

**HOT COMPRESSED WATER PRETREATMENT OF OIL PALM
RESIDUES TO ENHANCE GLUCOSE RECOVERY**

GOH CHUN SHENG

Universiti Sains Malaysia

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**HOT COMPRESSED WATER PRETREATMENT OF OIL PALM
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by

GOH CHUN SHENG

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requirements of the degree
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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BET	Brunauer–Emmett–Teller
CBU	Cellobiase unit
CCD	Central Composite Design
EFB	Empty Fruit Bunch
EGU	Endo-glucanase unit
FFV	Flexible Fuel Vehicles
FGB	First-Generation Bio-ethanol
HCW	Hot Compressed Water
NREL	National Renewable Energy Laboratory (USA)
OPF	Oil Palm Frond
RSM	Response Surface Methodology
SD	Standard deviation
SGB	Second-Generation Bio-ethanol
w/w	Weight per Weight

LIST OF SYMBOLS

		Unit
α	Axial distance from the center point	-
y	Response	-
x_i and x_j	Independent variables	-
$\beta_0, \beta_i, \beta_{ii}$ and β_{ij}	Intercept, linear, quadratic and interaction constant coefficients, respectively	-

PRA-RAWATAN SISA KELAPA SAWIT DENGAN AIR PANAS TERMAMPAT UNTUK MENINGKATKAN PEMULIHAN GLUKOSA

ABSTRAK

Penyelidikan ini melibatkan pra-rawatan sisa kelapa sawit dengan menggunakan air panas termampat (HCW) untuk meningkatkan pemulihan glukosa dalam hidrolisis enzim untuk penghasilan bio-ethanol generasi kedua. Sisa kelapa sawit yang dipilih dalam kerja ini ialah pelepah kelapa sawit (OPF) dan tandan buah kosong (EFB). Eksperimen dijalankan dalam satu sistem kelompok dengan menggunakan satu reaktor bertekanan 10 bar. Kaedah respons permukaan (RSM) bersama dengan rekabentuk statistik komposit tengah (CCD) telah digunakan bagi mengoptimumkan respons dan kesan suhu tindakbalas, masa tindakbalas dan nisbah cecair-pepejal ke atas proses pra-rawatan. Respons yang dikaji ialah pemulihan glukosa bagi OPF dan EFB masing-masing. Semua pemboleh ubah didapati memberi kesan yang bererti ke atas respons. Perubahan komposisi dengan keadaan tindak balas telah dikaji untuk memberi data sokongan untuk justifikasi respons eksperimen. Adalah didapati bahawa pada dasarnya kandungan selulosa dan hemiselulosa menurun dengan suhu tindak balas dan masa tindak balas tetapi meningkat dengan nisbah cecair pepejal. Secara umumnya, lignin menunjukkan trend yang sama tetapi menunjukkan perubahan yang sedikit berbeza pada suhu tinggi dan masa tindak balas yang panjang, di mana pemeluwapan lignin berlaku dan kandungan lignin dalam pulpa meningkat. Penghadaman pulpa juga yang berkaitan dengan komposisi, tetapi ia berbeza dari satu keadaan yang lain. Persamaan polinomial kuadratik digunakan untuk memodelkan respons dengan analisis berganda bagi OPF dan EFB masing-masing. Pemulihan glukosa tertinggi bagi OPF dan EFB didapati ialah 83.60 % (176

°C, 11.16 min, 8.00 mL/g) dan 74.80 % (150.00 °C, 11.86 min, 12.23 mL/g) masing-masing. Eksperimen pengesahan untuk keadaan optimum memberikan hasil glukosa yang selaras dengan nilai anggaran model. Didapati bahawa OPF and EFB berbeza dalam pra-rawatan HCW, dari segi perubahan komposisi, dan seterusnya keterhadaman dan pemulihan glukosa keseluruhan. Secara amnya, OPF mempunyai pemulihan yang lebih tinggi daripada EFB disebabkan perubahan komposisinya.

HOT COMPRESSED WATER PRETREATMENT OF OIL PALM RESIDUES TO ENHANCE GLUCOSE RECOVERY

ABSTRACT

This study presents the pretreatment of oil palm residues using hot compressed water (HCW) to enhance glucose recovery in enzymatic hydrolysis for the production of second generation bio-ethanol. Oil palm residues chosen in this work is oil palm fronds (OPF) and empty fruit bunch (EFB). The experiments were carried out in a batch system using a 10 bar pressurized reactor. Response surface methodology (RSM) in conjunction with central composite design (CCD) was used to optimize the response and effect of reaction temperature, reaction time and liquid-solid ratio on the pretreatment process. Response investigated is glucose recovery for OPF and EFB respectively. All variables were found to significantly affect the responses. Composition changes with reaction conditions were studied to provide supportive data for the justification of experimental response. It was found that basically cellulose and hemicellulose content decreased with reaction temperature and reaction time but increased with liquid-solid ratio. Generally lignin shows a similar trend but it shows a slightly different change at high temperature and long reaction time, in which re-condensation of lignin occurred and lignin content in the pulps increased. Digestibility of pulps was also related to the composition, but it varies from one condition to another. Quadratic polynomial equations were used to model the responses by multiple regression analysis for OPF and EFB, respectively. Maximum glucose recovery of OPF and EFB was found to be 83.60 % (176 °C, 11.16 min, 8.00 mL/g) and 74.80 % (150.00 °C, 11.86 min, 12.23 mL/g) respectively. Experimental verification of the optimum conditions gave glucose yields in good

agreement with the estimated values of the models. It was found that OPF and EFB behaved differently in HCW pretreatment, in terms of composition changes, and subsequently digestibility and overall glucose yield. Generally, OPF has higher yield than EFB due to its compositional changes.

