REACTIVE EXTRACTION OF SUGARS FROM OIL PALM EMPTY FRUIT BUNCH HYDROLYSATE USING NAPHTHALENE-2-BORONIC ACID

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2007

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by

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**Thesis submitted in fulfillment of the
requirements for the degree
of Master of Science**

SEPTEMBER 2007

ACKNOWLEDGEMENTS

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LIST OF SYMBOLS

A, B or C	Coded factors	
α	Alpha (axial distance from center point which makes the design rotatable)	
β_o	Constant coefficient	
β_i	Coefficient for the linear effect	
β_{ii}	Coefficient for the quadratic effect	
β_{ij}	Coefficient for the cross-product effect	
D	Distribution coefficient	
% E	Percentage of sugars from aqueous solution on mass basis	%
% Exyl	Percentage of xylose extraction calculated by model	%
% Egluc	Percentage of glucose extraction calculated by model	%
% E_R	Percentage of sugars contained in the organic phase transported into the strip solution in mass basis	%
ε	Error	
$[N_2B]_O$	Total concentration of N_2B in organic phase	mmol/L
(O/W)	Volume ratio of organic phase to aqueous phase	
p*ka*	Acid dissociation constant	
P_f	Final concentration of ethanol in the medium	g/L
Q^+	Ammonium cation	
R	Loading factor of the extractant	
R^2	Coefficient of determination	
$[S]a$	Concentration of sugar in aqueous phase	mmol/L
S_f	Final concentration of sugar in the medium	g/L
$[S]o$	Concentration of sugars in the organic phase	mmol/L

$[V]o$	Volume of organic phase	L
$[V]a$	Volume of aqueous phase	L
v/v	Volume ratio	
λ	Wavelength	nm
wt%	Weight Percentage	
Y	Response calculated by model (dependent variables)	
Yp/s	Product yield coefficient (g product per g substrate consumed)	g/g

LIST OF ABBREVIATIONS

ANOVA   Analysis of Variance

BOD   Biological oxygen demand

$Ca(OH)_2$ Calcium hydroxide

CCD   Central composite design

CCFC   Central composite face-centered design

CCRD   Central composite rotatable design

Cl^- Chloride ions

CV   Coefficient variation

DF   Degree of freedom

DNS   Dinitrosalicylic acid

EFB   Empty fruit bunch

g   gram

hr   hour

H_2SO_4 Sulfuric acid

HCl   Hydrochloric acid

L   litre

mM   milliMolar

MIBK   Methylisobutylketone

N_2B Naphthalene-2-boronic acid

NaOH   Sodium hydroxide

OH^- Hydroxide ions

O/W   Organic phase to aqueous phase ratio

RSM   Response surface methodology

SD   Standard deviation

TOMAC   Trioctymethylammonium chloride

PENYARIAN BERTINDAK BALAS BAGI GULA DARIPADA HIDROLISAT TANDAN KOSONG KELAPA SAWIT MENGGUNAKAN ASID NAFTALENA-2-BORONIK

ABSTRAK

Tandan kosong (EFB) kelapa sawit adalah salah satu bahan buangan utama yang dihasilkan oleh kilang minyak sawit. Ia merupakan satu bahan buangan yang murah, terdapat dibanyak tempat dan boleh diperbaharui. Tandan kosong (EFB) kelapa sawit dipilih sebagai bahan mentah untuk menghasilkan etanol kerana mengandungi kepekatan xilan yang tinggi dan boleh ditukar kepada xilosa; bahan permulaan untuk menghasilkan etanol. Hidrolisis ke atas EFB kelapa sawit menghasilkan xilosa dan glukosa sebagai komponen utama dan hasil sampingannya adalah asid asetik, furfural dan komponen-komponen fenolik. Hasil sampingan ini mempunyai potensi sebagai perencat kepada metabolisma yis. Penyarian bertindak balas dengan asid naftalena-2-boronik (N_2B) memberikan satu alternatif yang menarik untuk menyaring gula yang boleh ditapai daripada hidrolisat EFB kelapa sawit.

Penyarian gula sintetik dilaksanakan terlebih dahulu, diikuti dengan penyarian gula yang terhasil daripada hidrolisat EFB kelapa sawit. Tiga pelarut organik yang berbeza termasuk *n*-heptana, *n*-heksana: 1-oktanol (85:15, v/v) dan metilisobutilketon (MIBK) telah diuji dalam mod kelompok untuk penyarian xilosa dan glukosa daripada larutan akuas. Pelarut organik yang mengandungi N_2B dan TOMAC ditambah kepada larutan gula (7 g/L xilosa atau glukosa) pada suhu 27 ^0C dan diaduk pada 200 rpm selama 5 jam untuk mencapai keseimbangan. Pelarut campuran *n*-heksana: 1-oktanol (85:15, v/v) memberikan hasil terbaik bagi peratusan penyarian xilosa dan glukosa. Kajian pengoptimuman bagi proses penyarian bertindak balas merangkumi kesan-kesan pH (10.5-11.50), kepekatan N_2B (60-100 mM) dan nisbah isipadu fasa (O/W) (0.5-1.5) terhadap keberkesanan penyarian gula daripada fasa akuas dengan

n-heksana: 1-oktanol (85:15, v/v) sebagai pelarut organik. Metodologi Permukaan Sambutan (RSM) telah digunakan untuk proses pengoptimuman bagi memberikan peratus penyarian tertinggi iaitu sebanyak 95.44 % dan 97.68 % masing-masing bagi xilosa dan glukosa. Asid hidroklorik (HCl) telah digunakan sebagai larutan perlucut untuk mendapat kembali semua gula daripada daripada fasa organik kepada fasa air. Kepekatan HCl dan nisbah isipadu fasa (O/W) adalah dua parameter yang dioptimumkan melalui RSM. Keadaan optimum untuk proses perlucutan untuk mendapat kembali xilosa dan glukosa masing-masing menggunakan HCl berkepekatan 1 M dan 1.25 M pada nisbah isipadu fasa (O/W) 2. Keadaan ini membolehkan untuk mendapat kembali sebanyak 99 % dan 97.65 % masing-masing bagi xilosa dan glukosa.

Keadaan-keadaan optimum penyarian bertindak balas gula sintetik daripada RSM telah digunakan dalam proses penyarian yang melibatkan semua gula (6.84 g/L xilosa, 0.39 g/L glukosa dan 0.01 g/L arabinosa) yang terhasil daripada hidrolisat EFB kelapa sawit. Oleh itu, N_2B berkepekatan 83 mM, pada pH 10.8 dan nisbah isipadu fasa (O/W) 1.5 adalah keadaan paling ide untuk penyarian semua gula daripada hidrolisat EFB kelapa sawit. Hampir 100 % dan 92.97 % masing-masing glukosa dan xilosa telah disaring ke dalam fasa organik. Keadaan optimum yang dijangkakan menggunakan RSM telah digunakan bagi proses perlucutan untuk mendapat gula kembali. Fasa organik telah dicampur dengan 1 M HCl pada nisbah isipadu fasa (O/W) 2. Hampir 99.50 % gula dengan 8.44 g/L xilosa dan 0.52 g/L glukosa telah diperolehi kembali dalam fasa akuas. Kepekatan komponen-komponen perencat telah menurun sebanyak, 98.38 % bagi furfural dan 80 % bagi asik asetik secara serentak dalam hidrolisat EFB kelapa sawit. Kebolehlaksanaan penghasilan etanol oleh *P.stipitis* menggunakan gula daripada hidrolisat EFB kelapa sawit yang dirawat dengan penyarian bertindak balas menggunakan N_2B telah ditunjukkan. Apabila penapaian dijalankan selama 95 jam, 2.40 g/L etanol telah dihasilkan.

