LACTIC ACID PRODUCTION FROM MICROWAVE-ALKALI PRETREATED OIL PALM TRUNK BIOMASS USING SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

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requirements for the award of the degree of
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For Papa and Mom,

Thank you for always being there; your endless love, faith and encouragement never fail to strengthen me

and specially for my wife and son,

Thank you for always standing next to me

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I would like to take this opportunity to extend my sincere appreciation and gratitude to following people, without whom, the work would not have been possible.

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ABSTRACT

Oil palm trunk (OPT) has never been used as a substrate in simultaneous saccharification and fermentation (SSF) for lactic acid production due to the existence of lignin in lignocellulose which makes biomass difficult to be hydrolyzed by enzymes and microbes. Hence, when used as substrate, effective pretreatment method is necessary so as to release the cellulose from complex crystalline structure. Production of lactic acid *via* SSF required compromising circumstances as microbe and enzyme perform best at different operating conditions. Present study demonstrated the production of lactic acid from microwave-alkali (Mw-A) pretreated OPT biomass by using cellulase, 1,4-β-D-glucosidase and *Rhizopus oryzae* NRRL 395 through SSF process. The OPT biomass was treated using three different pretreatment methods: Mw-A, steam-alkali-chemical (SAC) and Mw-A + SAC techniques. Variations on physical and chemical constituents on OPT were analyzed. After pretreatment, results revealed higher amount of cellulose (g/100g biomass) was obtained for Mw-A sample, 71.88 as compared to Mw-A +SAC, 56.50 and SAC, 42.70. The 72 h enzymatic saccharification revealed that accumulated glucose amount (Mw-A sample) was 4.86-fold as compared to untreated substrate. The values of enzyme kinetics parameters: Lineweaver-Burk method (K_m=3.682 $g.L^{-1}$, V_{max}=4.750 $g.L^{-1}.min^{-1}$) were in close agreement with non-linear regression (K_m=3.422 $g.L^{-1}$, V_{max}=4.710 $g.L^{-1}.min^{-1}$), obeying Michaelis-Menten model. Experimental design on SSF was performed by response surface methodology (RSM) using face centered central composite design. The influence of three independent variables: temperature (32–42 °C), pH (4–6) and enzyme ratio (3:1–7:1) on lactic acid production were investigated. When temperature, pH and enzyme ratio were set to 36.11 °C, 4.56 and 5:1; experimental value was in good agreement with RSM model prediction where lactic acid production at 6.632 ± 0.032 $g.L^{-1}$ was achieved. By performing Mw-A pretreatment; treated OPT substrate was easy to be utilized in SSF process for the production of lactic acid.

ABSTRAK

Batang kelapa sawit (OPT) belum pernah digunakan sebagai substrat dalam fermentasi dan pensakaridaan serentak (SSF) untuk penghasilan asik laktik disebabkan kewujudan komponen lignin dalam lignoselulosa yang mengakibatkan biojisim sukar untuk dihidrolisiskan oleh enzim dan mikrob. Oleh itu apabila digunakan sebagai substrat, kaedah prarawatan yang berkesan diperlukan untuk membebaskan selulosa daripada struktur kompleks kristal. Penghasilan asid laktik melalui proses SSF memerlukan keadaan kompromi kerana mikrob dan enzim selulolitik bertindakbalas baik pada keadaan operasi yang berbeza. Kajian ini menunjukkan penghasilan asid laktik daripada biomas OPT terawat oleh mikrogelombang-alkali (Mw-A) dengan menggunakan selulase, 1,4-β-D-glukosidase dan *Rhizopus oryzae* NRRL 395 melalui proses SSF. Biomas OPT telah dirawat dengan tiga prarawatan berbeza: teknik Mw-A, stim-alkali-kimia (SAC) dan Mw-A + SAC. Variasi juzuk fizikal dan kimia pada OPT terawat telah dianalisa. Selepas prarawatan, keputusan menunjukkan jumlah selulosa yang tinggi (g/100g biomass) diperoleh dalam sampel Mw-A, 71.88 berbanding dengan Mw-A + SAC, 56.50 dan SAC, 42.70. Pensakaridaan enzim selama 72 jam mendedahkan bahawa pengumpulan glukosa (sampel Mw-A) adalah 4.86 kali ganda berbanding dengan substrat mentah. Nilai-nilai parameter kinetik enzim: kaedah Lineweaver-Burk (K_m=3.682 $g.L^{-1}$, V_{max}=4.750 $g.L^{-1}.min^{-1}$) adalah rapat dengan nilai regresi bukan linear (K_m=3.422 $g.L^{-1}$, V_{max}=4.710 $g.L^{-1}.min^{-1}$), mematuhi model Michaelis-Menten. Eksperimen ke atas SSF telah dilakukan dengan menggunakan kaedah gerak balas permukaan (RSM) berpusat muka reka bentuk komposit. Pengaruh terhadap tiga pembolehubah bebas: suhu (32–42 °C), pH (4–6) dan nisbah enzim (3:1–7:1) untuk pengeluaran asid laktik telah dikaji. Apabila suhu, pH dan nisbah enzim ditetapkan pada 36.11 °C, 4.56 and 5:1; nilai eksperimen yang diperoleh berada dalam persetujuan yang baik dengan ramalan dari model RSM di mana pengeluaran asid laktik adalah 6.632 ± 0.032 $g.L^{-1}$. Dengan melakukan prarawatan Mw-A, substrat OPT terawat lebih mudah digunakan dalam proses SSF bagi penghasilan asid laktik.