CHAPTER 1

INTRODUCTION

1.1 Renewable Energy

In 17th century, the discoveries of ways to exploit energy resources had ignited the remarkable revolution, namely Industrial Revolution. The use of coal and oil which have high energetic values greatly accelerated the development of technologies in transportation, construction, and communication technologies (Edinger and Kaul, 2000). With increase of food supply resulted from expansion in agriculture sector and improved living standard, the world population has risen sharply. At the same time, the energy demand in the world is rising exponentially with the soaring surge of population. Consequently, energy issues have directly or indirectly imposed great economic and social impacts. This was proven when the world was smacked by energy strike for three times.

Energy resources have become a crucial component for a country's development. The United States and strong powers in Europe are expanding their influence on oil and gas resources, through diplomacy, capitalists and military. However, there is still large percentage of these scarce resources controlled by traditional petroleum powers in Middle East, South America, Central Asia and Russia. At the same moment, the rise of China and India as two of the largest energy consumers has also complicated the situation.

Due to this tendency, policies were formulated to improve the stability of energy sector. Apart from competition in controlling limited oil resources, many countries had started to adopt credible plans to principally reduce oil dependency. In this context, development of renewable energy could be pivotal in curbing the issue. Recently, extensive researches of renewable energy are being carried out throughout the world. In Malaysia, most of the liquid fuels are utilized in transportation sector, accumulating up to 6.58×10^8 GJ annually and emitting nearly 19 % of total greenhouse gases emission in Malaysia (PTM, 2008).

However, it is challenging to search for a long-term solution for reliable and infinite source of clean energy supply in the future. Biofuel is one of the proposed solutions. It is generally regarded as carbon neutral because CO₂ released during combustion of biomass was in first instance fixed from the atmosphere, and (under sustainable conditions) is captured by newly-planted trees and crops again. Hence, it should therefore not contribute to the overall accumulation of carbon in the atmosphere and thus reduce the risk of greenhouse effect. Bio-ethanol is one of the examples of biofuels, which can be defined as ethanol derived from biomass instead of fossil resources. On top of that, the complete combustion of bio-ethanol only produces carbon dioxide and water. Hence, incorporation of bio-ethanol into transportation sector may effectively protect next generation against the calamity resulting from climate change caused by greenhouse effect in the future.

1.2 Perspectives and Challenges in Malaysia

1.2.1 Potential in Second-generation Bio-ethanol

Generally, bio-ethanol converted from edible source is called first-generation bio-ethanol (FGB). However, the drawback of FGB stems from the edible feedstock utilized, which includes corns and sugarcane. In this respect, second-generation bio-ethanol (SGB) offers better promise to replace fossil fuels without causing the feud of food-fuel supply as they are derived from non-edible sources such as lignocellulose biomass, which comprises mainly of cellulose, hemicellulose and lignin (Sun and Cheng, 2002). As the world's most exciting oil palm industry cluster, Malaysia generates a great deal of lignocellulosic biomass which has the potential to be converted to second-generation biofuels. These long-chain polysaccharides can be hydrolyzed to produce a mixture of pentoses and hexoses which can be further converted to ethanol. Agricultural intensification is not needed as in FGB where edible agricultural crops such as sugarcane and corn are used as feedstock (Tan *et al.*, 2008).

1.2.2 Biomass Availability

Malaysia is a country located at Southeast Asia, which has tropical and humid climate. Malaysia is blessed with agriculture resources such as oil palm and rubber in a land area of over 330,000 km². Over the past four decades, agriculture industry has significantly contributed to the economy. As one of the top exporters of palm oil, Malaysia generated foreign exchange of US\$ 20,000 million from export of palm oil & palm oil-based products in the year 2008 (DOS, 2009). Table 1.1 represents the

land area of crops planting and average production in Malaysia. According to the table, the area of oil palm cultivation is the highest among the other crops, which was 4,304,914 Ha in year 2007.

Table 1.1: Land area of crops planting and production in Malaysia for year 2007 (DOA, 2009a).

	Area of planting (Ha)	Production (MT)
Oil palm ^a	4,304,914	26,120,754
Rubber ^b	1,229,940	1,119,553
Paddy	676,111	2,375,604
Fruits	287,327	1,871,262
Vegetables	42,832	694,811
Field crops	12,979	129,302
Herbs	495	890
Pepper	4,896	43,932
Coconut	117,650	504,824
Coffee	7,100	30,550
Sugarcane	14,670	733,500
Tea	2,784	5,540

a. MPOB, 2009a (production of oil palm including all palm-based products)

b. MRB, 2008

At the same time, largest portion of total agricultural waste in Malaysia comes from oil palm fields (Ahmad, 2001; Misson *et al.*, 2009). The plantations generate huge amount of wastes such as chopped trunks, dead fronds, empty fruit bunches, shell and fibers. These wastes comprise of biomass in the form of lignocellulose which is suitable for SGB production (Sánchez and Cardona, 2008; Cardona and Sánchez, 2007). Yeoh and Lim (2000) proposed that oil palm trunks and fibers have the potential to produce glucose which could be further fermented to

ethanol. [Kelly-Yong et al. \(2007\)](#) reported that each hectare of oil palm plantation produces 10.88 tons of oil palm fronds on the average. Moreover, when the trees are chopped every 25 years, there are 2.515 tons of trunks generated from each hectare of oil palm cultivation. In the year 2007, total area of oil palm cultivation was 4,304,914 Ha, which means 46,837 thousand tons of fronds and 10,827 thousand tons of trunks were obtained. Besides that, paddy straw, rice husk, banana stem, sugarcane bagasse, coconut husk and pineapple residues are significant agriculture waste in Malaysia too.

The estimated production of lignocellulosic biomass in Malaysia in dry weight in the year 2007 is compiled in Table 1.2. In order to calculate the yield of other lignocellulosic residues in 2007, ratio method has been employed. Ratio of residues is calculated by dividing the quantity of residues to the production quantity of related commodity. Ratio of residues to production of commodity was also found from literature. By using this method, the estimated quantity of lignocellulosic biomass generated in Malaysia including those from agricultural wastes can be easily calculated. Dry mass of the residues was calculated by deducting the moisture content which values were taken from published data. According to [Wyoming Biomass Inventory \(2007b\)](#), water content in municipal solid waste organic waste such as food scraps, wood waste, papers and yard trimmings are 50 %, 20 %, 5 % and 40 %, respectively. Due to inadequate data in Malaysia, an average value of 57.5 % of moisture content was employed in this calculation.