REACTIVE EXTRACTION OF SUGARS FROM OIL PALM EMPTY FRUIT BUNCH HYDROLYSATE USING NAPHTHALENE-2-BORONIC ACID

ABSTRACT

Oil palm Empty Fruit Bunch (EFB) is one of the main wastes generated by palm oil mills. This waste which is cheap, widespread and renewable has been selected as the raw material for ethanol production as it contains high concentration of xylan which can be further converted to xylose; the starting material for ethanol production. Hydrolysis of oil palm EFB yielded xylose and glucose as the main component together with byproducts such as acetic acid, furfural and phenolics components. These byproducts are potential inhibitors to yeast metabolism. Reactive extraction with naphthalene-2-boronic acid (N_2B) provides interesting alternative to extract the fermentable sugars from oil palm EFB hydrolysate.

Synthetic sugar extraction was carried out, followed by extraction of the reduced sugar from oil palm EFB hydrolysate. Three different organic diluents including *n*-heptane, *n*-hexane; 1- octanol (85:15, v/v) and methylisobutylketone (MIBK) were examined in batch mode for the extraction of xylose and glucose from aqueous solution. The organic solvent containing N_2B and TOMAC were contacted with sugar solution (7 g/L xylose or glucose) at 27 ^{o}C and agitated at 200 rpm for 5 hrs to reach the equilibrium. Mixed diluent, *n*-hexane:1-octanol (85:15,v/v) showed the best result for xylose and glucose extraction. The optimization of the reactive extraction process study encompassed the effect of pH (10.5-11.50), N_2B concentration (60-100 mM) and phase volume ratio (O/W) (0.5-1.5) on the extraction efficiency of sugars from aqueous phase with *n*-hexane:1-octanol (85:15, v/v) as organic diluent. Response Surface Methodology (RSM) was employed for the optimization process to achieve the highest extraction percentages of 95.44 % and 97.68 % for xylose and glucose, respectively. Hydrochloric acid (HCl) was used as

stripping solution to recover the sugars from loaded organic phase to the aqueous phase. HCl concentration and phase volume ratio (O/W) were two parameters optimized by the RSM. The optimum conditions for stripping process of xylose and glucose recovery using 1M and 1.25 M of HCl solution, respectively at phase volume ratio (O/W) of 2, which led to a recovery of 99 % and 97.65 % for xylose and glucose respectively.

The optimum conditions of reactive extraction of synthetic sugar from RSM were used in the extraction process involving reduced sugars (6.84 g/L xylose, 0.39 g/L glucose and 0.01 g/L arabinose) from oil palm EFB hydrolysate. Therefore, 83 mM N_2B concentration, at pH 10.8 and 1.5 of phase volume ratio (O/W) were the ideal conditions for sugars extraction from oil palm EFB hydrolysate. Almost 100 % and 92.97 % of glucose and xylose were extracted into organic phase respectively. The optimum condition of stripping process to recover the sugar predicted by RSM was used. The organic phase was mixed with 1M HCl at a phase volume ratio (O/W) 2. About 99.5 % of sugars with 8.44 g/L of xylose and 0.52 g/L of glucose were recovered in the aqueous phase. The inhibitors concentration decreased as much as, 98.38 % for furfural and 80 % for acetic acid in the oil palm EFB hydrolysate simultaneously. The production of ethanol by *P. stipitis* using treated sugars from oil palm EFB hydrolysate by reactive extraction with N_2B was shown to be feasible. When the fermentation was operated for 95 hrs, 2.40 g/L ethanol was produced.

CHAPTER 1

INTRODUCTION

1.1 Oil Palm Industry in Malaysia

Oil palm tree, *Elaeis guineensis* which belongs to the *Palmae* family is one of the most versatile crops in tropical countries. The plant was presented as an ornamental crop and subsequently became a cultivation crop which is grown commercially since 1917 (Malaysian Palm Oil Promotion Council, 2005). In year 2005, the planted areas constitute about 63 % of the total agricultural land all over the country (Malaysia Palm Oil Board, 2005). Table 1.1 shows the total oil palm planted area in Malaysia which is expected to increase each year.

Table 1.1: Long – term projection of Malaysian palm oil production (2005-2020)

Year	New planting (ha)	Immature areas (ha)	Mature areas (ha)	Total planted areas (ha)
2005	135 000	578 000	3 592 000	4 170 000
2010	46 000	130 000	4 389 000	4 522 000
2015	63 000	291 000	4 616 000	4 907 000
2020	1000	74 000	4 841 000	4 915 000

(Ramli, 2003; Malaysia Palm Oil Board, 2005)

Presently, Malaysia is the world's largest producer and exporter of palm oil, contributing about 50 % of the world palm oil production and export more than 90 % of palm oil products (Malaysian Palm Oil Board, 2005). The palm oil exportation reached about RM31 billion increased by 8.54% or RM2.44 billion in 2006 compared to that in 2005 (Business Time, 2007). Therefore, some actions need to be taken in order to guarantee the sustainable development of palm oil production. However, in doing this the country generates tonnes of biomass during the process of getting the palm oil.

