

**PRETREATMENT OF OIL PALM FRONDS USING A SOLVENT-IONIC
LIQUID (BMIM)Cl TO ENHANCE GLUCOSE RECOVERY**

TAN HUI TENG

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**PRETREATMENT OF OIL PALM FRONDS USING A SOLVENT-IONIC
LIQUID (BMIM)Cl TO ENHANCE GLUCOSE RECOVERY**

by

TAN HUI TENG

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LIST OF ABBREVIATIONS

AFEX	Ammonia fiber explosion
ANOVA	Analysis of variance
[BMIM]Cl	1-butyl-3-methylimidazolium chloride
CCD	Central composite design
DF	Degrees of freedom
EFB	Empty fruit bunch
[EMIM]Ac	1-ethyl-3-methylimidazolium acetate
FGB	First-generation bio-ethanol
FTIR	Fourier transform infrared
HPLC	High-performance liquid chromatography
IL	Ionic liquid
LOI	Lateral order index
NREL	National renewable energy laboratory
OPF	Oil palm frond
RSM	Response surface methodology
SEM	Scanning electron microscopy
SGB	Second-generation bio-ethanol
TCI	Total crystallinity index

LIST OF SYMBOLS

		Units
α	Axial distance from the center point	-
$\beta_0, \beta_i, \beta_{ii}$ and β_{ij}	Intercept, linear, quadratic and interaction constant coefficients, respectively	-
C_o	Observed crystallinity index	-
W	Weight loss	%
$wt\%$	Weight percent	%
x_i and x_j	Independent variables	-
y	Response	-

**PRARAWATAN PELEPAH KELAPA SAWIT DENGAN MENGGUNAKAN
PELARUT- CECAIR IONIK (BMIM)Cl UNTUK PENINGKATAN
PEMULIHAN GLUKOSA**

ABSTRAK

Potensi pelepah kelapa sawit (OPF) sebagai sumber glukosa bagi penghasilan bahan api etanol telah dikaji dalam penyelidikan ini. Bahan mentah lignoselulosa telah diprarawat dengan pelarut cecair ionik (IL) 1-butil-3-metilimidazodium klorida ([BMIM]Cl) untuk memudahkan proses penukaran OPF ke gula boleh tapai (glukosa). Proses prarawatan dilaksanakan terlebih dahulu dengan merawat OPF dalam cecair ionik and diikuti dengan penjanaan semula selulosa dengan menggunakan anti-pelarut. Kesan beberapa pembolehubah prarawatan seperti suhu, masa tahanan dan kandungan pepejal terhadap komposisi dan kehabluran biojisim serta penghadaman oleh enzim dan pemulihan glukosa selepas prarawatan telah dikaji. Keputusan menunjukkan bahawa penghadaman biojisim dan pemulihan glukosa adalah dipengaruhi bukan sahaja oleh kehabluran biojisim tetapi oleh perubahan dalam struktur dan komposisi biojisim selepas prarawatan. Pembolehubah-pembolehubah prarawatan seterusnya dioptimumkan dengan menggunakan Kaedah Respons Permukaan (RSM) berdasarkan Rekabentuk Statistik Komposit Tengah (CCD). Pemulihan glukosa yang optimum iaitu 100 % didapati boleh dicapai dengan suhu prarawatan 80 °C, masa tahanan selama 15 min dan 10 %berat kandungan pepejal berbanding dengan pemulihan glukosa dari OPF mentah yang hanya mencapai 22.57 % di bawah keadaan yang sama. Oleh itu, ini membuktikan prarawatan biojisim lignoselulosa dengan menggunakan cecair ionik [BMIM]Cl merupakan salah satu teknik yang boleh menjanjikan peningkatan pemulihan glukosa untuk penghasilan bio-ethanol.

PRETREATMENT OF OIL PALM FRONDS USING A SOLVENT-IONIC LIQUID (BMIM)Cl TO ENHANCE GLUCOSE RECOVERY

ABSTRACT

The potential of oil palm frond (OPF) as glucose source for fuel ethanol production was investigated in this current work. Pretreatment of lignocellulosic feedstock with a solvent, ionic liquid (IL) 1-butyl-3-methylimidazolium chloride ((BMIM)Cl) was used to facilitate the conversion of OPF into fermentable sugar (glucose). The pretreatment was accomplished by first subjecting OPF to ionic liquid treatment, followed by regeneration of cellulose using an anti-solvent. The effects of several pretreatment variables, such as temperature, retention time and solid loading on the biomass composition, crystallinity as well as its digestibility by enzyme and glucose recovery after pretreatment were studied. Results showed that biomass digestibility and glucose recovery were strongly influenced by the changes in the biomass structure and composition after pretreatment but not by the crystallinity of the biomass alone. The pretreatment variables were further optimised using Response Surface Methodology (RSM) based on a factorial Central Composite Design (CCD). An optimum 100 % glucose recovery was found with pretreatment conditions of 80 °C, a 15 min retention time and 10 wt% solid loading as oppose to glucose recovery from raw OPF that was merely 22.57 % under the same conditions. Hence, pretreatment of lignocellulosic biomass using ionic liquid [BMIM]Cl is one of the promising techniques to enhance glucose recovery for the production of bio-ethanol.