Table 1.2: Estimated quantity of lignocellulosic biomass produced in Malaysia in the year 2007.

Types	Quantity (ktons)	Ratio	Source	Source (ktons)	Moisture content (% wt)	Dry Weight (ktons)
<i>Agriculture waste</i>						
Oil palm fronds	46,837	0.572 ^a	Oil palm	81,920	60.0 ^d	18,735
Empty fruit bunch	18,022	0.220 ^b	FFB	^c	65.0 ^b	6,308
Oil palm fibers	11,059	0.135 ^b			42.0 ^b	6,414
Oil palm shells	4,506	0.055 ^b			7.0 ^b	4,190
Oil palm trunks	10,827 ^{a*}	-	Replanting	-	75.9 ^e	2,609
Paddy straw	880	0.400 ^f	Paddy	2,375 ^g	11.0 ^h	783
Rice husk	484	0.220 ^f			9.0 ⁱ	440
Banana residues	530	2.000 ^j	Banana	265 ^k	10.7 ^l	473
Sugarcane bagasse	234	0.320 ^f	Sugarcane	730 ^g	50.0 ^m	117
Coconut husk	171 ⁿ	0.340	Coconut	505 ^g	11.5 ^q	151
Pineapple waste	48	0.700 ^o	Pineapple	69 ^p	61.2 ^r	19
<i>Forest residues</i>						
Logging residues	2,649	1.000 ^s	Logs	2,649 ^t	12.0 ^u	2,331
Plywood residues	2,492	1.000 ^s	Plywood	2,492 ^t	12.0 ^u	2,193
Sawmill residues	1,160	0.818 ^s	Sawn timber	1,418 ^t	12.0 ^u	1,021
<i>Municipal Solid Waste (MSW)</i>						
Organic waste	4,653	0.690 ^v	MSW	6,744 ^w	57.5 ^x	1,978

a. Jamil et al., 1987.

b. Yusoff, 2006; Gutiérrez, 2009.

c. MPOB, 2009b.

d. Supranto et al., 1999.

e. Yutaka et al., 2007.

f. Hashim, 2005.

g. DOA, 2009a.

- h. Abdel-Mohdy *et al.*, 2009.
- i. Nordin *et al.*, 2007.
- j. Jingura and Maitengaifa, 2008.
- k. DOA, 2009b.
- l. Bilba *et al.*, 2007.
- m. Akram *et al.*, 2009.
- n. Tan I.A.W. *et al.*, 2008.
- o. Kroyer, 1991.
- p. MPIB, 2008.
- q. Achaw and Afrane, 2008.
- r. Idris and Suzana, 2006.
- s. Suzuki and Yoshida, 2009.
- t. MTC, 2009. Density of wood: 0.57 ton/m³ (Sasaki *et al.*, 2009).
- u. Wyoming Biomass Inventory, 2007a.
- v. Hanssan *et al.*, 2001; Ngoc and Schnitze, 2009.
- w. Generation rate: 0.68 kg/cap/day (UNEP, 2002); Population: 27.17 million (DOS, 2008).
- x. Wyoming Biomass Inventory, 2007b.

* Calculated based on amount of trunks disposed from 1 ha of oil palm plantations / annum according to Jamil *et al.* (1987) with a value of 2.51 tons/ha/yr

1.3 Problem Statement

Malaysia has experienced steady escalation in energy consumption to a height of 2,693 kg of oil equivalent per capita in the year 2008 (World Bank, 2011). In Malaysia, the transportation sector contributes significantly to the total CO₂ emission with more than 19 % of the total emission. Although it has a relatively small population, the average emission in Malaysia was reported to be 7.32 tons of CO₂ per capita (World Bank, 2011). Hence, an indigenous, affordable and clean source of energy has become a high priority in Malaysia to surmount the issue of energy security and sustainability of human development. In Malaysia, most of the liquid fuels are utilized in transportation sector, accumulating up to 15,715 ktoe annually and emitting nearly 19 % of total greenhouse gases in Malaysia (PTM, 2008). Incorporation of bio-ethanol into the transportation sector could be an effective way

to mitigate global warming in the future. Bio-ethanol as one type of biofuels, is carbon 'neutral' and essentially free from sulfur and aromatics which are harmful to living organisms. However, FGB derived from edible sources has caused the feud of food-fuel supply. In contrast, SGB derived from non-edible lignocellulosic sources offers better promise without causing the food versus fuel issue. In this respect, agricultural intensification is not needed.

As one of the most important agricultural countries in the world with exports including palm oil, cocoa and rubber, Malaysia creates a substantial amount of lignocellulosic agriculture wastes. As far as oil palm is concerned, Malaysia produces an estimated 33 million tons of crop residue annually in the form of empty fruit bunch, fiber and shell (Mohamed and Lee, 2006). As the main exporter of palm oil in the world, utilization of oil palm biomass for the production of environmental friendly biofuel has become an attractive approach instead of creating pollution problems (Chew and Bhatia, 2008).

However, lignocellulosic biomass is difficult to be converted to bio-ethanol, and therefore an effective pretreatment is important to improve its feasibility (Liu and Shen, 2008). The high capital and operation cost of pretreatment process has become a significant problem to make the process viable. There are still large rooms for technical and engineering improvement and optimization. In this regard, hot compressed water has been proposed as a suitable medium for lignocellulose pretreatment. There are three main advantages of HCW. First, it does not involve the use of chemicals which may cause difficulty in reactor design, purification and effluent handling. Second, drying of raw materials is not needed. Third, with better

heating and mixing, it prevents the sugars from degradation. A comprehensive study on the HCW pretreatment of lignocellulosic oil palm residues for the production of SGB to achieve higher yield is necessary to understand the process and to optimize the operating conditions.

