SCREENING OF FACTORS AFFECTING THE ENZYMATIC HYDROLYSIS OF FIBER PRESSED OIL PALM FROND (FPOPF)

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SCREENING OF FACTORS AFFECTING THE ENZYMATIC HYDROLYSIS OF FIBER PRESSED OIL PALM FROND (FPOPF)

NUR FATIN BINTI MUHAMMAD

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
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JUNE 2015

SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

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Position :
Date :

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature :
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Dedication

To my family members and friends who are always by my side.

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In The Name of Allah, The Most Gracious and Merciful.
Alhamdulillah, praise to The Almighty Allah SWT who gave me the strength to complete this partial thesis. First and fore most, I would like to express my deepest gratitude to my supervisor, Dr. Wan Hafizuddin for the support towards this partial thesis. May the precious knowledge that I gain from them will be rewarded by Allah SWT.

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ABSTRACT

This paper reviews the alkaline pretreatment and factors that affecting the enzymatic hydrolysis oil palm frond (OPF). The factors studied in enzymatic hydrolysis are enzyme concentration, pH, temperature, reaction time and agitation speed. OPF is one type of agricultural wastes that consist of lignocellulosic material which comprised of cellulose, hemicellulose and lignin. Lignin is extremely strong component which resistant against chemical, enzymatic and bacteria breakdown. Thus, usage of lignocellulosic as a raw material involves alkaline pretreatment to be able to make the cellulose and hemicellulose fractions easily accessible for enzymatic hydrolysis. Dried OPF was pretreated by soaking into sodium hydroxide solution at 100°C for about 1 hour. For hydrolysis, the pretreated OPF was soaked into sodium acetate buffer and enzyme was applied too. The, the mixture was incubated in an incubator shaker. After that, the mixture was boiled in water bath for 10 minutes. The hydrolysates were quantified by using DNS method. After analyzed with Design Expert Software, pH gives the most contribution to the enzymatic hydrolysis. Lignocellulose material is known as a potential biomass for conversion into renewable sugar to produce value-added product such as biofuels, animal feeds, enzyme and healthy food products.

ABSTRAK

Kertas kerja ini mengkaji prarawatan alkali dan faktor-faktor yang memberi kesan kepada hidrolisis enzim pelepah kelapa sawit (OPF). Faktor-faktor yang dikaji dalam hidrolisis enzim adalah kepekatan enzim, pH, suhu, masa tindak balas dan kelajuan putaran. OPF adalah salah satu jenis bahan buangan pertanian yang terdiri daripada bahan lignoselulosa yang mengandungi selulosa, hemiselulosa dan lignin. Lignin adalah komponen yang amat kuat dan tahan terhadap kimia, enzim serta kerosakan bakteria. Oleh itu, penggunaan lignoselulosa sebagai bahan mentah melibatkan prarawatan alkali dapat membuat komponen selulosa dan hemiselulosa mudah diakses untuk hidrolisis enzim. OPF yang telah dikeringkan diprarawat dengan merendam ke dalam larutan natrium hidroksida pada suhu 100 ° C selama lebih kurang 1 jam. Untuk hidrolisis, OPF yang diprarawat telah direndam ke dalam buffer natrium asetat dan enzim telah diaplikasikan juga. Campuran tersebut telah dieram dalam inkubator. Selepas itu, campuran itu direbus dalam air selama 10 minit. The hidrolisat telah diukur dengan menggunakan kaedah DNS. Setelah dianalisis dengan Design Expert Software, pH memberikan sumbangan yang paling penting untuk hidrolisis enzim. Bahan lignoselulosa dikenali sebagai biomass yang berpotensi untuk dijadikan gula untuk menghasilkan produk seperti biofuel, makanan haiwan, enzim dan produk makanan yang sihat.

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LIST OF ABBREVIATIONS

°*C*	degree Celsius
g	gram
g/l	gram per liter
g/ml	gram per mililiter
ha	hectare
ml	mililiter
mm	milimeter
nm	nanometer
rpm	rotation per minute
w/w	weight per weight

Greek

α	coefficient of equation

LIST OF ABBREVIATIONS

CPO	crude palm oil
DNS	3-5-Dinitrosalicylic acid
DP	degree of polymerization
EFB	empty fruit bunch
FFB	fresh fruit bunch
FPOPF	fiber pressed oil palm frond
MPOB	Malaysian Palm Oil Board
OD	oven dried
OPEFB	oil palm empty fruit bunch
OPF	oil palm frond
OPT	oil palm tree
POME	palm oil mill effluent
TEM	Transmission Electron Microscopy
WPT	waste palm tree

Chapter 1 Introduction

1.1 *Background*

The major plantations of commercial crops including rubber, oil palm, cocoa and pineapple are the strength of agricultural production in Malaysia which engages almost all of the arable land. Of the commercial plantation crops, oil palm industry is the important contributor to Malaysian economy with more than 5.00 million hectares of planted areas in 2011; grow by 3% against 4.85 million hectares in 2010.Because of the rapid development of oil palm production in Malaysia, oil palm industry produced highest quantity of biomass for around 80 million dry tones in 2010.The different types of biomass from oil palm industry included empty fruit bunch (EFBs), palm oil mill effluent, fiber, shell, wet shell, palm kernel, fronds and trunks (A. Noraishah., 2012). In 2009, approximately 15.2 and 17.5 million tons (wet weight) of OPT and OPEFB were produced in Malaysia. In spite of this, the most abundant biomass from oil palm plantation is not OPEFB or OPT. The most produced oil palm biomass is oil palm frond (OPF), which amounted to 83 million tons (wet weight) every year and have been found to be a highly favorable source (MPOC, 2010).OPF is obtained in the middle of pruning for harvesting fresh fruit bunch (FFB) that is why it is obtainable daily. OPF is now under-utilized as the plantation owners believe that all the OPF is required for nutrient recycling and soil conservation (Hassan et al., 1994; Wan Zahari et al., 2002).Therefore; pruned fronds are simply left in the plantation. However, the study shows that OPF does not contain high metal contents as widely thought, but contain high carbohydrates in the form of simple sugars. Therefore, part of the OPF can be utilized for other purpose without scarifying the nutrient recycling process (M. Zahari, M. Zakaria, H. Ariffin et al., 2012).

1.2 *Problem statement*

An excessive amount of oil palm frond (OPF) from oil palm industry will not just caused the improper disposal problem, but also generally caused environmental pollution when the waste are left to go rotten on the grounds or burnt on the plantation sites (K. Lim, Z. Zainal, G. Quadir et al., 2000; N. Mohd Nor., 2008). In spite of this, the large numbers of oil palm fronds (OPF) containing lignocellulosic materials were potentially to be utilized as renewable material. Consumption of all these materials is not going to only fix the sufficient disposal, but as well as contribute side income for farmers and generate more job (O. Akpinor, K. Erdogan, S. Bostanci., 2009; R. Howard, E. Abotsi et al., 2003).

1.3 *Research objective*

This work aims to study the factors that affect the production of renewable sugar from fiber pressed oil palm frond (FPOPF) through enzyme hydrolysis using Design Expert Software.

1.4 *Research scope*

The scopes in this research are functioning as a guideline to achieve objective. The scopes are:

- To identify the effect of the pH (4 – 5.6), temperature (35 - 65°C), enzyme concentration (1.5 - 6%), reaction time (3 – 72 hours) and agitation speed (50 – 200 rpm) towards the enzymatic hydrolysis.
- To quantified glucose yield by applying DNS method.
- To analysed the experimental data by using Design Expert Software.

1.5 *Rationale and significance*

Based on the research scopes mentioned above, the following rationale and significance that we could get have been outlined.
i. It shall reduce the huge production of biomass residue.
ii. It shall reduce deforestation and environmental problem.
iii. Alternative way to produce valuable product from oil palm biomass residue.
iv. New substitute of raw material for renewable sugar production.
v. It shall reduce factory's waste disposal costs.
vi. It shall reduce termites' problem in plantation because of Zero Burning Policy.

1.6 *Organization of thesis*

A review of literature is presented in Chapter 2. This chapter review about the biomass which is oil palm frond. Chapters 3 describe the experimental work carried out in the thesis where focuses on the alkaline pretreatment and different parameters that affect the enzymatic hydrolysis.

The remaining chapters cover a number of additional investigations carried out as a part of this study:

- Chapter 4 contains result and discussion about the alkaline pretreatment and enzymatic hydrolysis. The results were analysed using Design Expert Software
- Chapter 5 is the conclusion of the study.

Chapter 2 Literature Review

2.1 *Lignocellulose Biomass*

Lignocellulose is a common term for describing the major components practically in most plants, which are cellulose, hemicelluloses, and lignin. Lignocellulose is a complex matrix, composed of a variety of polysaccharides, phenolic polymers and proteins. Cellulose is the main component of cell walls of land plants. Lignocellulosic biomass comprises of various materials with distinctive physical and chemical characteristics. It is the non-starch based fibrous part of plant material.

First-generation lignocellulose source is primarily from food crops such as grains, sugar beet and oil seeds. Their sustainable production is under scanner, as is the possibility of creating unnecessary competition for land and water used for food and fiber production, thus encroaching on fragile ecosystems like wetlands, forests, and shallow hills.

The cumulative effects of these issues have increased the interest in utilizing non-food biomass and agricultural residues. Feedstock from lignocellulose materials includes corn stover, cereal straw, bagasse, forest residues, and purpose-grown crops such as vegetative grasses and short rotation forests. These second-generation lignocellulosic could prevent many of the concerns facing first-generation lignocellulosic and potentially offer better cost reduction potential in the longer term. Importantly, lignocellulosic feedstock does not interfere with food security (Salman Zafar, 2014).Figure 2.1 shows the first and second generations of lignocellulosic sources.