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LIST OF SYMBOLS

A	-	Alfa
β	-	Beta
$Å$	-	Angstrom
Θ	-	Theta
$\pm$	-	Plus and minus
Sec	-	Second
min	-	Minute
H	-	Hour
G	-	Gram
Mg	-	Milligram
Cm	-	Centimeter
Mm	-	Millimeter
μm	-	Micrometer
Nm	-	Namometer
cm^{-1}	-	Reciprocal centimetre
L	-	Liter
Ml	-	Milliliter
μl	-	Microliter
M	-	Molarity
mM	-	Millimolar
^oC	-	Degree of Celsius
^oF	-	Degree of Fahrenheit
W	-	Watt
kV	-	kilo volt
mA	-	mili Ampere
%	-	Percentage
% T	-	Percentage of transmittance
(w/v)	-	weight per volume
(v/v)	-	volume per volume
$g.L^{-1}$	-	Gram per liter
$g.mol^{-1}$	-	gram per moles
$g.g^{-1}$	-	Gram per gram
CrI	-	Crystalline index
I_{002}	-	Maximum intensity of lattice diffraction (002) / primary peak
I_{18}	-	Intensity diffraction at 18^o / secondary peak
K_m	-	Michaelis-Menten constant
V	-	Initial reaction velocity

V_{max}	-	Maximum rate of reaction at infinite substrate concentration
$[S]$	-	Substrate concentration
$[E]_o$	-	Initial enzyme concentration
$[E]$	-	Enzyme concentration
K	-	Rate constant proportional to the diffusion coefficient
N	-	Structural diffusion resistance constant
P	-	Product
P_∞	-	Product at equilibrium
T	-	Time (min / hour)
pK_a	-	Acid dissociation constant
MPa	-	Megapascal
$mmHg$	-	Millimeter of Mercury
A	-	Temperature effect
B	-	pH effect
C	-	Enzyme ratio effect
A^2	-	Square effects of temp*temp
B^2	-	Square effects of pH*pH
C^2	-	Square effects of enzyme ratio*enzyme ratio
AB	-	Interactive effects of temp*pH
AC	-	Interactive effects of temp*enzyme ratio
BC	-	Interactive effects of pH* enzyme ratio
R^2	-	Regression coefficient
$Y_{Lactic\ acid}$	-	Lactic acid production response
$Y_{Glucose}$	-	Glucose accumulation response
$Y_{Protein}$	-	Protein formation response
G	-	Guaiacyl propane unit
S	-	Syringly propane unit
C	-	Carbon
$C5$	-	Pentose sugar
$C6$	-	Hexose sugar
H	-	Hydrogen
O	-	Oxygen
W_E	-	Weight of extractive
W_{SAE}	-	Weight of sample after extraction
W_{ADS}	-	Weight of air-dried sample
W_{ADHS}	-	Weight of air-dried holocellulose sample
W_{ADCS}	-	Weight of air-dried cellulose sample
Ws	-	Weight of sample
W_{FP}	-	Weight of filter paper
$W_{IniADHS}$	-	Initial weight of air-dried holocellulose sample
W_L	-	Weight of lignin
pH	-	Logarithm to base 10 of the activity of the hydrogen ion

LIST OF ABBREAVIATION

ADH	-	Alcohol dehydrogenase
AFEX	-	Ammonia fibre expansion
ANOVA	-	Analysis of variance
ATCC	-	America type culture collection
BSA	-	Bovine serum albumin
CBH	-	Cellobiohydrolases
CBU	-	Cellobiase unit
CMC	-	Carboxymethyl cellulose
CrI	-	Crystallinity index
DCW	-	Dry cell weight
DNS	-	Dinitrosalicylic acid
DO	-	Dissolve oxygen
DOE	-	Design of experiment
DP	-	Degree of polymerization
EC	-	Enzyme commission
EG	-	Edo-1,4-β-D-glucanases
etc.	-	Et cetera
FCCCD	-	Face centered central composite design
FCD	-	Face centered design
FDA	-	Food and Drug Administration
FESEM	-	Field Emission Scanning Electron Microscope
FPU	-	Filter paper unit
FT-IR	-	Fourier Transform Infrared
FUM	-	Fumarase
GDP	-	Gross domestic product
GRAS	-	Generally regarded as safe

