PRE-TREATMENT OF OIL PALM FRONDS BY OZONOLYSIS FOR LEVULINIC ACID PRODUCTION

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DEDICATION

To

Future ME-Be proud of your choice. Take a challenge. It's kind of satisfaction to do the impossible to be possible

Family -A big…big …Thank you. Without you, all the journey would sadly end.

Reader-let's share our knowledge and strike for a better future.

Supporter-Thank you, Thank you, and Thank you

Colleague-Every journey has a destination. Take a rest and start a new journey with a brave heart.

RJP090619WOJ always in my heart

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ABSTRACT

Oil palm fronds (OPF) is an attractive feedstock for levulinic acid (LA) production due to its availability and high content of cellulose, but requires a pre-treatment because of its heterogeneity structure. This study explored the potential of ozonolysis pre-treatment of OPF for LA production. The suitable design of experiment and response surface methodology (RSM) by Statistica software *ver.* 8.0 was employed to study the effect of process parameter and to determine the optimum condition. The lignin degradation and total reducing sugar (TRS) recovery were set as responses while ozonolysis pre-treatment condition i.e. particle size, moisture content, ozone flowrate, reaction time, ozone concentration and part of OPF were set as independent parameters. The multi-response optimization of lignin degradation and TRS recovery for ozonolysis pre-treatment were verified by Box-Behnken design with four selected independent process parameters. The physico-chemical properties of OPF and treated OPF (ODT) were analysed by thermal gravimetric analysis (TGA), Fourier transform infra-red (FTIR), X-ray diffractogram (XRD), N2-adsorption, scanning electron microscopy (SEM) and field emission scanning electron microscopy with energy dispersive X-ray spectroscopy (FESEM-EDX). LA was produced by conventional acid hydrolysis. An optimal region of study for lignin degradation was recommended at 25–40 wt.% moisture content, particle size bigger than 0.6 mm and ozone flow rate faster than 70 mL/min within 60 min. The TRS recovery is independent of lignin degradation. 75.8 % of TRS recovery of ODT was attained at 0.63 mm, 30 wt.%, and 60 mL/min compared to 46.7 % of OPF. FESEM and SEM depicted that the cell wall of OPF was broken, exposing the microfibril and cellulose rosette structure during the pre-treatment. Rising crystallinity index from 36.1 % (OPF) to 44.7% (ODT) from XRD confirmed the removal of amorphous lignin and hemicellulose component as shown by TGA and FTIR analyses. The decreasing surface area does not hinder the subsequence hydrolysis reaction; reducing crystal size up to 60.5%, and increasing pore diameter and volume gave advantage for the reaction. The particle size-moisture content interaction is important for lignin degradation while the moisture content-reaction time interaction is crucial for the TRS recovery. Larger OPF particle size increases lignin degradation and TRS recovery due to interfacial surface tension. The reaction and mass transfer in water film was controlled by moisture content and reaction time. The optimum lignin degradation (84.7 wt.%) and TRS recovery (99.9 %) were reached at 0.8 mm particle size, 40 wt.% moisture content, 75 min reaction time and 105 mL/min ozone flow rate with 19.5 % ozone consumption. The LA yield of ODT at 180 °C for 1 h and 4 wt. % H_2SO_4 increased up to 4.72 times than OPF and comparable to commercial microcrystalline cellulose. 8.7 wt.% of LA recovery was attained by ozonolysis pre-treatment. The findings from this study provide the insight background of the ozonolysis pre-treatment of OPF for the further stage of commercialization that could contribute to Malaysia's economy.

ABSTRAK

Pelepah kelapa sawit (OPF) adalah bahan mentah yang sangat menarik untuk penghasilan asid levulinik (LA) kerana ketersediaan dan kandungan selulosanya yang tinggi, tetapi memerlukan pra-rawatan disebabkan strukturnya yang heterogen. Kajian ini meneroka potensi pra-rawatan ozonolisis ke atas OPF untuk penghasilan LA. Reka bentuk eksperimen yang sesuai dan kaedah permukaan sambutan (RSM) oleh perisian Statistica *ver.* 8.0 digunakan untuk mengkaji pengaruh parameter proses dan untuk menentukan keadaan optimum. Kemerosotan lignin dan perolehan jumlah gula (TRS) ditetapkan sebagai sambutan manakala keadaan pra-rawatan ozonolisis iaitu saiz zarah, kandungan lembapan, kadar aliran ozon, masa tindak balas, kepekatan ozon dan bahagian OPF ditetapkan sebagai parameter bebas. Pengoptimuman multi-sambutan kemerosotan lignin dan perolehan TRS untuk pra-rawatan ozonolisis disahkan oleh reka bentuk Box-Behnken dengan empat parameter proses bebas yang dipilih. Sifat-sifat fiziko-kimia OPF dan OPF terawat (ODT) diteliti oleh analisis gravimetri terma (TGA), sinaran infra-merah jelmaan Fourier (FTIR), pembelauan sinar-X (XRD), penjerapan N_2, mikroskop elektron imbasan (SEM) dan medan pelepasan mikroskop elektron imbasan dengan spektroskopi penyebaran tenaga sinar-X (FESEM-EDX). LA dihasilkan melalui proses hidrolisis asid konvensional. Kawasan kajian optimum untuk kemerosotan lignin disarankan pada kandungan lembapan 25-40 %, saiz zarah lebih besar daripada 0.6 mm dan kadar aliran ozon lebih cepat daripada 70 mL/min dalam masa 60 minit. Perolehan TRS tidak bergantung kepada kemerosotan lignin. 75.8 % perolehan TRS untuk ODT dicapai pada 0.63 mm, 30% kandungan lembapan, dan 60 mL / min berbanding 46.7% untuk OPF. FESEM dan SEM menggambarkan dinding sel OPF pecah dan mendedahkan struktur mikrofibril dan roset selulosa semasa pra-rawatan. Peningkatan indeks kristaliniti dari 36.1% (OPF) kepada 44.7% (ODT) daripada analisis XRD mengesahkan penyingkiran komponen lignin dan hemiselulosa amorfus yang ditunjukkan oleh analisis TGA dan FTIR. Penurunan luas permukaan tidak menghalang tindak balas hidrolisis seterusnya; pengurangan saiz kristal hingga 60.5%, dan peningkatan diameter dan isipadu liang memberi kelebihan kepada tindak balas. Interaksi kandungan lembapan zarah penting untuk kemerosotan lignin manakala interaksi masa tindak balas kandungan lembapan–masa sangat penting untuk perolehan TRS. Saiz partikel OPF yang lebih besar meningkatkan kemerosotan lignin dan perolehan TRS kerana ketegangan permukaan antara muka. Tindak balas dan pemindahan jisim dalam lapisan air dikawal oleh kandungan lembapan dan masa. Kemerosotan lignin yang optimum (84.7% wt.) dan perolehan TRS (99.9%) dicapai pada ukuran zarah 0.8 mm, 40% kandungan lembapan, masa reaksi 75 minit dan kadar aliran ozon 105 mL / min dengan penggunaan ozon 19.5%. Hasil LA daripada ODT pada suhu 180 °C selama 1 jam dan 4 wt. % H_2SO_4 meningkat hingga 4.72 kali daripada OPF dan setanding dengan selulosa mikrokristal komersial. 8.7% perolehan LA dicapai dengan pra-rawatan ozonolisis. Dapatan daripada kajian ini memberikan pemahaman tentang latar belakang pra-rawatan ozonolisis OPF untuk tahap pengkomersialan selanjutnya yang menyumbang kepada ekonomi Malaysia.

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LIST OF ABBREVIATIONS

5-HMF	-	5-hydroxymethyl furfural
AFEX	-	Ammonia fiber explosion
AIL	-	Acid-insoluble lignin
AMIMCl	-	1-allyl-3-methylimidazolium chloride
ANOVA	-	Analysis of variance
ASL	-	Acid soluble lignin
ATR	-	Attenuated total reflection
BBD	-	Box behken design
BET	-	Brunauer-Emmett-Taylor
BMIMCl	-	1-butyl-3-methylimidazolium chloride
$Ca(OH)_2$	-	Calcium hydroxide
CBG	-	Coastal bermuda grass
CFD	-	Computational fluid dynamics
C-NMR	-	Carbon-13 nuclear magnetic resonance
CO_2	-	Carbon dioxide
CPO	-	Crude palm oil
CrI	-	Crystallinity index
CSTR	-	Continuous stir tank reactor
DA	-	Dilute acid
DF	-	Dilution factor
DI	-	Deionized
DNS	-	3,5-dinitro benzoic acid
DOE	-	Design of experiment
DP	-	Degree of polymerization

DW	-	Distill water
EDX	-	Energy dispersive X-ray spectroscopy
EFB	-	Empty fruit bunch
EU	-	European
FD	-	Factorial design
FELCRA	-	Federal land consolidation and rehabilitation authority
FELDA	-	Federal land development authority
FESEM	-	Field emission scanning electron microscopy
FFB	-	Fresh fruit bunch
FFD	-	Fractional factorial design
FTIR	-	Fourier transform infrared spectroscopy
GVL	-	γ-valerolactone
H_2O_2	-	Hydrogen peroxides
H_2SO_4	-	Acid sulphuric
HPLC	-	High performance liquid chromatography
IL	-	Ionic liquid
IR	-	Infrared
K	-	Kappa number
KBr	-	Potassium bromide
KESEDAR	-	South Kelantan Development Authority
KI	-	Potassium iodide
$KMnO_4$	-	Potassium permanganate
KOH,	-	Potassium hydroxide
LA	-	Levulinic acid
LAP	-	Laboratory analytical procedure

MDF	-	Medium density fibre board
MF	-	Mesocarp fiber
MIGHT	-	Malaysian Industry-Government Group for High Technology
MPOB	-	Malaysian palm oil board
MT	-	Metric tonnes
$NaClO_2$	-	Sodium Chlorite
NaOH	-	Sodium hydroxide
NREL	-	National Renewable Energy Laboratory
OD	-	Oven-dried
O_3	-	Ozone
OA	-	Orthogonal array
OBU	-	Ultrasound irradiation
ODR	-	Oven-dried residue
ODT	-	Oven-dried treated OPF
VAT	-	One factor at a time
OPF	-	Oil palm frond
OPT	-	Oil palm trunks
PBR	-	Packed bed reactor
PKS	-	Palm kernel shell
POME	-	Palm oil mill effluent
R^2	-	*R*-square
RISDA	-	Rubber industry and smallholder development authority
RSM	-	Response surface methodology
SEM	-	Scanning electron microscopy
SO2	-	Sulphur dioxide
SS	-	Sum of square
SSA	-	Specific surface area

SSCF	-	Simultaneous saccharification and co-fermentation
SSE	-	Sum of square residue
SSF	-	Simultaneous saccharification and fermentation
SSR	-	Sum square of the regression
TAPPI	-	Technical Association of the pulp and paper industry
TEM	-	Transmission Electron Microscopy
TGA	-	Thermal gravimetric analysis
TRS	-	Total reducing sugar
TRS	-	Total reducing sugar
USA	-	United State America
UV	-	Ultraviolet
WDM	-	Wet disk milling
WRV	-	Water retention value
XRD	-	X-ray diffraction