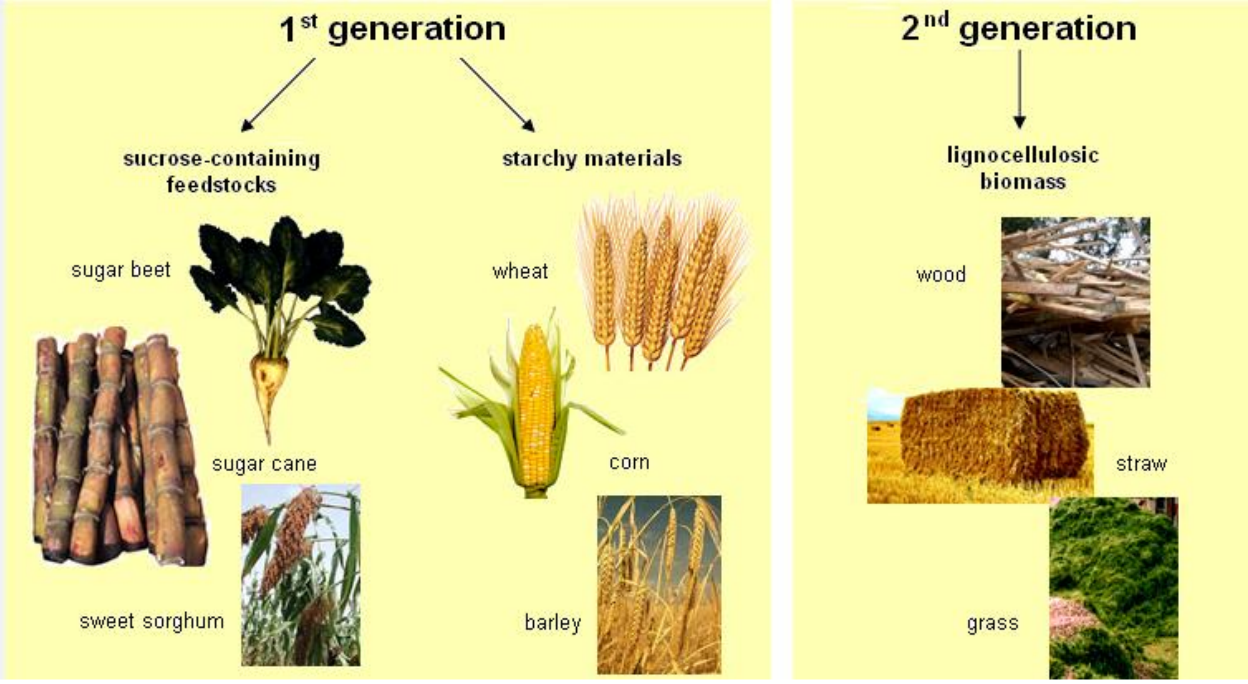


Figure 2.1: The first and second generations of lignocellulosic sources

2.2 *Oil Palm*

In Malaysia, the most beneficial plant is African oil palm Elaeisguineens which originated from the tropical rain forests of West Africa. It produces palm oil and palm kernel oil, which are commonly used in food as well as other industries such as detergents and cosmetics. In 1871 the oil palm was introduced through the Singapore Botanic Gardens as an ornamental tree. It was commercially exploited as an oil crop (Wan Rosli et al., 2007) only from 1911when the first oil palm estate was established.

In 1911, the first commercial planting was done at Tenammaran Estate; Kuala Selangor however the progress was initially very slow. It was only during the last 50 years that plantation development was accelerated through large scale investments in the cultivation of the oil palm (Yusof, 2007).

The oil palm tree bears fruit at the age of approximately for a couple of years. The fruit takes roughly five to six months to grow before it is ready for harvest. Its economic life is around 25-30 years, at which stage the tree is felled for replanting. The fruits are developed in large condensed infructescence and are often called fresh fruit bunches (FFB). The size and weight of every bunch varies considerably based on the age and growing conditions. The weight ranges from 8-16 kg per bunch. Table 2.1 shows the area under oil palm plantation (mature and Immature) by states in 2006.

Table 2.1: The area under oil palm plantation (mature and Immature) by states in 2006

State	Mature	Immature	Total
Johor	607,663	63,762	671,425
Kedah	71,705	4,624	76,329
Kelantan	75,825	18,717	94,542
Melaka	49,105	3,127	52,232
N. Sembilan	141,864	19,208	161,072
Pahang	551,713	71,577	623,290
P. Pinang	13,895	224	14,119
Perak	321,656	26,344	348,000
Perlis	258	0	258
Selangor	121,140	7,775	128,815
Terengganu	137,866	26,199	164,065
P. Malaysia	**2,092,690**	**241,557**	**2,334,247**
Sabah	1,139,535	99,962	1,239,497
Sarawak	471,029	120,442	591,471
Sabah/Sarawak	**1,610,564**	**220,404**	**1,830,968**
Malaysia	**3,703,254**	**461,961**	**4,165,215**

*Source:*MPOB

The fast increase in plantation area in Malaysia implies the economics significance of this plantation crop as well as the increasing world demand for palm oil. Malaysia is the world's largest producer and exporter of palm oil and also currently producing around 50 % of the world's supply of palm oil. Indonesia is the next largest world producer of palm oil producing around 30 % of world palm oil volume. Apart from palm oil, the industry also produces great quantities of lignocellulosic residues like trunks, fronds and the empty fruit bunches (EFB) with an estimated amount of 30 million tons (N. Mohd Nor, 2008).Figure 2.2 shows the photograph of oil palm tree.

Figure 2.2: The photograph of oil palm tree

2.3 *Oil Palm Biomass*

Waste palm tree (WPT) at 25 years of age consists of different physical parts (Figure 2.3). Table 2.2 shows the physical parts of the tree that will be acquired during felling with an estimated oven dried weight. The main part by fresh weight is the trunk (70%), followed by rachis (20.5%) and leaflets (6.53%). The moisture contents (depending on O.D. weight) of the various parts vary between 95% and 78%. Since one hectare of an oil palm plantation consists of between 136-140 trees, the sum of dry matter (tons/ha) of the various parts available during felling on a per hectare basis can also be predicted.

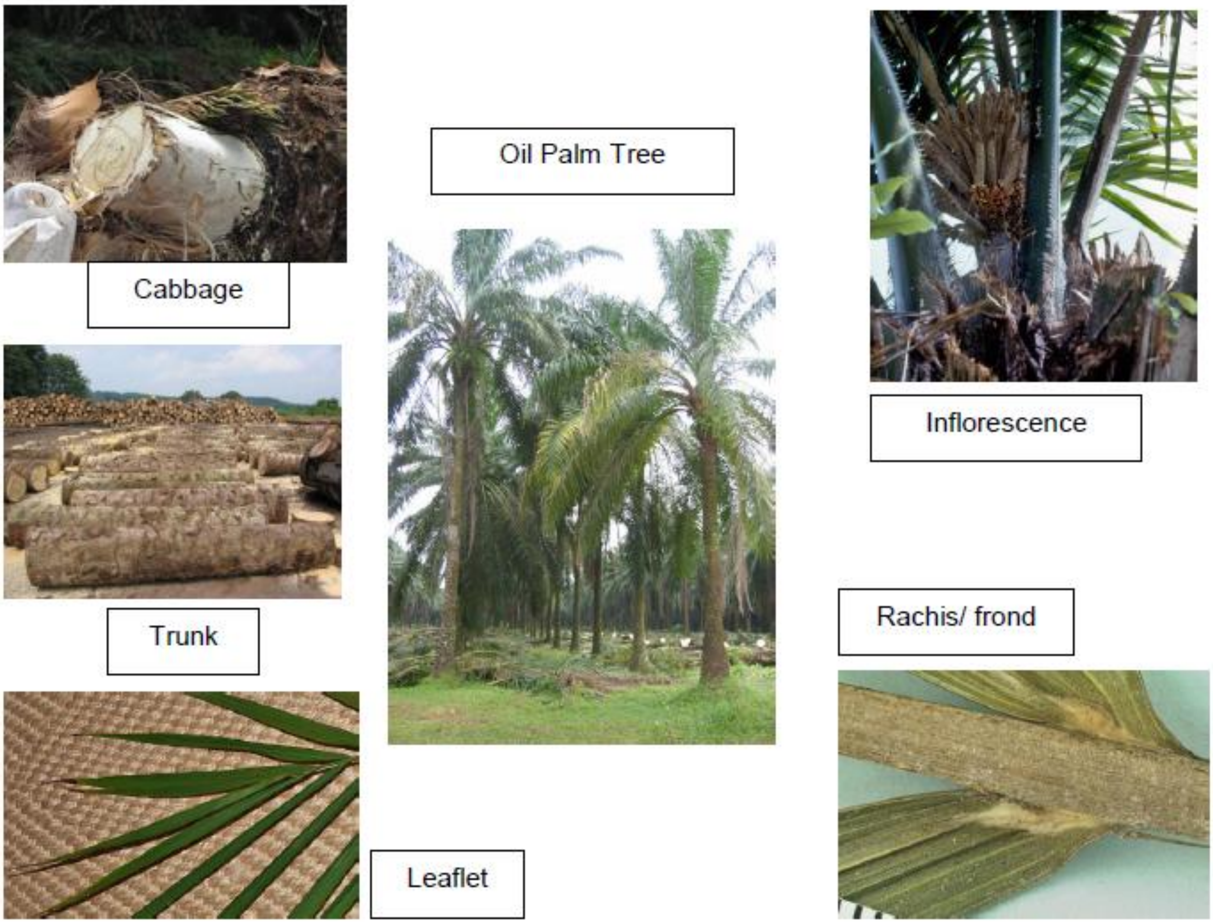


Figure 2.3: WPT at 25 years of age consists of different physical parts (*Sources:* FRIM)