LAB	-	Lactic acid bacteria
LDH	-	Lactate dehydrogenase
MDH	-	Malate dehydrogenase
MPOB	-	Malaysia Palm Oil Board
Mw-A	-	Microwave alkali
NAD	-	Nicotinamide adenine dinucleotide
OPBC	-	Oil Palm Board Commission
OPEFB	-	Oil palm emptry fruit bunch
OPF	-	Oil palm frond
OPT	-	Oil palm trunk
PK	-	Phosphoketolase
PKS	-	Palm kernel shells
PLA	-	Polylactic acid
PDA	-	Potato dextrose agar
PDC	-	Pyruvate decarboxylase
PYC	-	Pyruvate carboxylase
R.	-	*Rhizopus*
Rpm	-	Round per minute
RSM	-	Response surface methodology
SAC	-	Steam alkali chemical
SHF	-	Separate enzymatic hydrolysis and fermentation
Sp	-	Species
SSF	-	Simultaneous saccharification and fermentation
TCA	-	Tricarboxylic acid cycle
US	-	United States
viz.	-	Videlicet
XDH	-	Xylitol dehydrogenase
XI	-	Xyloseisomerise
XK	-	Xylose kinase
XOS	-	Xylo-Oligosaccharide
XR	-	Xylose reductase
XRD	-	X-Ray Diffractometer
3D	-	Three-dimensional

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Malaysia, the second world's largest palm oil industry tycoon possesses an oil palm plantation up to 5.076 million hectares (MPOB, 2012). Each year, there is an abundance of agricultural waste derived from palm oil industry. The major types of these lingocellulosic residues are palm kernel shells (PKS), oil palm empty fruit bunch (OPEFB) obtained from the mills, oil palm frond (OPF) obtained during routine pruning and oil palm trunk (OPT) derived from the field when the replanting is required.

The oil palm solid biomass wastes contributed to a total amount of 59 million tons annually (Chen and Danapal, 2012). These amounts are significant enough to consider the oil palm biomass residues as a complementary source of raw material in the production of bioethanol and other biochemical derived products, such as lactic acid. Figure 1.1 demonstrates the total projected biomass residue in Malaysia.

Converting these tremendous agricultural residues into higher value added products would benefit the nation economy. This is because oil palm biomass behaves like other lignocellulosic biomaterial which mainly consists of cellulose, hemicellulose and lignin. The cellulose and hemicellulose compounds are primarily made up of polymeric blocks consisting of hexose and pentose sugars entrenched in the phenolic polymer lignin matrix (Mass *et al.*, 2006).

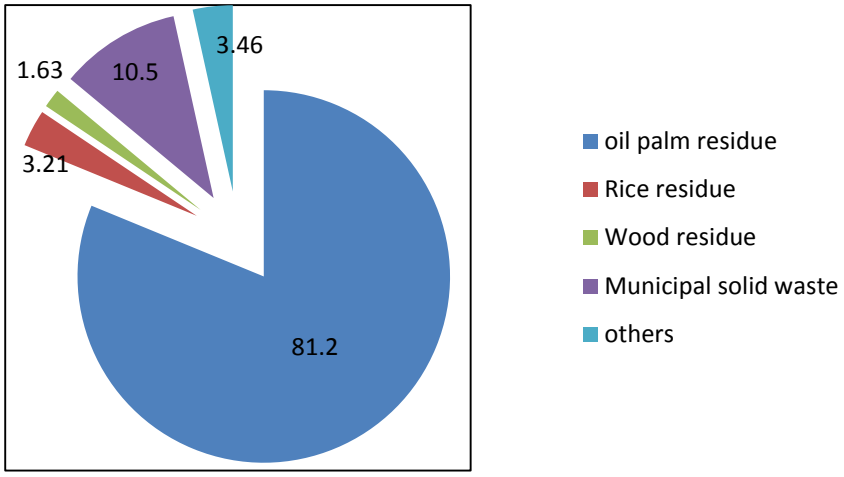

Figure 1.1 Total projected annual biomass availability in Malaysia (million tons, wet weight) (Tang, 2014)

The natural existing of pentose and hexose sugars in lignocellulose has made this waste residue, a beneficial substrate for a wide application particularly in fermentation industry. Therefore, the huge oil palm residue in Malaysia should be utilized efficiently. Table 1.1 illustrates the Malaysia scenario of oil palm biomass residue which includes oil palm trunk, oil palm frond and oil palm empty fruit bunch supplied from 2001 to 2014 and the forecasted supply for year 2015 to 2020.