LIST OF UNITS AND SYMBOLS

%	-	Percentage
$m^2 m^{-3}$	-	Square meter Per cubic meter
B_{hkl}	-	Full width half maximum (FWHM)
D_{hkl}	-	Size of crystallite
A_c	-	Spectral intensities of the filtrate
A_o	-	Spectral intensities of blank sample
Atm	-	Atmosphere
C	-	Celsius
cm	-	Centimeter
cm^{-1}	-	per centimeter
cm^3/g	-	Cubic centimeter per gram
FPU	-	Filter paper unit
G	-	Gram
g/L	-	Gram per litre
H	-	Hour
I_{002}	-	Scattered intensity at the main peak
I_{am}	-	Scattered intensity due to the amorphous portion
K	-	Scherrer constant
Kg	-	Kilogram
$kg\ m^{-3}$	-	Kilogram per cubic meter
$m\ s^{-1}$	-	Meter per second
m^2/g	-	Square meter per gram
Min	-	Minute
mL	-	Millilitre
mL/min	-	Millilitre per min

mm	-	Millimeter
mol/L	-	Mol per liter
mol/mol	-	Mol per mol
MT	-	Metric tonnes
nm	-	Nanometer
RPM	-	Revolution per minute
u_g	-	Gas velocity
v/v	-	Volume per volume
W	-	Weight
w/w	-	Weight per weight
wt.%	-	Weight percentage
B	-	Beta
Θ	-	Bragg angle
Λ	-	X-ray wavelength
α	-	Alpha

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CHAPTER 1

INTRODUCTION

1.1 Background of Research

The total plantation area in Malaysia for each state in 2016 is pictured in Figure 1.1. The oil palm plantation area in Malaysia reached 5.74 M Ha with 453 mills (Din, 2017a; Leong, 2015). The largest oil palm planted area is located in Sabah (1.55 M Ha, 27 %) with followed closely by Sarawak (1.51 M Ha, 26 %). The remaining 11 states located in Peninsular Malaysia contributes to 47 % (Din, 2017b). In 2018, the plantation area has been increased up to 5.849 M Ha planted area with 2.7 M Ha in peninsula Malaysia and 3.13 M Ha in the Borneo state of Sabah and Sarawak, leave 0.651 M Ha to be explored without exploring new permanent forest areas or peatland (Tan and Ho, 2019). The main product from the palm oil industry is crude palm oil (CPO) by exploited 10 % of palm oil tree (Loh, 2017). The CPO has been exported to India, China, European Union (EU), Pakistan, Turkey, Philippines, and United State of American (USA) as Malaysia being largest exporter. The exportation of CPO has ranking the Malaysia as second contributed to Malaysia economy (Ferdous Alam *et al.*, 2015; Din, 2017b). While the remaining 90 % settle as the oil palm waste (OPW) which is categorized as lignocellulosic biomass. The fully commercialized the OPW for high value product could contribute up to RM 30 billion gross net income (GNI) for Malaysia (Aziz, 2015; Agensi Inovasi Malaysia, 2013; Agensi Inovasi Malaysia, 2011).

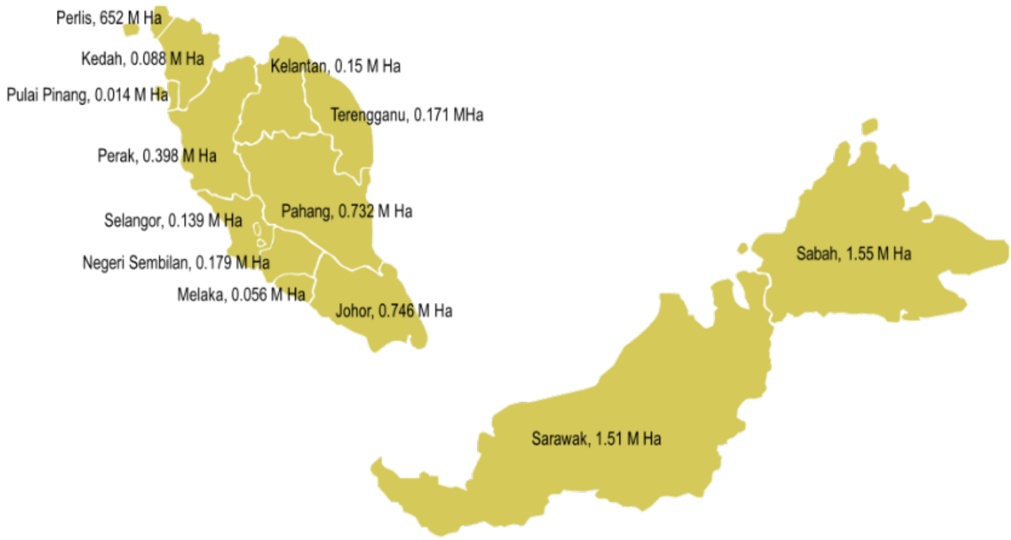

Figure 1.1 Oil palm planted area by state (Adapted from Din, 2017a)

The OPW is categorized as lignocellulosic biomass since it consists of three major components: lignin, hemicelluloses and cellulose. Figure 1.2 illustrates the composition of each component in OPW, respectively. Among of OPW, the OPF contains a fair amount of cellulose, hemicellulose and lignin that makes is attractive to be a feedstock for biorefinery (Awalludin *et al.*, 2015; Lai and Idris, 2013; Kumneadklang *et al.*, 2019; Loh, 2017).

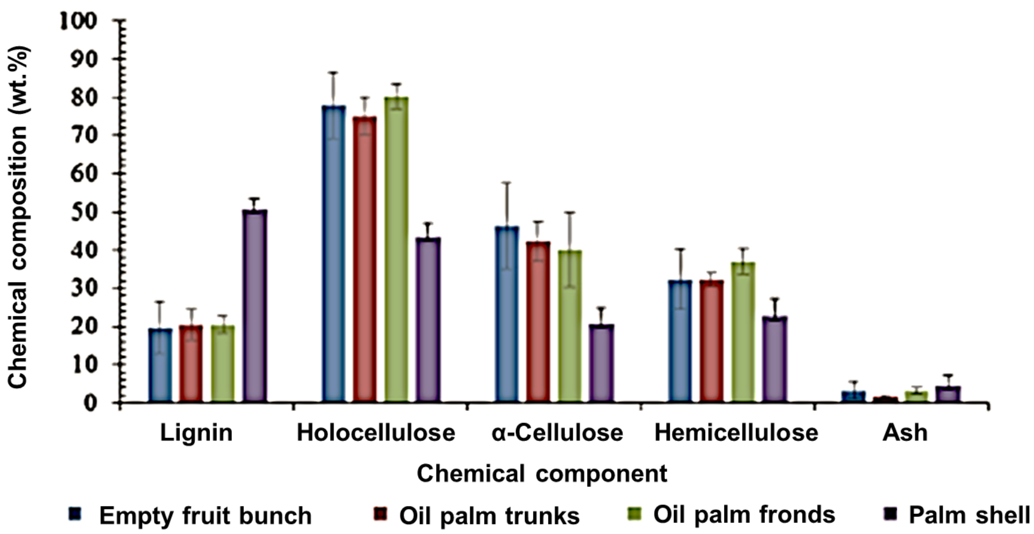

Figure 1.2 The fraction of lignocellulosic component in palm oil biomass (Reproduced from Loh, 2017)

Furthermore, OPF is identified as the largest biomass produced in Malaysia as illustrated in Figure 1.3. However, the OPF has been reported to be left to rot or burn in the plantation for the soil nutrient conservation (Aliyu *et al.*, 2015; Agensi Inovasi Malaysia, 2013; Awalludin *et al.*, 2015; Loh, 2017). These practices could contribute to an environmental and public health issue in the form of haze as has happened in 2015 (France-Presse, 2016; Nash, 2015; Ferdous Alam *et al.*, 2015). Therefore, utilization of the OPF for potentially high-value biomass–product in the downstream process is demanded. The OPF pellets, bio-alcohol, syngas, industrial sugar or chemicals, organic compost, biochar, and phytochemicals have been identified as the potential biomass-product from the OPF (MIGHT, 2013). However, optimizing the OPF as a raw material is hurdled by mobilization, competition for the other applications and the development of the technology (Agensi Inovasi Malaysia, 2013; Agensi Inovasi Malaysia, 2011; Aziz, n.d.; MIGHT, 2013). Instead of these factors, the biomass constituents are a major factor in the selection of profitable technology for commercialization purposes.

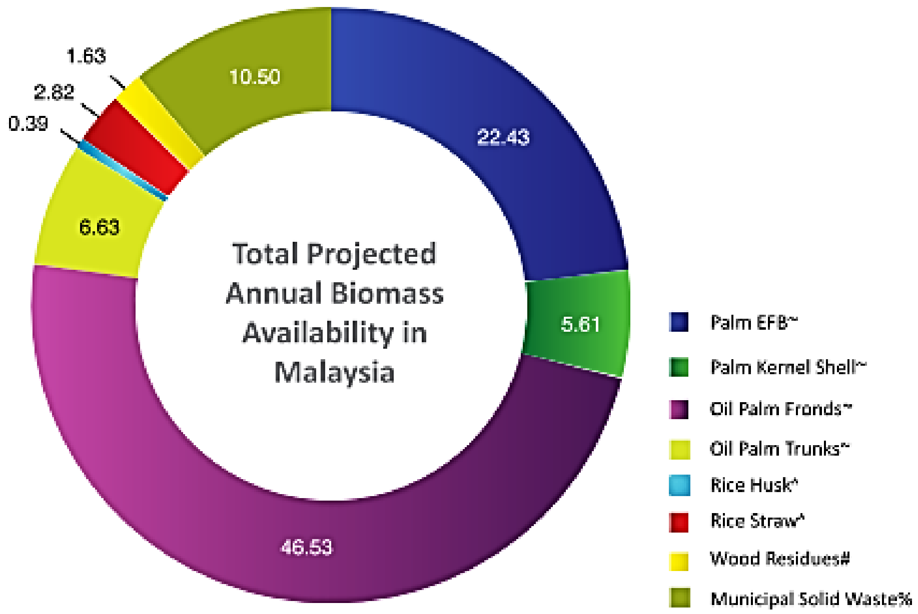

Figure 1.3 Total projected annual biomass availability in Malaysia (Million Metric Tonnes) (Reproduced from MIGHT, 2013)

Lignin is a resin-like polymer matrix with various phenolic compounds present. It is valuable for the production of surfactant, phenolic formaldehyde resin, antacids, and fertilizers. On the other hand, holocellulose layers consisting of hemicellulose and cellulose are carbohydrate-based polymers that can be degraded to sugar monomer by hydrolysis; such as xylose, arabinose, glucose, and fructose (Lima *et al.*, 2009). The sugars can be converted into a number of the high-value bio-based chemical as illustrated in Figure 1.4. The bio-based derived chemicals such as furfural and its derivative, 5-hydroxymethylfurfural (5-HMF) can be produced by partial dehydration of the monomer sugar. Furfural is produced from xylose or arabinose, while HMF is synthesized from fructose and glucose (Wang *et al.*, 2019; Lima *et al.*, 2009). HMF can then be converted to a platform chemical known as levulinic acid (LA) by acid hydrolysis. The valuable intermediate products, LA are very useful for fuel industries (Jeong, 2014; Jeong *et al.*, 2018). Besides, LA can be used as coating material, solvent, fragrant, and food flavouring agent (Ramli and Amin, 2014).