Table 2.2: The physical parts of the tree that will be acquired during felling with an estimated oven dried weight

WPT component	Average fresh weight (kg)	Weight percentage (%)	Estimated oven dried (OD) weight (kg/free)	Oven dried weight (ton/ha)
Trunk	1507.50	70.0	301.50	41.07
Leaflets	145.00	6.53	58.00	7.69
Rachis	452.50	20.5	117.70	16.00
Spears	42.75	1.92	9.40	1.28
Cabbage	44.50	2.00	4.50	0.60
Inflorescence	134.50	1.11	6.30	17.56
Total weight	2217.50	100.00	497.30	0.86

***Source:* FRIM**

In Malaysia the palm oil industry involves plantation (upstream) and mill (downstream) activities. These activities produce various kinds of residues that are also reported as the residues from the palm oil industries. In 1998 the type and amount of the biomass and residues produced from these activities and their level of usage are demonstrated in Table 2.3. The majority of these biomass and residues are utilized within the system for mulching/fertilizer and for energy production at the mill. From these, the biomass residues generated from replanting activities are just the trunks and fronds at replanting. Pruned fronds are available throughout the year during fruit harvesting.

Table 2.3: The type and amount of the biomass and residues produced from these activities and their level of usage

Biomass	Quantity produced (mil tons)	Quantity utilized (mil tons)	Utilized (%)	Method of utilization
Pruned fronds	27.20	25.83	95	Inter-row mulching in plantations
Trunks and fronds at replanting	1.38	1.10	80	Left to degrade in the fields as mulch to newly planted palms
Mesocarpfibre	3.56	3.20	90	Fuel
Palm kernel shell	2.41	2.17	90	Fuel
Palm oil mill effluent (POME)	1.43	0.50	35	Nutrient source & organic fertilizer
Empty fruit bunch (EFB)	3.38	2.20	65	Left to degrade in the fields as mulch and bunch ash
Crude palm oil (CPO)	39.36	35.00	-	-

Source: FRIM

Even though the main portions of the felled trunks and fronds are claimed being utilized as mulch, there were no reports on the amount actually needed by young palm trees, since fertilizers continue to be being used at the same rate for mulched and un-mulched trees. Mulching was reported as a method of soil surface moisture retention, and is also being done in oil palm plantations by means of cover crops. The other 20% of the WPT is most likely being wasted away when poisoning methods are employed to get rid of old palm trees. WPT is usually used by local communities for temporary structural use for example small bridges and for road maintenance around the village and plantations.

2.3.1 Oil Palm Frond

Oil palm frond has several sizes of vascular bundles which are widely imbedded in thin-walled parenchymatous ground tissue. Every bundle consists of a fibrous sheath, vessels, fibers, phloem and parenchymatous tissues. Xylem and phloem tissues are obviously distinguishable (Abdul Khalil et al., 2006). Phloem is split up into two separate areas in every bundle. Several vascular bundles also have several well-defined protoxylem elements. Protoxylem and metaxylem vessels in the bundle are split up by a layer of parenchyma cells. Within the stem and leaves, proto- and metaxylem vessels are split up by at least one layer of live parenchyma cells which form a living boundary to enable gas bubbles to transfer (Tomlinson et al., 2001). The Transmission Electron Microscopy (TEM) views of transverse sections of OPF are shown in Figure 2.4 and Figure 2.5.

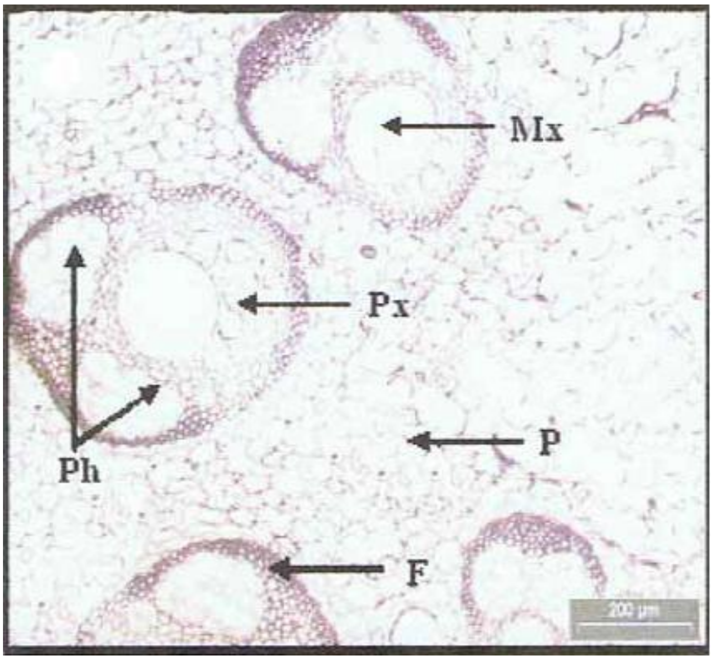


Figure 2.4: Transverse section of OPF at low magnification (4x). F = Fiber; P =Parenchyma; Mx = Metaxylem; Px = Protoxylem; Ph = Phloem (Source: Abdul Khalil *etal.*, 2006)

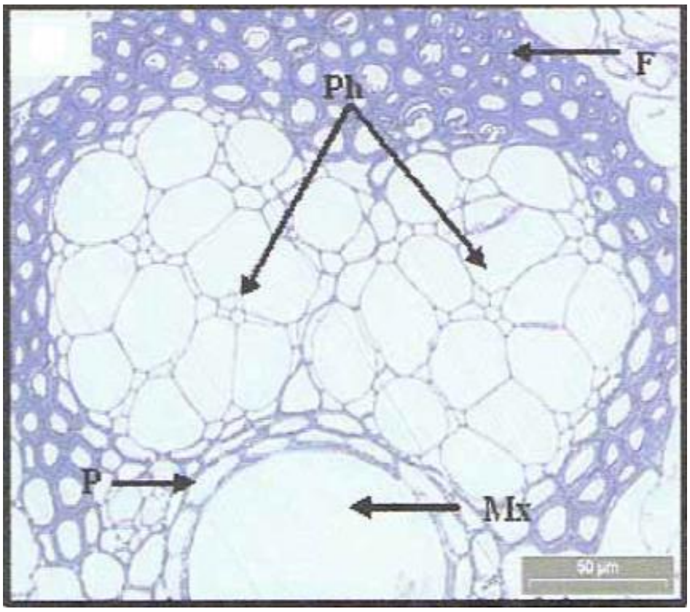


Figure 2.5: Transverse section of OPF at high magnification (20x). F = Fiber; P =Parenchyma; Mx = Metaxylem; Px = Protoxylem; Ph = Phloem (Source: Abdul Khalil *etal.*, 2006)

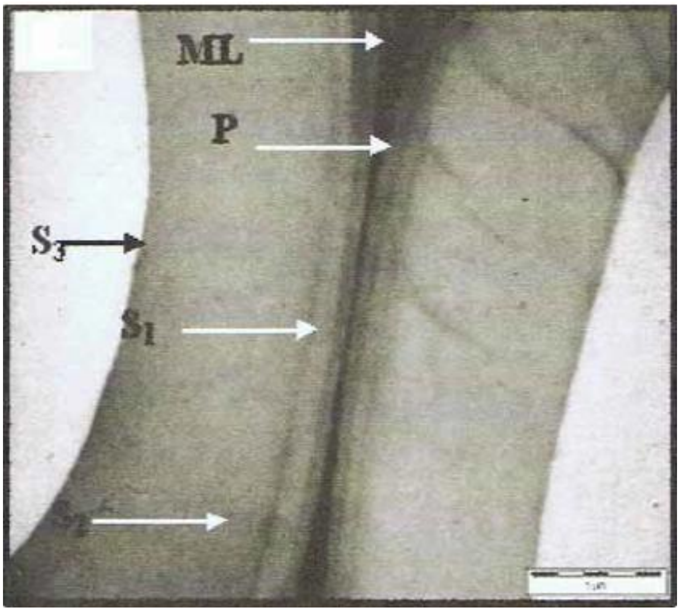


Figure 2.6: Transverse section of multi layered structure of OPF at high magnification (17 000x). ML = Middle lamella; P = Primary wall; S1, S2 & S3 = Secondary wall sub layers. (Source: Abdul Khalil *et al.*, 2006)

The transverse section of multi layered of OPF at high magnification is shown in Figure 2.6. The figure shows that the layered structure of OPF wall has primary (P) and secondary (S1, S2 and S3) wall layer. The primary wall seems as a solid boundary of the cell. The middle lamella indicates an obvious transition to the neighbouring primary wall layers. The S1 layer of OPF is well-defined and could be distinguished from the adjacent S2 layer, since it is the brightest layer than other layers. The existence of distinct S3 layer in the cell wall of OPF also observed using TEM micrograph.