1.2 Oil Palm Wastes

There are various forms of solid and liquid waste from the mills. Figure 1.1 shows the flowchart of the production of oil palm wastes from oil palm industry.
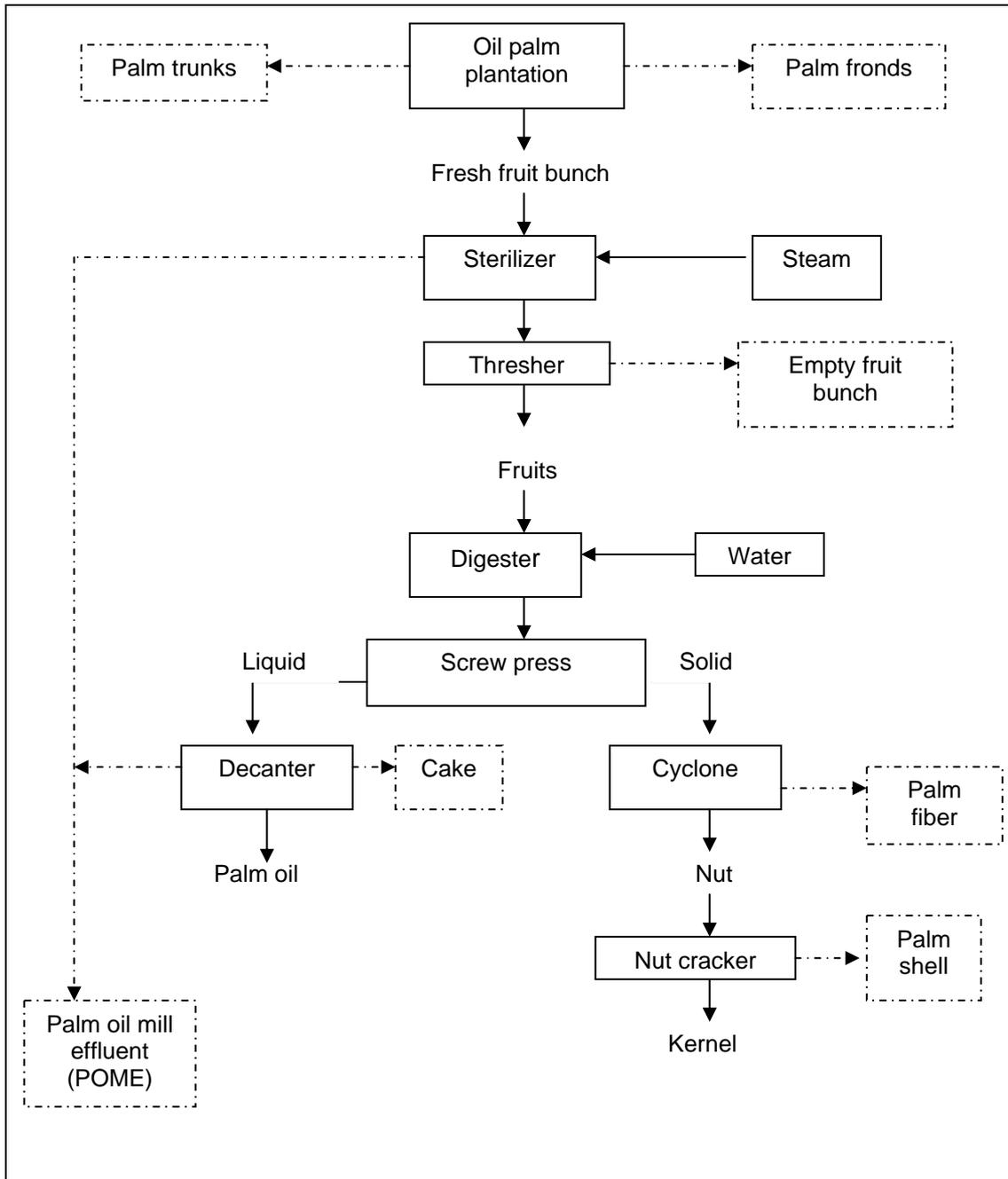
Figure 1.1: The production of oil palm wastes from oil palm industries (⎯⎯) process, (......) waste (Prasertsan and Prasertsan, 1996).

These include oil palm trunks (OPT), oil palm fronds (OPF), empty fruit bunch (EFB), palm press fiber, palm kernel, cake, palm kernel shell and palm oil mill effluent (POME). More than 70 % (by weight) of the processed fruit bunch in the palm oil mill was left over as the oil palm waste (Prasertsan and Prasertsan, 1996). The amount of oil palm wastes has significantly increased throughout the year simultaneously with the extensive growth of oil palm industry.

Today, the oil palm wastes are considered as the largest biomass resource in Malaysia. In 2004, approximately 18.6 tonnes of fresh fruit bunch were produced per hectare, which consist only 21.6 % (by weight) of fresh fruit bunch was the crude palm oil, leaving the remaining as by product including the palm kernel (6%) and solid waste which comprise of 23 % EFB, 13.5 % fiber and 5.5 % shell (Basiron, 2005; Yusof, 2006). Plate 1.1 shows the different types of solid wastes generated by palm oil mill.
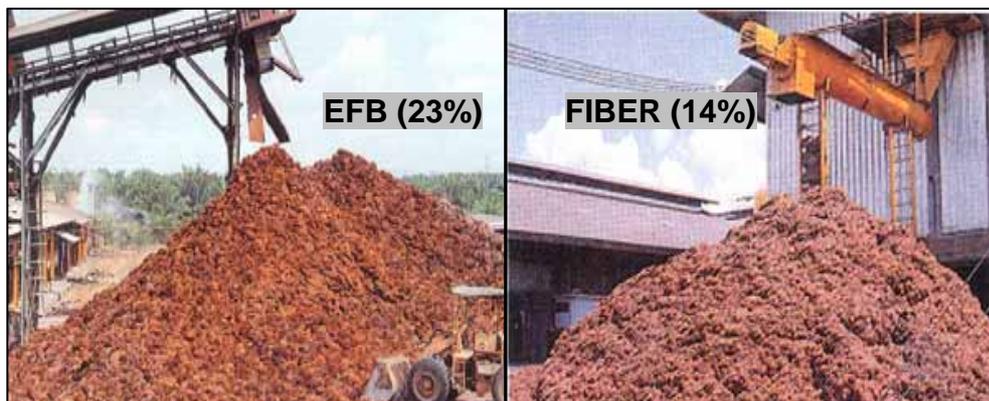
Plate 1.1: Solid wastes from oil palm industry.

EFB is the major component of all generated solid wastes. The amount of biomass waste supply from EFB itself is reaching approximately 1.34×10^7 tonnes in 2005. Steam from the sterilization process results in high moisture content (50-60 %) in the EFB makes it unsuitable as fuel. It differs with fibers and shells of the oil palm

fruit which composed of high colorific values and extensively used as boiler fuel to generate steam and electricity for mill's consumption. In some plantations, the EFB were left to decompose under the trees. Although it helps retain the moisture and returns organic matter to the soil but unfortunately they also attracted harmful insects. To prevent the environmental pollution such as smoke and smell problems the EFB was used as mulch. The EFB is placed around the young palms to prevent erosion. However, due to current labor shortage and the transportation, distribution of EFB in the field is getting more expensive.

Other solid wastes are palm fronds and palm trunks. Approximately 10.4 tonnes/hectare (dry matter) of palm fronds are obtained from annual routine pruning activities, 14.4 tonnes/ hectare and 75.5 tonnes/hectare of felled trunks and palm fronds through the replanting program (Yusoff, 2006). The production of palm oil also generated a large amount of polluted wastewater, referred as palm oil mill effluent (POME). On average about 53 million cubic meters POME per year is being produced in Malaysia (Malaysia palm oil Production Council, 2006). POME is commonly treated by ponding system in most oil palm mills. The system is relatively reliable, stable and capable of producing a final discharge with BOD of less then 100 mg/L (Abdullah, 1995).

1.3 Renewable Energy

The world economy is continuously growing. Thus, demand for energy would continue to grow in tandem with it as the public demand for better quality of life would also grow in the same proportion. However, as the current global scenario cannot ensure continuous oil supply due to various reasons such as political and civil war, the oil price is expected to remain high. With the rapid depletion of the oil and gas resources in the world, serious attention are now given to alternative sources of energy such as renewable energy. Malaysia has announced its Fifth Fuel Policy in

2001, which incorporates renewable energy as one of the major source of energy in addition to oil, natural gas, coal and hydro. A target of 5% renewable energy of the total installed electricity capacity has been set by the year 2010 (Malaysia Palm Oil Board, 2005). Renewable energy such as solar photovoltaic (PV), wind, geothermal and biomass residues are some of the options to supplement the energy needs.

Renewable energy sources for the gasoline alcohols have gained importance. These are also clean energy sources and can be obtained from the biomass alcohols with low carbon like ethanol. Due to the high evaporation heat, high octane number and high flammability temperature, ethanol has positive influence on the engine performance and increases the compression ratio. The engine power, break thermal efficiency and volumetric efficiency are increased by 8.3 %, 9 % and 7 %, respectively when the ethanol blended fuels are used (Yucesu *et al.,* 2006). The emission and toxicity of ethanol is lower than those of petroleum (Wyman, 2007). As a result, today, ethanol is blended by more than 30 % of the gasoline sold in the United State (Renewable Fuels Association 2006).