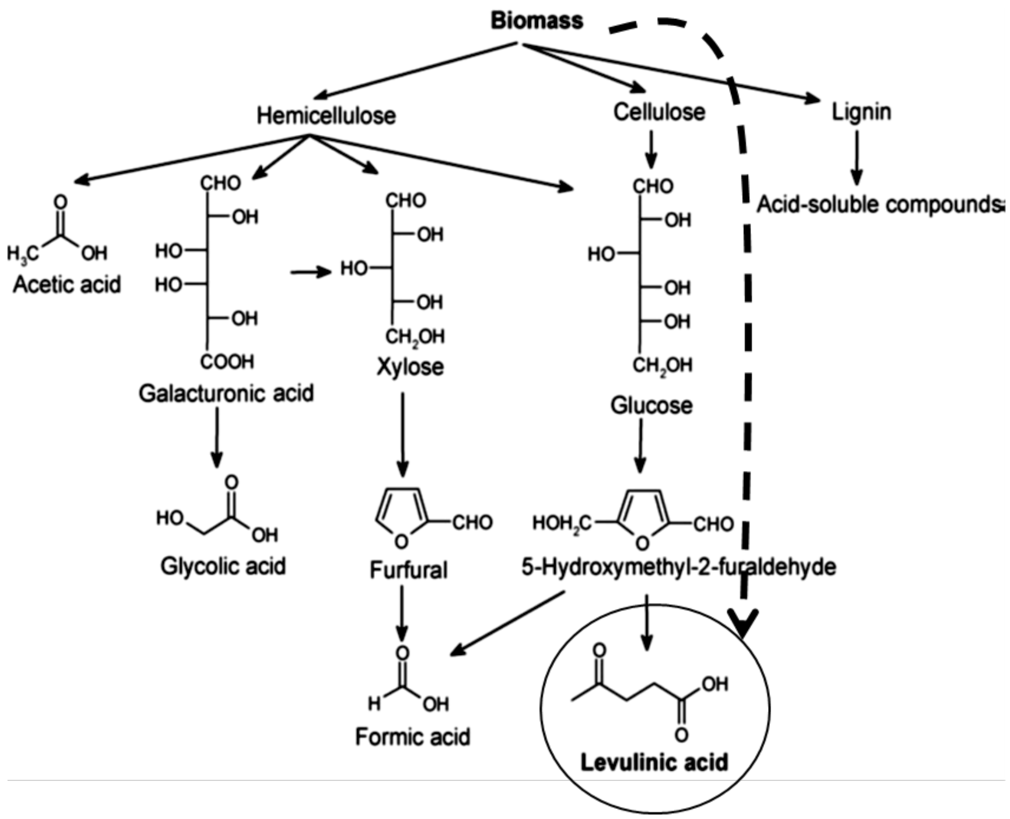

Figure 1.4 Possible pathways and products of hydrolysis of a typical lignocellulosic material (Modified from Girisuta *et al.*, 2006)

However, the presence of lignin hindered many of the biomass conversion processes as it trapped the sugar monomer. A layer of lignin functions as a support and can protect the plants against microbial attack (Conde-Mejía *et al.*, 2012; Kumar *et al.*, 2009). Therefore, the deconstruction of biomass to remove the lignin layer is needed so that the holocellulose layer would be exposed for the next processing step (Figure 1.5). The process of biomass deconstruction is known as a pre-treatment.

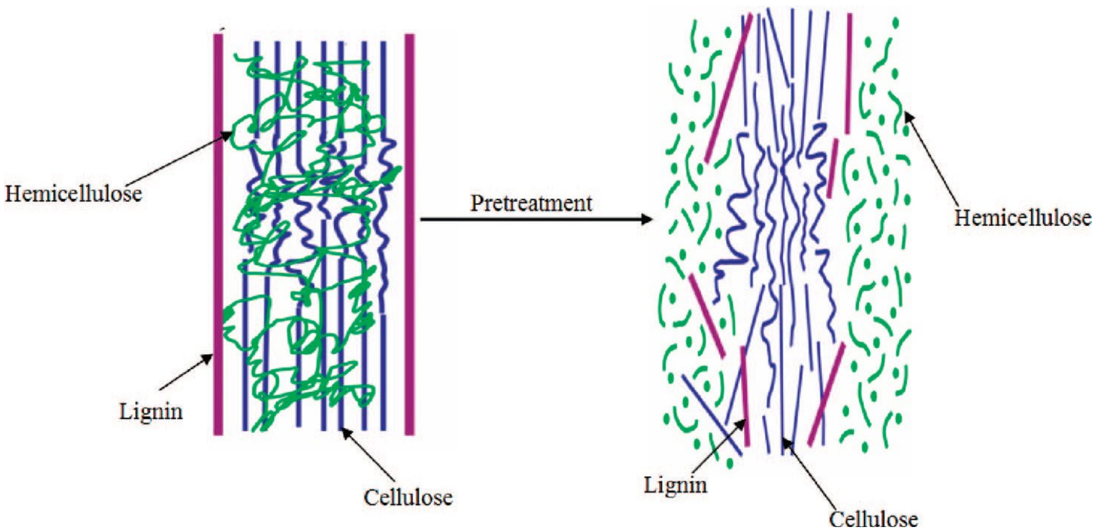

Figure 1.5 Schematic of role biomass pre-treatment (Reproduced from Kumar *et al.*, 2009)

Biomass can be pretreated by physical (milling and grinding), physicochemical (steam pre-treatment/autohydrolysis, hydrothermolysis, and wet oxidation), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), biological, electrical or a combination of these techniques (Kumar *et al.*, 2009; Taherzadeh and Karimi, 2008; Wang *et al.*, 2019). However, the thermal chemical pre-treatments are necessary to degrade the lignin and expose the plant cell wall microfibrils to open an access into cellulose for LA production.

Some of biomass pre-treatment method known as ammonia fiber explosion (AFEX), acid and alkaline hydrolysis, and organosolv can degrade/remove the lignin (Kumar *et al.*, 2009; Taherzadeh and Karimi, 2008). However, the drawbacks of this method deter the commercialization process. AFEX is not efficient for biomass with high lignin content. Alkaline hydrolysis requires long residence time besides forming irrecoverable salts and incorporates into biomass. Acid hydrolysis presents a very high capital cost due to the need corrosiveness of the reagent, not to mention the toxic by-products coming out of the process, an issue also shared by the organosolv methods.

Recently, the emerging technologies for biomass processing such as non-ionizing radiation (microwaves), ionizing radiation (*gamma*-ray, electron beam), pulsed-electric field, high-pressure (high hydrostatic pressure, high-pressure homogenization) and ultrasound are promising for commercial purpose. However, the technologies are a relatively high cost for development in biorefinery (Hassan *et al.*, 2018). Besides the cost and environment issues, the physico-chemical properties of biomass are affected negatively or positively during the treatment process. Some of the pre-treatment methods could reduce the crystallinity of cellulose and increase the porosity of the lignocellulosic biomass, which is advantageous for the next biorefinery process (Kumar *et al.*, 2009).

Among the green chemistry pre-treatment method, ozonation of biomass has appeared as the most promising method for pre-treatment. The ozonation of biomass can degraded the lignin by giving only a slight effect on hemicellulose and almost no effect on cellulose at all (Hendriks and Zeeman, 2009; Kumar *et al.*, 2009; Pandey *et al.*, 2015). Most of studied reported that the sugar yield increased after ozonolysis pre-treatment (Pereira *et al.*, 2013; García-cubero *et al.*, 2012; Mardawati *et al.*, 2019). In addition, Perrone *et al.* (2017) stated the ozonolysis pre-treatment of sugar cane bagasse increase the crystallinity and surface area.

Moreover, the ozonolysis process is an energy-efficient because it operates under ambient temperature and pressure (Galletti and Antonetti, 2012; Travaini, Martín-Juárez, *et al.*, 2016; Travaini, Barrado and Bolado-Rodriguez, 2016; García-cubero *et al.*, 2012). Furthermore, the reaction uses a non-corrosive chemical, and the excess of ozone is converts into oxygen before discharged into the atmosphere. This led to a zero-waste production system. Herein, the ozonolysis treatment is acknowledged as a green technology. Therefore, the possibility to employ the ozonolysis method for pre-treatment of OPF for LA production is prominent.

1.2 Statement of Problem

As the second-largest palm oil plantation in the world, the amount of biomass produced has reached 123 M tonnes per year and assumed to increase more in 2020 (Agensi Inovasi Malaysia, 2013; Awalludin *et al.*, 2015). The bulk of the biomass is undergoing substandard management by converted into a low-value product such as medium density fibreboard (MDF), plywood, briquettes, and torrefied pellet in addition to burning for soil nutrient or electricity generation (Aziz, n.d.; MIGHT, 2013). An alternative and sustainable energy and chemicals derived from OPW by biorefinery could be a potential key to overcome the waste management crisis and creates an opportunity for Malaysia to generate income in this sector. One of promising chemical product is LA that produces from glucose conversion (Kang *et al.*, 2018; Ramli *et al.*, 2014; Ramli and Amin, 2016). The first pilot commercial-scale for 2G-Sugar biomass conversion plant is developed in Segamat, Johor and is scheduled to be operational by 2021 (Agensi Inovasi Malaysia, 2013). This pilot plant will provide the most economic route to produce cellulosic sugars and convert it into LA from biomass. But, it is still challenging to reduce the cost of production and increase the sustainability since the rigid and complex structure of OPF unfavorably obstructs the conversion into valuable chemical products (Figure 1.6). Thus, the pre-treatment process is required to assist OPF dissolution and conversion processes by deconstruct the OPF constituents to be efficiently feedstocks for downstream biorefinery processing.

One of the promising pre-treatment methods of biomass is the ozonolysis. To date, the feasibility of ozonolysis pre-treatment of biomass was carried out for sugar release by enzymatic hydrolysis for ethanol production. The ozonolysis method has been proven to obtain the high sugar yield by enzymatic hydrolysis and the lignin is degraded by the previous study. (Bhattarai *et al.*, 2015; Travaini *et al.*, 2014; Eqra *et al.*, 2014; Panneerselvam, Sharma-Shivappa, *et al.*, 2013; Panneerselvam, Sharma-shivappa, *et al.*, 2013; Travaini, Barrado and Bolado-Rodríguez, 2016; García-cubero *et al.*, 2012). Besides, the effect of washing prior to subsequent step investigated by Al jibouri *et al.*, (2015) explained that the ozonolysis could separate the lignin from biomass component. However, the method has not yet been explored for Malaysia's biomass such as OPF. Besides, the efficacy of the ozonolysis pre-treatment on thermochemical conversions, such as acid hydrolysis as an alternative pathway for glucose production is not yet investigated. Hence, the potential of ozonolysis pre-treatment of OPF for LA production by acid hydrolysis is highly encouraged to be investigated.

Up till now, the study of ozonation is mostly done in a slurry semi-batch or fixed bed reactor. The slurry semi-batch reactor was usually employed for the slurry mixing of biomass and ozone. Therefore, the reactor is not suitable for biomass with low moisture content (20-40 wt.%). Meanwhile, the distribution of ozone was reportedly not scattered uniformly in fixed bed reactor according to García-Cubero *et al.* (2012) which led to loading the biomass on plate bed with 1 cm thickness (Bhattarai *et al.*, 2015). However, the plate bed reactor also unrealizable to be commercialized since it need a bigger scale. One of the most common reactors, continuous stir tank reactor (CSTR) concept are applied in this study. Whereas, the OPF sample is loading to the reactor that equipped with stirrer prior to the reaction. The ozone is then supplied into the reactor within the time desired. This semi-batch reactor type could promise the ozone is distributed evenly in the reactor.