2.3.1.1 Oil Palm Frond Juice

Oil palm frond (OPF) juice contains high sugars content and the OPF are readily available frequently which make it a potential industrial fermentation substrate. Recently, there was a report on the probability of obtaining OPF juice just by pressing the OPF petiole. OPF juice has great amount of sugars, which makes it a potential fermentation feedstock for several beneficial products including polyhydroxyalkanoates (PHA), bioethanol, biobutanol, lactic acid, and succinic acid. Earlier research has proven that OPF juice works well to be utilized as fermentation feedstock as there was no inhibition on microbial growth or product formation, there were no impurities, it was easy to be operated, and it also had no risk on health and safety. OPF juice was proved to be a good fermentation substrate for lab-scale PHA production (M. Zahari, M. Zakaria, H. Ariffin et al., 2012).

2.4 *Lignocellulose component in Lignocellulose Biomass*

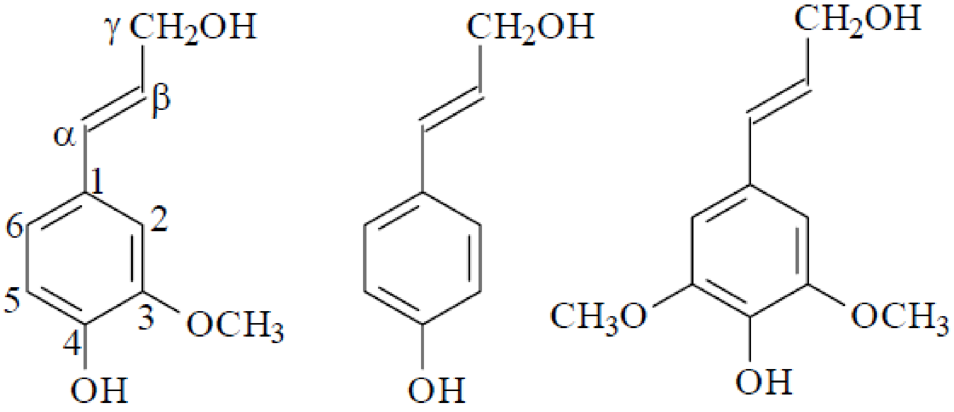
Lignocellulose is the main component of plant cell walls which is basically composed of lignin, cellulose and hemicelluloses. Xylan which consists of 20 – 30 % by weight of wood and agricultural wastes is the main part of the hemicelluloses portion. Xylan therefore represents a preferable resource of renewable biomass that can be applied as a substrate for the making of several beneficial products for instance fuel, solvents and chemicals. However, the industrial usage of such materials has been compromised by a few aspects for example the close association present among the three major parts of the plant cell wall; cellulose, hemicellulose and lignin, as well as the low efficiency by which lignocellulosic substrates are converted via biological processes such as hydrolysis and fermentation.

2.4.1 Lignin

Lignin is a long-chain, heterogeneous polymer comprised mostly of phenyl propane units normally linked by ether bonds and strongly insoluble in water. Lignin is in charge of the structural adhesion of the plant cell wall components because it excludes water when it forms networks by cross-linking along with other saccharide-type molecules in plants. It is presented in every lignocellulosic biomass; hence, any ethanol production process will have lignin as a residue. Lignin is really resistant against chemical, enzymatic and bacterial breakdown, however, just a few organisms, such as rot-fungi as well as some bacteria are able to degrade it. It is an extremely strong component and is most associated with the structural stability of wood. Lignin content in wood can differ from 20 to 35 %. Lignin prevent hydrolysis by protecting cellulose surfaces or by adsorbing and inactivating enzymes. It had been understood that the close union between lignin and cellulose avoided swelling of the fibers, therefore influencing enzyme accessibility to the cellulose. To fix this condition, various research shows that removing lignin magnifies cellulose hydrolysis (Y. Corredor, 2008; N. Mohd Nor, 2008).

The structural units of lignin are different derivatives of phenyl propane with several bonding combinations (Erdtman, 1957).Lignin is made up by oxidative coupling of three main C6 – C3 (phenylpropanoid) units, known as syringyl alcohol, guaiacyl alcohol, and p–coumaryl alcohol, which forms a randomized structure in a tri–dimensional network inside the cell walls. Two major types of lignin units are guaiacyl (with one methoxyl group in phenol ring) and syringyl (with two methoxyl groups).

The structure of lignin is known to differ between cell wall layers and between separate morphological parts of the tree. Commonly inner lamella and outer cell wall layers contain a lot more guaiacyl units compared to the secondary cell wall of hardwoods. The lignin of softwoods is mainly (almost exceptionally) guaiacyl units whereas hardwoods consist of both guaiacyl and syringyl. Apart from the 20 various kinds of bonds found within the lignin alone, lignin is apparently particularly associated with the hemicellulosic polysaccharides. Figure 2.7 shows the lignin precursors; (I) = p-coumaryl; (II) = coniferyl and (III) = sinapyl alcohol.



1 coniferyl alcohol

2 *p*-coumaryl alcohol

3 sinapyl alcohol

Figure 2.7: The lignin precursors; (I) = p-coumaryl; (II) = coniferyl and (III) = sinapyl alcohol

The conversion of cellulose and hemicellulose to fuels and chemicals produces lignin as a by-product. Such by-product can be burned to supply heat and electricity, or utilized to manufacture a variety of polymeric materials. There are several publications on microbial breakdown of lignin; on the other hand, due to high complexity of the problem, an enormous amount of study must be carried out.

2.4.2 Hemicellulose

Hemicelluloses were initially known to be intermediates in the biosynthesis of cellulose. Nowadays it is acknowledged, however, that hemicelluloses are part of a group of heterogeneous polysaccharides which are developed via biosynthetic paths not the same as that of cellulose. Compared to cellulose which is a homopolysaccharide, hemicelluloses are heteropolysaccharides. Hemicelluloses are heterogeneous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids (Figure 2.8). They are usually specified based on the major sugar residue in the backbone, e.g., xylans, mannans, and glucans, with xylans and mannans. Hemicellulose, due to the branched, amorphous character, is relatively easy to hydrolyze (Hamelinck et al., 2005). A few hemicelluloses have mainly xylan, while others have mainly glucomannans (Y. Corredor, 2008).

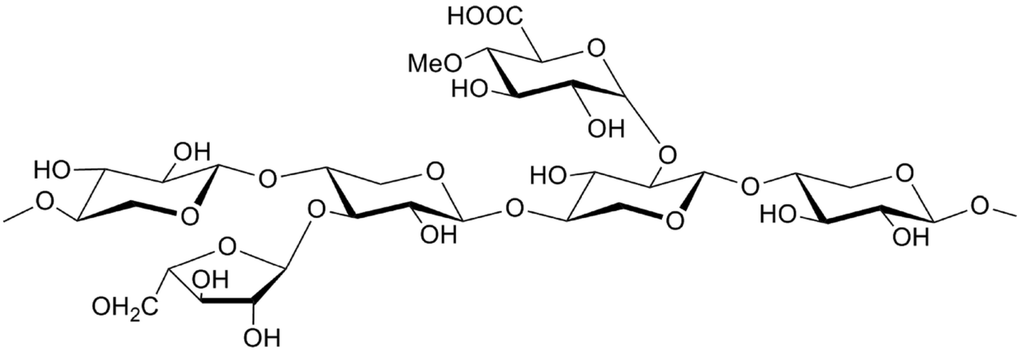


Figure 2.8: Hemicellulose

The hemicellulose content of softwoods and hardwoods vary significantly. Hardwood hemicelluloses are mainly made up of highly acetylated heteroxylans, commonly categorized as 4-O-methylglucuronoxylans. Hexosans are also found however in small quantities as glucomannans. Hardwood xylans are generally labile to acid hydrolysis and may undergo autohydrolysis under relatively moderate situations. On the other hand, softwoods hemicelluloses comprise of glucomannan, galactoglucomannan, arabinan including a few arabino-(4-O methylglucurono)-xylan. Softwood xylans are free from acetyl groups, there are split up as a gel from acidic solutions, one of them, in methylated form, being presented as a robust film, indicating the xylan's linear structure.Due to this fact, softwood hemicelluloses (mainly hexosans) are very proofed against acid hydrolysis rather than hardwood hemicelluloses (mainly pentosans). The average amount of hemicelluloses in wood is 25 – 35 %. Hardwood species contain in average 1.5 times extra hemicelluloses compared to softwood species (N. Mohd Nor, 2008).

In contrast to cellulose, hemicelluloses have lower DP in a range of 50 to 300 with side groups on the chain molecule and are basically amorphous which have a lot more branches and are less crystalline than cellulose. They are easily hydrolysable mainly to xylose when it comes to hardwood and to mannose when it comes to softwood.

2.4.3 Cellulose

In nature, the most numerous organic compound is cellulose which consist more than 50% of all the carbon in vegetation. Regardless of the source, cellulose is believed to be similar in chemical composition, it is insoluble in water and aqueous solutions in alkalis.

Cellulose is a linear homopolymer that contains glucose (D-glucose) units linked by β-(1-4) glycosidic bonds. Usually, the size of cellulose molecule is depends on degree of polymerization (DP). Cellulose chains in primary plant cell walls have DP of in the range of 5,000 to 7,500 glucose monomer units. Cellulose from wood has DP around 10,000, while cotton is around 15,000.

Cellulose molecules are completely linear with a strong probability to form intra and intermolecular hydrogen bonds. Long molecules of cellulose from micro fibrils build the structure of a cell wall (fiber wall) where highly organized (crystalline) parts alternate with less organized (amorphous) parts. The crystalline part wherein the linear molecules of cellulose are bonded laterally by hydrogen bonds is distinguished by the cellulose lattice which broadens over the whole cross-section of the micro-fibrils. This crystalline part is bordered by a layer of cellulose molecules that show several degrees of parallelism.