Table 1.1: Malaysia scenario of oil palm biomass waste supply from 2001 to 2020

	Year					
Biomass waste (tones per year, dry weight)	**2001-2003**	**2004-2006**	**2007-2010**	**2011-2013**	**2014-2016***	**2017-2020***
Oil palm trunk	3,993,442	4,020,852	3,234,164	4,283,082	3,583,803	2,971,934
Oil palm frond	7,412,074	7,025,525	6,890,233	6,803,260	7,044,853	7,141,490
Empty fruit bunch	2,870,148	2,860,194	2,823,695	2,830,311	2,906,647	2,863,512

* Projection (Fazlena, 2012)

The large quantity of the palm oil wastes causes a disposable problem, as the bulk density need to be stored or processed before discharging. The environmental health risk such as dengue fever can also occur because farmers tend to leave the cut oil palm residues in the plantation site. In most cases, they are burnt; however the open burning of bulk quantity of palm oil wastes is prohibited and banned by government and environmentalists as this may create serious air-pollution (Ethaya, 2010).

In order to make use of oil palm biomass, one of the promising technologies is to convert this abundance and renewable biomass to sugar monomers using enzymes. This is then followed by microbes to convert the fermentable sugars into desired products. Meanwhile, the processing of lignocellulosic biomass into useful bioproducts would require a few steps. First, it usually involves the pretreatment of lignocellulosic biomass followed by enzymatic saccarification. The third step is normally the fermentation process and finally the product isolated from the fermentation broth.

Numerous pretreatment methods have been developed since 1970s to pretreat the recalcitrant lignocellulosic biomass. The apparent objectives of pretreatment are: i) to alter the structure of lignocellulose so as to make cellulose more accessible to enzymes that convert the carbohydrate polymers into fermentable sugars (monosaccharide) and ii) to break the lignin seal and disrupt the crystalline structure of cellulose (Ewanick, 2010). The destruction of cell wall has led to the loosening of the lignocellulosic complex and eventually resulted in lesser lignin and hemicellulose but increase released of cellulose in treated sample. The pretreated biomass would aid in enzymatic saccharification latter.

Nowadays, none of the pretreatment protocol is universal and economically viable to pretreat different cellulosic biomass. Therefore, the proper pretreatment methods should be identified in order to maximize the efficiency of cellulose recovery (released from lignocellulosic structure). The most popular used protocol such as hydrothermal, thermochemical, solvent fractionation, dilute and concentrated acid or alkali treatment, enzymatic hydrolysis and biological treatment have recently

been investigated in-depth by research scientists. The pretreated solid biomass is then used directly in the fermentation process as a carbon source to achieve the bioprocess economic. Nowadays, the interest in lignocellulosic biomass has drawn a great attention. From the scientific point of view, the lignocellulose could be used to replace the expensive refined carbon source due to its abundance, cheap and renewable character (Zheng *et al*., 2009). Thus, application of the biomass waste as a raw material in chemical process has become a new challenge to the researcher in industrial sector.

Lately, with the state-of-the-art biotechnology technique, there is a growing trend in conversion of lignocellulosic biomass into value added products such as single cell protein, bioethanol, xylitol, organic acid (such as poly-lactic acid) and etc. Figure 1.2 displays the potential products that can be generally produced from lignocellulosic biomass. Table 1.2 tabulates the compositions of cellulose, hemicellulose and lignin in the different parts of oil palm tree by different researchers. According to Table 1.2, highest cellulose content was found in OPT; thus bioconversion of OPT into high market demand product such as lactic acid is attractive.

Recently, scientists have attempted to synthesize lactic acid from various wood based or lignocellulosic feedstock in order to replace the expensive pure sugar as a result of the increased demand of the lactic acid in the world market. The global demand for lactic acid was 800,000 tons in 2013 (NARA, 2013). Generally, lactic acid could be produced via fermentation route by using either bacteria or fungus strain. The production of lactic acid from cellulosic materials as reported using various sources such as wheat straw (Saito *et al*., 2012), corncob (Shen and Xia, 2006), corn stover (Garrett *et al*., 2015) and oil palm empty fruit bunch (Hamzah *et al*., 2009), respectively. Therefore, the objective of this research is to utilize the OPT biomass efficiently for the conversion of cellulose into lactic acid.