1.4 Research Objectives

The principle objective of this research project is to perform experimental studies on hot compressed water (HCW) pretreatment of oil palm residues to enhance glucose recovery for the production of second-generation bio-ethanol. The measurable objectives in this study are:

1. To carry out characterization work on oil palm frond (OPF) and empty fruit bunch (EFB) to determine its chemical composition;
2. To determine the effect of reaction temperature, reaction time and liquid-solid ratio on HCW pretreatment of oil palm residues in a batch system by investigating the changes in composition and digestibility;
3. To analyze, model and optimize the functional relationships between operating variables on the desired response (glucose recovery) based on the reliability of response surface methodology (RSM) in conjunction with central composite design (CCD).

1.5 Thesis organization

The thesis was divided into 5 main chapters. Chapter One describes the current trend of energy exploitation and development. In this background, the status, perspectives and challenges of bio-ethanol development in Malaysia as a new source of renewable energy were discussed. The problem statement, objectives, and organization of the thesis were also given in this chapter.

Chapter Two is a literature review that provides a background study on the chemistry of biomass constituent and various kinds of pretreatment methods proposed by the researchers. The brief explanation on the chemistry of the pretreatment reactions was also included. This review then concentrates on hot compressed water pretreatment which was used in this project. Firstly, there was a brief view on HCW and its properties. The reaction mechanisms of lignocellulose in HCW were roughly given, and subsequently the general effect of process parameters was elaborated.

Chapter Three provides the lists of all the materials and explanation of optimization and experimental procedures. Firstly, chemicals and gases used were listed, followed by preparation of oil palm residues as raw materials for the study. Characterization, pretreatment and enzymatic hydrolysis, analytical methods were described and fully explained. Finally, the chapter was completed with a detail description on design of experiment and process optimization.

Chapter Four presents the experimental results and discussion. Generally, this chapter was divided into two main parts: (1) optimization study of HCW pretreatment for the case of OPF; and (2) for the case of EFB. It presents the results from characterization and experimental work on the study of HCW pretreatment of oil palm biomass. Then, it was followed by detail analysis and discussions on the results obtained, including a detail discussion on the effects of process parameters on pulps composition and digestibility, and model development as well as model analysis using ANOVA. Important statistical terms and calculations were explained. Besides tables, the explanation was supported by various types of graphical presentations including bar chart, two-dimensional plots, and three-dimensional surface plots. Numerical optimization study was discussed for HCW pretreatment to complete the study.

Chapter Five presents the overall conclusion of the research. In the final part, recommendations were given to further expand the scope of the research to obtain a more comprehensive result.

CHAPTER 2

LITERATURE REVIEW

2.1 Chapter Overview

This chapter basically elaborates the review of important components in this study, including materials, methods, reaction mechanism and effects of parameter. A detail literature study was made, referring to the latest development in the related field.

2.2 Biochemistry of Lignocellulose

Lignocellulose composed of cellulose, hemicellulose and lignin. It is the most abundant organic material on earth. Basically, cellulose microfibrils are encrusted in the lignocellulose matrix.

Lignin is an unusual biopolymer because its structure is heterogeneous. It does not have a defined primary structure. It also consists of various types of substructures which appear to repeat in a haphazard manner. There are various types of lignin depending on the means of isolation. Lignin forms covalent bonds with hemicellulose and thereby cross links with cellulose. It provides mechanical strength to the plant. Unlike polysaccharides, it is more hydrophobic and not permeable to water. Figure 2.1 represents a small molecular structure of lignin. Lignin provides support through strengthening of wood in plants.

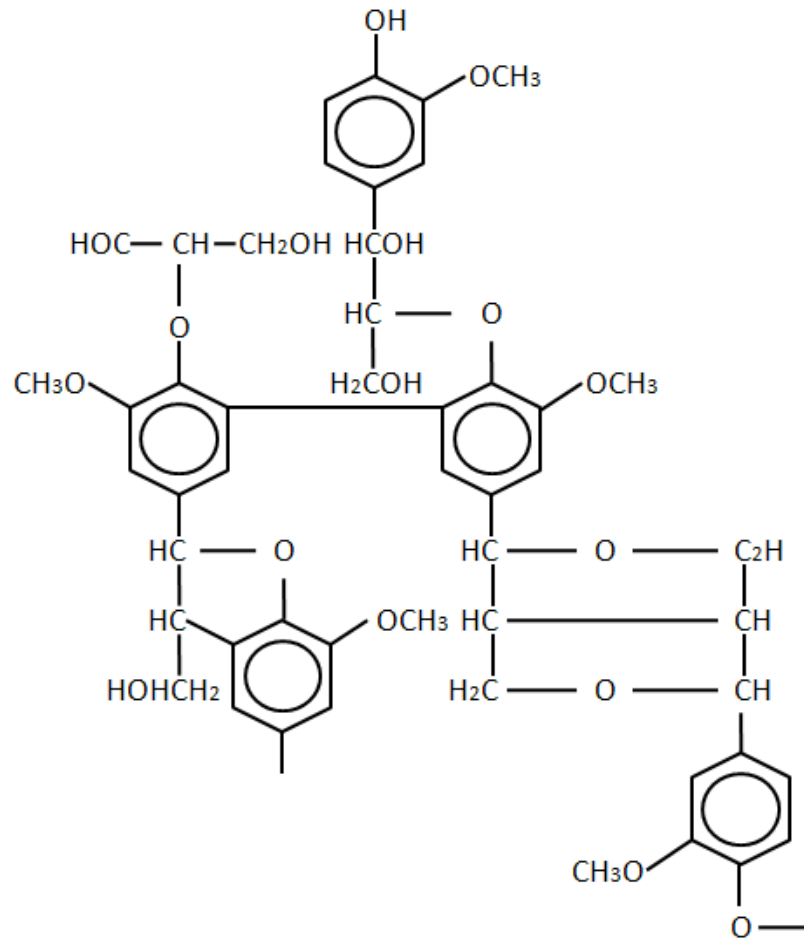


Figure 2.1: A molecular structure of lignin (Glazer and Nikaido, 1995).

