SUCCINIC ACID PRODUCTION VIA SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF OIL PALM EMPTY FRUIT BUNCH

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requirements for the award of the degree of
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To my beloved father and mother,

Muhammad Israr & Shahida Nasreen,

To my lovely wife,

Nur Fasihah Abdul Kadir,

My cute princess,

Nur Ruhi Junaid Akhtar.

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ABSTRACT

Oil palm empty fruit bunch (EFB), a plentiful agricultural waste in Malaysia has never been utilized for the production of succinic acid via simultaneous saccharification and fermentation (SSF). The presence of lignin and hemicellulose makes the biomass difficult to be hydrolysed by enzymes and microbes. Hence, effective pretreatment method is required to release cellulose from the crystalline complex structure of lignocellulose. The novelty of this study is the production of succinic acid via SSF from EFB by a rumen bacteria *Actinobaccilus succinogenes* ATCC 55618. The effect of three different methods; autoclave/alkali (AA), dilute acid (DA) and sequential dilute acid microwave/alkali (DA-MwA) pretreatment on the physical and chemical properties of EFB were analysed and their influence on enzymatic hydrolysis and SSF process were also assessed. Results revealed that maximum amount of cellulose (86.8 g/100g) was achieved for DA-MwA as compared with AA (53.3 g/100g) and DA (46.7 g/100g). The highest glucose concentration among all pretreated EFB was DA-MwA (20.3 gL^{-1}) method using *cellulase* enzyme. The effect of different *cellulase*: *cellobiase* ratios on enzymatic hydrolysis of DA-MwA pretreated EFB showed that ratio of 7:1 produced 34.45 % higher glucose as compared when only *cellulase* was used. Succinic acid concentration via SSF from DA-MwA (33.4 gL^{-1}) was the highest followed by AA (20.9 gL^{-1}) and DA (14.4 gL^{-1}). The SSF media for the succinic acid production was optimized using Full Factorial Design (FFD) by varying the EFB loading (10-70 gL^{-1}), yeast extract (0-20 gL^{-1}) and corn steep liquor (0-20 gL^{-1}) followed by Face Central Composite Design. The best concentration of succinic acid (39.14 gL^{-1}) was obtained when the values of EFB, yeast extract and corn steep liquor were set at 70 gL^{-1}, 30 gL^{-1} and 10 gL^{-1}, respectively. The influence of three independent SSF process variables: enzyme loading (10-70 FPU/g), temperature (36-40 ^{o}C) and pH (5-8) were investigated for succinic acid production using FFD. When the enzyme loading was set at 40 FPU/g, temperature 36 ^{o}C and pH 5; the experimental values were in good agreement with the predicted Response Surface Methodology model where the best succinic acid production of 42.5 gL^{-1} was achieved. The present study revealed that using DA-MwA pretreated EFB, cellulose was utilized by *cellulase* and *cellobiase* enzymes via optimized SSF conditions resulting in optimum production of succinic acid.