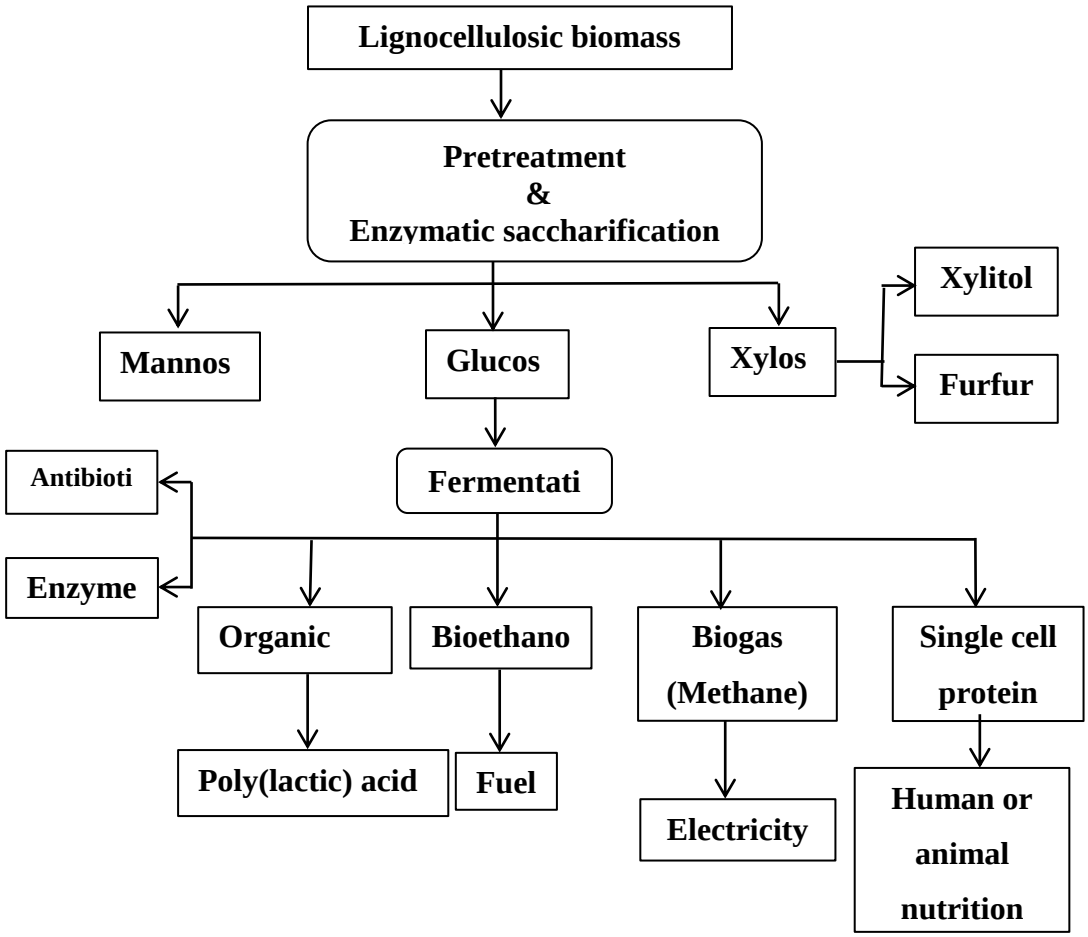

Figure 1.2 Products synthesis from lignocellulosic biomass

Table 1.2: Chemical compositions in oil palm biomass (%)

Part of plants	Cellulose	Hemicellulose	Lignin	Reference
Oil palm empty fruit bunch (OPEFB)	44.2	33.5	20.4	Astimar *et al*. (2002)
	43.8	35.0	16.4	Hamzah *et al*. (2011)
	43.7	28.6	16.2	Aanifah *et al*. (2014)
	40.4	20.2	23.1	Zakaria *et al*. (2015)
Oil palm frond (OPF)	32.7	22.5	15.2	Zakaria *et al*. (2015)
	25.1	24.1	18.5	Tan *et al*. (2011)
Oil palm trunk (OPT)	47.5	31.0	18.4	Chin *et al*. (2010)

Note: Minor components are not listed there, these numbers do not sum to 100 %

1.2 Lactic Acid Production

In general, lactic acid could be produced by conventional fermentation processes which include a few stages where the starchy material usually undergoes the gelatinization and liquefaction pretreatment at high temperature (90 – 130 $°C$) for a short duration (15 min). It is then followed by enzymatic saccharification which mainly produces monosaccharide, i.e. glucose which is then consumed by particular microbes to produce lactic acid, cell mass and carbon dioxide formation. This type of fermentation path is called separate hydrolysis and fermentation (SHF). Figure 1.3 illustrates the conventional SHF and simultaneous saccharification and fermentation (SSF) approaches for lactic acid production.