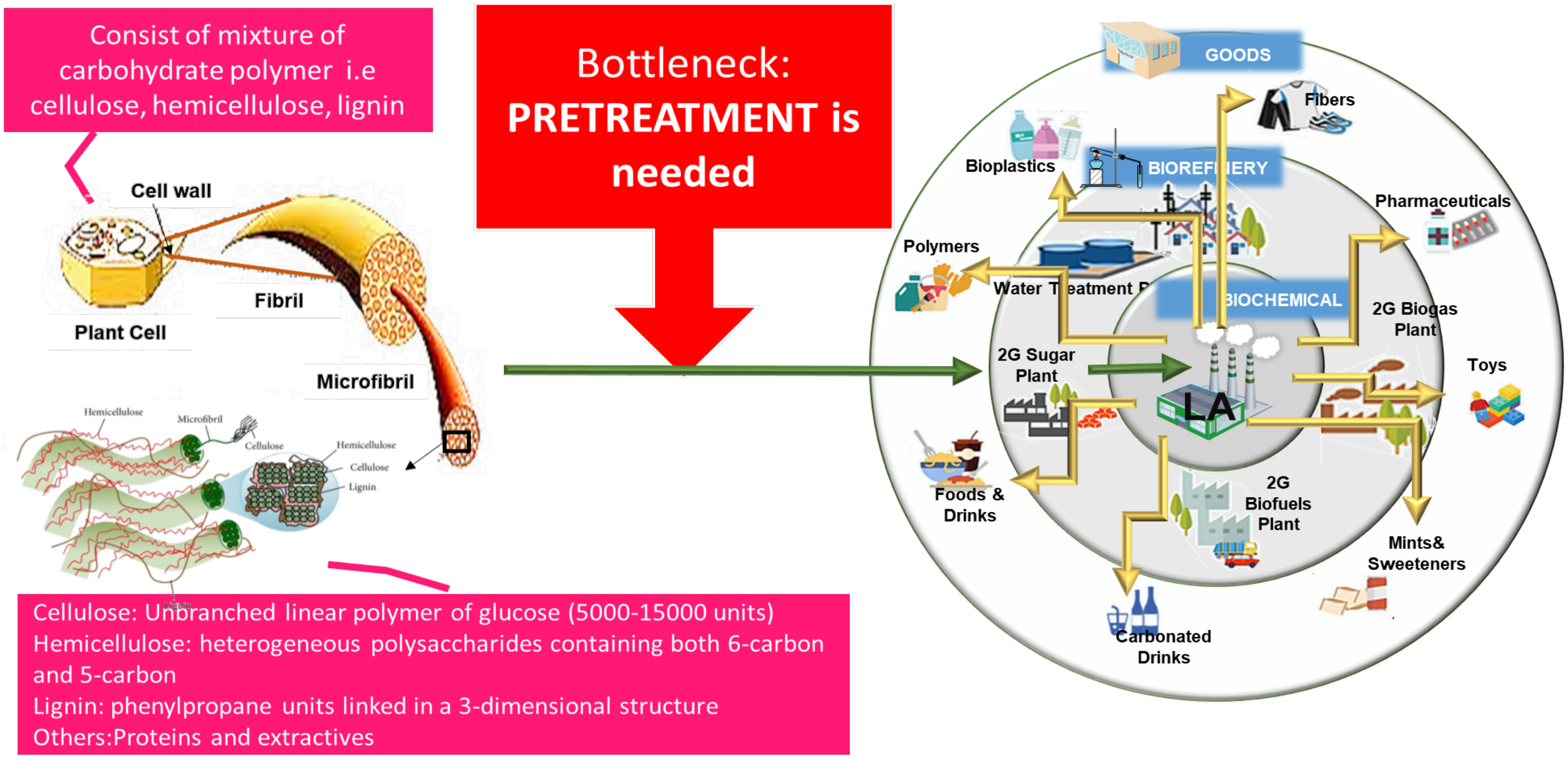

Figure 1.6 The bottleneck of LA production from lignocellulosic biomass

On the other hand, there report on effect of parameter on ozonolysis reaction is limited to foreign local type feedstock such as rye straw, wheat straw, sugar cane bagasses, and grass which had low lignin content and categorized as softwood. Since OPF is quite different than this softwood biomass, the investigation on process parameter is need to be carried out. Despite that, the crucial discussion of the process parameter on lignin degradation is scarcely explained since most of researcher focus on the effect of pre-treatment on sugar release yield and bioethanol production (García-Cubero *et al.*, 2009; Travaini *et al.*, 2014; Perrone *et al.*, 2017). In addition, mostly the researcher used one factor at the time (OFAT) approach to carried out the experiment. The approach requires large number of homogenous feedstocks and consumes a lot of time. Herein, the design of experiment (DOE) by applied factorial design (FD) is suggested to investigate the effect of the process parameter on lignin degradation due to feedstock limitation.

In addition, the effect of pre-treatment on physico-chemical properties of biomass is also barely reported (Perrone *et al.*, 2016; Orduña Ortega *et al.*, 2019; Bule *et al.*, 2013; Perrone *et al.*, 2017). Perrone *et al.* (2017, 2016) reported and discussed the effect of ozonolysis and combination with ultrasonic on functional group, crystallinity, and morphology of treated sugar bagasse. Meanwhile, Orduña Ortega *et al.*, (2019) focused on effect of soaking and ozonolysis of sugar cane straw on FTIR study. On the other hands, the characterization of lignin is analyzed by Bule *et al.* (2013). Thus, the effect of ozonolysis pre-treatment on oil palm waste physico-chemical properties should be investigated to understand the mechanism of the reaction.

Moreover, no report on the optimization of the ozonation condition using optimization tools such as response surface methodology (RSM) has been found until recently (Al jibouri *et al.*, 2015; Mardawati *et al.*, 2019). Al Jibouri *et al.* (2015) investigated the effect of two stage of ozonolysis treatment on enzymatic hydrolysis for bioethanol production from wheat straw. While, Mardawati *et al.* (2019) inspected the effect of pre-treatment condition on sugar production by enzymatic production from EFB. Herein, optimization of OPF ozonolysis pre-treatment on lignin degradation and TRS recovery needs to carried out to explore more on the reaction.

Consequently, the details investigation on the feasibility of ozonolysis pre-treatment on OPF before acid hydrolysis for sugar and LA production needs to be performed due to limited knowledge on the reaction. The study should include the process screening and effect on the physico-chemical properties of OPF in order to understand the reaction as a fundamental knowledge prior to further optimization. The optimization of the ozonation pre-treatment condition is advantageous to minimize the cost of production for commercialization purpose.

1.3 Hypothesis of Research

The ozonolysis pre-treatment would appear as a new promising pre-treatment method for the delignification of OPF and enhance total reducing sugar (TRS) recovery and LA production. The ozonolysis pre-treatment of OPF would be successfully carried out in the semi-batch process by introducing the ozone into the moist OPF. During the process, the ozone would attack the lignin without affecting the cellulose component. The particle size, moisture content, ozone flow rate, ozone concentration, and reaction time would be recognized as the important process parameters that induce lignin degradation. The fractional factorial design (FFD) would suffice to elucidate the activities of the process.

Furthermore, the physical properties of biomass such as crystallinity and porosity would change in the way that gives advantages for subsequent hydrolysis reaction for sugar monomer yield and TRS recovery. The thermal gravimetric analysis (TGA) and fourier transform infrared (FTIR) could prove the component of OPF after the pre-treatment. On the other hand, X-ray diffractometer (XRD), scanning electron microscopy (SEM) and field emission scanning electron microscopy (FESEM) would show how the ozonolysis pre-treatment changes the physical structure of OPF. In addition, the sugar yield and TRS recovery would increase after pre-treatment due to the physical changes of OPF.

Moreover, the response surface methodology (RSM) is an efficient tool for optimization. The Box-Behnken design (BBD) would design a sufficient set of a run for optimization of the lignin degradation and TRS recovery simultaneously. The RSM would predict the optimum condition that could maximize the lignin degradation and TRS recovery simultaneously by using the desired probability function. Moreover, the RSM approach could describe the influence of each process parameters and their interaction meticulously. Besides, the treated OPF is ready for subsequent biorefinery processes i.e LA production. The LA yield would increase after pre-treatment. The ozonolysis is expected to increase the sustainability of feedstock supply.

1.4 Objective of Research

This research is carried out to reach the following objectives:

1. To screen the process parameters (i.e: particle size, moisture content, ozone concentration, ozone flow rate, reaction time and part of OPF) on lignin degradation of oil palm frond (OPF) and to evaluate the effect of the pre-treatment on physico-chemical properties of biomass.
2. To find and investigate the optimum process parameters during ozonolysis pre-treatment of OPF for sugar monomer production by response surface methodology (RSM) approach.
3. To inspects the potential of ozonolysis pre-treatment for levulinic acid (LA) production.

1.5 Scope of Research

In scope 1, the preliminary study of ozonolysis pre-treatment is carried out to screen the process parameters on lignin degradation of OPF. Figure 1.7 illustrated the flow of study within the scope. The parameters i.e. particle size, moisture content, ozone concentration, ozone flow rate, reaction time, and part of OPF are considered. The two-level fractional factorial design with resolution III is employed to design the experiment matrix. The lignin degradation of treated OPF, known as ODT samples is analysed by the Kappa number test. Meanwhile the physical properties of OPF and ODT such as crystallinity, BET surface area, and functional group are investigated using XRD, BET, and FTIR. In addition, the image of morphological of OPF and ODT are captured by SEM. The composition of lignin, hemicelluloses and cellulose of the OPF and ODT samples are measured using standard gravimetric methods.

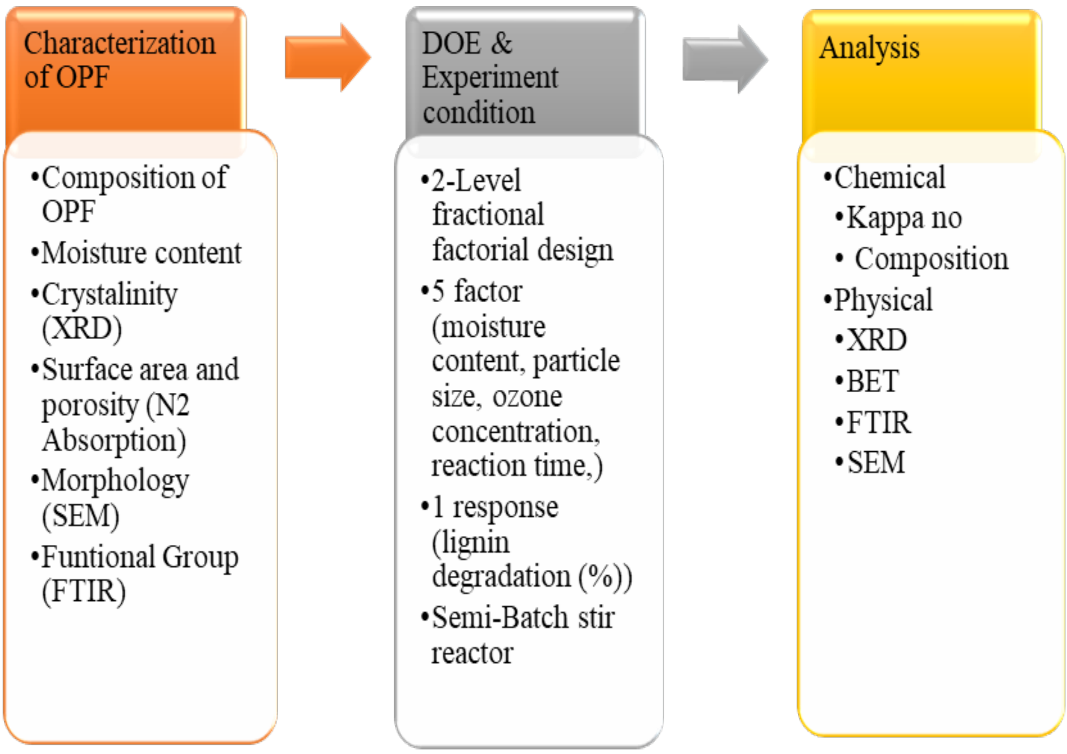

Figure 1.7 Preliminary study of ozonolysis treatment on lignin degradation and physical properties of OPF process flow

The parameter screening of ozonolysis pre-treatment on lignin degradation of OPF is re-investigated in order to determine the optimum region. The parameters i.e. particle size, moisture content, ozone flow rate, reaction time, and part of OPF as blocking parameter are considered. The two-level factorial design with resolution IV is employed to design the experiment matrix. Kappa number test is performed to determine the lignin degradation. In addition, the effect of ozonolysis on total reducing sugar (TRS) recovery is investigated. The TRS is produced from two-step acid hydrolysis and analysed by DNS method. Meanwhile the crystallinity, BET surface area, functional group and thermal stability of OPF and treated is scrutinized for more detail investigated using XRD, BET, FTIR and TGA. In addition, FESEM-EDX is using to capture the morphological of OPF and ODT. Moreover, the physical properties of OPF and ODT is tested by swelling properties. The summary of the research activities in this scope is show in Figure 1.8.