ABSTRAK

Tandan kosong buah kelapa sawit (EFB), adalah sisa pertanian yang banyak di Malaysia dan tidak pernah lagi digunakan bagi penghasilan asid suksinik melalui penapaian dan pensakaridaan serentak (SSF). Kehadiran lignin dan hemiselulosa menjadikan bahan biojisim sukar untuk dihidrolsis oleh enzim dan mikrob. Oleh itu, kaedah pra-rawatan adalah diperlukan untuk membebaskon selulosa daripada struktur kompleks kristal lignoselulosa. Penemuan baru bagi kajian ini adalah penghasilan asid suksinik melalui SSF daripada EFB oleh bakteria rumen *Actinobaccilus succinogenes* ATCC 55618. Kesan daripada tiga kaedah yang berbeza; autoklaf/alkali (AA), asid cair (DA), dan siri asid cair gelombang mikro/ alkali (DA-MwA) ke atas sifat-sifat fizik dan kimia bagi EFB telah dikaji dan pengaruh mereka terhadap proses SSF dan hidrolisis enzim juga telah dinilai. Hasil kajian menunjukkan bahawa kuantiti maksima selulosa (86.8 g/100g) diperolehi melalui kaedah DA-MwA berbanding AA (53.3 g/100g) dan DA (46.7 g/100g). Kepekatan glukosa yang maksima dicapai antara pra-rawatan EFB adalah melalui kaedah DA-MwA (20.3 gL^{-1}) menggunakan enzim *selulase*. Kesan dari purata berbeza *selulase* dan s*elobiase* bagi pra-rawatan EFB kaedah DA-MwA memberi hasil glukosa yang paling tinggi 34.45 % pada nisbah 7:1 berbanding penggunaan *selulase* sahaja. Kepekatan asid suksinik melalui SSF iaitu daripada DA-MwA (33.4 gL^{-1}) adalah yang tertinggi diikuti oleh AA (20.9 gL^{-1}) dan DA (14.4 gL^{-1}). Media SSF bagi penghasilan asid suksinik telah dioptimum menggunakan Reka bentuk Faktor Penuh (FFD) dengan mengubah kuantiti muatan EFB (10-70 gL^{-1}), ekstrak yis (0-20 gL^{-1}) dan likuor tusuk jagong (0-20 gL^{-1}), diikuti oleh Reka bentuk Komposit Muka Pusat. Kepekatan asid suksinik yang paling optimum (39.14 gL^{-1}) telah diperoleh apabila kuantiti EFB, ekstrak yis dan likuor tusuk jagong ditetapkan masing - masing pada 70 gL^{-1}, 30 gL^{-1} dan 10 gL^{-1}. Pengaruh pembolehubah bebas: muatan enzim (10-70 FPU/g), suhu (36-40 ^oC) dan pH (5-8) telah dikaji untuk penghasilan asid suksinik menggunakan FFD. Apabila muatan enzim ditetapkan pada 40 FPU/g, suhu 36 ^oC dan pH 5; asid suksinik mencatatkan hasil pada 42.5 gL^{-1} melalui model ramalan Kaedah Gerak Balas Permukaan. Kajian ini mendedahkan bahawa pra-rawatan melalui kaedah DA-MwA, selulosa telah digunakan dengan baik oleh enzim *selulase* dan *selobiase* melalui pengoptimuman keadaan SSF yang menghasilkan pengeluaran asid suksinik yang optimum.

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LIST OF SYMBOLS

A	-	Weight of lignin in grams
a_3	-	Weight. of crucible with oven dry cellulose
MT	-	Metric ton
α	-	Alfa
β	-	Beta
€ /MT	-	Euro per metric ton
^{o}C	-	Degree Celsius
%	-	Percentage
FPU/g	-	Filter paper unit per gram
CBU/g	-	Cellobiase unit per gram
cm	-	Centimeter
cm^{-1}	-	Reciprocal centimeters
$	-	Dollar sign
USD	-	United States dollar
MPa	-	Megapascal
mg	-	Milligram
mgL^{-1}	-	Milligram per liter
L	-	Liter
g	-	Gram
h	-	Hour
M	-	Molar
gL^{-1}	-	Gram per liter
kg^{-1}	-	Per kilogram
mM	-	Millimolar
mL	-	Milliliter
min	-	Minute