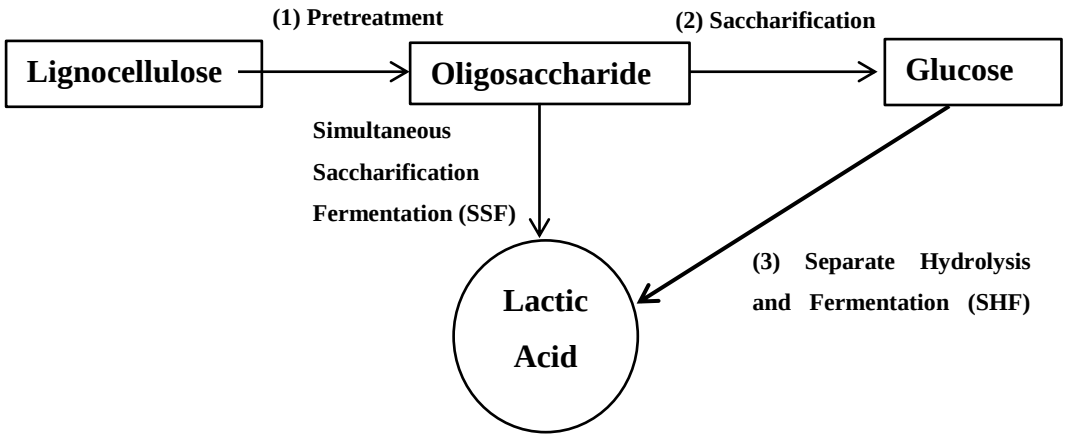

Figure 1.3 Different fermentation approaches for lactic acid production

The involvement of several SHF steps makes the fermentation of lactic acid production unattractive and uneconomically viable (Huang *et al*., 2005). This may be attributed to high energy consumption as two separate stages are involved and eventually add-on to the production cost. In contrast, the SSF process as shown in Figure 1.3 is able to initiate the fermentation process with the introduction of enzymes and microbes simultaneously in a single step. The SSF has been attracting much attention recently as it is envisaged to save time, cost of production, low contamination risk, reduce the number of reactor vessels and reactor volumes.

Furthermore, the SSF process can increase the productivity by eliminating the inhibition effect caused by glucose accumulation (Satriyo *et al*., 2014). Recently, Zhang *et al*. (2015) has reported that higher L-(+)-lactic acid titer (60.3 $g.L^{-1}$) was obtained in SSF process from corncob waste residue. The finding shows that the yield of lactic acid in SSF is 43.6 % higher than in SHF.

The lactic acid could be produced by either bacteria or fungus strain. Generally, the bacteria from *Lactobacillus* and *Lactococcus* exhibit fast growing rate and high product yield. Current industrial production of lactic acid uses homolactic acid bacteria, culture in enriched media with glucose substrate. The supplementation of adequate nutrient such as yeast extract to culture broth is also essential. This make the overall of lactic acid production suffer from high raw materials and purification cost. Although *Lactobacillus sp.* is able to synthesize lactic acid but the concentration was relatively low, i.e. 10–30 $g.L^{-1}$ (Park *et al*., 2004).

Thus, an alternative substitution for lactic acid bacteria has resulted in the use of fungus species. For example, the *Rhizopus oryzae*, a fungus strain is known to produce only the pure L-(+)-form lactic acid, unlike bacteria which generate mix isomers in either D-(–)- or L(+)-form (Zheng *et al*., 2009). As reported, the L-(+)-type lactic acid is an essential component to form the polylactic acid (PLA), which is a precursor to mass produce the biodegradable plastic (Yin *et al*., 1997). Another advantage of using *R. oryzae* for lactic acid production is the ability to utilize various carbon sources including pentose and hexose sugars in lignocellulosic biomass and it requires low nutrient to grow (Skory *et al*., 1998). Recent studies have revealed that *R. oryzae* could produce lactic acid for at least 24 – 60.3 $g.L^{-1}$ in SSF process by using paper pulp or corncob as a substrate (Vially *et al*., 2010; Zhang *et al*., 2015).

Moreover, the ability of self-immobilized character of *R. oryzae* has enhanced the rate of mass transfer. As stated by Liao *et al*. (2007b), the pelletized *R. oryzae* has produced lactic acid up to 60 $g.L^{-1}$ as compared to clump morphology, 20 $g.L^{-1}$ for 30 h fermentation by using pure glucose as a substrate. Besides, the pelletized morphology also makes the separation process easier during downstream processing.

To best of our knowledge, the lactic acid produced from OPEFB was done by Hamzah *et al*. (2009). Limited data has been reported on lactic acid production from other parts of oil palm such as OPF and OPT. For this reason, the OPT biomass was selected as a substrate candidate for L-(+)-lactic acid production from *Rhizopus oryzae* via simultaneous saccharification and fermentation.