CHAPTER 1

INTRODUCTION

1.1 Lignocellulosic biomass

Lignocellulosic biomass is the fibrous material that forms the cell walls, leaves, stems, stalks and woody portions of plants. Lignocellulosic materials are natural renewable resources and abundantly available as residues biomass from agriculture and forestry such as corn stover, sugarcane bagasse, wheat straw, paper mill residues, municipal waste as well as oil palm residues. Lignocellulose is composed of three major components: lignin, hemicellulose and cellulose with varying composition for different biomass. Cellulose, a major constituent of biomass is extensively used for paper-making, textile manufacturing and production of value added chemicals, cellulose derivatives. Cellulose derivatives are used in a wide range of products such as cigarette filters, photographic films, coating materials, foils, sponges, moulded articles, detergent, adhesive, foods stabilizer and thickener as well as membrane in pharmaceutical industry (Clark and Deswarte, 2008; Iller *et al.*, 2007).

Apart from that, the use of cellulose also focused on glucose recovery for other bio-based chemicals production. In pharmaceutical industry, glucose is used as a precursor to make vitamin C in the Reichstein process. Gluconic acid, a biodegradable chelating agent and an intermediate in food and pharmaceutical industry can be derived from oxidation of glucose. Glucose obtained from starch or cellulose hydrolysis also can be hydrogenated to sorbitol which is a commodity product used in food, pharmaceutical and chemical industries (Gallezot, 2007).

However, recently the use of glucose has been focused for the production of bio-ethanol, one of the renewable energy that has a high demand today due to the depletion of conventional energy resources as well as environmental issues.

1.2 Energy scenarios

Energy is apparently the lifeblood of economic growth in major countries worldwide. In 2007, world energy consumption, which totaled 495 quadrillion Btu, is projected to grow by 49 % to 739 quadrillion Btu in 2035, driven by strong long-term economic growth in the developing nations of the world (EIA, 2010a). Currently, around 88 % of total energy sources are contributed by conventional resources such as non-renewable oil, gas and coal (Ong *et al.*, 2011). Unfortunately, these conventional resources, which have taken three million years to form, are likely to deplete soon. The global proven oil and gas reserves are estimated to last for just 41.8 and 60.3 years, respectively with current rates of consumption (Ong *et al.*, 2011). However, coal is likely to last a little over 200 years (Yilmaz and Uslu, 2007). Furthermore, energy production from these non-renewable resources can significantly contribute to environment degradation, especially the emission of carbon dioxide, the most important greenhouse gas which brings serious changes in global climate nowadays. The nature carbon cycle has been disrupted when the carbon stored in fuels is released to atmosphere at a rate faster than the rate at which these fuels were created. Currently, the increase in carbon dioxide in atmosphere is happening at an alarming rate, which is rising by over 40 % from 20.7 GT in year 1990 to 29.9 GT in year 2008 (United Nations, 2011). In this context, development

of alternative energy sources that are renewable and environmentally friendly is of utmost importance.

In fact, development of alternative renewable energy sources is no longer a new issue but extensive effort has been carried out in the past. Today, renewable energy comprises 16 % of the total global energy used, including energy generated from wind power, solar energy, hydropower, geothermal, biomass, biofuels and etc. (REN 21, 2011). While the effort in finding alternative energy sources increase progressively, rapid growth has been seen in liquid fuels. This is due to the fact that the largest fraction of world energy sources was contributed by liquid fuels, accounting for 35% of the total energy demand in 2007 (EIA, 2010b). Apart from the concern over depletion of resources and emission of greenhouse gases associated with fossil fuels, soaring petroleum prices and oil supply disruption implicated by exclusive monopoly by politically volatile countries also provoked the interest in exploiting alternative sources of energy to enhance the energy security of a country.

1.2.1 First-generation bio-ethanol (FGB)

Amongst the liquid biofuels developed nowadays, bio-ethanol is one of the most widely used biofuels. Bio-ethanol is an alcohol-based alternative motor fuel produced by fermenting simple sugars from agricultural feedstock. Ethanol is most commonly blended with gasoline and used to power automobiles to improve the emissions quality of gasoline. To some extent, bio-ethanol can be regarded as a vital solution to issues regarding volatile crude oil prices, high carbon intensity from the transportation sector, and the attendant impacts on climate change. Bio-ethanol or

more specifically first-generation bio-ethanol (FGB) derived from food crops, such as wheat, corn, sugar beet, sorghum and sugarcane, is currently the dominant biofuel produced on a global scale, accounting for 19.5 billion gallons of fuel ethanol in 2009 (RFA, 2010). Despite the benefit of mitigating environmental issues entailed by the supersession of fossil fuels with FGB as a source of energy supply, the use of food sources as feedstocks for biofuel production has sparked vehement controversy over the food-fuel supply issue. In response to this, researchers are now working to produce second generation bio-ethanol (SGB).

1.2.2 Second-generation bio-ethanol (SGB)

Second-generation bio-ethanol is derived from agricultural waste, i.e. lignocellulosic biomass instead of food crops. Lignocellulosic biomass is natural renewable resources and abundantly available as residual biomass from agriculture and forestry such as corn stover, sugarcane bagasse, wheat straw and paper mill residues. Apart from solving the food-fuel supply issue, ethanol derived from lignocellulosic biomass has environmental and resource conservation advantages. SGB has near zero net carbon emissions when considered over their full life cycle. During the combustion of SGB in vehicle engines, carbon stored in the biomass is released to the atmosphere. Subsequently, the carbon emitted will eventually be taken up by plants through photosynthesis process. Thus, the carbon dioxide emitted from SGB combustion is therefore re-utilized for the new growth of biomass and renders lower net greenhouse gas (GHG) emission compared to both petroleum-based fuel and food crop-derived ethanol (Hahn-Hägerdal *et al.*, 2006; Tan *et al.*,

2008). In addition, utilization of biomass reduces the disposal problem especially when the by-products, such as lignin, can also be used as energy sources.