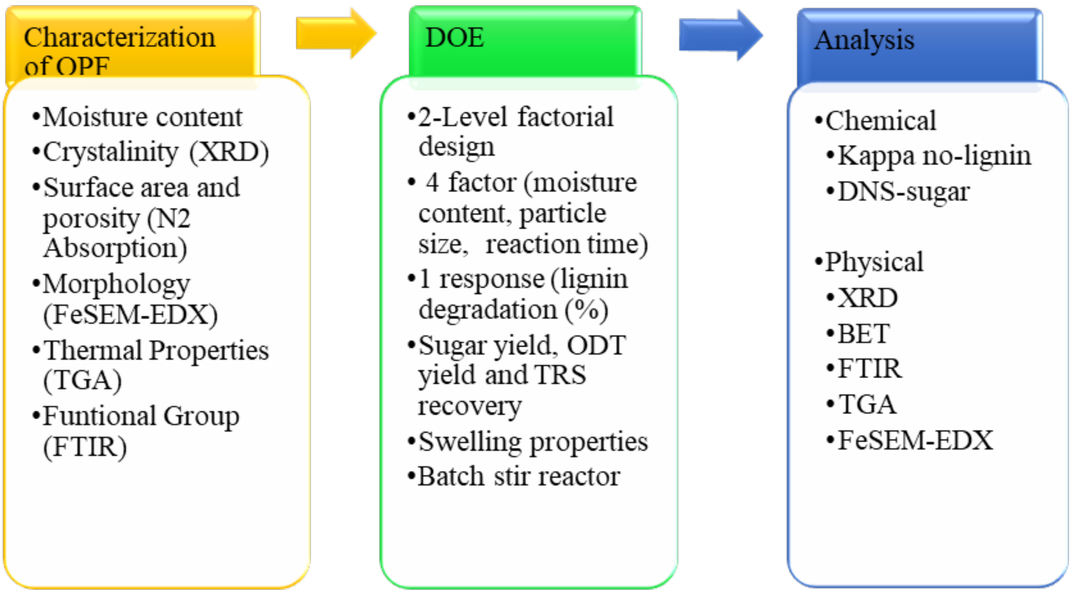

Figure 1.8 Parameter screening and effect of ozonolysis treatment on sugar production and physical properties of OPF process flow

Next, the process parameters during ozonolysis reaction for lignin degradation and TRS recovery are optimized by the response surface methodology (RSM). Figure 1.9 shows a summary of the process flow. The important parameters that have been determined in the preliminary study i.e particle size, moisture content, ozone flowrate and reaction time are re-investigate using Box-Behnken design (BBD) at different region of study. Desirability function is used for the simultaneous optimization of lignin degradation and TRS recovery. *STATISTICA* software is employed as a tool in this part.

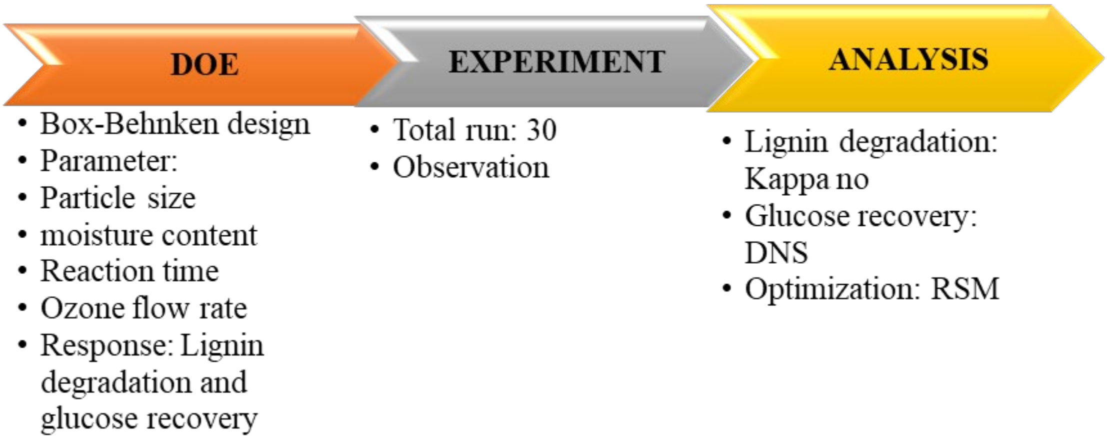

Figure 1.9 The process flow of the optimization of ozonolysis pre-treatment

The feasibility of the ozonolysis pre-treatment on acid hydrolysis reaction for levulinic acid (LA) synthesis is studied in next scope. The selected ODT from Phase 2 and the OPF is used as a feedstock for acid hydrolysis (Figure 1.10). Also, commercial cellulose is used as a benchmark for the study. LA is produced by acid hydrolysis at 180°C for 1 h. LA concentration is analysed using HPLC.

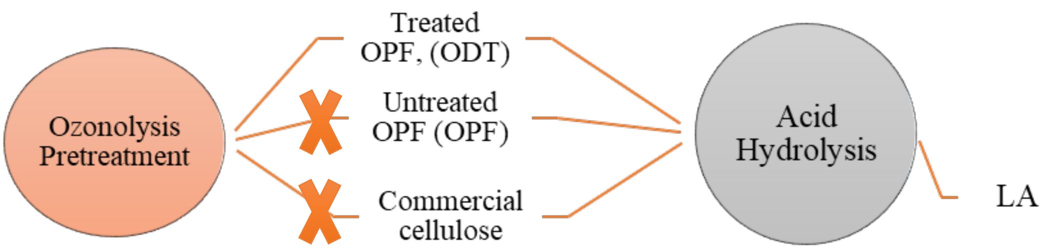

Figure 1.10 Study the effect of ozonolysis of OPF on LA production

1.6 Significant of Research

In general, the research provides fundamental information for the ozonolysis pre-treatment of Malaysia's biomass especially OPF. The implementation of the ozonolysis as the pre-treatment method in Malaysia's biorefinery could contribute to Malaysia's economy as elucidated in the National Biomass Strategy (Agensi Inovasi Malaysia, 2013; Agensi Inovasi Malaysia, 2011).

1.7 Outline of the Thesis

This dissertation consists of seven chapters including introduction, literature review, research methodology, results and discussions, which are divided into three main chapters, and lastly conclusion and recommendation.

Chapter 1 introduces the background of the research as the guideline of the work. The detailed information of the background knowledge related to the research is discussed in Chapter 2. Then, Chapter 3 provides the detail of the method for each procedure including the diagram of each set up for each process.

The results and discussion are reported in Chapters 4, 5 and 6 for each scope of the study, respectively. In Chapter 4, the fundamental of the ozonolysis reaction is reported and discussed. The discussion included the observation made during the experiment as well as process screening to find the significant parameters and the region of each parameter for optimization study. Meanwhile, Chapter 5 discusses more details on process screening. The effect of the pre-treatment on sugar recovery is reported in this chapter. Besides that, the effect of the pre-treatment on the physico-chemical properties of OPF is scrutinized and supported by investigation on swelling activity. The finding in Chapter 4 and 5 would give a fundamental knowledge of technology and would be a great help for the next stage of the study.

Chapter 6 reports and discusses the optimization study of the ozonolysis pre-treatment by the RSM approach to maximize the lignin degradation and sugar recovery. The influence of particle size, moisture content, reaction time, and ozone flow rate, and their interactions in the ozonolysis pre-treatment of OPF on lignin degradation and sugar recovery are carefully investigated and reported. The empirical mathematical model is developed from the RSM approach. The model would elucidate the effect of the process parameters on the response. Meanwhile, multi-objective responses for lignin degradation and TRS recovery of OPF for the ozonolysis pre-treatment are optimized simultaneously using desirability function in *STATISTICA* software tools. The recommended optimum condition for the ozonolysis pre-treatment of OPF is verified experimentally. Additionally, the application of ozonolysis product for bio-based chemical product i.e. levulinic acid (LA) is assessed in this chapter. Lastly, Chapter 7 give the overall conclusion and recommendation for future study.

REFERENCES

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., and Levin, D.B. (2011). 'Biomass pretreatment: Fundamentals toward application.' *Biotechnology Advances* 29, 675–685.

Agensi Inovasi Malaysia (2013). *National Biomass Strategy 2020: New Wealth Creation for Malaysia' s Palm Oil Industry*. Kuala Lumpur, Malaysia.

Agensi Inovasi Malaysia (2011). *National Biomass Strategy 2020: New Wealth Creation for Malaysia' s Palm Oil Industry*. Kuala Lumpur, Malaysia.

Al jibouri, A.K.H., Turcotte, G., Wu, J., and Cheng, C.H. (2015). 'Ozone Pretreatment of Humid Wheat Straw for Biofuel Production.' *Energy Science & Engineering* 3, 541–548.

Anis, M., Siti Nadrah, A.H., Kamaruddin, H., Astimar, A.A., and Mohd Basri, W. (2011). 'Isolation and Functional Properties of Hemicelluloses from Oil Palm Trunks'. *Journal of Oil Palm Research* 23, 1178–1184.

Antoy, J. (2014). *Design of Experiments for Engineers and Scientists*. USA. 2nd revised ed. Elsevier Ltd.

Awalludin, M.F., Sulaiman, O., Hashim, R., and Nadhari, W.N.A.W. (2015). 'An Overview of the Oil Palm Industry in Malaysia and Its Waste Utilization through Thermochemical Conversion, Specifically via Liquefaction'. *Renewable and Sustainable Energy Reviews* 50, 1469–1484.

Aziz, A.A. (2015). Driving National Biomass Agenda. *Biomass Conference 2015*. 10th June 2015. Perdana Hall, MIDA Sentral, Kuala Lumpur,Malaysia.

Barrera-Martínez, I., Guzmán, N., Peña, E., Vázquez, T., Cerón-Camacho, R., Folch, J., Honorato Salazar, J.A., and Aburto, J. (2016). 'Ozonolysis of Alkaline Lignin and Sugarcane Bagasse: Structural Changes and their Effect on Saccharification.' *Biomass and Bioenergy* 94, 167–172.

Barros, R. da R.O. de, Paredes, R. de S., Endo, T., Bon, E.P. da S., and Lee, S.-H. (2013). 'Association of Wet Disk Milling and Ozonolysis as Pretreatment for Enzymatic Saccharification of Sugarcane Bagasse and Straw.' *Bioresource Technology* 136, 288–294.

Bhatia, S.K., Jagtap, S.S., Bedekar, A.A., Bhatia, R.K., Patel, A.K., Pant, D., Rajesh Banu, J., Rao, C. V., Kim, Y.G., and Yang, Y.H. (2020). 'Recent Developments in Pretreatment Technologies on Lignocellulosic Biomass: Effect of Key Parameters, Technological Improvements, and Challenges.' *Bioresource Technology* 300, 122724.

Bhattarai, S., Bottenus, D., Ivory, C.F., Gao, A.H., Bule, M., Garcia-Perez, M., and Chen, S. (2015). 'Simulation of the Ozone Pretreatment of Wheat Straw'. *Bioresource Technology* 196, 78–87.

Binder, A., Pelloni, L., and Fiechter, A. (1980). 'Delignification of Straw with Ozone to Enhance Biodegradability'. *European Journal of Applied Microbiology and Biotechnology* 11, 1–5.