The conventional production of bio-ethanol utilizes sugar resources supplied by sugar cane or corn grain (Wayman, 2007). The production may not be practical in the long run since the use of current sources may be competing with the need for human food. The economics of ethanol production from sugar beet is the worst source due to the requirement to buy the sugar beet (Murphy and McCarthy, 2005). Hence, the production of ethanol using sugar from lignocellulose materials would seem a favorable option. Lignocellulosic materials are an attractive raw material for the production of fuel ethanol due to their extensive distribution, renewable character and availability in great quantities at low cost. The ability to produce ethanol from low-cost biomass will be the key to making it competitive as a gasoline additive and fuel. The use of oil palm residues i.e EFB as a feedstock for the production of ethanol is an

attractive option since Malaysia generates a large amount of biomass waste by palm oil mills during palm oil extraction process as described earlier.

1.4 Problem Statement

The waste management problem created by EFB is still a burden to the oil palm industry. As solid wastes disposal had become a stringent waste disposal problem for the industry, the use of EFB fiber for the synthesis of sugar feedstock for the production of ethanol seemed to be valuable and an attractive option. The low-cost lignocellulosic material will allow cost effective conversion of biomass into fuels and chemicals.

The typical production of bio- ethanol from lignocellulosic materials consists of two sequential steps; the processing of lignocellulosic materials into reducing sugars and followed by fermentation of the liberated sugars to ethanol. Cellulose and hemicellulose fractions would be hydrolyzed to their sugar constituents. The sugars produced in the hydrolysate may present themselves as a cheap carbon source for the microbiological fermentation and production of ethanol. On the other hand, the process has to be efficient with pure and high conversion yield of sugars from lignocellulosic material.

The major problem encountered in this process is that the EFB hydrolysate contains not only fermentable sugars, but also a wide spectrum of by-products including furfural, acetic acid and phenolic compounds (Rahman *et al.,* 2007; Mohamad Ibrahim *et al.,* 2004). Furfural, acetic acid and phenolics are potential inhibitors to yeast metabolism. When these compounds are present in the hydrolysate, they inhibit the fermentation process causing cell morphological change or ultimate death of the organism (Luo *et al.*, 2002; Nigam, 2002; Palmqvist and Hahn-Hagerdal, 2000b). The inhibitory effect has become one of the major impediments to the commercialization of ethanol from biomass. According to

Delgenes *et al.* (1996) since the toxic components effects are cumulative, efficient methods must be developed to overcome either the single or recombined effect resulting from even low residual concentration of individual components. Figure 1.2 shows the overall process of ethanol production using sugars form lignocellulosic material.
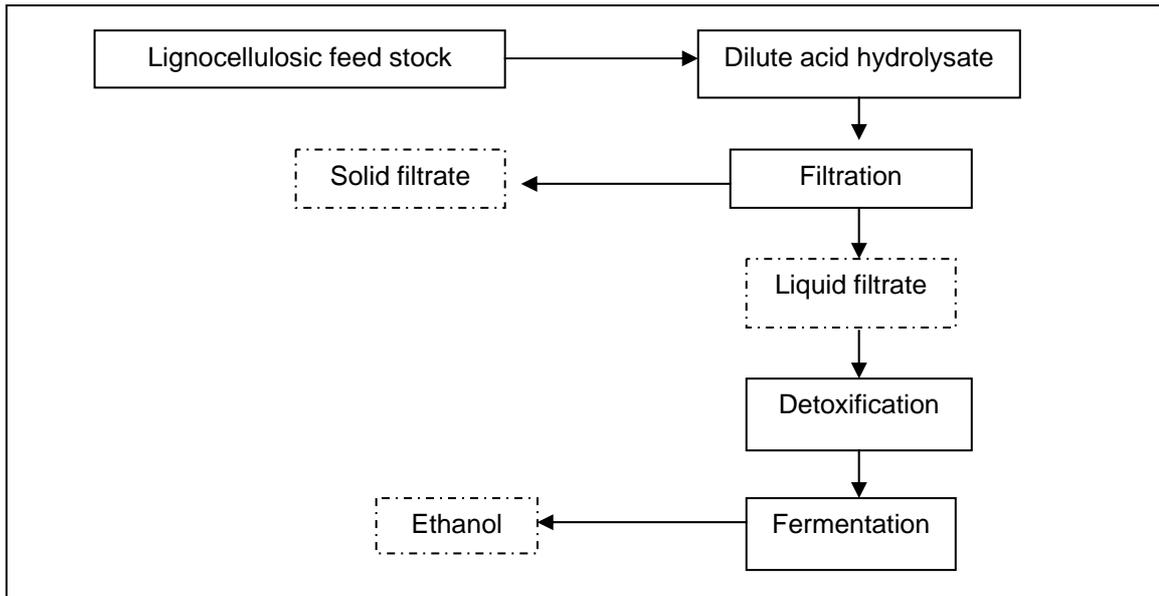
Figure 1.2: Overall process of ethanol production from lignocellulosic materials.

To enhance the yield of ethanol several biological, physical and chemical methods have been employed for detoxification of lignocellulosic hydrolysates. Different detoxification methods cannot be strictly compared when different lignocellulosic hydrolysate and different microorganisms have been used. Lignocellulosic hydrolysates vary in their degree of inhibition, and different microorganisms have different inhibitor tolerances (Palmqvist and Hahn-Hagerdal, 2000). Different detoxification methods have different effects on the hydrolysate, and in most instances the inhibitors are only partly removed; others have been reported to give rise to sugar loss (Mussatto and Roberto, 2004). Some treatments show an improvement on ethanol productions but were still considerably less then observed in

a simulated synthetic hydrolysate medium with sugar composition mimic to the hydrolysate.

The purification and concentration of fermentable sugars are important since the presences of the inhibitors are toxic to the fermenting microorganisms. Alternative process based on chemical affinity of sugar has been proposed, which utilizes boronates to form complex with sugars. This process is reversible and the sugar complex is able to decompose in acidic solution thus releasing the sugar bound in aqueous phase (Griffin and Shu, 2004).

Lipophilic boronic acids are the best known extractants for sugars extraction (Matsumoto *et al.,* 2005; Takeuchi *et al.,* 1996; Malmary *et al.,* 1995). These boronate carriers can form reversible covalent complexes with diols group, but it is highly selective towards glucose and xylose which is the main fermentable sugars. These boronate carriers can also form reversible complexes with other sugars like arabinose, fructose, sucrose and mannose (Matsumoto, 2005). Hence, complete fermentable sugars reduced from acid hydrolysis could be extracted.

In this present study reactive solvent extraction with naphthalene-2-boronic acid (N_2B) ion-pairing with trioctylmethylammonium chloride (TOMAC) was investigated in order to purify and concentrate the fermentable sugars from EFB hydrolysate. The use of N_2B in the sugar cane baggase hydrolysate has been reported to be 65 % extraction with Shellsol as organic diluent. N_2B has large number of conjugate and may form the most stable anionic species when bound to sugar molecules (Griffin and Shu, 2004). The stability of anionic species between organic and aqueous interface would bring an efficient reactive extraction of sugar complex into organic phase.