1.3 General process for production of SGB

Generally, bio-ethanol production involved extraction of reducing sugars from biomass feedstock containing cellulose and hemicellulose through hydrolysis process followed by sugar fermentation into ethanol. While the production of FGB from sugar and starch is more straightforward, SGB production from lignocellulosic material creates additional technical challenges, such as the need for pretreatment. Unlike starchy material, lignocellulosic biomass are recalcitrant to biological degradation due to the biomass matrix where hemicellulose and cellulose are bound together by lignin. Lignin is hydrophobic and highly resistant towards chemical and biological degradation, thus the access of cellulose is relatively difficult by the cellulose enzyme and pretreatment is required prior to sugar extraction process.

In general, pretreatment step is required to overcome the resistance of biomass by breaking up its structure and reducing its crystallinity. Numerous pretreatment methods, such as acid pretreatment, steam explosion, hot water pretreatment and oxidative pretreatment, have been developed and reported in the literature (Kim *et al.*, 2008; Nouredini and Byun, 2010). Each pretreatment method has different ways of interaction and effects on different biomass. In this study, a relatively new pretreatment method, ionic liquid pretreatment, was investigated for bio-ethanol production from lignocellulosic palm biomass. Ionic liquid pretreatment involves dissolving cellulose from the biomass and then regenerate with the addition of an

anti-solvent. Through the dissolution and regeneration process, the biomass structure was destroyed and regenerated cellulose exhibits significantly reduced crystallinity and increased porosity, which enhance the accessibility of the material (Zhu *et al.*, 2006; Kuo and Lee, 2009; Li *et al.*, 2009).

Subsequently, pretreated biomass was subjected to enzymatic hydrolysis where polymeric carbohydrates are hydrolyzed into monomeric form which is fermentable sugar. Among existing hydrolysis technologies, enzymatic hydrolysis is advantageous although the process is slower because of the specific reactions of the enzyme used which only catalyze certain reactions, hence leading to no side reactions and degradation products of the sugar formed (Alzate and Toro, 2006; Ballesteros *et al.*, 2008). After the hydrolysis process, sugar solution obtained was further fermented by yeast to ethanol. *Saccharomyces cerevisiae* is one of the yeast strains that is generally used for ethanol fermentation. Finally, the resulting fermentation broth, also called “beer” is a mixture of ethanol with variety of impurities such as water, cell mass, unreacted biomass residues and probably side products. Separation step is therefore the last important step before pure bio-ethanol can be obtained for use as liquid biofuel. Schematic diagram for the production route of SGB is shown in Figure 1.1 below.

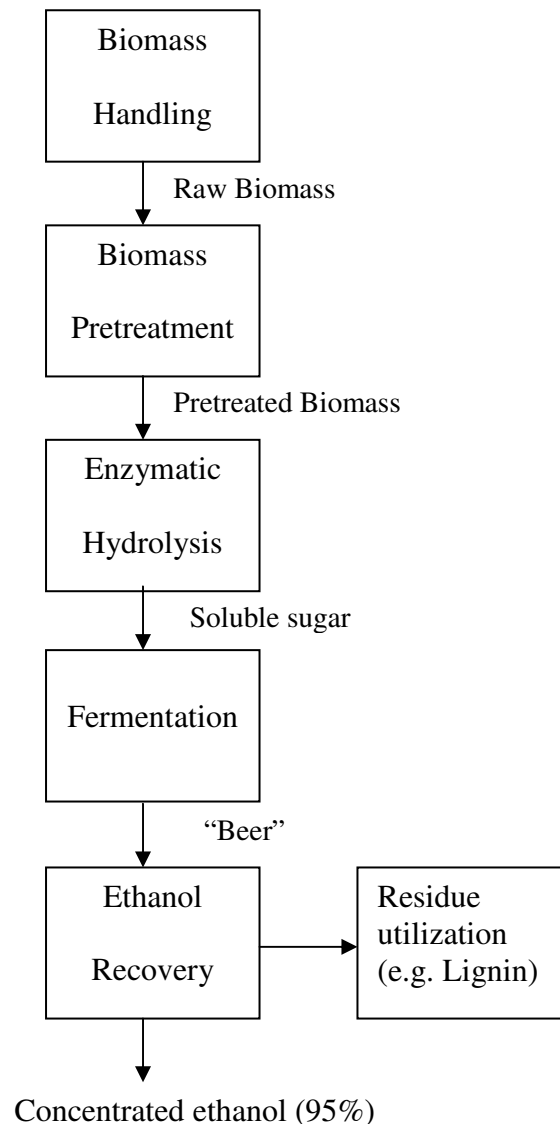


Figure 1.1 Production route for SGB

1.4 Problem Statement

Concerns about petroleum supply in the forthcoming years and scenarios of rising average earth temperature in the last few years have led to a dramatic increase in the production and use of bio-based fuels around the world, with the objective to ensure sustainability and economic growth. In spite of that, Malaysia, which possesses the total energy consumption record of 1.6 quadrillion Btu (Shuit *et al.*, 2009), is still lacking on practices of biofuel utilizations due to the abundance of

domestic energy resources, particularly the oil and natural gas endowed by the country. Moreover, biodiesel produced from palm oil, which is a major established agricultural product in Malaysia, is primarily for export purposes instead of domestic use. Nevertheless, the production of second-generation bio-ethanol is considered conceivable in Malaysia owing to the availability of bountiful sources of under-utilized residues in the form of lignocellulosic materials from palm oil industries in the country.