1.3 Problem Statement

The main components of lignocellulosic biomass are composed of cellulose, hemicellulose and lignin. Among these, lignin is a major obstacle for efficient cellulosic sugars conversion. The existence of lignin impedes the enzymatic hydrolysis and affects the sugars yield. This is because lignin acts as a barrier, shields the cellulose by preventing it binds to enzyme (Henning *et al*., 2007). Therefore, in cases when the substrate used is lignocellulose, a suitable pretreatment method needs to be identified so as to release the cellulose from the complex crystalline structure before it can be effectively hydrolyzed by enzyme and microorganisms. In view of this, different pretreatment methods (Mw-A, SAC and Mw-A +SAC techniques) were used to pretreat OPT so as to determine the most efficient one.

In SSF process, substrate, enzyme and inoculum are introduced simultaneously in a same reactor. The SSF outperformed SHF due to its high product yield and less energy consumption (Vially *et al*., 2010; Saito *et al*., 2012; Zhang *et al*., 2015). However, the only drawback of using SSF is enzymes and fermentation organisms perform best at different operating conditions. It is difficult to preset the SSF conditions since saccharification and fermentation required different settings of pH and temperature. For instance, lower pH < 5 and high temperature > 40 $°C$ would promote enzymatic hydrolysis but show adverse effect on the lactic acid production and fungal cell growth in SSF process (Huang *et al.*, 2005). This leads to low product yield at the end of fermentation.

On this account, the compromising circumstance between enzyme and microbe use in SSF process must be pre-determined in order for maximize product yield. In order to overcome this, current study employed the response surface methodology (RSM) to identify the best SSF conditions for optimum lactic acid production. By analyzing the RSM results, the concession SSF conditions for optimizing lactic acid production in between producer, enzymes and those tested parameters were established.

1.4 Objectives and Scopes of Study

The main aim of this study is to produce L-(+)-lactic acid from microwave-alkali pretreated OPT biomass via SSF route using cellulase, 1,4-β-D-glucosidase and *Rhizopus oryzae* NRRL 395. In order to ensure its achievement, the objectives include the following.

1) To investigate the suitable pretreatment methods for OPT.

2) To perform enzymatic saccharification study so as to determine the effectiveness of glucose formation.

3) To produce L-(+)-lactic acid from OPT biomass.

4) To determine the optimum conditions for lactic acid fermentation using response surface methodology (RSM) method.

The scopes of the study are within the following.

1) The influence of three different types of pretreatment protocol: i) microwave-alkali, Mw-A; ii) steam-alkali-chemical, SAC and iii) combination of microwave-alkali followed by steam-alkali-chemical, Mw-A + SAC techniques on compositional contents of OPT biomass were studied. The morphological

tests such as FESEM, FT-IR and XRD analyses of pretreated OPT samples were thoroughly investigated. Finally, the crystallinity index relates to crystalline and amorphous region of cellulose for all pretreated OPT were also examined.

2) The effectiveness of soluble glucose formation from Mw-A OPT substrate with various physiological effects: pH, temperature, enzyme ratio and substrate mass were determined. The pre-selected best conditions were used to rerun enzymatic saccharification reaction for all treated and raw OPT substrate so as to evaluate the comparatively glucose formation.

3) The enzyme kinetics parameters, K_m and V_{max} on single saccharification of Mw-A OPT substrate were estimated using linearized and non-linearized regression. The enzymatic saccharification on Mw-A treated OPT was fitted into Michaelis-Menten model. The Chrastil kinetics equation was employed to understand the effect of structural alternation on Mw-A OPT substrate.

4) The pelletized *Rhizopus oryzae* NRRL 395 was used in L-(+)-lactic acid production via SSF process. The preliminary study on 96 h of SSF reaction was performed in order to ensure the *R. oryzae* is able to consume Mw-A OPT substrate and produces the target product viz. L-(+)-lactic acid.

5) The response surface methodology (RSM) was computed based on three independent variables like temperature, pH and enzyme ratio to optimize the lactic acid response. All factors were statistically judged using analysis of variance (ANOVA). The RSM predicted optimal conditions for lactic acid production was validated by confirmation experiments.

1.5 Significant of Study

The cost for the production of lactic acid via SSF process is very much dependent on the raw materials. This can be achieved by using readily available low cost lignocellulose wastes derived from oil palm industry. The utilize of oil palm

trunk biomass as a substrate in SSF process not only reduce the production cost at up-stream level but at the same time also tackles the disposal problem. The OPBC (2012) report revealed that bioconversion of oil palm residue into value added products would contribute 5 % of today's Malaysian GDP up to RM 663 billion. If the oil palm residues can be transformed into lactic acid, this would increase the profits and competitiveness of this industry. Besides, the use of lignocellulose waste to produce lactic acid is smarter choice than food-based cellulose as its unwanted characteristic possesses no compete to any edible food produced.