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The choice of organic diluent for sugar extraction using boronic acid has received some attention but the results are still conflicting. Takeuchi *et al.* (1996) conclude that polar organic diluents would produce high sugar extraction at appropriate pH. Meanwhile, Matsumoto (2005) indicates that most efficient reactive extraction of sugars occurred using non polar mixed with slightly polar organic diluent like *n*-hexane:1-octanol (85:15, v/v). Therefore, it was considered attractive to study the sugar extraction using N_2B in both types of organic diluent.

Diluted hydrochloric acid (HCl) was used to strip the sugar complex in organic phase then released the sugar molecule in aqueous phase for sugar recovery. Griffin and Shu (2004) reported that all sugar present in the organic phase could be recovered with sufficient acid concentration in the strip solution. More interesting, the xylose solution from baggase hydrolysate increased three times from the original with 1:8 (aqueous:organic) phase ratio while reducing the concentration of the undesirable acid soluble lignin by over 90%. Coupled with high sugar concentration achieved, this reactive extraction may provide a great lignocellulosic hydrolysate detoxifying methods for producing a suitable sugar for direct fermentation.

Synthetic sugar extraction has been carried out first, followed by reduced sugars from oil palm EFB hydrolysate at optimum extraction process condition. The best type of organic diluent and optimum N_2B concentration, pH and phase volume ratio condition were investigated in order to achieve the highest xylose and glucose extraction percentages. The concentrations of HCl and phase volume ratio for stripping process were also investigated to recover and concentrate the sugars significantly. In order to show that enhancement took place in ethanol production, a feasibility study of ethanol production by fermentation using treated sugars was also carried out using *Pichia stipitis. P. stipitis* has been selected as it shows the best ability of producing ethanol rapidly from both commercial xylose and raw wood

material such as water-hyacinth hydrolysate (Nigam, 2002), wheat straw hydrolysate (Nigam, 2001) and apparently produces no xylitol

1.5 Research Objectives

The present research has the following objectives:

- To determine the effect of the process parameters involved in reactive extraction of synthetic sugars (xylose and glucose) using N_2B ion-pairing with TOMAC by varying parameters such as N_2B concentration, type of organic diluent, agitation speed, pH and phase volume ratio. Additional parameters studied for sugar stripping process are effect of HCl concentration and phase ratio in batch system.

- To analyze, model and optimize reactive extraction process for sugar (pH, N_2B concentration and phase volume ratio) and sugar stripping process (HCl concentration and phase volume ratio) using Response Surface Methodology (RSM).

- To identify optimum conditions for the reactive extraction of reduced sugars from oil palm EFB hydrolysate and the stripping process.

- To carry out the feasibility study on microbiological conversion of treated sugars from oil palm EFB hyrolysate to ethanol using *pichia stipitis* as fermentative organism.

1.6 Organization of Thesis

This thesis is divided into five chapters as follows;

Chapter 1 (Introduction) describes the development of palm oil industry in Malaysia, waste generation in the palm oil mill and renewable energy. This chapter focused on the problem statement and the objectives of the project.

Chapter 2 (Literature review) describes literature review from other researchers and methods applied in the present days for reactive extraction process, lignocellulosic hydrolysate treatment and pretreatment process that are related to the present study. Modeling of extraction and stripping process using statistical method are also covered.

Chapter 3 (Materials and methods) describes the detail of the materials and chemicals used in the present study. Then, overall experimental flowchart is presented. This is followed by the detail experimental procedures and analysis required for batch experiments for sugar extraction and stripping process. Finally the use of RSM for optimization process of sugar reactive extraction and stripping process has been described.

Chapter 4 (Results and Discussion) presents the results obtained from experimental runs and discuss on every effect of parameters on the reactive extraction of sugar for direct fermentation. This is followed by the detailed discussion on the developed empirical model and optimum conditions from RSM for sugar reactive extraction and stripping process.

Chapter 5 (Conclusion) concludes the findings from the current studies and recommendation for the improvement in future research.

CHAPTER 2

LITERATURE REVIEW

2.1 Empty Fruit Bunch Waste

The oil palm empty fruit bunch (EFB) waste is produced regularly during harvesting and processing of the fruit for the oil. Table 2.1 shows the biomass waste supply outlook in Malaysia from 2007 to 2020. The amount of biomass supply by EFB itself is reaching approximately 2.8 million tones in the year 2020.

Table 2.1: Oil palm biomass supply in Malaysia from 2007 to 2020

Biomass supply (tones/year, dry weight)	Year 2007-2010	Year 2011-2013	Year 2014-2016	Year 2017-2020
OPT	3,234,164	4,283,082	3,583,803	2,971,934
EFB	2,832,695	2,830,331	2,906,647	2,863,512
Pruned fronds	6,890,233	6,803,260	7,044,853	7,141,490

Source: Hassan et al. (1997)

The bulky nature of the EFB causes a high land-fill disposal cost. Therefore most of the mills disposed the EFB by burning in the incinerator. The ash produced thereafter contain a high mineral especially potassium and had extensively been used as soil conditioner for the oil palm trees. However, the ash is highly alkaline in nature (pH 11) thus required careful handling and disposal (Tay and Show, 1995). Black smoke would be emitted when the EFB was overloaded inside the incinerator. The black smoke contains particulates and gaseous (SO_2, CO_2, CO and NO_x). This method is no longer acceptable because it pollutes the environment and would pose a severe impact towards human health. Due to the air pollution created from the EFB combustion, the Department of Environment (DOE), under the Environmental Quality Clean Air Regulation Act, 1978 has restricted the incineration process of EFB (Yusoff, 2006). Malaysian government still spends large amount of money for handling these amount of biomass waste. Hence, the utilization of EFB as raw material for the production of value added commodity is very much needed.

Oil palm EFB fiber is a lignocellulosic biomass. Lignocellulosic biomass is one of the most renewable and inexpensive sources that could be converted to chemical and biological product. Oil palm fiber from palm oil tree is categorized as a hard wood plant consists of about 50.40 % cellulose, 21.90 % hemicellulose, 10.0 % lignin and 0.50 % ash as reported by Umikalsom *et al.* (1997). Extensive research has been done on conversion of lignocellulosic materials to ethanol in the last two decades (Sun and Cheng, 2002).

Dilute acid hydrolysis of oil palm EFB fiber produced xylose as the main sugar from hemicellulose while other byproducts include glucose, acetic acid and furfural (Rahman *et al.,* 2006; Rahman *et al.,* 2007). The present research is about preparation and detoxification of reduced sugars from oil palm EFB hydrolysate from oil palm industry for the production of ethanol. This waste has been selected as raw material because of its abundance in Malaysia and it has high content of sugars.