Among the palm oil biomass produced from Malaysia's palm oil industry, oil palm frond (OPF) has large potential as feedstock for bio-ethanol. This is mainly due to the abundance of OPF generated during the harvesting of fresh fruit bunches. Approximately 51 million tons of OPF was produced in 2008, accounting for 53 % of the total palm biomass (Goh *et al.*, 2010; MPOB, 2009). Unlike other palm biomass, such as palm shells, palm fibres, palm kernels, palm trunks and empty fruit bunches, OPF is still under-utilised and discarded on the plantation as waste. Hence, OPF has great potential as a sustainable biomass resource for the production of biofuel in Malaysia.

Due to the fact that lignocellulosic biomass is recalcitrant to biological degradation, pretreatment step is required to alter the OPF structure in order to significantly increase the biomass digestibility in the bioconversion process. Up to now, various types of pretreatment methods have been considered for the production of SGB from various types of biomass to meet the increasing requirement for bio-ethanol around the world. Although the existing pretreatment methods significantly increase the biomass digestibility through delignification, however, they also

substantially increase the formation of compounds, which then act as inhibitors in the subsequent hydrolysis and fermentation processes. Some of the pretreatment approaches require severe operating conditions and others use corrosive reagents which require costly construction materials and lead to environment pollution upon discharge. The limitations and problems associated with the existing pretreatment methods have urged the development of new pretreatment technology for the lignocellulosic ethanol conversion process. On this account, a relatively new pretreatment method using ionic liquid 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) as an excellent option of pretreatment medium has been proposed. Ionic liquid has the ability to substantially increase the biomass digestibility by enzyme through alteration of the biomass structure and its crystallinity during the dissolution and regeneration process.

Although the use of ionic liquid for pretreatment has been shown to facilitate the conversion of lignocellulosic biomass into reducing sugar (Li *et al.*, 2010; Liu and Chen, 2006; Sun *et al.*, 2009), however, the efficiency of ionic liquid pretreatment on palm biomass has not been studied so far. In addition, the understanding on the effect of ionic liquid pretreatment on the biomass constituent and its impact towards the subsequent process is still lacking. Hence, in conjunction with the investigation of OPF for its potential as feedstock for bio-ethanol production, this work is proposed also to study of compositional and structural change of biomass after pretreatment as well as optimisation study to optimise pretreatment conditions for optimum yield of glucose recovery.

1.5 Research Objective

The principle objective of this research project is to perform experimental studies on pretreatment of oil palm fronds using ionic liquid [BMIM]Cl to remove lignin from the biomass matrix and reduce the crystallinity of cellulose in order to enhance cellulose digestibility and glucose recovery. The measurable objectives in this study are:

1. To evaluate the effects of different ionic liquid pretreatment parameters (temperature, retention time and solid loading) on pretreatment efficiency with respect to the chemical composition, crystallinity, cellulose digestibility and glucose recovery of pretreated biomass.
2. To optimize the pretreatment conditions for maximum glucose recovery in enzymatic hydrolysis using Response Surface Methodology (RSM).

1.6 Scope and significance of study

This study aimed to evaluate the effectiveness of ionic liquid [BMIM]Cl as a media for pretreatment of lignocellulosic biomass. At the same time, the study also aimed to identify the potential of OPF as a promising glucose source. Throughout the work, pretreatment of OPF using ionic liquid [BMIM]Cl will be carried out under different pretreatment conditions and followed by the study of changes in biomass constituents and biomass crystallinity after pretreatment. A fundamental understanding of ionic liquid pretreatment can be obtained by investigating the

changes of biomass during pretreatment. Subsequently, pretreated biomass will be subjected to enzymatic hydrolysis in order to study the impacts of ionic liquid pretreatment on cellulose digestibility and glucose recovery. Finally, the pretreatment parameters will be optimized for greatest glucose recovery using Response Surface Methodology (RSM).

1.7 Thesis Organization

This thesis is divided into 5 main chapters. Chapter One gives an overview regarding the use of cellulose and glucose from biomass, energy scenario and importance of second-generation bio-ethanol as an alternative source of energy which is more sustainable and environmental friendly. General conversion processes of SGB are illustrated together with an introduction on the new pretreatment technology investigated in this work, ionic liquid pretreatment. Next, problem statement, research objectives, scope and significance of study and organization of thesis are clearly stated in this chapter.

Chapter Two is literature review which provides a thorough study on the knowledge, ideas and technologies regarding the production of lignocellulosic bio-ethanol. The chemistry of lignocellulosic biomass constituent, utilization as well as its constraints are first introduced and followed by discussion on various types of biomass pretreatment technologies available nowadays. In addition, the review also focuses on the introduction of ionic liquid together with their utilization as pretreatment solvent. Last but not least, the mechanism of dissolution and effect of

process parameter (temperature, time, solid loading, particle size and water content) on the pretreatment efficiency are also included in the chapter.