gg^{-1}	-	Gram per gram
$gL^{-1}h^{-1}$	-	Gram per liter per hour
rpm	-	Rounds per minute
pH	-	Measure of hydrogen ion concentration
V_{max}	-	Maximum rate of reaction at infinite substrate concentration
V_{emax}	-	Maximum velocity
$[S]$	-	Substrate concentration
R^2	-	Regression coefficient
$[E]_o$	-	Initial enzyme concentration
$[E]$	-	Enzyme concentration
K_m	-	Michaelis-Menten constant
K_e	-	Half saturation constant of substrate
v	-	Initial hydrolysis velocity
w/v	-	Weight per volume
W	-	Watt
W	-	Oven-dry weight of test specimen in grams
W_1	-	Weight of sample
W_2	-	Weight of dish
W_3	-	Weight of dried sample plus dish
W_3X	-	Weight. Of oven dried sample (thanmble)
Z_3	-	Weight of cellulose which can be calculated by (air dry holocellulose with crucible-weight of crucible)
(f)	-	Function
μmol	-	Micromoles
μm	-	Micrometer
θ	-	Theta

LIST OF ABBREVIATIONS

AA	-	Autoclave alkali
ANOVA	-	Analysis of variance
ARP	-	Ammonia recycle percolation
ATCC	-	American Type Culture Collection
CCD	-	Central composite design
CFH	-	Corn fiber hydrlyzate
CMCase	-	Endoglucanase
CO_2	-	Carbon dioxide
CO_3^{2-}	-	Carbonate ion
CSH	-	Corn straw hydrolysate
CSL	-	Corn steep liquor
$CaCl_2$	-	Calcium chloride
$CaCO_3$	-	Calcium carbonate
$Ca(OH)_2$	-	Calcium hydroxide
$C_4H_6O_4$	-	Succinic acid
DA	-	Dilute acid
DA-MwA	-	Dilute acid-microwave alkali
DNS	-	3, 5-dinitrosalicylic acid
DOE	-	Department of Energy
DoE	-	Design of expert
EFB	-	Empty fruit bunch
EDX	-	Energy Dispersive X-ray analysis
etc.	-	Et cetera
et al.	-	And others
FESEM	-	Field Emission Scanning Electron Microscope
FFB	-	Fresh fruit bunch

FT-IR	-	Fourier Transform Infrared spectroscopy
Fpase	-	Cellobiohydrolase
HCl	-	Hydrochloric acid
HCO_3^-	-	Hydrogen carbonate
HPLC	-	High Performance Liquid Chromatography
H_2O	-	Water, ice or steam
H_2O_2-	-	Hydrogen peroxide
H_2SO_4	-	Sulfuric acid
IU	-	International unit
KOH	-	Potassium hydroxide
K_2HPO_4	-	Dipotassium phosphate
KH_2OH_4	-	Potassium dihydrogen phosphate
LAPS	-	Laboratory testing analytical procedures
LOF	-	Lack of fit
$MgCO_3$	-	Magnesium carbonate
$MgCl_2$	-	Magnesium chloride
$MgSO_4.7H_20$	-	Magnesium sulphate heltahydrate
$Mg(OH)_2$	-	Magnesium hydroxide
MwA	-	Microwave alkali
NREL	-	National Renewable Energy Laboratory
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
NaH_2PO_4	-	Sodium dihydrogen phosphate
OPF	-	Oil palm frond
OPT	-	Oil palm trunk
O_2	-	Oxygen
PEP	-	Phosphoenolpyruvate
PEPCK	-	Phosphoenolpyruvate carboxykinase
PKS	-	Palm kernel shell
pKa	-	Acid dissociation constant
RM	-	Malaysian Ringgit
RSM	-	Response surface methodology
SAA	-	Aqueous ammonia

SEM	-	Scanning electron microscope
SHF	-	Separate enzymatic hydrolysis and cultivation
SP	-	Steam pretreatment
SSF	-	Simultaneous saccharification and cultivation or Simultaneous saccharification and fermentation
US	-	United States
USA	-	United States of America
XRD	-	X-ray powder diffraction
YE	-	Yeast extract
YSH	-	Yeast cell hydrolyzate
$ZnSO_4.7H_20$	-	Zinc sulphate heltahydrate
A	-	Actinobacillus
M	-	Mannheimia
3D	-	Three dimentional