2.2 Lignocellulosic Material

Lignocellulosic material refers to plant made up by three main polymer group; cellulose, hemicellulose and lignin; the contents vary according to plant species. The complex structure of lignocellulosis in plants forms a protective barrier to cell destruction by bacteria and fungi. Lignocellulosic materials include wood, grass, forestry waste and agriculture residues. The typical substrate contains primarily cellulose, a polymer of 6 carbon sugar as major component in wood (40-45 %) surrounded by lignin (15-20 %) as encrust and hemicellulose consisting of 5 carbon and 6 carbon sugar (25-35 %) as a matrix. A typical plant-cell is illustrated in Figure 2.1. Lignocellulosic materials are an attractive raw material for the production of chemicals and fuels due to their extensive distribution, renewable character and availability in great quantities at low cost.
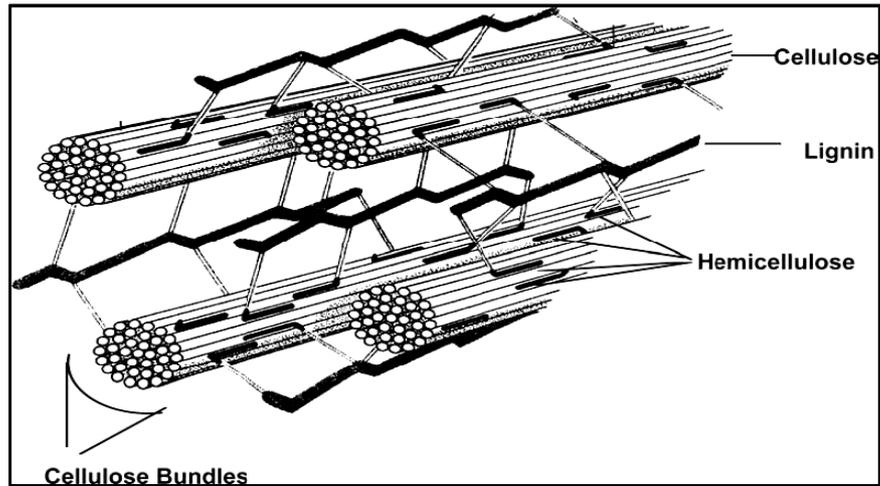
Figure 2.1: Typical plant cell wall arrangement (Murphy and McCarthy, 2005).

2.2.1 Cellulose

Cellulose is a homopolysaccharide which is highly stable chain composed of 12,000 of β-D-glucopyranose units linked together by (1→ 4) glycosidic bonds. Each β-D-glucopyranose unit is oriented at an angle of 180° from the succeeding glucose unit (Kitani and Hall, 1989). Cellulose becomes the main constituent in most wood species and is located in the secondary cell wall. The highly ordered structure of the cellulose and the intermolecular hydrogen bonds are the basis of high tensile strength and generally insoluble nature (Kitani and Hall, 1989). Cellulose is one of the most abundant organic materials in nature that can be hydrolyzed to glucose. Glucose produced can be converted to ethanol as fuel additive (Nigam, 2002; Sun and Cheng, 2002).

2.2.2 Hemicelluloses

Hemicelluloses are chemically complex, straight chain and brunched polysaccharides consisting of the pentose; D-xylose and L-arabinose, and the hexoses; D-mannose, D-glucose, D-galactose, uronic acids and acetyl group (Kitani and Hall, 1989). Hemicelluloses consisted of 100-200 units of pentose and hexose

sugars in their molecular backbones (Thomas, 1977). The variable composition and structure of individual hemicellulose polymers prohibit the close packing of hemicellulose. Thus, native hemicelluloses are not crystalline and partially soluble in water (Kitani and Hall, 1989). Hardwood hemicellulose comprised of O-acetyl-4-O-methylglucuronoxylan and glucomannan. O-acetyl-4-O-methylglucuronoxylan, simply call xylan is the major hemicellulose in hardwoods. Rahman *et al.* (2007) estimated that oil palm EFB fiber (on an oven-dry basis) consist of 24.01 % xylan. Hydrolysis of xylan produce mainly xylose in the hydrolysate which can further be used as starting material for ethanol production (Nigam, 2002; Helle *et al.,* 2004).

2.2.3 Lignin

Lignin is an amorphous polyphenolic material arising from an enzyme-mediated dehydrogenates polymerization of three major phenylpropanoid monomers, which are coniferyl, sinapyl and p-coumaryl alcohol. The lignin structural elements are linked by carbon-carbon and ether bonds to form tri-dimensional network associated with the hemicelluloses polysaccarides inside the cell wall. Lignin is usually insoluble in all solvents and can only be degraded by physical or chemical treatments (Mohamad Ibrahim *et al.,* 2004).

2.3 Acid Hydrolysis of Biomass

Biotechnological conversion of biomass into fuels and chemicals requires hydrolysis of polysaccharide fraction into monomeric sugars. The first discovery of cellulose conversion using concentrated sulphuric acid by Braconnet in 1819 has attracted most researchers' attentions toward the hydrolysis of polysaccharides of biomass material. This process became an important method in recovery of fuels and chemicals from lignocellulosic biomass which provided the soluble sugars as intermediate components for a wide variety of fermentation products. Hydrolysis is a conversion process of a complex substance into simple units of substance in the

presence of a strong acid catalyst such as the conversion of sugar complex polymer into the simple sugars like xylose, glucose, and arabinose. As the hydrolysis reaction by water alone is very slow, acid catalyst is employed to accelerate the reaction. Sulphuric and hydrochloric acids are commonly employed as a catalyst (Rahman *et al.,* 2007; Mohagheghi *et al.*, 2006; Neureiter *et al.*, 2004; Herrera *et al.*, 2004; Herrera *et al.,* 2003; Singh *et al.*, 1984). These acids are stronger than organic acids due to their lower pK_a values and completely ionized in water (Christian, 1994). The more hydrogen ions formed in the solution, the more rapid hydrolysis reaction could be.

Figure 2.2 shows the main degradation pathways during acid hydrolysis of lignocellulosic. When hemicellulose is degraded, xylose, mannose, acetic acid, galactose and glucose are liberated. Cellulose is hydrolysed to glucose. At high temperature and pressure xylose is further degraded to furfural (Canettieri *et al.*, 2007).
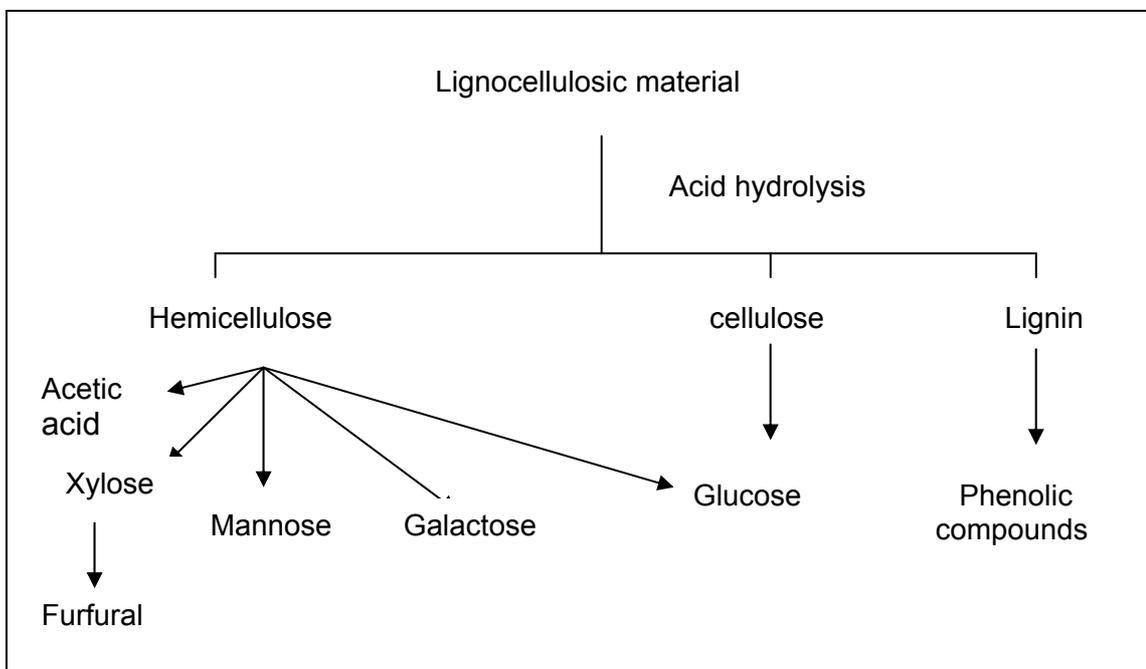
Figure 2.2: Reaction occurring during hydrolysis of lignocellulosic materials (Palmqvist and Hagerdal, 2000b).