Chapter Three clearly presents the materials and chemicals used in this work. A detail description of experimental procedures and data calculation procedures are also included in the following sections. Lastly, the chapter concludes with the explanation on the procedures of the optimization studies using Response Surface Methodology (RSM).

The full results analysis and discussion are presented in Chapter Four. The chapter begins with the result for composition analysis of raw material, oil palm frond, used in this work. Subsequently, analysis and discussions on the effects of pretreatment parameters on the biomass composition, cellulose crystallinity, cellulose digestibility and overall glucose recovery are presented according to sections in this chapter together with graphical presentations which relate the respond to different pretreatment conditions. At the end of the chapter, the optimum pretreatment conditions for optimum glucose recovery obtained from the optimization studies in this work using RSM are described. Finally, the discussion on morphology changes on the biomass after pretreatment using SEM images are included as well.

Chapter Five concludes on the finding of current studies and provides several suggestions for future research on the production of second-generation bio-ethanol using ionic liquid pretreatment method.

CHAPTER TWO

LITERATURE REVIEW

2.0 Summary

The background of research project in the current work was thoroughly studied in this section for a better understanding on the knowledge, ideas and technologies which have been established regarding the production of lignocellulosic bio-ethanol nowadays. In order to make a proper flow of information and subsequently provide a clearer picture of overall process, the chemistry of lignocellulosic biomass was introduced in the first section followed by discussion on various types of biomass pretreatment technologies available nowadays including their limitations and disadvantages. Next, information about the solution ionic liquid which is deemed important to the research was detailed together with the review on their usage as pretreatment solvent. Meanwhile, explanation on the mechanism of dissolution and effect of process parameter (temperature, time, solid loading, particle size and water content) on the pretreatment efficiency were also included.

2.1 Lignocellulosic biomass

Lignocellulose is the fibrous material that forms the cell walls, leaves, stems, stalks and woody portions of plants. Lignocellulosic materials are natural renewable resources and abundantly available as biomass residues from agriculture and forestry such as corn stover, sugarcane bagasse, wheat straw, paper mill residues, municipal waste as well as oil palm residues. Energy that accounts for 10 - 20 % of today's

world energy demand could be obtained from the conversion of all lignocellulosic waste produced in the world (Lange, 2007). Lignocellulosic biomass has been recognized as a potential source for large scale production of liquid transportation fuels mainly because it does not compete with food industries, low in cost and readily available regardless of geographical locations.

Lignocellulose is composed of three major components: lignin, hemicellulose and cellulose with varying composition for different biomass. Generally, cellulose is the major constituent of biomass which accounts for 35 - 50 %, followed by hemicellulose (20 - 35 %) and lignin (15 - 35 %) (van Spronsen *et al.*, 2011; Várnai *et al.*, 2010). The remaining fraction makes up by a variety of plant-specific chemicals called extractives and minerals that will turn into ash when biomass is burned. The compositions of various lignocellulosic biomasses based on data reported in literature are shown in Table 2.1. The three types of lignocellulosic biomass shown in the table have different composition. Woody materials (softwood and hardwood) contain a higher percentage of cellulose and lignin content compared to agricultural residues, which are characterised by slightly lower lignin and cellulose contents. Percentage of extractives for some agricultural residues such as corn stover, switchgrass and empty fruit bunch are significantly higher than extractives content of woody materials. In addition, agricultural residues also carry a greater amount of inorganic species, which contribute to higher concentration of ash in biomass compared to softwood and hardwood.

Table 2.1 Compositions of various lignocellulosic biomasses on dry basis (wt%)

Components	Agricultural residues				Softwood		Hardwood	
	Wheat Straw ^a	Corn Stover ^b	Switchgrass ^c	EFB ^d	Pine ^e	Spruce ^e	Eucalyptus ^e	Oak ^f
Lignin	19.10	20.18	18.35	20.40	28.43	27.00	27.71	25.90
Hemicellulose	36.40	22.95	25.50	33.50	22.50	25.00	13.07	21.00
Cellulose	38.20	38.42	32.37	44.20	45.78	46.00	49.50	42.30
Extractives	1.80	14.63	17.75	11.60	2.96	0.60	4.27	9.50
Ash	2.80	3.82	6.02	3.50	0.33	1.40	1.26	1.30

^a Zhang *et al.* (2010)

^b Wan and Li (2010)

^c Balat and Balat (2009)

^d Khalid *et al.* (2008)

^e Várnai *et al.* (2010)

^f Ashori and Nourbakhsh (2010)

2.1.1 Cellulose

Cellulose, a major constituent of the lignocellulose is considered to be the most abundant organic compound on the earth. It is a high molecular weight and linear polymer of glucose which is formed by joining thousands of anhydroglucose molecule together by β (1,4)-glycosidic linkages. Cellobiose, where two anhydroglucose units arrange in 180° relative to each other bound together by the linkage is established as the repeating unit for cellulose chain. Figure 2.1 shows the structure of the repeating unit. The regular cellulose chains are held rigidly together

by intermolecular and intramolecular hydrogen bonds to form microfibril bundles which are embedded in the matrix of lignin and hemicellulose (Huang *et al.*, 2009). Due to the intramolecular hydrogen bonds between the hydroxyl group on C-3 and the neighbouring oxygen in the pyranose ring and also between the hydroxyl groups on C-6 and C-2 of the adjacent glucose monomer, cellulose chains exist as helical twist (Richardson and Gorton, 2003). On the other hand, intermolecular hydrogen bond of cellulose matrix occurred between the hydroxyl groups on C-6 and C-3 of another cellulose chain to form a sheet structure where cellulose chains are located in parallel to each other. The overall view of the hydrogen bonds within and between cellulose chains are illustrated in Figure 2.2.