Bule, M. V., Gao, A.H., Hiscox, B., and Chen, S. (2013). 'Structural Modification of Lignin and Characterization of Pretreated Wheat Straw by Ozonation'. *Journal of Agricultural and Food Chemistry* 61, 3916–3925.

Burhenne, L., Messmer, J., Aicher, T., and Laborie, M. (2013). 'The Effect of the Biomass Components Lignin, Cellulose and Hemicellulose on TGA and Fixed Bed Pyrolysis'. *Journal of Analytical and Applied Pyrolysis* 101, 177–184.

Canavos, G.C., and Koutrouvelis, I.A. (2009). *An Introduction to the Design & Analysis of Experiments*. US. Pearson Education.

Capareda, S. (2013). *Introduction to Biomass Energy Conversions.* U.S. CRC Press.

Cardona, C.A., Sánchez, Ó.J., and Gutiérrez, L.F. (2010). *Process Synthesis for Fuel Ethanol Production*. New York. CRC Press Taylor and Francis Group.

Carrillo, F., Colom, X., Suñol, J.., Saurina, J., Adapa, P.K., Karunakaran, C., Tabil, L.G., and Schoenau, G.J. (2004). 'Qualitative and Quantitative Analysis of Lignocellulosic Biomass using Infrared Spectroscopy'. *European Polymer Journal* 40, 1–20.

Centi, G., and van Santen, R.A. (2007). Conclusions, Perspective and Roadmap. In: Centi, G., and van Santen, R.A. (Eds.), *Catalysis for Renewables: From Feedstocks to Energy Production*. Weinhem. WILEY-VCH. 387–410.

Chai, X.S., and Zhu, J.Y. (2002). *Method for Rapidly Determining a Pulp Kappa Number using Spectrophotometry*. US6475339. US

Chen, H., Liu, J., Chang, X., Chen, D., Xue, Y., Liu, P., Lin, H., and Han, S. (2017). 'A Review on the Pretreatment of Lignocellulose for High-Value Chemicals'. *Fuel Processing Technology*, 160, 196–206.

Cheng, Q. Wang, J. McNeel, J.F. and Jacobson, P.M., (2010). 'Water Retention Value Measurements of Cellulosic Materials Using a Centrifuge Technique'. *BioResources*, 5(3), 1945–1954.

Cherubini, F., (2010). 'The Biorefinery Concept: Using Biomass Instead of Oil for Producing Energy and Chemicals'. *Energy Conversion and Management*, 51(7), 1412–1421.

Chuck, C.J. Parker, H.J. Jenkins, R.W. and Donnelly, J., (2013). 'Renewable Biofuel Additives from the Ozonolysis of Lignin'. *Bioresource Technology*, 143, 549–554.

Chundawat, S.P.S., Pal, R.K., Zhao, C., Campbell, T., Teymouri, F., Videto, J., Nielson, C., Wieferich, B., Sousa, L., Dale, B.E., Balan, V., Chipkar, S., Aguado, J., Burke, E., and Ong, R.G. (2020). 'Ammonia Fiber Expansion (AFEX) Pretreatment of Lignocellulosic Biomass'. *Journal of Visualized Experiments*, 2020 (158), 1–8.

Chundawat, S.P.S. Venkatesh, B. and Dale, B.E., (2007). 'Effect of Particle Size Based Separation of Milled Corn Stover on AFEX Pretreatment and Enzymatic Digestibility'. *Biotechnology and Bioengineering*, 96 (2), 219–231.

Clark, J.H. and Deswarte, F., (2008*). Introduction to Chemicals from Biomass*, U.K. Wiley.

Conde-Mejía, C. Jiménez-Gutiérrez, A. and El-Halwagi, M., (2012). 'A Comparison of Pretreatment Methods for Bioethanol Production from Lignocellulosic Materials'. *Process Safety and Environmental Protection*, 90(3), 189–202.

Cornell, J.A., (1990). *How to Apply Response Surface Methodology*. US: Am Soc Qual Control: Statistic Devision.

Curbelo Hernández, C. Véliz-Lorenzo, E. and Ameneiros Martínez, J.M., (2019). 'Alternative Pretreatments of Rice and Tobacco Wastes for the Production of Fermentable Sugars'. *Revista Facultad de Ingenieria,* (91), 24–30.

Damartzis, T. and Zabaniotou, A., (2011). 'Thermochemical Conversion of Biomass to Second Generation Biofuels through Integrated Process Design—A Review'. *Renewable and Sustainable Energy Reviews*, 15(1), 366–378.

Demirbas, A., (2009). *Biohydrogen: For Future Engine Fuel Demands*. Turkey, Springer.

Demirbas, A., (2007). 'Progress and Recent Trends in Biofuels.' *Progress in Energy and Combustion Science*, 33(1), 1–18.

Din, A.K., (2017a). Malaysian Oil Palm Industry Performance 2016 and Prospects for 2017. *Palm Oil Economic Review & Outlook Seminar.* 17 Jan 2017, Kuala Lumpur, Malaysia.

Din, A.K., (2017b). *Overview of the Malaysian Oil Palm Industry 2016*. MPOB, Malaysia. Retrieved on 25 May 2018 from https://www.palmoilis.mpob.gov.my

Domański, J. Marchut-Mikołajczyk, O. Polewczyk, A. and Januszewicz, B., (2017). 'Ozonolysis of Straw from Secale Cereale L. for Anaerobic Digestion'. *Bioresource Technology* 245 (Part A), 394-400

Effendi, A. Gerhauser, H. and Bridgwater, A. V, (2008). 'Production of Renewable Phenolic Resins by Thermochemical Conversion of Biomass: A Review'. *Renewable and Sustainable Energy Reviews*, 12(8), 2092–2116.

Eqra, N. Ajabshirchi, Y. and Sarshar, M., (2014). 'Effect of Ozonolysis Pretreatment on Enzymatic Digestibility of Sugarcane Bagasse'. *Agricultural Engineering International: CIGR Journal*, 16(1), 151–156.

Ferdous Alam, A.S.A. Er, A.C. and Begum, H., (2015). 'Malaysian Oil Palm Industry: Prospect and Problem'. *Journal of Food, Agriculture and Environment*, 13(2), 143–148.

France-Presse, A., (2016). Haze from Indonesian Fires May Have Killed More than 100,000 People – Study. *The Guardian*. Retieved on 29 October 2019 from https://www.theguardian.com/

Galletti, A.M.R. and Antonetti, C., (2012). Biomass Pretreatment: Separation of Cellulose,Hemicellulose,Amd Lignin- Existing Technologies and Perpectives. In: Aresta, M., Dibenedetto, A. and Dumeignil, F., (eds.) *Biorefinery: From Biomass to Chemicals and Fuels*. Berlin, De Gruyter, 101–122.

Gallezot, P., (2007). Process Option for the Catalytic Conversion of Renewables into Bioproduct. In: Centi, G. and van Santen, R.A., (eds.) *Catalysis for renewables: from feedstocks to energy production*. Weinhem, WILEY-VCH. 53–73.

Gan, L., Wang, C., You, K., Liu, J., and Long, M. (2019). 'Development of Pretreatment of Lignocellulose for Bioenergy'. *Journal of Glycobiology*, 8, 1–3.

García-Cubero, M.T., González-Benito, G., Indacoechea, I., Coca, M., and Bolado, S (2012). 'An Analysis of Lignin Removal in a Fixed Bed Reactor by Reaction of Cereal Straws with Ozone'. *Bioresource Technology* 107, 229–234.

García-cubero, M.T., Palacín, L.G., González-benito, G., Bolado, S., Lucas, S., and Coca, M. (2009). 'Effect of Ozonolysis Pretreatment on Enzymatic Digestibility of Wheat and Rye Straw'. *Bioresource Technology*, 100(4), 1608–1613.

Girisuta, B. Janssen, L.P.B.M. and Heeres, H.J., (2006). 'Green Chemicals: A Kinetic Study on the Conversion of Glucose to Levulinic Acid'. *Chemical Engineering Research and Design*, 84(5 A), 339–349.

Hassan, Shady S Williams, G.A. and Jaiswal, A.K., (2018). 'Emerging Technologies for the Pretreatment of Lignocellulosic Biomass'. *Bioresource Technology*, 262, 310–318.

Heinimö, J. and Junginger, M., (2009). 'Production and Trading of Biomass for Energy – An Overview of the Global Status'. *Biomass and Bioenergy*, 33(9), 1310–1320.

Hendriks, A.T.W.M. and Zeeman, G., (2009). 'Pretreatments to Enhance the Digestibility of Lignocellulosic Biomass'. *Bioresource Technology*, 100(1), 10–18.

Hong, L. Ibrahim, D. and Omar, I., (2008). 'Microscopic Studies of Oil Palm Frond During Processing for Saccharification'. *The Internet Journal of Bioengineering*, 4(2), 1–7.

Hu, F. and Ragauskas, A., (2012). 'Pretreatment and Lignocellulosic Chemistry'. *Bioenergy Research*, 5(4), 1043–1066.

Hussin, M.H. and Hamidon, T.S., (2020). 'Overview of Pretreatment Methods Employed on Oil Palm Biomass in Producing Value-Added Products: A Review'. *BioResources*, 15 (4), 9935-9997.

Jeong, G.-T., (2014). 'Production of Levulinic Acid from Glucosamine by Dilute-Acid Catalyzed Hydrothermal Process'. *Industrial Crops and Products*, 62(0), 77–83.

Jeong, H., Park, S.Y., Ryu, G.H., Choi, J.H., Kim, J.H., Choi, W.S., Lee, S.M., Choi, J.W., Choi, I.G., (2018). ‘Catalytic Conversion of Hemicellulosic Sugars Derived from Biomass to Levulinic Acid’. *Catalysis Communications*, 117, 19–25.

Al jibouri, A.K.H. Turcotte, G. Wu, J. and Cheng, C.-H., (2015). ‘Ozone Pretreatment of Humid Wheat Straw for Biofuel Production’. *Energy Science & Engineering*, 3(6), 541–548.

Kafle, K., Shin, H., Lee, C.M., Park, S., and Kim, S.H., (2015). ‘Progressive Structural Changes of Avicel, Bleached Softwood, and Bacterial Cellulose during Enzymatic Hydrolysis’. *Scientific Reports*, 5, 15102.

Kang, K.Y., Hwang, K.R., Park, J.Y., Lee, J.P., Kim, J.S., and Lee, J.S., (2018). ‘Critical Point Drying: An Effective Drying Method for Direct Measurement of the Surface Area of a Pretreated Cellulosic Biomass’. *Polymers*, 10(676).

Kang, S. Fu, J. and Zhang, G., (2018). ‘From Lignocellulosic Biomass to Levulinic Acid: A Review on Acid-Catalyzed Hydrolysis’. *Renewable and Sustainable Energy Reviews*, 94, 340–362.

Kawamura, F., Saary, N.S., Hashim, R., Sulaiman, O., Hashida, K., Otsuka, Y., Nakamura, M., and Ohara, S., (2014). ‘Subcritical Water Extraction of Low-Molecular-Weight Phenolic Compounds from Oil Palm Biomass’. *Japan Agricultural Research Quarterly*, 48(3), 355–362.

Kenneth Jr, L.R., (1973). *Oxidation and Reduction of Organic Compounds.* N.J. Prentice-hall, Inc.

Khan, Z., Yusup, S., Ahmad, M., Vui Soon, C., Uemura, Y., and Sabil, K., (2010). ‘Review on Hydrogen Production Technologies in Malaysia’. *International Journal of Engineering and Technology*, 10(2), 111.

Kim, J.S. Lee, Y.Y. and Kim, T.H., (2016). ‘A Review on Alkaline Pretreatment Technology for Bioconversion of Lignocellulosic Biomass’. *Bioresource Technology*, 199, 42–48.