Cellulose exists as structural component in primary cell wall of green plants. Cellulose is a straight chain polysaccharide consisting of a linear chain of several hundred to over ten thousand β -1,4 linked D-glucose units with the formula $(C_6H_{10}O_5)_n$. In fact, cellulose is a linear polymer consists of cellobiose as repeating units, in which cellobiose is double sugar of glucose connected by β -1,4-glycosidic bond. It has no coiling or branching. Hydrogen bonds are formed between hydroxyl groups to hold neighbour chains firmly together side-by-side. Crystallinity of cellulose is higher in comparison to other glucose based polysaccharides. There are several types of crystallinity, corresponding to the location of hydrogen bonds between and within strands. Figure 2.2 shows the basic structure of cellulose.

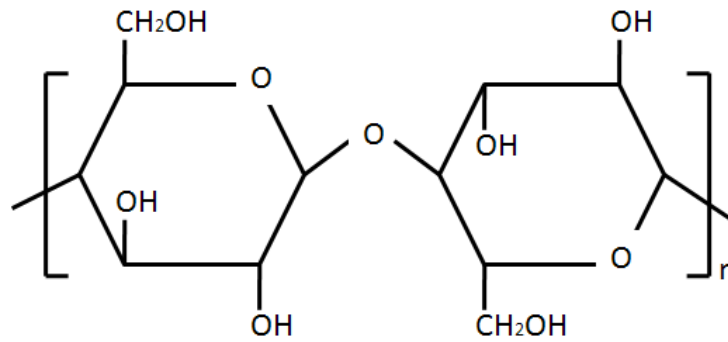


Figure 2.2: Structure of cellulose (Brown *et al.*, 2003).

Hemicellulose plays the role as connector between microfibrils. Hemicellulose structure is shown in Figure 2.3. It presents along with cellulose but its structure is very different from cellulose. It has a random and amorphous structure and consists of shorter branched chains. It contains different sugar monomers, mainly D-pentoses like xylose, mannose, galactose, rhamnose and arabinose. Hemicellulose consists of short chains formed by 500-3,000 sugar units, which is shorter than cellulose chains with 7,000 - 15,000 glucose molecules per polymer. In addition, cellulose is unbranched but hemicellulose is a branched polymer.

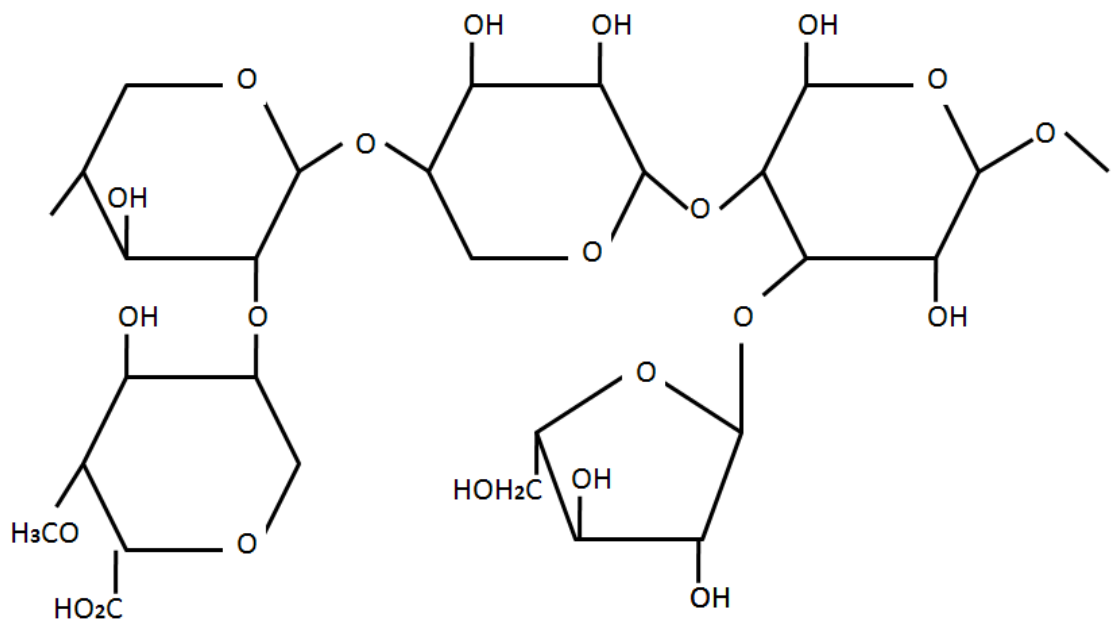


Figure 2.3: Structure of hemicellulose (Laine, 2005).

2.3 Production of Bio-ethanol from Lignocellulose

Lignocellulosic biomass has complicated structure which is more difficult to be converted to fermentable sugar like other sugar energy storage compounds such as starch (Liu and Shen, 2008). Extra cost is added in equipments and process operation since lignocellulose is not meant to be readily accessible as a sugar source. The high capital and operation cost of pretreatment process has become a significant challenge to make the process viable. Apart from that, the enzymatic hydrolysis process to depolymerize the cellulose into fermentable sugars would be a considerable cost component. Until today, there are still technical and engineering constraints in this stage which prevent large-scale commercial use of lignocellulosic biomass. The process has not been feasible due to relatively expensive cellulases. However, the effort from National Renewable Energy Laboratory (NREL) has made the advances toward cost reduction of cellulases. Under a contract with NREL, Novozymes Biotech has reduced the cost of making one gallon of bio-ethanol from > US\$5.00 to < 50 cents (Focus on catalyst, 2004).

Second-generation bio-ethanol requires an extra step to hydrolyze lignocellulosic biomass in comparison with first-generation bio-ethanol. Basically, the transformation of lignocellulose into ethanol can be completed in three stages (biochemical route): (1) pretreatment to liberate cellulose from the lignocellulosic complex; (2) hydrolysis of carbohydrate polymer to produce free sugars; and (3) fermentation to ethanol from pentoses and hexoses liberated in the second step. Figure 2.4 illustrates the process flow diagram.