The conversion of lignocellulosic materials through acid hydrolysis is mainly performed in three different processes. They are dilute acid hydrolysis, concentrated acid hydrolysis and two-stage acid hydrolysis. The dilute acid hydrolysis is carried out at high pressure and temperature (Rahman *et al.,* 2007). In contrast concentrated acid hydrolysis is conducted at moderate temperature with high acid concentration (Sing *et al.,* 1984). Although the concentrated acids are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous. Concentrated acid hydrolysis operations require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible (Sun and Cheng, 2002). In the two-stage hydrolysis, the process is performed in a sequential order where dilute acid hydrolysis is employed as the prehydrolysis in the first stage. The process is then, followed by the main hydrolysis reaction with high acid concentration and operating temperature (Yan *et al.*, 1996).

Amount of sugars recovered from raw material is independent of dry matter but fully dependent on the acid concentration, temperature and reaction time (Neureiter *et al.*, 2004; Rahman *et al.,* 2006; Rahman *et al.,* 2007). In dilute acid hydrolysis, acid concentration was found to be the most important parameter affecting the sugar yield (Rahman *et al.,* 2007) while reaction temperature gives the highest impact on the formation of sugar degradation products (Canettieri *et al.,* 2007). Up till now, dilute acid hydrolysis process is selected as it is more promising than others (Sun and Cheng, 2005). However dilute acid hydrolysis has some limitations. If higher temperatures or longer residence time are applied, the hemicellulosic derived monosaccharides will degrade and give rise to fermentation inhibitors like furfural, acetic acid and phenolics compounds. Table 2.2 shows the optimum conditions of acid hydrolysis of different lignocelullosic material.

Table 2.2: Acid hydrolysis of lignocellulosic material

Lignocelullosic material	Optimum acid hydrolysis condition	Product (g/L)	Reference
Sorghum straw	6% HCl, 100 ^{o}C, 83 min (batch process)	21.3 xylose 4.70 glucose 0.80 furfural 2.80 acetic acid	(Herrera *et al.,* 2004)
Eucalyptus grandis	0.65% H$_2$SO$_4$, 157 ^{o}C, 20 min, (1.4 L pilot scale)	13.65 xylose 1.65 glucose 1.55 arabinose 1.23 furfural 3.10 acetic acid	(Canettieri *et al.,* 2007)
Wheat straw	1.85% (w/v) H$_2$SO$_4$, 90 ^{o}C, 18 hr	12.83 xylose 1.73 glucose 2.62 arabinose 2.73 acetic acid	(Nigam, 2001)
Oil palm empty fruit bunch	6% H$_2$SO$_4$, 120 ^{o}C, 15 min (batch process)	29.40 xylose 2.34 glucose 0.87 furfural 1.25 acetic acid	(Rahman *et al.,* 2007)

2.4 Inhibitors in Hydrolysate

During hydrolysis of lignocellulosic materials a wide range of compounds which are inhibitory to microorganisms are formed or released. Based on their origin the inhibitors are usually divided in three major group; weak acids, furan derivatives and phenolic compounds (Palmqvist and Hahn-Hagerdal, 2000b). The inhibitory effect has become one of the major impediments to the commercialization of ethanol from biomass. Furfural and hydromethylfurfural (furan derivative) generated from pentose and hexose degradation while acetic acid is liberated from acetylated hemicelluloses were significantly inhibiting the microbial growth used in ethanol production depending on the type of microbial strain (Taherzadeh *et al.,* 2000; Luo *et al.,* 2002; Nigam, 2002; Helle *et al.,* 2003). The intensity of inhibition is closely related with the initial concentration of inhibiting molecules. A high inhibition values indicated high toxicities of the compounds or more severe inhibition effects in the fermentation (Delgenes *et al.,* 1996).

2.4.1 Effect of Acetic Acid

Acetic acid derived from acetyl groups present in the hemicellulose is discharged into the hydrolysate during the hydrolytic process. When the pH of the medium is low, acetic acid (pK_a=4.75) appears in the undissociated form, is liposoluble and diffuses across the plasma membrane. Once the cell interior is at pH 7.4, this acid dissociates and accumulates in the cytoplasm, discharging protons. As a consequence, the internal pH drops inhibiting cell activity and even causing death (Mussatto and Roberto, 2004; Palmqvist and Hahn-Hagerdal, 2000b).

Effect of acetic acid on the growth of microorganism also depends on type of microbial strain and pH of the media. Acetic acid concentrations of about 2 g/L to 5 g/L inhibit the growth of *pichia stipitis* and *pachysolen tannophilus* (Van Zyl *et al.,* 1991). The growth rate decreased by 15 % while the ethanol yield was decreased by 50 % when 1.5 g/L acetic acid at pH 5 was present during fermentation of *saccharomyces cerevisiae* 259ST with xylose as carbon source (Helle *at al.,* 2003). Parajo *et al.* (1997) reported that acetic acid is an inhibitory compound to yeast in the range from 0.5 to 9 g/L.

Effect of acetic acid on the ethanol production also depends on type of sugar as carbon source. The ethanol yield was decreased by 50% when 1.5 g/L acetic acid at pH 5 was present during fermentation of *Saccharomyces cerevisiae* 259ST with xylose as carbon source. In contrast, the ethanol yield on glucose is relatively unchanged until acetic acid concentration of 4 g/L is obtained (Helle *et. al.,* 2003). Therefore ethanol yield on xylose is more sensitive to acetic acid; inhibition commences at lower acetic concentration and decreases at a greater rate with increasing acetic acid.

2.4.2 Effect of Furfural

During hydrolysis, furfural can be formed easily under high acid concentration and reaction temperature from degradation of xylose (Rahman *et al.,* 2006; Palmqvist and Hagerdal, 2000b). Most of the microbial metabolized the furfural under aerobic condition. The presence of furfural delays the fermentation process and causes the cell death or damage during fermentation process. There was no or very little growth of the yeast until after complete conversion of furfural (Nigam, 2001; Parajo *et al.,* 1997). During fermentation furfural reduction to furfuryl alcohol occurs with high yields. The alcohol dehydrogenises is responsible for the reduction of furfural to furfuryl alcohol where the furfural inhibits the respiration process and the furfuryl alcohol affects the microbial growth rate (Parajo *et al.,* 1997; Taherzadeh *et al.,* 1999; Nigam, 2001).

Martinez et *al.* (2000) observed that ethanol production by *E.coli* from sugarcane bagasse hydrolysate started to be affected by furfural when its concentrations were higher than 0.9 g/L. Nigam (2001) and Delgenes *et al.* (1996) reported 1.5 g/L of furfural markedly reduce the ethanol yield about 90 % and100 % respectively by directly inhibiting respiration and growth of *P. stipitis.* When the furfural concentration was higher than 1.5 g/L, the growth process is completely stopped with 99 % reduction in ethanol production.