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CHAPTER 1

INTRODUCTION

1.1 Overview

Succinic acid ($C_4H_6O_4$), a dicarboxylic acid, was isolated for the first time from microbial fermentation in 1546. Its traditional name is amber acid, but it is also known as butanedioic acid. It is formed by plants, animals and microorganisms, but its maximum production is obtained through anaerobic fermentation by microbes. Succinic acid originates from carbohydrate fermentation and is extensively used in chemical markets and industries which are producing food, green solvents and biodegradable plastics and ingredients used for the stimulation of plant growth (Zeikus *et al.*, 1999). Succinate is the feedstock for several industrial products including tetrahydrofuran, adipic acid, 1,4-butanediol, and aliphatic esters (Willke and Vorlop, 2004).

Succinic acids were previously produced from refined sugar, maleic anhydride and petrochemicals. The production cost was high because of the expensive substrate, so a low price substrate lignocellulosic biomass was used to produce a high yield, concentration and productivity of organic acids such as lactic acid, succinic acid, acetic acid, citric acid and bioethanol (Fitzpatrick *et al.*, 2010). The maleic anhydride price and the environment is adversely impacted, therefore, the need has been felt in developing alternative resources and environmentally friendly technologies. This lead to the development of alternative cheaper biochemical processes which can complement and replace the conventional chemical methods (Pinazo *et al.*, 2015).

Lignocellulosic production is about 180 million tons, from terrestrial plants 1.3 x 10^{10} metric tons and coal is 7 x 10^{9} metric tons per year which are equal to fulfill two- thirds of world energy requirements (Demain *et al.*, 2005). Lignocellulosic biomass such EFB, oil palm trunks (OPT), oil palm fronds (OPF), rice straw, wheat straw, corn stem, corn husk, corn cobs, palm etc. contain high amounts of cellulose components. It has been calculated that cellulose production from biomass was around 1.5 trillion tons per year. High value-added and environmentally friendly product such as succinic acid can be produced from these low prices and inexhaustible source of lignocellulosic raw materials (Kim *et al.*, 2006). In order for the raw material to be considered a good substrate for the production of organic acids it must have the following characteristics: available abundantly throughout the year, renewable, cheap, produce less amounts or no by-products formation, stereospecific, high production rate, low level of contaminants and no competing food value (John *et al.*, 2006b).

Research on lignocellulosic biomass has gained interest because it can replace existing expensive raw materials and are abundantly available, renewable and cheap (Zhang *et al.*, 2007). Another reason that has made the re-use of lignocellulosic more attractive is the environmental issue. Before this lignocellulosic biomass especially EFB was burned and produced ash which consists of 30% potassium. Due to environmental pollution issue, these open combustion was forbidden to minimize emission of greenhouse gases (Suhaimi and Ong, 2001). Hence to achieve zero emission of greenhouse gases and maintain a healthy environment, production of high value-added products from this lignocellulosic biomass such as EFB are also aggressively considered. The utilization of lignocellulosic biomass opens a new field for the researchers to utilize it for the production of industrially important compounds such as succinic acid.

Malaysia is the world second largest producer of oil palm with approximately 59 million tons/year of the total lignocellulosic biomass agricultural waste. The oil palm biomass comprised of empty fruit bunches (EFBs), oil palm fronds (OPFs), palm kernel shells (PKSs) and oil palm trunks (OPTs). These biomass wastes constitute 26. 2 million tons of oil palm fronds and 23% of empty fruit bunch (EFB) per ton of fresh fruit bunch (FFB), 7.0 million tons of oil palm trunks. In 2010 EFB that was processed were about 88.74 MT while palm biomass that was extracted from trunks and fronds were approximately 87 MT. There are many studies that suggest on the use of lignocellulosic biomass especially empty fruit bunches for the production of industrially important organic acids and biofuels (Hamzah *et al.*, 2009). The amount of biomass waste supply in Malaysia from 2001-2020 (tones per year, dry weight) is depicted in Table 1.1.