Kojima, Y. and Yoon, S.L., (2008). ‘Improved Enzymatic Hydrolysis of Waste Paper by Ozone Pretreatment’. *Journal of Material Cycles and Waste Management*, 10(2), 134–139.

Kumar, B., Bhardwaj, N., Agrawal, K., Chaturvedi, V., and Verma, P., (2020). Current Perspective on Pretreatment Technologies Using Lignocellulosic Biomass: An Emerging Biorefinery Concept', *Fuel Processing Technology*, 199, 106244.

Kumar, P. Barrett, D.M. Delwiche, M.J. and Stroeve, P., (2009). 'Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production'. *Industrial and Engineering Chemistry Research*, 48(8), 3713–3729.

Kumar, R. Mago, G. Balan, V. and Wyman, C.E., (2009). 'Physical and Chemical Characterizations of Corn Stover and Poplar Solids Resulting from Leading Pretreatment Technologies'. *Bioresource Technology*, 100(17), 3948–3962.

Kumneadklang, S. O-Thong, S. and Larpkiattaworn, S., (2019). 'Characterization of Cellulose Fiber Isolated from Oil Palm Frond Biomass'. *Materials Today: Proceedings*, 17, 1995–2001.

Lai, L.W. and Idris, A., (2013). 'Disruption of Oil Palm Trunks and Fronds by Microwave-Alkali Pretreatment'. *BioResources*, 8(2), 2792–2804.

Lange, J.P., (2007). Lignocellulosic Conversion: An Introduction to Chemistry, Process and Economics. In: Centi, G. and van Santen, R.A., (eds.) *Catalysis for renewables: from feedstocks to energy production.* Weinhem. WILEY-VCH.

Lee, J.M. Jameel, H. and Venditti, R.A., (2010). 'Effect of Ozone and Autohydrolysis Pretreatments on Enzymatic Digestibility of Coastal Bermuda Grass'. *BioResources*, 5(2), 1084–1101.

Leong, K.M., (2015). Potential for Waste-to-Energy in Malaysia Focus: Biomass. *Waste to Energy in East Malaysia Program*. 15 Sept 2015, Frankfurt, Germany.

Li, C., Wang, L., Chen, Z., Li, Yongfu, Wang, R., Luo, X., Cai, G., Li, Yanan, Yu, Q., and Lu, J., (2015). 'Ozonolysis Pretreatment of Maize Stover: The Interactive Effect of Sample Particle Size and Moisture on Ozonolysis Process'. *Bioresource Technology*, 183, 240–247.

Lima, S., Neves, P., Antunes, M.M., Pillinger, M., Ignatyev, N., and Valente, A.A., (2009). 'Conversion of Mono/Di/Polysaccharides into Furan Compounds Using 1-Alkyl-3-Methylimidazolium Ionic Liquids'. *Applied Catalysis A: General*, 363(1–2), 93–99.

Ling, Z., Chen, S., Zhang, X., Takabe, K., and Xu, F., (2017). 'Unraveling Variations of Crystalline Cellulose Induced by Ionic Liquid and Their Effects on Enzymatic Hydrolysis'. *Scientific Reports*, 7(1), 1–11.

Loh, S.K., (2017). 'The Potential of the Malaysian Oil Palm Biomass as a Renewable Energy Source'. *Energy Conversion and Management*, 141, 285–298.

Mahmood, H. Moniruzzaman, M. Iqbal, T. and Khan, M.J., (2019). 'Recent Advances in the Pretreatment of Lignocellulosic Biomass for Biofuels and Value-Added Products'. *Current Opinion in Green and Sustainable Chemistry*, 20, 18–24.

Mamleeva, N.A. Autlov, S.A. Bazarnova, N.G. and Lunin, V. V, (2009). 'Delignification of Soofwood by Ozonation'. *Pure and applied chemistry*, 81, 2081–2091.

Mandal, A. and Chakrabarty, D., (2011). 'Isolation of Nanocellulose from Waste Sugarcane Bagasse (SCB) and Its Characterization'. *Carbohydrate Polymers*, 86(3),1291-1299.

Mardawati, E., Herliansah, H., Suryadi, E., Hanidah, I.I., Siti Setiasih, I., Andoyo, R., Sukarminah, E., DJali, M., Rialita, T., and Cahyana, Y. (2019). 'Optimization of Particle Size, Moisture Content and Reaction Time of Oil Palm Empty Fruit Bunch Through Ozonolysis Pretreatment'. *Journal of the Japan Institute of Energy*, 98, 132–138.

Mbachu, R. A D. and Manley, R.S.J., (1981). 'Degradation of Lignin by Ozone I:. The Kinetics of Lignin Degradation of Ozone'. *Journal of Polymer Science: Polymer Chemistry Edition*, 19, 2053–2063.

MIGHT, (2013). *Malaysian Biomass Industry Action Plan 2020: Driving SMEs Towards Sustainable Future,* Kuala Lumpur, Malaysia.

Misson, M. Haron, R. Kamaroddin, M.F.A. and Amin, N.A.S., (2009). 'Pretreatment of Empty Palm Fruit Bunch for Production of Chemicals via Catalytic Pyrolysis'. *Bioresource Technology*, 100 (11), 2867–2873.

Miura, T. Lee, S.-H. Inoue, S. and Endo, T., (2012). 'Combined Pretreatment Using Ozonolysis and Wet-Disk Milling to Improve Enzymatic Saccharification of Japanese Cedar'. *Bioresource Technology*, 126, 182–186.

Mohan, M. Banerjee, T. and Goud, V. V., (2015). 'Hydrolysis of Bamboo Biomass by Subcritical Water Treatment'. *Bioresource Technology*, 191, 244–252.

Montgomery, D.C., (1991). *Design and Analysis of Experiments 3rd ed*., New York. John Wiley & Sons, Inc.

Morris, M., (2010). *Design of Experiments: An Introduction Based on Linear Models*, US. Chapman and Hall.

Mousdale, D.M., (2010). *Introduction to Biofuels*, US. CRC Press Taylor,

Nadia, A. Sunardi, S. and Rodiansono, R., (2018). 'Hydrothermal Pretreatment of Oil Palm Fronds for Increasing Enzymatic Saccharification'. *AIP Conference Proceedings*, 1 (2021),03002.

Nag, A., (2008). *Biofuel Refining and Performances*, US. McGraw Hill,

Nash, J., (2015). The Current Haze Over Southeast Asia Could Be Among the Worst Ever. *TIME*. Retrieved on 16 July 2016 from http://www.*time.com*

Neely, W.C., (1984). 'Factors Affecting the Pretreatment of Biomass with Gaseous Ozone'. *Biotechnology and Bioengineering*, 26(1), 59–65.

Noorshamsiana, A.W. Nur Eliyanti, A.O. Fatiha, I. and Astimar, A.A., (2017). 'A Review on Extraction Processes of Lignocellulosic Chemicals from Oil Palm Biomass'. *Journal of Oil Palm Research*, 29(4).

Ogiwara, Y. and Arai, K., (1968). 'Swelling Degree of Cellulose Materials and Hydrolysis Rate with Cellulase'. *Textile Research Journal*, 38(9), 885–891.

Olivier-Bourbigou, H. Magna, L. and Morvan, D., (2010). 'Ionic Liquids and Catalysis: Recent Progress from Knowledge to Applications'. *Applied Catalysis A: General,* 373(1–2), 1–56.

Onoja, E., Chandren, S., Abdul Razak, F.I., Mahat, N.A., and Wahab, R.A. (2019). 'Oil Palm (Elaeis Guineensis) Biomass in Malaysia: The Present and Future Prospects'. *Waste and Biomass Valorization*, 10(8), 2099–2117.

Orduña Ortega, J., Mora Vargas, J.A., Perrone, O.M., Metzker, G., Gomes, E., da Silva, R., and Boscolo, M. (2019). 'Soaking and Ozonolysis Pretreatment of Sugarcane Straw for the Production of Fermentable Sugars'. *Industrial Crops and Products,* 145, 111959.

Owolabi, A.F. Khalil, H.P.S.A. Fazita, M.R.N. and Haafiz, M.K.M., (2016). 'Isolation and Characterization of Microcrystalline Cellulose from Oil Palm Fronds Using Chemomechanical Process'. *Wood and fiber science: Journal of the Society of Wood Science and Technology*, 48, 1–11.

Palm Morphology. Retrieved on 12 Dec 2017 from http://idtools.org/id/palms/palmid/morphology.php,

Pandey, A. Negi, S. Binod, P. and Larroche, C., (2015). *Pretreatment of Biomass: Processes and Technologies*. Netherlands Elsevier Science.

Panneerselvam, A., Sharma-shivappa, R.R., Kolar, P., Clare, D.A., and Ranney, T., (2013a). 'Hydrolysis of Ozone Pretreated Energy Grasses for Optimal Fermentable Sugar Production'. *Bioresource Technology*, 148, 97–104.

Panneerselvam, A., Sharma-Shivappa, R.R., Kolar, P., Ranney, T., and Peretti, S. (2013b). 'Potential of Ozonolysis as a Pretreatment for Energy Grasses'. *Bioresource Technology*, 148.

Pardo, L.M.F. Mendoza, J.G.S. and Galán, J.E.L., (2019). 'Influence of Pretreatments on Crystallinity and Enzymatic Hydrolysis in Sugar Cane Residues. Brazilian' *Journal of Chemical Engineering*, 36(1), 131–141.

Pereira, J.D.C., Travaini, R., Gomes, E., Bolado, S., and Martins, D.A.B., (2013). 'Enzymatic Hydrolysis of Ozone Pretreated Sugarcane Bagasse with Cellulases of a New Isolated Thermophilic Fungus Myceliophtora Sp JCP 1-4'. *Current Opinion in Biotechnology*, 24, S140–S141

Pereira, J.D.C., Travaini, R., Paganini Marques, N., Bolado-Rodríguez, S., and Bocchini Martins, D.A., (2016). 'Saccharification of Ozonated Sugarcane Bagasse Using Enzymes from Myceliophthora Thermophila JCP 1-4 for Sugars Release and Ethanol Production'. *Bioresource Technology*, 204, 122–129.

Perrone, O.M., Rossi, J.S., Moretti, M.M. de S., Nunes, C. da C.C., Bordignon, S.E., Gomes, E., Da-Silva, R., and Boscolo, M., (2017). 'Influence of Ozonolysis Time during Sugarcane Pretreatment: Effects on the Fiber and Enzymatic Saccharification'. *Bioresource Technology*, 224, 733–737.

Perrone, O.M., Colombari, F.M., Rossi, J.S., Moretti, M.M.S., Bordignon, S.E., Nunes, C. da C.C., Gomes, E., Boscolo, M., and Da-Silva, R. (2016). 'Ozonolysis Combined with Ultrasound as a Pretreatment of Sugarcane Bagasse: Effect on the Enzymatic Saccharification and the Physical and Chemical Characteristics of the Substrate'. *Bioresource Technology* 218, 69–76.

Quesada, J. Rubio, M. and Gómez, D., (1999). 'Ozonation of Lignin Rich Solid Fractions from Corn Stalks'. *Journal of Wood Chemistry and Technology*, 19(1–2), 115–137.

Ramli, N.A.S., (2010). *Levulinic Acid Production from Glucose, Empty Fruit Bunch and Kenaf.* Degree Thesis. Universiti Teknologi Malaysia, Johor, Malaysia.

Ramli, N.A.S., (2015). *Renewable Levulinic Acid Production Catalyzed by Iron Modified HY Zeolite and Functionalized Ionic Liquid.* PhD Thesis, Universiti Teknologi Malaysia, Johor, Malaysia.