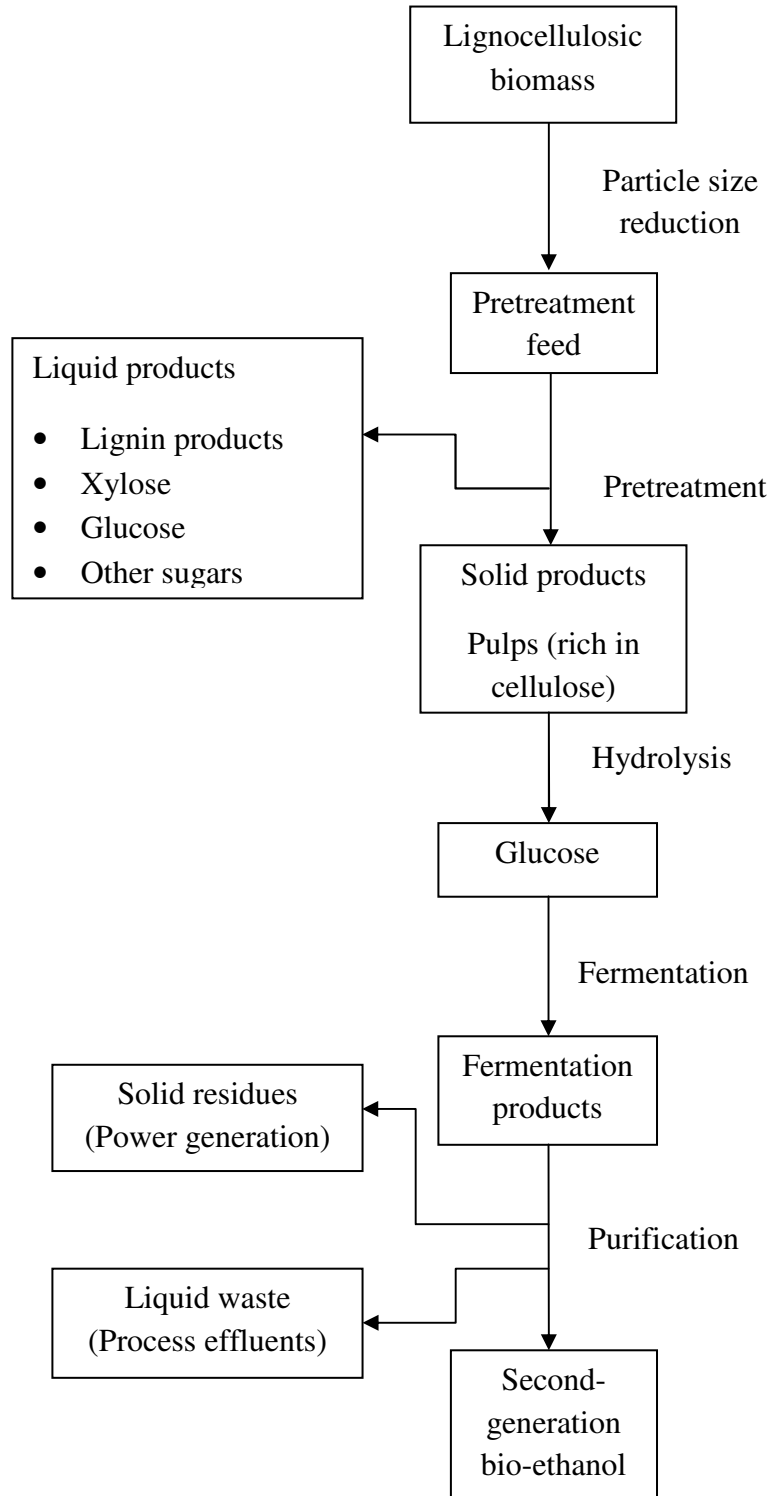


Figure 2.4: Process flow diagram of conversion of lignocellulose into second-generation bio-ethanol.

2.4 Pretreatment of Lignocellulose: A Review

The major challenge to obtain sugar from the biomass is to increase the accessibility of enzymes to the lignocellulose. Lignin and hemicellulose in the biomass are the major barriers of enzymatic hydrolysis of cellulose into its monomers. Generally, there are three methods to remove the barriers by pretreating the biomass (i) thermally, (ii) chemically and (iii) biologically. The objective of pretreatment is to solubilize the lignin and hemicellulose to make the cellulose better accessible (Hendriks and Zeeman, 2009). Besides that, pretreatments also decrystallize the cellulose into amorphous form to ease the hydrolysis by enzymes later in hydrolysis stage. Figure 2.5 shows the schematics of pretreatment process.