Table 1.1: The amount of biomass waste supply from 2001 to 2020 (tons per year of dry weight) in Malaysia (Hassan *et al.*, 1997)

	Year					
Biomass Supply	2001-2003	2004-2006	2007-2010	2011-2013	2014-2016	2017-2020
Empty Fruit Bunches	**2,870,148**	**2,860,194**	**2,823,695**	**2,830,331**	**2,906,647**	**2,863,512**
Oil Palm Fronds	7,412,074	7,025,525	6,890,223	6,803,260	7,044,853	7,141,490
Oil Palm Trunk	3,933,442	4,020,852	3,234,164	4,283,082	3,583,803	2,971,934

Currently, many researchers are working to synthesize succinic acid from lignocellulosic biomass to replace expensive pure sugar because of succinic acid demand in the world market. Production of succinic acid from corn fiber, corn stalk hydrolysate, rapeseed and cane molasses etc. has been studied in detail. The purpose of this research is to produce high value added product succinic acid from EFB, instead of burning, to solve environmental pollution issue.

Production of biosuccinate from renewable resources with the optimized condition is a focus point for the last two decades. There are several problems faced in biosuccinic acid production such as substrate requirements, auxotrophy and complex medium conditions and low production rate (Beauprez *et al.*, 2010). For commercial and economic point of view, biosuccinic process must compete with a chemical process. The price of biobased succinic acid is 85 to 1040 € /MT, while that produced from maleic anhydride is ∼2550 €/MT. Biobased succinic acid price is even lower than maleic anhydride itself which cost 1059 €/MT. Chemical and oil industry were highly interlaced with each other in the previous century and this price will be tripled in the coming years because of the increasing oil price. Therefore, the need will be for the cheaper biobased production of succinic acid from renewable sources that will replace the chemical method (Pinazo *et al.*, 2015)

Lignocelluloses conversion into the cellulose fraction and the production of succinic acid can be done by two methods, separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF). SHF refers to the process in which lignocellulosic bioconversion occurs in two steps; enzyme hydrolysis followed by fermentation in two different reactors. SSF is a one step process where enzymatic hydrolysis and fermentation occur within the same bioreactor (Lynd *et al.*, 2005, Xu *et al.*, 2009). Several studies (Mckinlay and Vieille, 2008) revealed that production of succinic acid from cellulose can be done more effectively by combining the two steps: enzymatic hydrolysis and microbial fermentation into a single step known as SSF.

SSF of lignocellulosic biomass are a novel technique, a time and cost effective process and can replace the two step fermentation process SHF, because it can reduce costs by replacing high amount of biomass consumption and also achieve high productivity by controlling the release of sugar. Process efficiency can be enhanced and brings it to the level equal to *cellulase* enzyme by the use of thermotolerant organisms. Also, the effect of glucose inhibition of enzymes is minimized (John *et al.*, 2009). Figure 1.1 clearly shows the process for succinic acid production through simultaneous saccharafication and fermentation (SSF) and the two-step process (SHF).

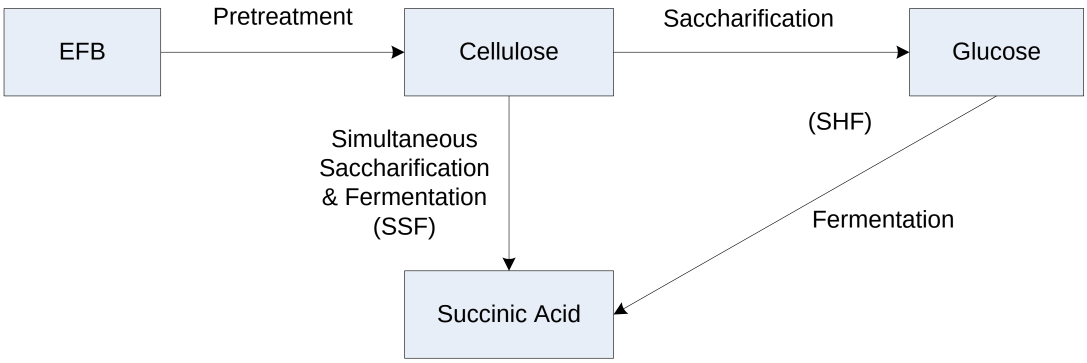

Figure 1.1 Schematic diagram for SHF and SSF of succinic acid production from EFB.