The less organized part is known as the paracrystalline or amorphous part. The disorganized part enables disintegration of the cellulose by hydrolysis into rod-like particles with aqueous, non-swelling, strong acid (Y. Corredor, 2008). These structures make cellulose a rigid, strong, dense, partly crystalline, chemically and enzymatically resistant features.

Yet somehow, a number of cellulose which is roughly 10 % can also be found in an amorphous state. The cellulose fibrillous structure is shown in Figure 2.9.

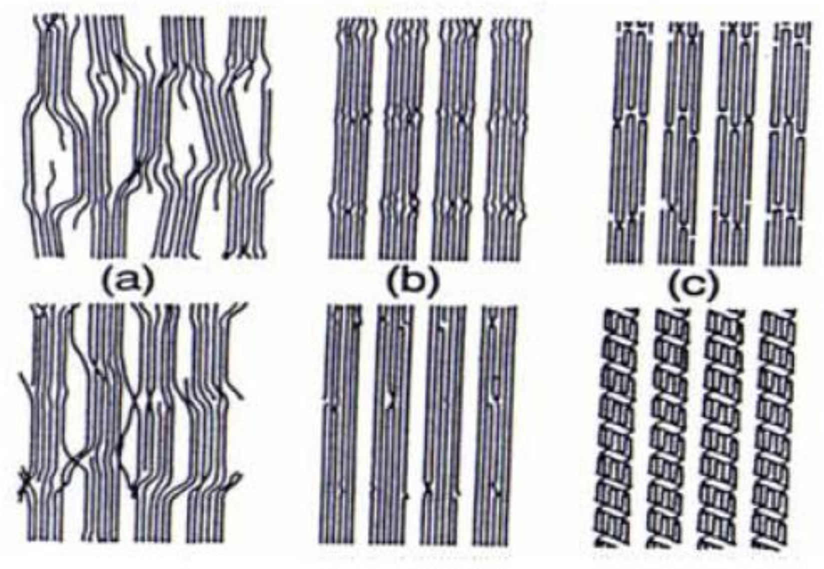


Figure 2.9: Cellulose fibrillous structures: (a) low crystallinity; (b) high crystallinity; (c) folded models (Source: N. Mohd Nor, 2008)

The crystalline structure of cellulose can be destroyed and turned into amorphous form under over 250°C of water or strong acid. The enzymatic attack needs particular pretreatment methods, or else the saccharification yields are dramatically low (N. Mohd Nor, 2008).

2.5 *Application of Lignocellulose Component*

Biomass is generally defined as the mass of organic material from any existing biological material, and by extension, any large mass of biological matter. Numerous types of biomass resources can be found (Table 2.4) on earth for conversion into bioproducts. These may involve entire plants, plant parts, plant constituents, processing byproducts, materials of marine origin and animal byproducts, municipal and industrial wastes (Smith et al., 1987). These resources are useful to generate new biomaterials and this will need a passionate knowledge of the composition of the raw material whether it is entire plant or constituents, to ensure that the required functional elements is available for bioproduct production.

Table 2.4: Types of lignocellulosic materials and their current uses

Lignocellulosic material	Residues	Competing use
Grain harvesting		
Wheat, rice, oats barley and corn	Straw, cobs, stalks, husks	Animal feed, burnt as fuel, compost, soil conditioner
Processed grains		
Corn, wheat, rice, soybean	Waste water, bran	Animal feed
Fruit and vegetable harvesting	Seeds, peels, husks, stones, rejected whole fruit and juice	Animal and fish feed, some seeds for oil extraction
Fruit and vegetable processing	Seeds, peels, waste water, husks, shells, stones, rejected whole fruit and juice	Animal and fish feed, some seeds for oil extraction
Sugarcane other sugar products	Bagasse	Burnt as fuel
Oils and oilseed plants		
Nuts, cotton seeds, olives, soybean etc.	Shells, husks, lint, fibre, sludge, press cake, wastewater	Animal feed, fertilizer, burnt fuel
Animal waste	Manure, other waste	Soil conditioners
Forestry-paper and pulp		
Harvesting of logs	Wood residuals, barks, leaves etc.	Soil conditioners, burnt
Saw and plywood waste	Woodchips, wood shavings, saw dust	Pulp and paper industries, chip and fiber board
Pulp and paper mills	Fiber waste, sulphite liquor	Reused in pulp and board industry as fuel
Lignocellulose waste from communities	Old newspapers, paper, cardboard, old boards, disused furniture	Small percentage recycled, others burnt
Grass	Unutilized grass	Burnt

Source: Smith et al., 1987

2.5.1 Chemicals

Bioconversion of lignocellulosic wastes could make an important contribution to the production of organic chemicals. More than 75% of organic chemicals are produced from five primary base-chemicals which are ethylene, propylene, benzene, toluene and xylene that utilized to synthesis other organic compounds. These organic compounds often are utilized to produce a variety of chemical products such as polymers and resins. The aromatic compounds might be produced from lignin while the low molecular mass aliphatic compounds can be derived from ethanol produced by fermentation of sugar generated from the cellulose and hemicellulose.

2.5.2 Bio-fuel

The demand for ethanol has the most important market where ethanol is either utilized as a chemical feedstock or as an octane enhancer or petrol additive. The production of ethanol from sugars or starch effects unfavourably on the economics of the process, hence making ethanol more costly rather than fossil fuels. This is why the technology development focus for the production of ethanol has shifted to the utilization of residual lignocellulosic materials to reduce production costs.

2.5.3 Other high-value bioproducts

Nowadays a lot of products including organic acids, amino acids, vitamins and a variety of bacterial and fungal polysaccharides like xanthan are made by fermentation using glucose as the basic substrate. However in theory these same products could be produced from “lignocellulose waste”. There are possible value added products that can be derived from lignin. Vanillin and gallic acid are the two most regularly discussed monomeric possible products which have attracted interest. Vanillin can be used for different applications like being an intermediate in the chemical and pharmaceutical industries for the production of herbicides, anti-foaming agents or drugs such as papaverine, L-dopa as well as anti-microbial agent, trimethoprim. Additionally it is applied in household products including air-fresheners and floor polishes.

Hemicelluloses are of particular industrial attraction because these are easily available bulk source of xylose from which xylitol and furfural can be derived. Xylitol utilized rather than sucrose in food as a sweetener, has odontological purposes for example teeth hardening, remineralisation, and as an antimicrobial agent, it is applied in chewing gum and toothpaste formulations. The yield of xylans as xylitol by chemical signifies is just around 50-60% making xylitol production pricey. A variety of bioconversion methods are studied for the production of xylitol from hemicellulose using microorganisms or their enzymes. Furfural is employed in the production of furfuralphenol plastics, varnishes and pesticides.

2.6 *Availability of Lignocellulose Source (OPF)*

2.6.1 Total oil palm plantation area

Malaysia consists of Peninsular Malaysia (West Malaysia) and the States of Sabah and Sarawak (East Malaysia). Establishment of oil palm plantations started in Peninsular Malaysia in 1917 and the plantation area has now achieved close to capacity which is 2.5 million ha in 2010. During 2010 the area planted with oil palms continues to be expanding in Sabah and Sarawak with total of 1.4 million ha and 839,748 ha respectively, because of the availability of larger potential areas. The distribution of oil palm plantation hectares in Peninsular Malaysia, Sabah and Sarawak from 1975 until 2008 is shown in Figure 2.10.

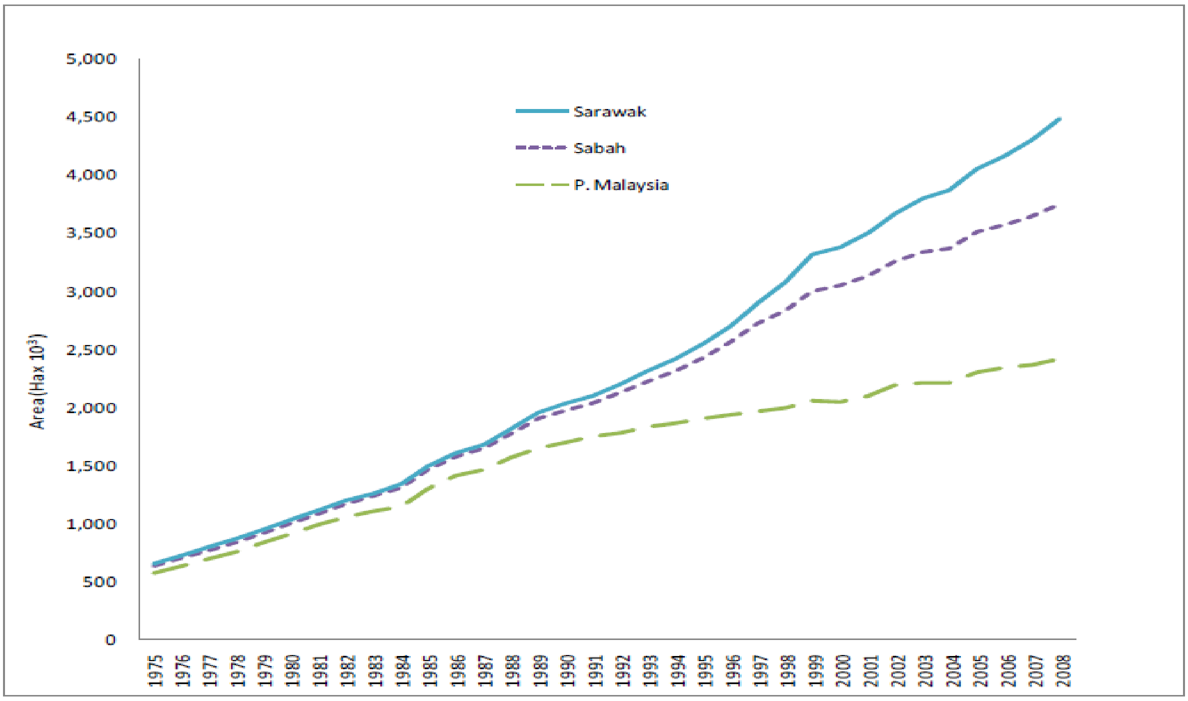


Figure 2.10: The distribution of oil palm plantation hectares in Peninsular Malaysia, Sabah and Sarawak from 1975-2008

Malaysian Palm Oil Board has published the area of oil palm plantation data yearly which enables the computation of palm trees that have reached replanting age (25 years). These are the trees that were planted from 1986 through 2007, and will be referred to as potential WPT. The potential WPT area from years 2011 – 2032 is shown in Figure 2.11. The area of potential WPT available annually in Peninsular Malaysia, Sabah and Sarawak is shown in Figure 2.12.