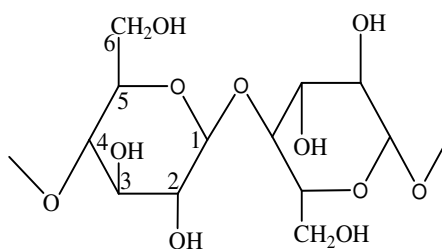


Figure 2.1 Schematic structure of cellulose repeating unit

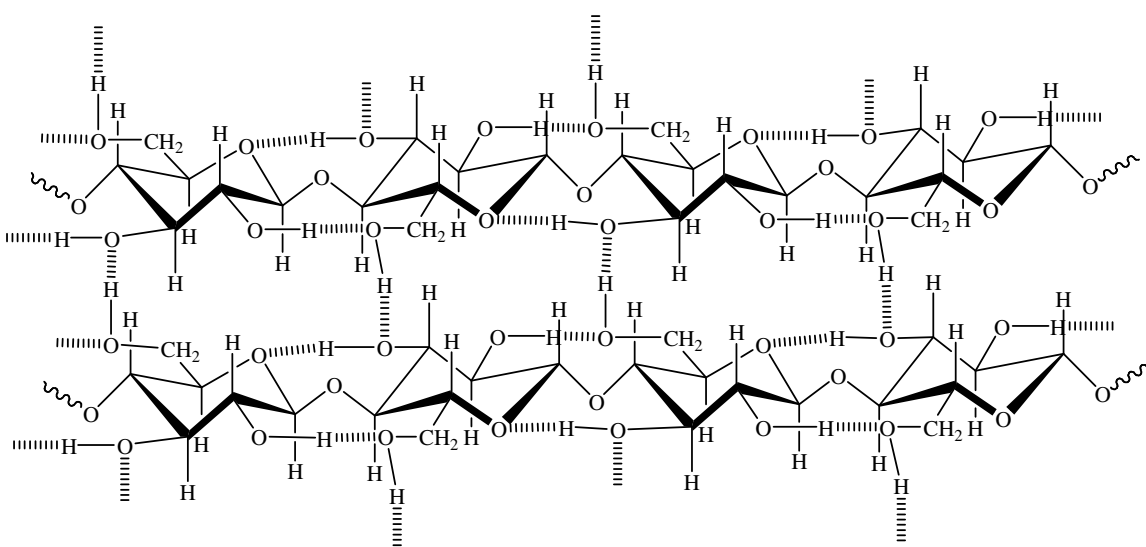


Figure 2.2 Intramolecular and intermolecular hydrogen bonding within and between cellulose chains

2.1.2 Hemicellulose

Hemicellulose has a lower molecular weight than cellulose. It is a highly branched heteropolymer containing various sugar units such as hexoses (D-glucose, D-galactose, L-galactose, D-mannose, L-rhamnose, L-fucose), pentoses (D-xylose, L-arabinose), and uronic acids (D-glucuronic acid, D-galacturonic acid) (Zhang *et al.*, 2011). Structure and constituents of hemicellulose vary significantly according to different species of biomass and even in different parts within one biomass. For example, dominant component in hemicellulose from hardwood and agricultural residues is xylan (xylose polymer) while for the case of softwood is glucomannan (polymer with D-mannose and D-glucose in a ratio of 1.6:1) (Hendriks and Zeeman, 2009). Hemicellulose serves as a connection between lignin and cellulose in the biomass. It forms covalent linkages (ether linkages) with lignin while hydrogen bonded to cellulose and thus builds up a more rigid network that provides the structural backbone for mechanical support to plant cell wall (Zhang *et al.*, 2011).

2.1.3 Lignin

Lignin is heteropolymer of three different phenylpropane units: *p*-hydroxyphenyl (H) units from the precursor *p*-coumaryl alcohol, guaiacyl (G) units from coniferyl alcohol and syringyl (S) units from sinapyl alcohol. These units are held together by different kind of linkages (Buranov and Mazza, 2008). The composition of lignin varies widely with biomass species. However, it is known that softwood lignin contains mainly guaiacyl units while hardwood lignin contains almost equal amount of guaiacyl and syringyl units. The monomeric *p*-

hydroxyphenyl, guaiacyl and syringyl unit of lignin are shown in Figure 2.3. Lignin is a complex, hydrophobic, amorphous and cross-linked aromatic polymer. It is always associated with hemicellulose through covalent bonds and sheath around cellulose. Essentially, lignin fills the space between cellulose, hemicellulose and other components which may exist in the biomass. The important role of lignin is to impart further strength to the plant structure, create impermeability, and also provide oxidative stress and resistance against pests and diseases attack (Hendriks and Zeeman, 2009). Overall view for the complex chemical structure of lignocellulosic biomass is shown in Figure 2.4.