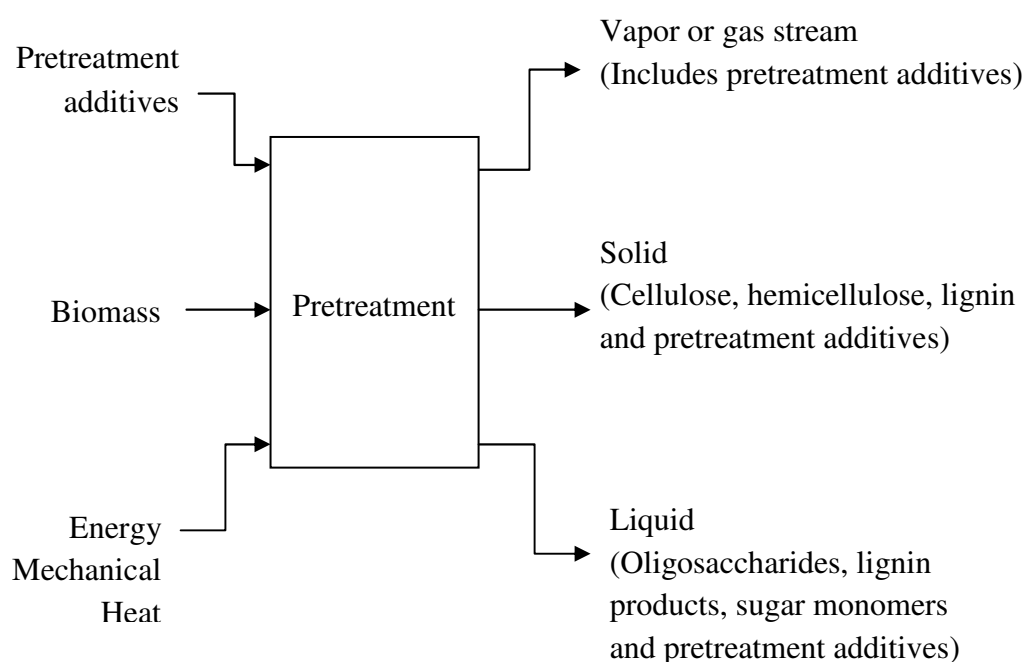


Figure 2.5: Schematic diagram of Pretreatment Process (Mosier *et al.*, 2005).

2.4.1 *Physical Pretreatment*

Before lignocellulosic biomass is treated to remove the lignin and hemicellulose, the particle size must be reduced through milling. It can be done with mechanical stress such as dry, wet vibratory, and compression based ball milling procedures (Sdiras and Koukios, 1989; Tassinari *et al.*, 1980; Alvo and Belkacemi, 1997). Smaller size of particles provides a higher surface area to volume ratio. In some cases, degree of polymerization and crystallinity of cellulose is also reduced. Consequently, the enzyme performance is improved (Fan *et al.*, 1981; Palmowski and Muller, 1999). It is important to have a proper particle size reduction in order to overcome mass and heat transfer problems. However, particle size reduction beyond a certain size is not economically feasible and reduction below 40 mesh has little effect on hydrolysis yield (Chang and Holtzapple, 2000).

2.4.2 *Thermal Pretreatment*

Lignocellulosic biomass is heated in this pretreatment. Basically it can be divided into three types: steam pretreatment, hot compressed water pretreatment and catalytic pyrolysis. Steam pretreatment solubilizes the hemicellulose to make the cellulose suitable for enzymatic hydrolysis and to avoid the formation of inhibitors. During steam pretreatment the biomass is charged with steam at high temperature (temperatures up to 240 °C) and pressure. After a few minutes, the steam is discharged and the biomass is cooled down quickly (Hendriks and Zeeman, 2009). Wu *et al.* demonstrated the steam explosion of Douglas fir heartwood and sapwood chips to optimize the ease of enzymatic hydrolysis of the water-insoluble fraction.

The digestibility recorded 97.9 % at high severity environment ($\log R_0 = 4.21$) (Wu *et al.*, 1999). Hot compressed water or also called as subcritical water, is another medium used to pretreat lignocellulosic biomass. It will be discussed in details in section 2.5.

2.4.3 Chemical Pretreatment

Chemical pretreatment of lignocellulose is divided into seven major categories depending on the types of chemical used: solvent extractions, acid pretreatment, alkaline pretreatment, oxidative pretreatment, ammonia pretreatment, carbon dioxide pretreatment and combined pretreatment. These pretreatments modify the cell wall chemically and ultrastructurally with different types of chemistries and mechanisms (da Costa Sousa *et al.*, 2009).

2.4.3.1 Solvent extraction

Solvent extraction in lignocellulose pretreatment is a process of disruption of the hydrogen bonding between microfibrils. The principle of this pretreatment is differential solubilization and partitioning of various components of the plant cell wall, including cellulose (Heinze and Koschella, 2005). Organosolv process, phosphoric acid fractionation, and ionic liquids based fractionation were reported as the most attractive options among the solvent similar methodologies available (da Costa Sousa *et al.*, 2009). In organosolv process, organic solvents such as alcohols are employed to extract lignin from lignocellulosic biomass in the presence of an acidic catalyst (Pan *et al.*, 2006). The process operates at temperature range from

90 °C to 220 °C and reaction time range from 25 to 100 mins, depending on the types of lignocellulosic biomass used. [Pan *et al.* \(2006\)](#) reported that more than 85 % of glucose recovery was achieved after the poplar was pretreated with ethanol at optimum conditions. The solvent can be separated by distillation and recycled.

Based on differential solubility of various plant cell wall components in different solvents, phosphoric acid, acetone, and water can be used to fractionate the cell wall into cellulose, hemicellulose, lignin, and acetic acid at 50 °C and atmospheric pressure ([Zhang *et al.*, 2007](#)). The key advantage of the method is its mild operating condition and possible to decrystallize cellulose fibers and remove most of the hemicelluloses and lignin. The highest digestibility reported is 97 % when the enzymatic hydrolysis was carried out for 24 hours ([Zhang *et al.*, 2007](#)). Despite of the high yield, costly recovery of phosphoric acid has restricted the economical consideration ([Moxley *et al.*, 2008](#); [da Costa Sousa *et al.*, 2009](#)).

Recently, ionic liquids have been considered as a green and energy-efficient process recently to be employed in pretreatment due to its capability to form hydrogen bonds with cellulose at high temperatures. Ionic liquids consist of entirely ions, including anions like chloride, formate, acetate, or alkyl phosphonate. It represents a new class of solvents with high polarities ([Wasserscheid and Keim, 2000](#)). [Zavrel *et al.* \(2009\)](#) reported that 1-ethyl-3-methylimidazolium acetate, [EMIM][Ac], turned out to be the most efficient solvent for cellulose according to the obtained dissolution profiles. The quantity of ionic liquid used is about 10 times the weight of biomass and the temperature range from 100 to 150 °C. Water, methanol and ethanol can be used as antisolvents to precipitate the soluble biomass.

The major drawback of ionic liquids is their detrimental effects on cellulase activity (Zhao *et al.*, 2009). For this reason, ionic liquids residues should be eliminated entirely during cellulose regeneration. Ionic liquids hold strong potential as alternative reagents for lignocellulosic biomass pretreatment to increase the cellulose digestibility up to 90% (Lee *et al.*, 2008).