Current industrial production of lactic acid uses homolactic acid bacteria; majority uses *Lactobacillus sp*. through fermentation (Zhang *et al*., 2015). Also, the lactic acid generated from bacteria fermentation was in two isomer forms which required further purification steps to obtain pure L-(+)-lactic acid. Hence, the alternative is to use fungus strain like *Rhizopus orzyae*. Reports revealed that lactic acid produced from *R. oryzae* mainly used the refined sugar, i.e. glucose as a main substrate (Yang *et al*., 1995; Liao *et al*., 2007a and Wu *et al*., 2011) as well as some lignocellulosic biomass such as paper pulp, wheat straw and corncob (Vially *et al*., 2010; Saito *et al*., 2012; Miura *et al*., 2004). On these accounts, *R. oryzae* NRRL 395 was fed with Mw-A OPT substrate as a carbon source during SSF process. Throughout state-of-the-art innovation, this research could contribute primary data for future investigation particularly for large scale production of commercial polylactic acid. The new information and idea were developed as compared to bacterial lactic acid fermentation.

In short, the novelty of present study is to produce L-(+)-type lactic acid from OPT substrate via SSF process. To the best of our knowledge, industrially lactic acid production was made using bacteria strains and the substrate used was derived from food based sources, refined sugars and starchy materials. The present study demonstrates the utilization of OPT as a substrate to substitute the expensive carbon source. Also, OPT has no food competing values and can be obtained cheaply from local oil palm plantation.

1.6 **Organization of Thesis**

This thesis is divided into five main chapters. Chapter 1 outlines the research background regarding the Malaysia oil palm industry, pretreatment protocol and global lactic acid demand. The lactic acid production using SHF and SSF was also discussed. Next, the problem statement, objectives and scopes of study, significant of study and organization of thesis were clearly mentioned.

Chapter 2 is literature review. It provides a thorough study and related works done by previous researchers on the knowledge, ideas and technologies. The related topics included lignocellulosic substrate, pretreatment protocols, role of cellulolytic enzyme, enzyme kinetics and fungus used in lactic acid fermentation as well as the RSM approach were discussed.

Chapter 3 details the methodology of the study. This chapter described all materials, enzymes, chemicals and reagents as well as instrumentation used in the present study. Besides, all the experiment methods were clearly explained in this section.

Meanwhile the results and discussion section was presented in Chapter 4. This chapter is sub-divided into four sections: the pretreatment of OPT biomass, enzymatic saccharification study, enzyme kinetics and lactic acid production via SSF path. All obtained results include tables and figures were elaborated in-depth.

The final chapter provides the summary and conclusion of the whole study. It also includes the recommendations for future work. Figure 1.4 shows the overall flow of the present study.

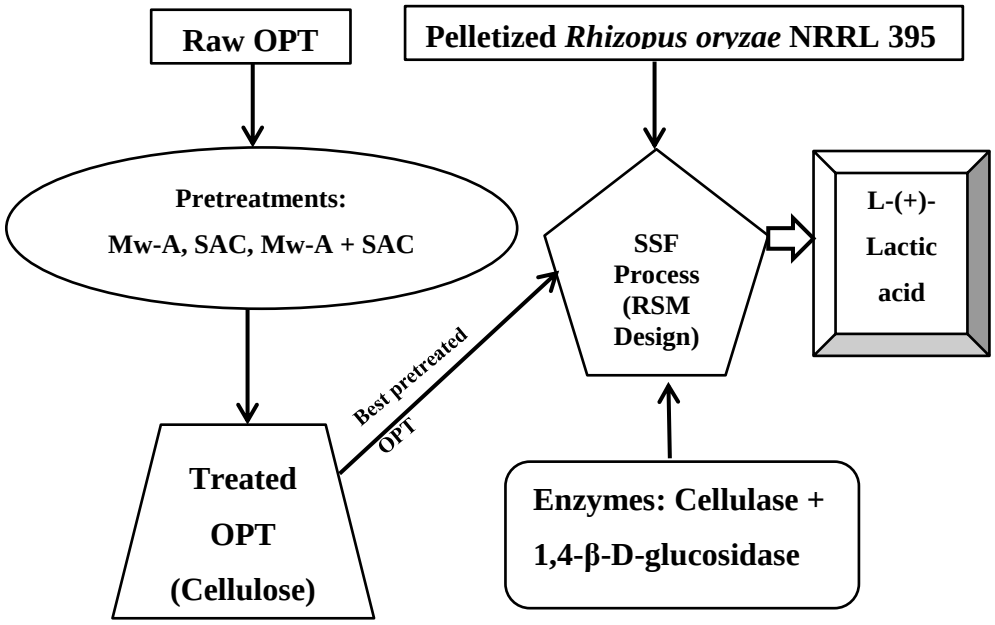

Figure 1.4 Flowchart of the overall process in present study

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