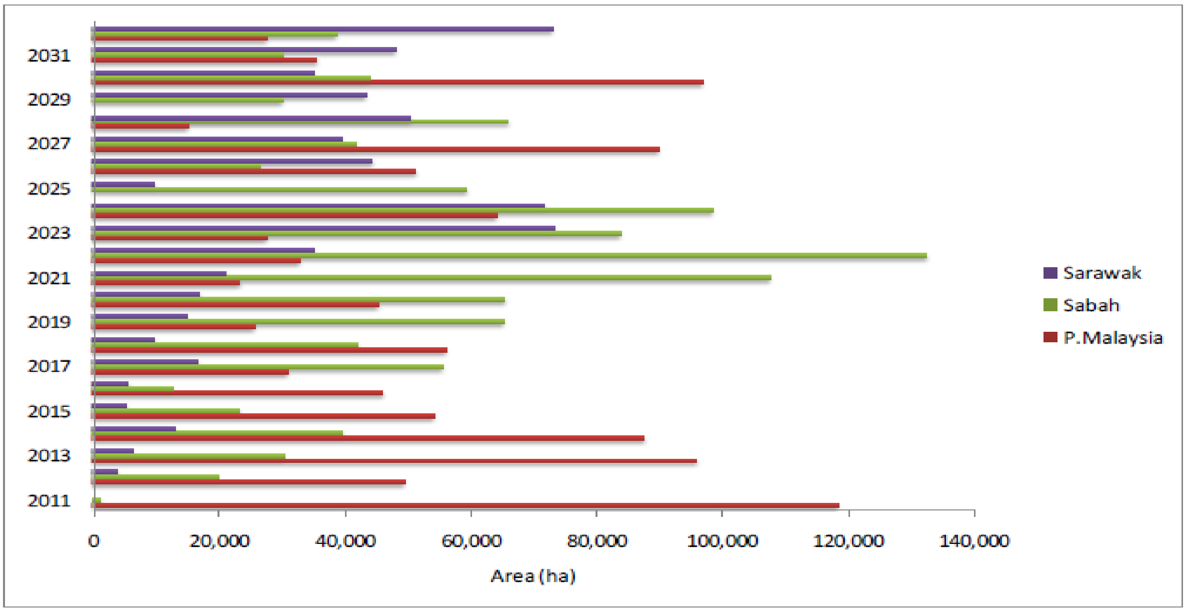


Figure 2.11: The potential WPT area from years 2011 – 2032

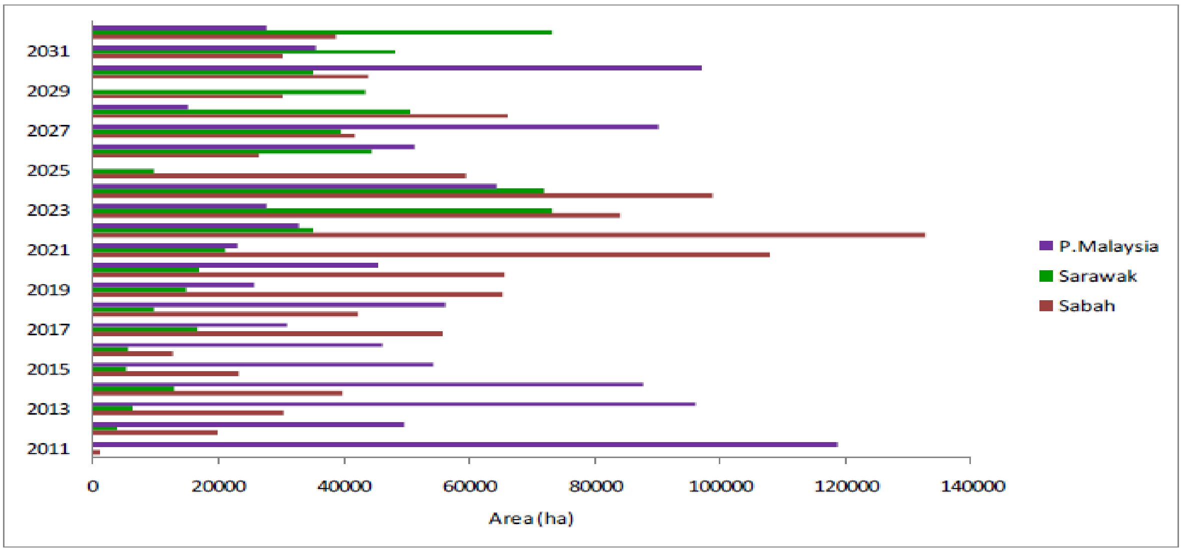


Figure 2.12: The area of potential WPT available annually in Peninsular Malaysia, Sabah and Sarawak

According to the fact that one hectare of plantation area contains approximately 140 trees, it is possible to figure out the amount of WPT available within the country, and hence the total available dry biomass. Figure 2.13 shows the number of potential WPT available annually in Peninsular Malaysia, Sabah and Sarawak. The largest amount of WPT will be available in Sabah (18,561,060 trees in year 2022) followed by P. Malaysia (16,593,360 and 13,580,280 trees in years 2011 and 2030 respectively).

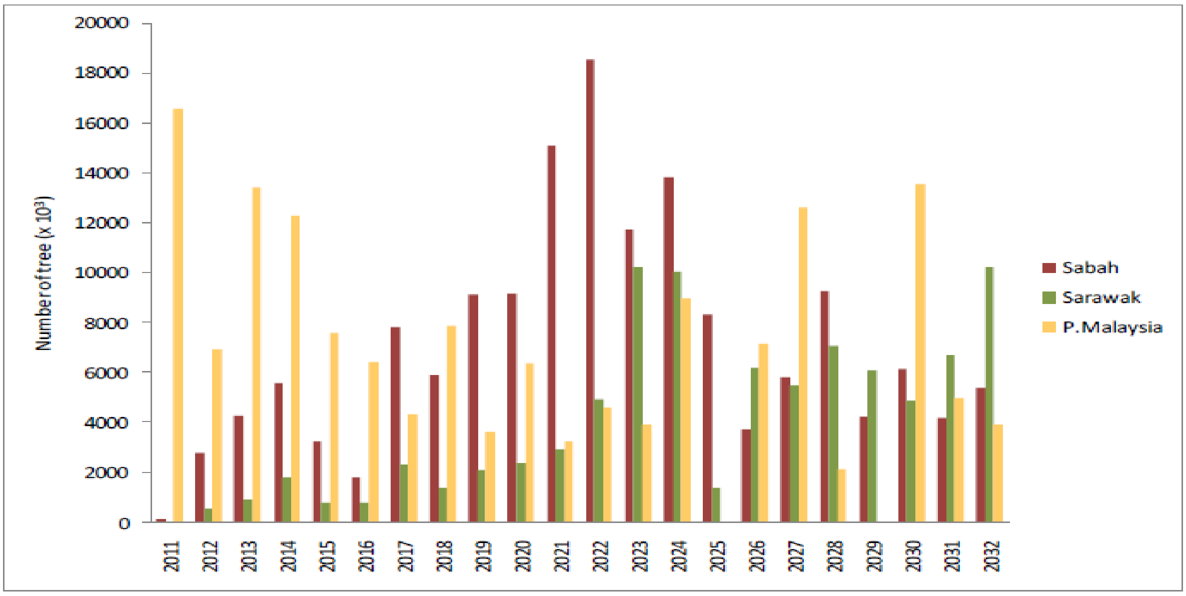


Figure 2.13: The number of potential WPT available annually in Peninsular Malaysia, Sabah and Sarawak

2.6.2 Frond availability from waste palm trees

From these potential WPT, fronds also contribute to biomass generation during felling. Fronds are produced as well during harvesting of the fresh fruit bunches (harvesting). This kind of frond is known as fronds produced during pruning. Frond length steadily increases over the first couple of years, and from around year 4, the stem begins to grow to be taller, at approximately 30 – 60 cm/year. The fronds are structured in two opposing sets of spirals. Normally every 8th frond falls on one spiral and every 13th on the opposing set.