Ramli, N.A.S. and Amin, N.A.S., (2014). 'Catalytic Hydrolysis of Cellulose and Oil Palm Biomass in Ionic Liquid to Reducing Sugar for Levulinic Acid Production'. *Fuel Processing Technology*, 128.

Ramli, N.A.S. and Amin, N.A.S., (2016). 'Optimization of Biomass Conversion to Levulinic Acid in Acidic Ionic Liquid and Upgrading of Levulinic Acid to Ethyl Levulinate'. *BioEnergy Research*, 1–14.

Ramli, N.A.S. Amin, N.A.S. and Ware, I., (2014). 'Optimization of Oil Palm Fronds Pretreatment Using Ionic Liquid for Levulinic Acid Production'. *Jurnal Teknologi*, 71(1).

Rowell, R.M., (2005). *Handbook of Wood Chemistry and Wood Composites*, US. Taylor & Francis.

Sankaran, R., Parra Cruz, R.A., Pakalapati, H., Show, P.L., Ling, T.C., Chen, W.H., and Tao, Y. (2020). 'Recent Advances in the Pretreatment of Microalgal and Lignocellulosic Biomass: A Comprehensive Review'. *Bioresource Technology,* 298, 122476.

van Santen, R.A., (2007). Renewable Catalytic Technologies - A Perspective. In: Centi, G. and van Santen, R.A., (eds.) *Catalysis for Renewables: From Feedstocks to Energy Production*. Weinhem. WILEY-VCH.

Schultz-Jensen, N., Kádár, Z., Thomsen, A., Bindslev, H., and Leipold, F., (2011). 'Plasma-Assisted Pretreatment of Wheat Straw for Ethanol Production.' *Applied Biochemistry and Biotechnology*, 165(3–4), 1010–1023.

Shefet, G. and Ben-Ghedalia, D., (1982). 'Effect of Ozone and Sodium Hydroxide Treatments on the Degradability of Cotton Straw Monosaccharides by Rumen Microorganisms'. *European Journal of Applied Microbiology and Biotechnology*, 15(1), 47–51.

Shi, F. Xiang, H. and Li, Y., (2015). ‘Combined Pretreatment Using Ozonolysis and Ball Milling to Improve Enzymatic Saccharification of Corn Straw’. *Bioresource Technology*, 179, 444–451.

Sindhu, R. Pandey, A. and Binod, P., (2015). Alkaline Treatment. In: Pandey, A., Negi, S., Binod, P. and Larroche, C., (eds.) *Pretreatment of Biomass: Processes and Technologies*. Netherlands, Elsevier Science.

Sitter, R.R. Chen, J. and Feder, M., (1997). ‘Fractional Resolution and Minimum Aberration in Blocked 2 n—k Designs’. *Technometrics*, 39(4), 382–390.

Sofla, R. K. M. Brown, R.J. Tsuzuki, T. and Rainey, T.J., (2016). ‘A Comparison of Cellulose Nanocrystals and Cellulose Nanofibres Extracted from Bagasse Using Acid and Ball Milling Methods’. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 7(3).

StatSoft, Inc., (2011). *Electronic Statistic Textbook*, Tulsa,OK, StatSoft.

Sulaiman, S.A., Balamohan, S., Moni, M.N.Z., Atnaw, S.M., and Mohamed, A.O., (2015). ‘Feasibility Study of Gasification of Oil Palm Fronds’. *Journal of Mechanical Engineering and Sciences*, 9, 1744–1757.

Sun, Y. and Cheng, J., (2002). ‘Hydrolysis of Lignocellulosic Materials for Ethanol Production: A Review’. *Bioresource Technology*, 83(1), 1–11.

Taherzadeh, M.J. and Karimi, K., (2008). ‘Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review’. *International Journal of Molecular Sciences*, 9(9), 1621–1651.

Tan, H.T. and Lee, K.T., (2012). ‘Understanding the Impact of Ionic Liquid Pretreatment on Biomass and Enzymatic Hydrolysis’. *Chemical Engineering Journal*, 183, 448–458.

Tan, X.Y. and Ho, S.H., (2019, March 8). Oil Palm Planted Areas to Be Capped at 6.5 Million Hectares. *The Edge Financial Daily*. Retrieved on 19 Jan 2019 from http://theedgemarkets.com

Tejado, A., Peña, C., Labidi, J., Echeverria, J.M., and Mondragon, I., (2007). ‘Physico-Chemical Characterization of Lignins from Different Sources for Use in Phenol–Formaldehyde Resin Synthesis’. *Bioresource Technology*, 98(8), 1655–1663.

Thommes, M., Kaneko, K., Neimark, A. V, Olivier, J.P., Rodriguez-Reinoso, F., Rouquerol, J., and Sing, K.S.W., (2015). 'Physisorption of Gases, with Special Reference to the Evaluation of Surface Area and Pore Size Distribution (IUPAC Technical Report).' *Pure and Applied Chemistry,* 87(9-10), 1051-1069.

Travaini, R., Otero, M.D.M., Coca, M., Da-Silva, R., and Bolado, S., (2013). 'Sugarcane Bagasse Ozonolysis Pretreatment: Effect on Enzymatic Digestibility and Inhibitory Compound Formation'. *Bioresource Technology*, 133, 332–339.

Travaini, R., Pereira, J.D.C., Zavarizi, F., Martins, D.A.B., Gomes, E., and Bolado, S.R., (2014). 'Studies on the Application of Myceliophthora Thermophila JCP1-4 Cellulases Cocktail on Sugarcane Bagasse Pretreated by Different Methods'. *Journal of Biotechnology*, 185, S122–S123.

Travaini, R., Marangon-Jardim, C., Colodette, J.L., Morales-Otero, M., and Bolado-Rodríguez, S., Chapter 7 - Ozonolysis. In: Negi, S., Binod, P. and Larroche, C., (eds.) Pretreatment of Biomass. Amsterdam, Elsevier, 105–135.

Travaini, R. Barrado, E. and Bolado-Rodriguez, S., (2016a). 'Effect of Ozonolysis Pretreatment Parameters on the Sugar Release, Ozone Consumption and Ethanol Production from Sugarcane Bagasse'. *Bioresource Technology*, 214, 150–158.

Travaini, R. Barrado, E. and Bolado-Rodríguez, S., (2016b). 'Effect of Ozonolysis Parameters on the Inhibitory Compound Generation and on the Production of Ethanol by Pichia Stipitis and Acetone-Butanol-Ethanol by Clostridium from Ozonated and Water Washed Sugarcane Bagasse'. *Bioresource Technology*, 218, 850–858.

Travaini, R. Martín-Juárez, J. Lorenzo-Hernando, A. and Bolado-Rodríguez, S., (2016c). 'Ozonolysis: An Advantageous Pretreatment for Lignocellulosic Biomass Revisited'. *Bioresource Technology*, 199, 2–12.

Utami, S.P. and Amin, N.S., (2013). 'Optimization of Glucose Conversion to 5-Hydroxymethylfulfural Using [BMIM]Cl with Ytterbium Triflate'. *Industrial Crops and Products*, 41, 64–70.

Vidal, P.F. and Molinier, J., (1988). 'Ozonolysis of Lignin — Improvement of in Vitro Digestibility of Poplar Sawdust'. *Biomass*, 16(1), 1–17.

Wan Omar, W.N.N. (2011). *Alkaline Modified Zirconia Based Catalyst for Biodiesel Production from Waste Cooking Palm Oil*. Master Thesis, Universiti Teknologi Malaysia, Johor, Malaysia.

Wang, H., Zhu, C., Li, D., Liu, Q., Tan, J., Wang, C., Cai, C., and Ma, L. (2019). 'Recent Advances in Catalytic Conversion of Biomass to 5-Hydroxymethylfurfural and 2, 5-dimethylfuran'. *Renewable and Sustainable Energy Reviews* 103, 227–247.

Wertz, J.L., Bédué, O., and Mercier, J.P. (2010). *Cellulose Science and Technology*. Lausanne, Switzerland. EFPL Press.

Wijaya, Y.P., Putra, R.D.D., Widyaya, V.T., Ha, J.M., Suh, D.J., and Kim, C.S. (2014). 'Comparative Study on Two-step Concentrated Acid Hydrolysis for the Extraction of Sugars from Lignocellulosic Biomass'. *Bioresource Technology* 164, 221–231.

Wood, I.P., Elliston, A., Ryden, P., Bancroft, I., Roberts, I.N., and Waldron, K.W. (2012). 'Rapid Quantification of Reducing Sugars in Biomass Hydrolysates: Improving the Speed and Precision of the Dinitrosalicylic Acid Assay'. *Biomass and Bioenergy* 44, 117–121.

Yang, H., Yan, R., Chen, H., Lee, D.H., and Zheng, C. (2007). 'Characteristics of Hemicellulose, Cellulose and Lignin pyrolysis'. *Fuel* 86, 1781–1788.

Yeoh, S.Y. (2013). *Optimization of Ozonolysis Pretreatment of Lignocellulosic Biomass.* Degree Thesis, Universiti Teknologi Malaysia, Johor, Malaysia.

Zaikov, G.E., and Rakovsky, S.K. (2009). *Ozonation of Organic & Polymer Compounds*. Shropshire, United Kingdom. iSmithers.

Zhang, Z., O'Hara, I.M., Doherty, W.O.S. (2013). 'Pretreatment of Sugarcane Bagasse by Acidified Aqueous Polyol Solutions'. *Cellulose* 20, 3179–3190.

Zhu, Y., Huang, J., Sun, S., Wu, A., and Li, H. (2019). 'Effect of Dilute Acid and Alkali Pretreatments on the Catalytic Performance of Bamboo-derived Carbonaceous Magnetic Solid Acid'. *Catalysts* 9, 245.

LIST OF PUBLICATIONS

1. Wan Omar WNN, Amin NAS (2020), Fractionation of Oil Palm Fronds (OPF) by Ozonolysis for Enhanced Sugar Production. Chemical Engineer Transaction.
2. Wan Omar WNN, Amin NAS (2016), Multi response optimization of oil palm frond pre-treatment by ozonolysis. Ind Crops Prod 85:389–402. doi: http://dx.doi.org/10.1016/j.indcrop.2016.01.027. (Q1: if=3.884)
3. Wan Omar WNN, Amin NAS (2015), *Pre-treatment and Fractionation of Oil Palm Frond by Ozonolysis Process for Enhancing Reducing Sugars.* 6th Conference on emerging energy and process technology 2017 (CONCEPT 2017),27th-28th November 2017.
4. Wan Omar WNN, Amin NAS (2015), *Parameter Screening of Ozonolysis Treatment and Product Characterization*. 2nd Int. Conf. 2015 (CONCEPT 2016)
5. Wan Omar WNN, Amin NAS (2015), *Characterization of Solid Products from Ozonolysis Pre-treatment of Oil Palm Fronds*. 1st Int. Conf. Oleo PetroChemical Eng. (ICOOPChE 2015).
6. Wan Omar WNN, Amin NAS (2015) *Optimization ozonolysis pre-treatment of oil palm frond* (2ND UTM-UTP SEMINAR), UTM SEMARAK, KUALA LUMPUR Malaysia.
7. Wan Omar WNN, Amin NAS (2014) *Pre-treatment of Oil Palm Oil via Ozonolysis: Parameter Screening and Treated Product Characterization* (1st UTM-UTP SEMINAR), Bukit Jalil, KUALA LUMPUR Malaysia.
8. Wan Omar WNN, Amin NAS (2015), *Ozonolysis pre-treatment of oil palm frond. 2015* (Probiorefine 2015), UTM, Johor, Malaysia.
9. Wan Omar WNN, Amin NAS (2013), *Parameter screening of OPF lignin degradation using ozone*. (IScHE 2013), PWTC, KUALA LUMPUR Malaysia