2.4.3.2 Acid pretreatment

Dilute or strong acid can be used to hydrolyze the hemicellulose into monomers. Meanwhile, dilute acid method is considered a mature technology that allows the hydrolysis of the main part of hemicellulose as well as an adequate level of amorphous cellulose (Gutiérrez *et al.*, 2009). The heated residue was sprayed or agitated with acid in a reactor (Mosier *et al.*, 2005). Gutiérrez *et al.* (2009) reported a study of diluted acid pretreatment with the reactor operating at 190 °C and 12.2 atm. All cellulose and lignin have almost remained as solid whereas the hemicellulose was hydrolyzed into pentoses and hexoses which dissolved in the liquid fraction together with the products of the thermal degradation of these sugars and lignin. Nevertheless, there is a risk on the formation of volatile degradation products which causes loss of fermentable sugars for the conversion to ethanol. Therefore, detoxification step is required for acidic pretreatment to remove the inhibitory substances by using ion exchange column (Gutiérrez *et al.*, 2009). However, in acidic condition, solubilized lignin will quickly condense and precipitate (Liu and Wyman, 2003). Furthermore, the condensation and precipitation of solubilized lignin components are undesired reactions because it decreases digestibility. Although the effect of strong acid is more pronounced, it has the risk to form inhibiting compounds such as furfural and

hydroxymethylfurfural (HMF) (Hendriks and Zeeman, 2009). Sun and Cheng (2005) reported a maximum glucose yield of 81 % from rye straw can be achieved using sulfuric acid in the absence of enzymatic hydrolysis.

2.4.3.3 Alkali Pretreatment

In alkaline pretreatment, solvation and saponification occur as preliminary reactions, causing a swollen state of the biomass and makes it more accessible for enzymes and microbes (Hendriks and Zeeman, 2009). It is believed that the swelling and hydrolysis of lignin and other hemicellulose occur during the pretreatment (Sutcliffe and Saddler, 1986). Carrilloa *et al.* (2005) investigated the effect of alkali pretreatment by comparing the hydrolysis of untreated and treated wheat straw with 37.5 g/L cellulase enzyme. About threefold total sugar yield was obtained on the enzymatic hydrolysis after the NaOH alkali pretreatment. Koullas *et al.* (1993) reported that 70-100% of hydrolysis of wheat straw was achieved after alkaline delignification. Microwave-assisted alkali pretreatment is another alternative for lignocellulose. Lower enzyme loading and shorter reaction time could achieve higher ethanol concentration and yield in the microwave-assisted alkali pretreatment. Under the optimum condition, the ethanol yield reached 64.8 % (Zhu *et al.*, 2006). When strong alkali was employed, 'peeling' of end-groups and decomposition of dissolved polysaccharides took place. Similar to strong acid pretreatment, strong alkali caused formation of lower molecular compounds, which is an advantage for later conversion but also have the risk on degradation and loss of carbon. Misson *et al.* (2009) claimed that the chemical pretreatment using NaOH was found to be an effective method to degrade almost 100% of lignin in empty palm fruit bunches.

2.4.3.4 Oxidative Pretreatment

Oxidative agents such as hydrogen peroxide or peracetic acid also can be added to the biomass suspended in water to carry out pretreatment process. Reactions like electrophilic substitution, displacement of side chains, cleavage of alkyl aryl ether linkages or the oxidative cleavage of aromatic nuclei occur in this process (Hon and Shiraishi, 2001). Teixeira *et al.* (1999) claimed that peracetic acid is very lignin selective and no significant carbohydrate losses occurred when it is used at ambient temperatures as a pretreatment method for hybrid poplar and sugarcane bagasse. An average theoretical ethanol yield of 92.3% was achieved for hybrid poplar pretreated with 6% NaOH/15% peracetic acid through SSFC using a recombinant *Z. mobilis* CP4/pZB5.

2.4.3.5 Ammonia Pretreatment

Ammonia and carbon dioxide pretreatment require relatively higher temperature and pressure. Ammonia fiber/ freeze explosion pretreatment works moderately well on hardwoods but not on softwoods (McMillan, 1994). Mass ratio of ammonia to biomass is around 1 to 1. The process temperature is inversely proportionate to the time used. At ambient temperature, ammonia freeze explosion requires 10-60 days to complete the reaction but at temperature up to 120 °C, several minutes is enough for complete process. It was reported that elimination of lignin from switchgrass and swelling of cellulose increase the yield of enzymatic hydrolysis up to six-fold and consequently a 2.5-fold of ethanol yield after fermentation. 93 % digestibility of treated pulps was achieved in comparison with that of untreated

switchgrass, which was only 16 %. (Alizadeh *et al.*, 2005). Apart from that, the carbohydrate has become more accessible after ammonolysis of glucuronic cross-links (Lin *et al.*, 1981).

2.4.3.6 Carbon Dioxide Pretreatment

The high-pressure carbon dioxide at high temperatures of up to 200 °C is also employed to pretreat lignocellulosic biomass. Kim and Hong (2001) reported that when the lignocellulose with moisture content 73 % (w/w) was pretreated by supercritical carbon dioxide at 3100 psig and 165 °C for 30 min, the theoretical maximum sugar yield of 84.7±2.6 % (w/w) and 27.3±3.8 % (w/w) for aspen and southern yellow pine were obtained, respectively.

2.4.3.7 Combined Pretreatment

Innovative and creative approaches are being put forward by researchers all over the world. Combinations of several techniques were investigated such as thermal pretreatment in combination with chemical pretreatment. By adding external acid, the optimal pretreatment temperature was lowered and a better enzymatic hydrolysable substrate was obtained (Gregg and Saddler, 1996). However, significant amount of furfural was produced when 0.5 % sulfuric acid was added during steam pretreatment at temperatures of 160 °C (Grohmann *et al.*, 1985). Instead of acidic approach, Chang *et al.* (2001) reported that alkaline thermal pretreatment of switchgrass and corn stover at temperatures of 100 - 150 °C with lime addition of approximately 0.1 g calcium hydroxide per g substrate did not inhibit the enzymatic