This will be a great chance to utilize the lignocellulosic biomass or by-products of the oil palm, like fronds. Oil palm frond (OPF) is part of the group of fibrous crop residues, which also contains by products for example rice straw. Currently, OPF are left rotting between the rows of palm trees, mostly for soil conservation, erosion control and ultimately the long- term advantage of material recycling. The huge amounts of fronds generated by a plantation each year render these a very promising source of valuable lignocellulosic materials (N. Mohd Nor, 2008). The amount of dry matter weight of the fronds that will be generated from the WPT at time of felling is presented in Table 2.5.

Table 2.5: The amount of dry matter weight of the fronds that will be generated from the WPT at time of felling

Year	***Private Estate***	***FELDA***	***FELCRA***	***RISDA***	***State Scheme***	***Smallholders***	***Total Malaysia***
2011	10,163*	2,648	641	318	1,226	1,838	16,835
2012	6,395	1,666	404	200	771	1,157	10,593
2013	11,566	3,014	730	362	1,395	2,092	19,159
2014	12,226	3,186	772	383	1,474	2,211	20,252
2015	7,207	1,878	455	226	869	1,304	11,938
2016	5,613	1,462	354	176	677	1,015	9,297

2017	9,009	2,347	569	282	1,086	1,630	14,923
2018	9,412	2,452	594	295	1,135	1,702	15,590
2019	9,221	2,403	582	289	1,112	1,668	15,275
2020	11,135	2,901	703	349	1,343	2,014	18,445
2021	13,231	3,447	835	414	1,596	2,393	21,917
2022	17,456	4,548	1,102	547	2,105	3,158	28,916
2023	16,085	4,191	1,015	504	1,940	2,910	26,644
2024	20,453	5,329	1,291	640	2,466	3,700	33,880
2025	5,500	1,433	347	172	663	995	9,111
2026	10,636	2,771	671	333	1,283	1,924	17,618
2027	14,886	3,879	939	466	1,795	2,693	24,657
2028	11,457	2,985	723	359	1,382	2,072	18,979
2029	6,371	1,660	402	199	768	1,152	10,553
2030	15,304	3,988	966	479	1,846	2,768	25,351
2031	9,897	2,579	625	310	1,193	1,790	16,393
2032	12,144	3,164	766	380	1,464	2,197	20,117

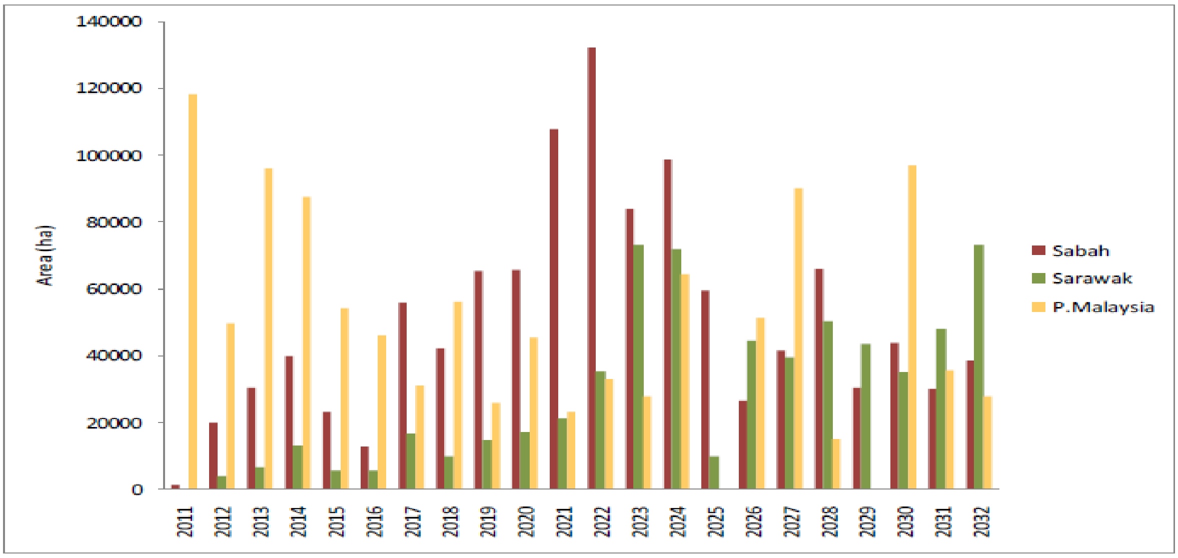


Figure 2.14: Amount of dry matter weight of fronds available annually from potential WPT in Peninsular Malaysia, Sabah and Sarawak

2.7 *Pretreatment*

Pretreatment is a significant tool for practical cellulose conversion processes. The primary purpose for pretreatment is to boost the reactivity of biomass fibers by breaking the lignin seal, solubilize hemicellulose, and disrupt the crystalline structure of cellulose, particularly to the enzymes applied during hydrolysis which mean it is essential before enzymatic hydrolysis can take place, efficiently. It is necessary as a way to modify the structure of cellulosic biomass, to render cellulose more accessible to enzymes that convert carbohydrate polymers into fermentable sugars. A little sugars from the hemicellulose may also be discharged throughout the pretreatment process (Taherzadeh et al., 2004). Figure 2.15 illustrates the alterations in the structure of biomass components whenever going through pretreatment.

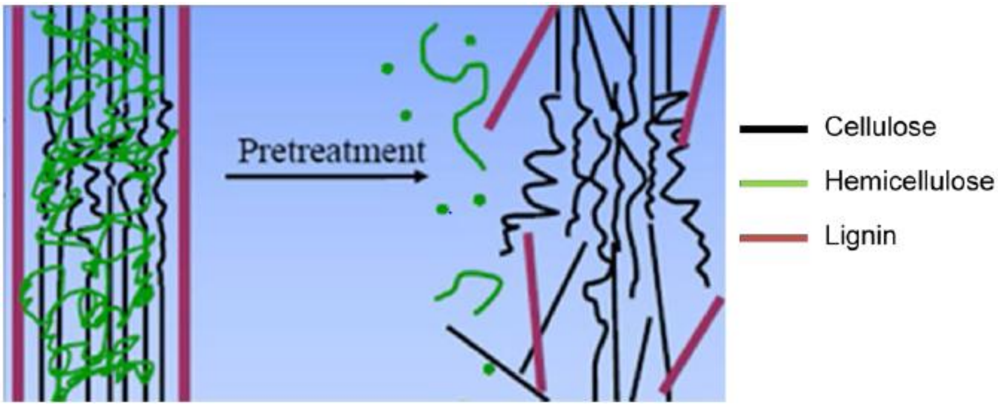


Figure 2.15: Schematic representation of effect of pretreatment on biomass (Mosier, 2005)

To fractionate, solubilize, hydrolyze, and distinguish cellulose, hemicellulose, and lignin components, numerous pretreatment choices are available now. Pretreatment is generally categorized into two types. They are chemical and physical pretreatment. Chemical pretreatment is usually more efficient, however it may lead to costly downstream maintenance. Physical pretreatment does not usually generate by-products, however it is more energy intensive. It is often necessary to apply a combination of the two (physicochemical methods) to increase the pretreatment performance. In each choice, the biomass is treated to decrease its size and open its structure. Pretreatment usually hydrolyzes hemicellulose to the sugars such as xylose, L-arabinose, and others that are water soluble (Malherbe & Cloete, 2002).

Temperature, pressure, particle size of substrate, as well as the existence of catalysts (catalyst concentration) are major parameters that will affect the pretreatment process. Temperature affects the degree of lignin elimination in two methods; it impacts the structure of lignin, according to the glass transition point which is differs between types of lignin, and it affects the kinetics of the delignification process. Furthermore, high temperature usually favours the dissolution of hemicellulose, however may also lead to degradation of these sugars, which is unfavourable to enzymatic hydrolysis and fermentation. Pressure affects the phase where the pretreatment takes place. The liquid phase will need higher pressure, correspond with the necessary temperature for the similar performance regarding delignification, hemicellulose elimination, and cellulose activation. Lastly, the addition of catalysts enables the pretreatment to be performed at lower temperatures. This is advantageous as it may minimize the degree of generation of degradation products. The major factors for an excellent pretreatment process include sugar recovery yield, chip size needed, and low energy utilize; eventually, an effective pretreatment enables optimum enzymatic hydrolysis yield and will not unfavourably influence downstream fermentation (Yang & Wyman 2008).

2.7.1 Physical treatment

2.7.1.1 Mechanical milling

Mechanical milling employs shear to lessen the particle size of the biomass. Usually, the biomass is obtained in chipped or even chopped form. In spite of this, it usually is needed to further grind the chips to maximize the surface area and guarantee proper impregnation of chemicals. The energy utilization against the degree of size reduction is the primary tradeoff that must be analyzed. Besides that, side impacts from extensive milling were examined because of deform of cellulose fiber, as well as coagulation of fine particles (Talebnia et al. 2010).

2.7.2 Chemical treatment

2.7.2.1 Acid treatment

Acid treatment consists of two types, concentrated and dilute. The acid performs as a catalyst and provides a higher interaction with hemicellulose and lignin than cellulose. Concentrated acid triggers high glucose yield throughout enzymatic hydrolysis, yet somehow causes problems when it comes to equipment protection, and generation of highly inhibitory products. From a process perspective dilute acid is much more beneficial in contrast to concentrated acid. The major benefit is the solubilization of lignin and hemicellulose while potentially hydrolyzing xylan into xylose; with fewer xylan to dissolve, more enzymes can work for breaking down the cellulose that is still in polymeric form.

2.7.2.2 Alkaline treatment

Alkaline treatment concentrates on lignin elimination as reducing the sugar degradation observed when acid treatment or hydrothermal treatment is utilized. It is believed that the saponification of intermolecular ester bonds that cross-link xylan and lignin is the primary route for the solubilization of lignin.