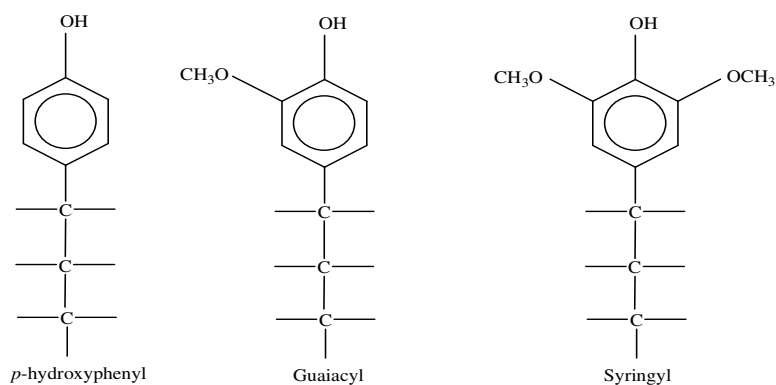


Figure 2.3 Schematic illustrations of *p*-hydroxyphenyl, guaiacyl and syringyl units of lignin

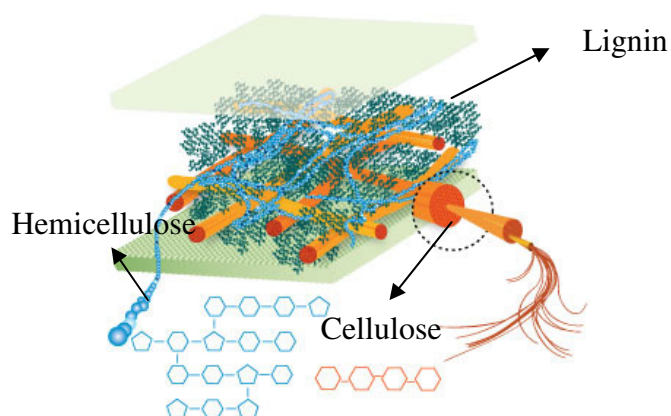


Figure 2.4 Schematic diagram of lignocellulose chemical structure (adopted from Ceres (2011))

2.2 Utilization of lignocellulosic biomass and its constraints

Potential utilizations of lignocellulosic biomass have evolved from being a source of animal food to one of the promising low cost feedstock for many industrial processes such as sawmill, pulp and paper, particle and fibre board industries. In recent decades, lignocellulosic biomass has also become renewable resources for the production of biofuel, bioenergy and added value bio-based chemicals. Leftover residues from the process are converted into heat and electricity to supply the energy demand of the industry. Different types of component in biomass have its own potential application in different areas.

Apart from being used for paper-making and textile manufacturing, the main constituent in lignocellulosic biomass, cellulose, is extensively used as source of material for conversion to a wide range of cellulose derivatives, mainly cellulose esters and ethers. Typical examples of cellulose esters are cellulose acetate, cellulose carbamate, and of cellulose ethers are methylcellulose, carboxymethylcellulose and hydroxypropylcellulose (Iller *et al.*, 2007). Cellulose esters are used in a wide range of products such as cigarette filters, photographic films, coating materials, foils, sponges, moulded articles (spectacle frames) and as membrane in pharmaceutical industry. On the other hand, cellulose ethers also find numerous applications in the production of detergent, adhesives, foods stabilizer and thickener (Clark and Deswarte, 2008; Iller *et al.*, 2007). They are also used as drilling fluid in oil and gas wells. However, recently the use of cellulose has been focused on sugar recovery for the production of bio-ethanol, one of the renewable liquid transportation fuels that

has a high demand. Cellulose is converted into ethanol through hydrolysis to release their fermentable sugars and subsequently subjected to fermentation process.

Hemicellulose fraction can also be used for ethanol production. However, the process is more complicated due to the difficulties in fermentation of xylose, the dominant component in hemicellulose. Therefore, utilization of hemicellulose is more towards the production of value added biochemicals. As a source of xylose and xylo-oligosaccharides molecules, hemicellulose is used for the production of furfural, xylitol and lactic acid which have many uses in chemical, food and pharmaceutical industries. Furfural is used mostly for production of furfuryl alcohol (to make foundry core resins and coatings), aromatic substances (preservatives, disinfectants and herbicides), tetrahydrofuran (THF) solvent, urethanes and nylon polymers. Xylitol is a polyol sweetener used in food or as sugar substitute in medicines for treatment of diabetes and other diseases, whereas lactic acid is used as an acidulent, preservative in food industry and as a precursor for the production of biodegradable thermoplastic polyester (Delgenes *et al.*, 1998; Garde *et al.*, 2002; Riansa-ngawong and Prasertsan, 2011).

The unique properties of lignin such as dispersing, binding, complexing and emulsifying have bring about the possibility of its use as a very effective and economical adhesive component in composite industry. Other commercial applications of lignin are like dispersants, emulsifiers, ion-exchange resins and as raw material in the production of vanillin which is a compound widely used as an ingredient in food flavors, in pharmaceuticals and as a fragrance in perfumes and odor-masking products. In addition, lignin resulted from kraft pulping process which

is known as kraft lignin is usually burned as fuel to supply part of the energy demand in the process (Tejado *et al.*, 2007).

