisiae* at 30 °C for 24 to 60 h. The study disclosed that the bioethanol yield was increased from 4.0 g L⁻¹ to 7.23 g L⁻¹ as the fermentation process proceeded from 10 h to 24 h, then the yield was observed to decrease as the time was extended to 60 h. The highest fermentation efficiency (i.e.; 79.40 %) was attained at 24 h. Meanwhile, the juice extracted from OPF (i.e.; petiole part without leaves) can be directly fermented into bioethanol by using *S.cerevisiae* as reported by Zani et al.[60]. The study divulged that the pH of the fermentation medium and incubation temperature affected the bioethanol yield. The fermentation process was conducted at 27 °C to 37 °C and the pH of the fermentation medium was adjusted from pH 5 – pH 9. About 19 g L⁻¹ of bioethanol was attained at pH 5 and the bioethanol yield was slightly increased to 22 g L⁻¹ at pH 7. The effect of temperature showed that the production of bioethanol from OPF juice steadily increased as temperature was increased from 27 °C to 32 °C, where above 32 °C bioethanol yield was observed to

gradually decrease. In summary, the optimization study suggested that the optimum condition of the fermentation process of OPF juice was at 32 °C and pH 7.

2.1.4 Analysis of bioethanol *via* Gas Chromatography-Flame Ionization Detector (GC-FID)

The most common method used to analyze and quantify bioethanol content after the fermentation process is by using gas chromatography (GC) as it offers fast, precise and reliable analysis[61]. The separation of analytes such as ethanol and methanol is preferable to be performed by using GC coupled with a flame ionization detector (FID) due to the high sensitivity of the FID detector for volatile compounds[61, 62]. In food analysis, Paolini et al.[62], divulged that the determination of volatile compounds in alcoholic beverages (i.e.: wine and spirit drinks) were attained by direct injection of beverage samples into the GC-FID system. The study revealed that about 17 compounds was successfully separated with 7 min of running time by using CP-wax 57 CB column (25 m x 0.25 m x 0.25 μ m), where ethanol exhibited the highest peak intensity indicating that ethanol is the main component in the beverage samples.

While direct injection of the sample into the GC-FID system is possible for the food samples, but for the fermentation broth is not highly recommended due to the high risk of contamination to the column and the possibility of harm to the detector[63]. This was due to the presence of adequate amounts of sugar and protein compounds in the fermentation broth. To overcome this issue, a sample preparation must be done to separate ethanol from the undesirable compounds which can be achieved by using organic extraction[63, 64]. An organic extraction employs the extraction of ethanol from the aqueous solution by using an organic extractant such as ethyl acetate with the addition of an internal standard such as butanol[63]. As reported by Grechman et

al.[63], the organic extraction of fermentation broth resulted in a mixture of the top organic layer and aqueous phase at the bottom layer.

The top organic layer containing ethanol was injected into the GC-FID system and the peak separation between ethanol (EtOH), ethyl acetate (EtOAc) and butanol (*n*-BuOH) analytes was attained by using Restec MXT-1 column (60 m x 0.53 mm, 5 μ m) as shown in Figure 2.3. Apart from ethyl acetate, other organic extractant such as diethyl ether and chloroform also can be used for the extraction of ethanol from the fermentation broth[64]. The selection of organic extractant and internal standard that have low volatility and low solubility in water is vital to ensure an efficient extraction process and to obtain a good peak resolution and separation between analytes and internal standard[63].

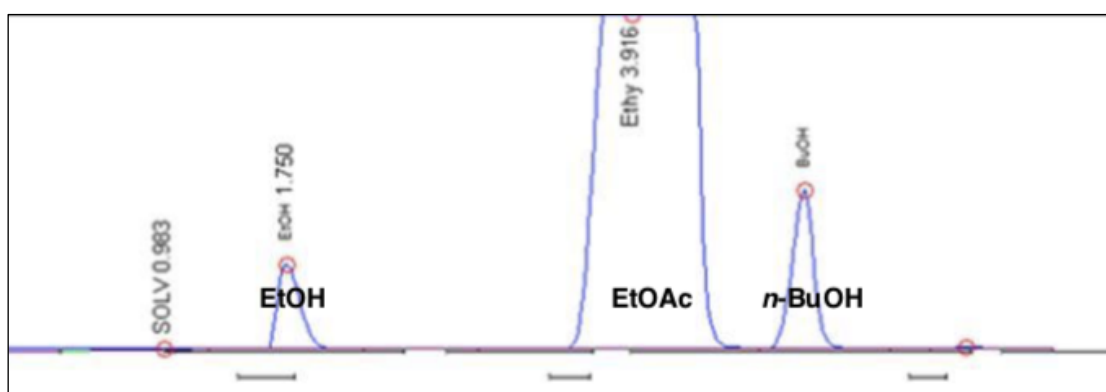


Figure 2.3: GC-FID chromatogram of the extracted organic layer from the fermentation broth showing three apparent peaks for ethanol (EtOH), ethyl acetate (EtOAc) and butanol (*n*- BuOH) [63]

2.2 Fuel cells

A fuel cell is a versatile energy conversion device that converts chemical energy to electrical current to generate energy in form of electricity[65]. To date, the fuel cell system is considered an uprising future technology capable of supplying electricity to power homes and vehicles. Generally, fuel cell can be classified into various kinds

according to the type of reactant used and the kind of electrolyte employed[4]. Currently, widely available fuel cells are solid oxide fuel cell, alkaline fuel cell, microbial fuel cell and direct alcohol fuel cell[66, 67]. The basic concept of fuel cell system in generating electricity involve the occurrence of redox reaction at the cathode and anode electrodes, where the reaction release electrons and these electrons travel through the external circuit generating current to the cell.

For instance, a solid oxide fuel cell is widely used in the industrial sector as it usually operates at a high temperature conditions of 500-1200 °C by employing solid electrolyte mainly made from non-porous ceramic materials[68]. Solid oxide fuel exhibits about 60 % fuel efficiency in converting fuel to electricity. Moreover, due to its operation at high-temperature conditions, heat is produced as a by-product that can be used for the co-generation of electricity, thus producing a total of 85 % fuel efficiency[66]. In 2021, a solid oxide electrolysis stack was developed by the National Aeronautics and Space Administration (NASA) research team and was implemented in the Mars Oxygen In-Situ Resource Utilization (ISRU) on the Perseverance rover for the Mars exploration investigation[69]. NASA has extensively used solid oxide fuel technology to support ISRU processes such as the generation of H₂ and O₂, and to also provide power emergencies for limited times[70]. Also, Nissan motor corporation recently have developed a trial stationary electrical power generation by using solid oxide fuel cell application[6]. The developed solid oxide fuel cell stack is paired with a reformer that converts hydrogen derived from bioethanol into electricity.

Meanwhile, the alkaline fuel cell system is one of the first types of fuel cell technology used in space exploration programs by NASA for the generation of electrical energy and to produce water on board spacecraft[66]. Alkaline fuel cell utilize alkaline solution such as NaOH and KOH as an electrolyte. Oxygen is reduced at the cathode