In SSF, cellulose was hydrolyzed to produce glucose and the glucose formed is simultaneously converted to succinic acid. The SSF process eliminates saccharafication of fermentable sugars step before fermentation. Hence, it is capable to substantially decrease the utilization of the enzyme loading. From the industrial view point this is important, as faster saccharification rate can be achieved in a reduced reactor volume. Besides, the SSF process also eliminates the use of different reaction vessels for both of the process. In SHF, the glucose produced was capable to competitively inhibit the *cellulase* and resulted in a low hydrolysis rate. Findings of studies revealed that the saccharification rate in SSF is faster than single saccharification process. Consequently, the higher saccharification rate increases productivity and reduce reactor volume and capital cost (Zhang *et al.*, 2007). Hence, the SSF process is seen to be a more comprehensive yet a simple process for utilization of lignocellulosic material.

However, it is also observed in SSF process the concentration and productivity achieved tend to be lower than SHF. The concentration of succinic acid attained using corn fiber as a substrate by *A. Succinogenes* via SHF process was 70.3 gL^{-1}. However, when the same substrate and bacteria in SSF process was used, the succinic acid concentration reduced to 47.4 gL^{-1}. However the metabolic engineering of the strains and fermentation conditions can solve this problem and increase the product concentration in SSF (Chen *et al.*, 2011d).

1.2 Problem Statement

Typical lignocellulosic biomasses contain 40% - 60% cellulose, 20% - 40% hemicelluloses and 10% - 25% lignin. (Mosier *et al.*, 2005). The potential of using the lignocellulosic material in this case of EFB as a substrate for organic acid production is due to the cellulose content that can be degraded into glucose subunits through hydrolysis process. However, due to the presence of lignin and hemicellulose which are entwined and closely attached to each other hindered the enzymatic hydrolysis and fermentation process. Hence, different pretreatment processes need to be performed to disrupt the lignocellulosic structure so as to remove maximum hemicellulose and lignin, thus exposing cellulose for enzymatic digestion.

Several pretreatment processes such as chemical, physical or both were performed for lignocellulosic materials and the choice for the process is very dependent on the type of lignocellulosic biomass used. Each pretreatment process has its advantages and disadvantages; steam pretreatment is effective for hardwoods but not for the softwood lignocellulose. Strong acid can produce high sugar yields but corrosive and has several disadvantages such as production of furfural (Ramos, 2003). Thus there is a need to find a suitable pretreatment method for EFB so that a high yield of succinic acid is achieved.

Succinic acid can be produced by two step process separate enzymatic hydrolysis and fermentation (SHF) and single step process simultaneous saccharification and fermentation (SSF). SSF process is quite complex and different substrates require different media composition, temperature and pH. Many studies suggest that enzymatic hydrolysis is favorable in low pH <5 and high temperature $> 45^{o}C$ while, fermentation is favorable at high pH > 6 and temperature below $<40\ ^{o}C$ (Chen *et al.*, 2011d, Li *et al.*, 2011, Li *et al.*, 2010, Li *et al.*, 2010b, Liu *et al.*, 2008a, Zheng *et al.*, 2009). Thus there is a need to determine the suitable process conditions when performing SSF for new substrates such as EFB.

1.3 Objective

1) To investigate the effect of different pretreatment methods on the morphology and chemical composition of EFB.