It is undeniable that lignocellulosic biomass represents one of the most important, most useful and renewable resource materials for numerous applications as well as most sustainable alternative to substitute fossil fuel when cellulose is utilized for the production of biofuel. However, the key challenges to process lignocellulose come from its complex chemical structure where cellulose, hemicellulose, and lignin are closely associated to each other. Lignin is naturally recalcitrant to chemical process or biological attack owing to its covalent bonds to hemicellulose and cross-linking to cellulose microfibrils; this explains, in part, the difficulty to access the embedded cellulose for hydrolysis. In addition, intermolecular and intramolecular hydrogen bonds between cellulose chains build up a tightly packed crystalline structure and also hinder the accessibility of reactivity sites (Vaca-Garcia and Borrendon, 1999). All of these again impede the chemical reactivity and solubility of cellulose. Consequently, pretreatment of lignocellulosic biomass to remove lignin and hemicellulose as well as to reduce the crystallinity of cellulose is indeed an essential step to counteract the recalcitrance of biomass before the biomass is further processed into valuable products. In the following sections, numerous methods used for pretreatment of lignocellulose will be discussed.

2.3 Methods for pretreatment of lignocellulosic biomass

Pretreatment is a crucial process for effective conversion of lignocellulosic materials to chemicals and fuels. The goal of pretreatment is to break up the biomass

structure by removal of lignin seal and disruption of the crystalline structure of cellulose which in turn improve the reactivity of biomass especially hydrolysis of cellulose into fermentable sugars. In the past few years, a number of pretreatment approaches that could result in as high as 90 % conversion of sugars have been proposed, investigated and developed (Kumar *et al.*, 2009). Basically, pretreatment approaches can be divided into different categories according to the different forces or medium employed in the pretreatment process. There are physical, chemical, physicochemical, biological and irradiation pretreatment or a combination of these.

2.3.1 Physical pretreatment

Physical pretreatment does not involve the use of chemical agents. The pretreatment processes are accomplished by size reduction or thermal treatment with steam and water under high pressure and temperature. Examples of physical pretreatment include comminution, extrusion and hydrothermolysis (steam explosion and liquid hot water).

2.3.1.1 Mechanical comminution

The particle size of biomass materials is reduced through a combination of chipping, grinding or milling. The size of particle is usually 10 - 30 mm after chipping while 0.2 - 2 mm after grinding or milling. It was reported that comminution of biomass disrupts cellulose crystallinity, reduces the degree of polymerization, and increases the specific surface area. Consequently, as reported, increasing the accessibility of cellulose for hydrolysis by 5 - 25 % depending on type

of biomass, type of milling, and duration of the milling (Alvira *et al.*, 2010; Hendriks and Zeeman, 2009). However, power requirement for mechanical comminution is relatively high causing the process to be expensive. In addition, it is also time-consuming. Therefore, this method is not economically feasible and seldom used at present as a pretreatment exclusively (Kumar *et al.*, 2009).

2.3.1.2 Extrusion (without chemicals)

Extrusion process provides a continuous reactor environment for a combination of thermo-mechanical pretreatment of lignocellulosic biomass. The biomass is subjected to heating, mixing and shearing during the passage through the extruder, resulting in physical and chemical modifications. According to Lamsal *et al.* (2010), total reducing sugar yield from wheat bran after extrusion pretreatment ranged from 22 - 32 % which is higher than that after comminution pretreatment (18 - 20 %) but lower than chemical or other type of pretreatments. Thus, further improvement on this relatively novel technique is required for a better efficiency.

2.3.1.3 Steam explosion (uncatalyzed)

Steam explosion is recognized as one of the most cost-effective pretreatment processes for lignocellulosic biomass that have advanced to pilot scale demonstration and commercialized application. In this method, biomass is rapidly heated by high-pressure saturated steam at a temperature of 160 - 270 °C for several seconds to a few minutes to allow hydrolysis of hemicellulose, and then suddenly depressurized to cause the biomass to undergo an explosive decompression. Hemicellulose is hydrolyzed by the acetic acid released from biomass during high temperature

pretreatment. Besides, at high temperature, water itself also possesses certain acidic properties which further promote hemicellulose hydrolysis (Zheng *et al.*, 2009). The action mode of steam explosion basically involves removal of hemicellulose due to hydrolysis and redistribution of lignin due to melting and depolymerization/repolymerization reactions. Owing to the sudden explosive decompression, mechanical effects cause the opening up of the biomass structure. However, degradation of sugars might happen depending on the severity of pretreatment conditions (Kumar *et al.*, 2009). Changes on the biomass structure will bring about the exposure of cellulose and increase the accessible surface area of pretreated sample thus enhances the digestibility of biomass. The effectiveness of steam explosion as pretreatment method to improve sugars yield for ethanol production has been proven in several studies. The enzymatic hydrolysis yields of sunflower stalks was improved from 18 to 72 % after subjected to steam explosion pretreatment at 220 °C for 5 min (Ruiz *et al.*, 2008). Whereas almost 100 % of enzymatic hydrolysis yield can be obtained when herbaceous agricultural waste (*Brassica carinata*) was pretreated using steam explosion technique at 210 °C for 4 min (Ballesteros *et al.*, 2002).

2.3.1.4 Liquid hot water

Liquid hot water pretreatment is another hydrothermal pretreatment which does not require any catalyst or chemicals but pressure to maintain the water in liquid phase at elevated temperatures (160 - 240 °C) (Alvira *et al.*, 2010). The action mode of liquid hot water pretreatment is solubilization of mainly hemicellulose from biomass as oligosaccharides which can be further hydrolyzed for recovery of pentose