2.4.3 Effect of Phenolic Compounds

A large variety of compounds (aromatic, polyaromatic, phenolic and aldehydic) are released from lignin during hydrolysis of lignocellulosic materials. Phenolic compounds have a considerable inhibitory effect on the fermentation of lignocellulosic hydrolysate and those with low molecular weight are the most toxic. Phenolic compounds cause a partition and loss of integrity of biological membranes, thereby affecting their ability to serve as selective barriers and enzyme matrices. As a

consequence, both cell growth and sugar assimilation are reduced (Palmqvist and Hahn-Hagerdal, 2000b).

2.4.4 Effect of Synergistic

Synergistic effect refers to combination of more than one inhibitor compound (Mussato and Roberto, 2004). The maximum concentration of each inhibitor that a microorganism can withstand cannot be established because inhibition strongly depends on factors such as the kind of microorganism, its adaptation to medium, the fermentative process employed, the number of inhibitors present in the medium and their synergistic effect (Mussato and Roberto, 2004). Since a lignocellulosic acid hydrolysate is complex and expected to contain more than one inhibitor, the complete removal of inhibitors must be carried out in order to improve the cell growth and to ensure the success of ethanol production.

2.5 Detoxification of Lignocellulosic Hydrolysate

When compared with the fermentation of commercial sugars, the fermentation of non-detoxified hydrolysate is characterized by slow kinetics, very limited yield and low productivity (Nigam, 2002; Mussato and Roberto, 2004). Therefore, the lignocellulosic substrates need to be pretreated and neutralized to attain the fermentation pH, thereby becoming more suitable for microorganism metabolism. The choice of the best hydrolyzate detoxification method is important for improving the efficiency of the fermentative process. A variety of biological, physical and chemical techniques have been proposed to reduce the concentration of several inhibitory compounds in lignocellulosic hydrolysate. However, the efficiency of any detoxification method depends both on the hydrolysate composition, which differs according to the raw material used and on the hydrolysis condition employed (Mussatto and Roberto, 2004).

2.5.1 Biological Methods

Adaptation of microorganism to the hydrolysate is a biological method for improving the fermentation of hemicellulose hydrolysate media (Nigam, 2001, Amartey and Jeffries, 1996). Adaptation of *P. stipitis* by three times subculturing to the inhibitors in the treated corn cob hydrolysate and this case resulted in the strain capable of producing ethanol at rates, yield and concentration significantly higher than the parent strain (Amartey and Jeffries, 1996). Nigam (2001) has developed a mutant from *P. stipitis NRRL-7124* tolerant of high acetic acid concentration. The selected mutant is capable of fermenting both hexoses and pentose present in red oak wood hydrolysate compared with the parent strain. The ethanol yield and productivity were increased 1.6 and 2.1 folds respectively and also showed detectable growth and ethanol production about 30 hr earlier than the parent culture. However, the adaptation sequences need to continue for more than 6 weeks to make the adapted culture able to tolerate the high acid hydrolysis. The culture however could not be adapted on semi-solid medium (agar slant) but could only grow in a shake flask. Therefore, for biological methods one of the critical issue will be time consuming. Better technique need to be found which allows an effective and economical method for detoxification of byproduct components in hydrolysis.

2.5.2 Physical Methods

Hydrolysate concentration by vacuum evaporation is a physical detoxification method for reducing the contents of volatile compounds such as furfural and hydromethylfurfural present in hydrolysate. However, this method moderately increases the concentration of non-volatile toxic compounds (extractive and lignin derivatives) and consequently the degree of fermentation inhibition.

Parajo *et al.* (1997) used this method with wood hydrolysate and observed an increase in the concentration of lignin derivatives and extractives. The volume of the

hydrolysate was reduced to about 1/3 and the fermentation time necessary for the yeast to consume about 90 % xylose increased from 24 hr to 94 hr. Rodrigues *et al.* (2001) employed the vacuum-evaporation method either before or after treating sugarcane bagasse hemicellulose hydrolysate with activated charcoal. The result was that 98 % of furfural was removed whereas acetic acid was only partially eliminated because this compound is volatile in its undissociated form. The hydrolysate concentration by vacuum evaporation is considered efficient on furfural removal. Unfortunately, other inhibitors like acetic acid and phenolic compounds are still maintained in the hydrolysate. There is a need to find an efficient method that can detoxify the inhibitors from the lignocellulosic hydrolysate for a better subsequent fermentation process.

2.5.3 Chemical Methods

Detoxification of lignocellulosic hydrolysate using alkali treatment by increasing the pH to 9-10 with $Ca(OH)_2$ (overliming) and readjustment to 5.5 with sulfuric acid has been described as early as 1945 by Leonard and Hajny. The detoxifying effect of overliming is due to both the precipitation of toxic components and to the instability of some inhibitors at high pH (Palmqvist and Hanh-Hagerdal, 2000b). Palmqvist and Hahn-Hagerdal (2000a), reported the used of NaOH and $Ca(OH)_2$ to adjust the pH to 10 to the dilute-acid hydrolysate of spruce and obtained about 20 % reduction of furfural concentration but the concentration of acetic acid remained unchanged. In contrast to what has been reported by Amartey and Jeffries, (1996), corn cob hydrolysate treatment with $Ca(OH)_2$ resulting in the loss of acetic acid (43 %), xylose (4 %), glucose (14 %) and arabinose (8 %) thus increasing the ethanol production compared to untreated hydrolysate.

Sugar loss in conversion of sugars to unfermentable compounds is known to be a potentially important issue with overliming (Mussatto and Roberto, 2004).

Mohagheghi *et. al.* (2006) reported using Ca(OH)$_2$ to adjust the pH of corn corb hydrolysate to pH 11 was highly fermentable, but xylose losses were greatest at this condition up to 34 %. Martinez *et al.* (2000) observed the final ethanol concentrations were reduced in treatments above pH 10 at 25 ^{o}C or pH 9 at 60 ^{o}C. Sugar losses due to base destruction and reduced the ethanol production (Mohagheghi *et. al.,* 2006; Martinez *et al.*, 2000). Overall result shows that too high a pH would destroy some of the available sugars and reduces overall ethanol yield. Therefore, pH in overliming critically needs to be kept as low as possible while making the hydrolysate fermentable. Furthermore, alternative detoxification methods should be able to remove inhibitors but still allows the hydrolysate to be fermentable.

Adsorption on ion-exchange resins is also an effective technique, but its cost is high compared to the cost of other treatment. According to Corvalho *et al.* (2004), sugarcane bagasse hemicellulosic hydrolysate was detoxified by a combination of four ion-exchange resins. They used A-103S, A-860S, Applexion cation and Applexion anion. Adsorption with anion-exchange resins was more efficient for the removal of phenols and furans than treatment with cation-exchange resins. This treatment resulted in a removal of 82.1 % furfural, 66.5 % hydromethylfurfural, 61.9 % phenolic compounds, 100 % heavy metals and 0% acetic acid. On the other hand, the removal of acetic acid was not significant.

2.5.4 Combined Treatments

The choice of a detoxification method has to be based on the degree of microbial inhibition caused by the compounds. As each detoxification method is specific to certain types of compounds, better results can only be obtained by combining two or more different methods (Mussatto and Roberto, 2004). Calvalho *et al.* (2005) compared several different detoxification methods for eucalyptus hemicellulosic hydrolysate including concentration by vacuum evaporation and