2) To study the influence of differently pretreated EFB on the enzymatic hydrolysis in terms of glucose produced.

3) To determine the effect of different pretreated EFB on succinic acid production via SSF.

4) To optimize SSF conditions such as media composition, temperature, pH, enzyme and substrate loadings using Design of Experiment.

1.4 Scope of the Study

The research was conducted within the following limits:

1) The influence of three different pretreatment methods: i) dilute acid (DA) pretreatment, using 8% Sulphuric acid, ii) autoclave alkali (AA) pretreatment using 20% NaOH, iii) sequential DA-MwA pretreatment, DA pretreatment followed by microwave alkali (MwA) pretreatment on composition of EFB were investigated. The morphological tests such as FESEM, XRD and FT-IR analysis of differently pretreated EFB samples were thoroughly examined.

2) The effect of differently pretreated EFB on enzymatic hydrolysis was investigated using *Cellulase* enzyme 25 FPU/g. The best pretreated method was selected to study different ratios of *cellulase* and *cellobiase* (10 CBU/g) (1:0, 1:1, 1:2, 2:1, 5:1, 7:1 and 10:1) to evaluate the comparatively glucose formation. The enzymatic hydrolysis were carried out in 150ml flasks having 50 ml of citrate buffer solution at 50°C, pH 4.8, 100 rpm in a water bath with 70 gL^{-1} of the substrate loading.

3) Succinic acid production via SSF was performed using rumen bacteria *A. Succinogenes* in the 150ml flasks containing 50ml of the fermentation medium for 48 h. *Cellulase* and *cellubiase* (Novozyme 188) were added into the fermentation together with *A. succinogenes*. Samples were taken at 6, 12, 24, 36 and 48 h for analysis of the products by HPLC.

4) SSF media composition were optimized by varying substrate loading, yeast extract and corn steep liquor using full factorial design followed by central composite design. SSF process conditions were also optimized based on three independent variables like enzyme loading, pH and temperature using full factorial design: All factors were statistically judged by analysis of variance (ANOVA). The optimal conditions of RSM for succinic acid production were validated by confirmation experiments.

1.5 Significance of the Study

Oil palm EFB was used for the first time to produce succinic acid via SSF. Succinic acid is largely produced from refined sugars, petrochemical and maleic anhydride which is quite expensive. EFB an agriculture waste is a suitable substrate for the production of succinic acid because it is easily available in the local milling area, low price, non-starchy and is rich in cellulose content.

The optimal temperature and pH to achieve a high succinic acid production in the one step SSF process using rumen bacteria *A succinogenes* were determined and this contribute to scientific research and advancement of data, as no work was performed to produce succinic acid from EFB via SSF. The effect of different pretreatment methods on the morphology and chemical composition of EFB and subsequently a succinic acid yield were disclosed. Similarly, various parameters of microwave/alkali (MW-A) pretreatment of EFB will be studied in detail to attain high amount of cellulose and remove maximum hemicellulose and lignin.

1.6 Organization of the Thesis

Chapter 1 gives a general overview of bioconversion of lignocellulose EFB to succinic acid. The chapter also focused on the SSF process, problem statement, objectives, scope and significance of the study. Chapter 2 gives a general overview of literature related to work done by the previous researchers on conversion of lignocellulose to succinic acid via SHF and SSF. The chapter also describes importance of succinic acid factor affecting SSF and optimization of SSF. Chapter 3 describes the methodology used in the study which includes pretreatment, enzymatic saccharification, SSF and optimization of SSF conditions. Chapter 4 explains the effect of pretreatment on the morphology and chemical composition. The effect of enzymatic saccharification of EFB was studied to attain maximum glucose accumulation and effect on enzyme kinetics. Chapter 5 briefly explains about SSF process, optimization of SSF media, factors affecting SSF. Chapter 6 reveals the conclusion of the present study and suggests recommendations for improvement in future studies.

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