Sodium hydroxide (NaOH) is the typical alkaline agent. Besides solubilization of lignin, NaOH leads cellulose fibre to swell, hence maximizing surface area, at the same time minimizing crystallinity of cellulose (Taherzadeh & Karimi 2008).

The major benefit of alkaline treatment is that it can be optimized between two process parameters, that is residence time with the range between 1hr and 24hr and temperature which at room temperature or elevated above 100°C. In recent times, it was discovered that even cold (-5°C to -20°C) NaOH solutions enhanced hydrolysis yield.

2.7.2.3 Organosolv

Organosolv employs solvents to extract lignin without reacting with the sugars, and frequently produces highly digestible fiber from all sorts of biomass (Tomás-Pejó et al. 2011). This treatment normally happens between 100°C and 250°C. Usual solvents utilized are ethanol and acetone. Besides that, addition of acid catalysts has been proven to boost rate of delignification and cellulose reactivity (Zhao et al. 2009). It is possible to acquire non-degraded lignin by employing organosolv treatment; the lignin-solvent can then be assorted and the isolated lignin can be used to produce valued-added products. Disadvantages include additional energy necessity to totally eliminate the solvent before enzymatic hydrolysis (Tze Wei Sun, 2013).

2.7.2.4 Ionic liquid

Ionic liquids are salts that are comprised of a huge organic cation and a small inorganic anion. Examples involve 1-n-butyl-3-methylimidazolium chloride and 1-ethyl-3-methylimidazolium acetate. Because of their potential to break down the hydrogen bonds within the cellulose fibre, and so minimizing the crystallinity, they have gained a lot interest. Several benefits of ionic liquids include their particular affinity to different compounds, which can be tuned to focus on a bond. Additionally, in contrast to organosolv, the low volatility of ionic liquids generates less environmental problems during disposal. Increases in enzymatic hydrolysis yield up to 50-fold have been discovered (Dadi, Varanasi & Schall, 2006). Nevertheless, considering its novel nature and high costs, more studies in process economics and development in recycling is needed before it can be implemented on an industrial-scale.

2.7.3 Physicochemical treatment

2.7.3.1 Autohydrolysis (steam explosion)

Steam explosion requires treatment of ground biomass with high-pressure saturated steam, accompanied by a quick reduction of steam pressure to achieve an explosive decompression (Taherzadeh, 2004). Steam pretreatment efficiently boosts the conversion rate of carbohydrates into fermentable sugars (Varga, Reczey & Zacchi, 2004). It also increases accessibility of the cellulose and increases enzymatic hydrolysis yield (Fernandes-Bolanos, 2001). Residue from steam explosion consists of cellulose and lignin. The lignin can be extracted with solvents like ethanol, butanol, or formic acid. However, the drawback is loss of free sugar because of washing and purification (Dien et al., 2004).

Optimum conditions of the steam explosion procedure differ with type of feedstock. Very extreme reaction conditions can lead to full elimination of the hemicelluloses and provide highly digestible solids, along with partly solubilizing the cellulose fraction. In spite of this, it may also lead to sugar degradation. Too mild conditions, on the contrary, may generate a low yield of oligomeric-hemicellulose-derived sugars that require further hydrolysis before fermentation, and a cellulose fraction that is still resistant to hydrolysis.

2.7.3.2 Liquid hot water

Liquid hot water is a hydrothermal pretreatment to induce modifications in lignin structure but results in minimal lignin solubilisation. The pretreatment is generally conducted at a temperature between 160°C and 240°C, with a range of residence time from seconds to hours. Because of the high temperature (160°C to 240°C), almost all hemicellulose is solubilized, which enhances accessibility of cellulose. The benefit of liquid hot water treatment is economical since zero catalyst is needed, however other cost is associated with additional equipment and additional energy utilized to keep water in liquid state.

2.8 *Enzymatic hydrolysis*

The next step in the production of ethanol from lignocellulosic materials is enzymatic hydrolysis. It requires cleaving the polymers of cellulose and hemicellulose utilizing enzymes. The hemicellulose consists of polymers of a few sugars like mannan, xylan, glucan, galactan, and arabinan, while cellulose normally consists of only glucans. Consequently, the major hydrolysis product of cellulose is glucose, while the hemicellulose generates numerous pentoses and hexoses. But then, high amount of lignin blocks enzyme accessibility where results in end-product inhibition, and lessens the rate and yield of hydrolysis. Besides lignin, cellobiose and glucose also behave as strong inhibitors of cellulases (Y. Corredor, 2008).

Enzymatic hydrolysis of cellulose and hemicellulose has already been learned intensively. The structure of xylan is more complex compared to cellulose and needs a number of different specificities for complete hydrolysis. However, the polysaccharide does not form closely packed crystalline structures like cellulose and in turn, easier to make it to enzymatic hydrolysis (Y. Corredor, 2008). Highest cellulase and β-glucosidase activities happen at 40-60 °C and pH of 4.0 to 5.0. But then, optimal conditions may vary with hydrolysis residence time.

Enzymatic hydrolysis needs moderate conditions and a long time. The performance of hydrolysis of cellulosic materials can be improved by combining pretreatment including dilute acid with high temperature. The hydrolysis can be carried out within a short time with addition of acid. One other issue of enzymatic hydrolysis is that the sugars discharged inhibit the enzyme activities during hydrolysis.

Chapter 3 Materials and Methodology

3.1 *Feedstock collection and preparation*

Oil palm fronds (OPF) were collected from ground of the local palm oil plantation. The OPF residue was then ground and sieved into smaller than 1 mm particle size and kept in plastic bag at room temperature before using for further analysis (Siti Normah et al., 2012).

3.2 *Enzyme*

Both Cellic® CTec2 and the experimental accessory enzymes were obtained from Novozymes A/S (Bagsvaerd, Denmark). Cellic® CTec2 and the experimental enzyme cocktails were stored at 4°C and −30°C, respectively, until needed for hydrolysis of pretreated OPF (Ye Chen et al., 2013).

3.3 *Alkaline pretreatment*

Alkaline pretreatment of OPF were conducted in a 500 ml beaker. The beaker was loaded with material loading of 0.15 g/ml including 60 g of OPF that had been dried for 3 to 4 hours at 105°C and 400 ml of 4.42% (w/w) sodium hydroxide solution. Beaker contents were mixed thoroughly to achieve a total solid loading and the desired alkali loading. The mixture was heat at 100°C for about 1 hour. The treated OPF were then washed intensively with deionized water until neutrality. The washed OPF was dried at 105°C for 24 hours and stored (Ye Chen et al., 2013).

3.4 *Enzymatic hydrolysis*

The pretreated OPF was performed for further enzymatic hydrolysis in 250 Erlenmeyer flask. 1.5 g of alkali pretreated OPF was mixed with 20 ml of 0.2 M sodium acetate buffer with different pH which 4 and 5.6 supplemented with varying enzyme's concentration of 1.5% and 6%. The reaction mixture was hydrolyzed in an incubator shaker at rotation speed of 50 rpm and 200 rpm for 3 hours and 72 hours as the reaction time. After the reaction time, the OPF mixture was taken out and the enzyme reaction was stopped by boiling it for 10 minutes. Triplicate sample for enzyme hydrolysis were set up for each of experiment (Siti Normah et al., 2012; Ye Chen et al., 2013).

3.5 *Reducing sugar analysis*

The hydrolysates were quantified by DNS method. An amount of 3 ml DNS reagent was added to 3 ml of sample. The mixture was boiled in a water bath at 100°C for 10 min. The mixture was cooled at room temperature and 1 ml of potassium sodium tartrate was added into the mixture. The reducing sugar was analyze using UV-Vis spectrophotometer with the absorbance of 575 nm. Standard curve of glucose was used to quantify the concentration of sugar and expressed as gram per litre (g/L).

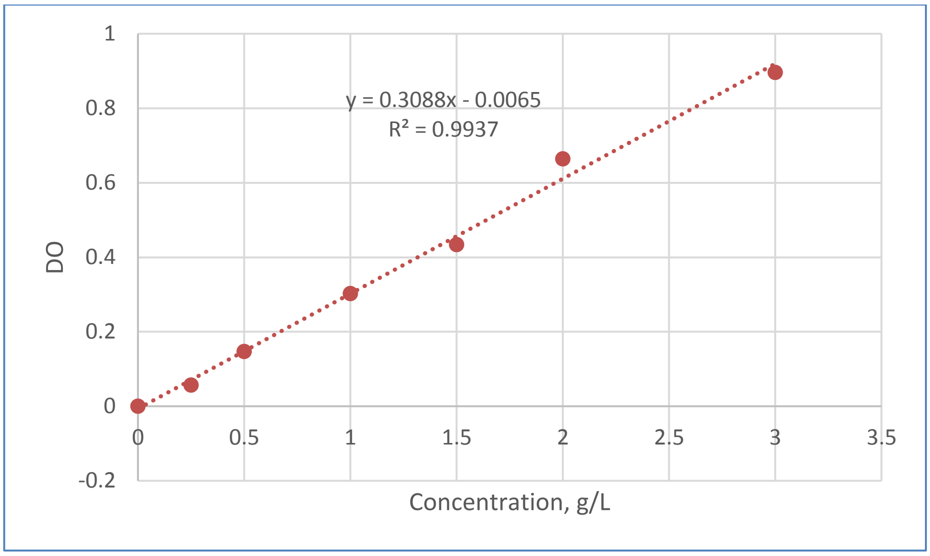
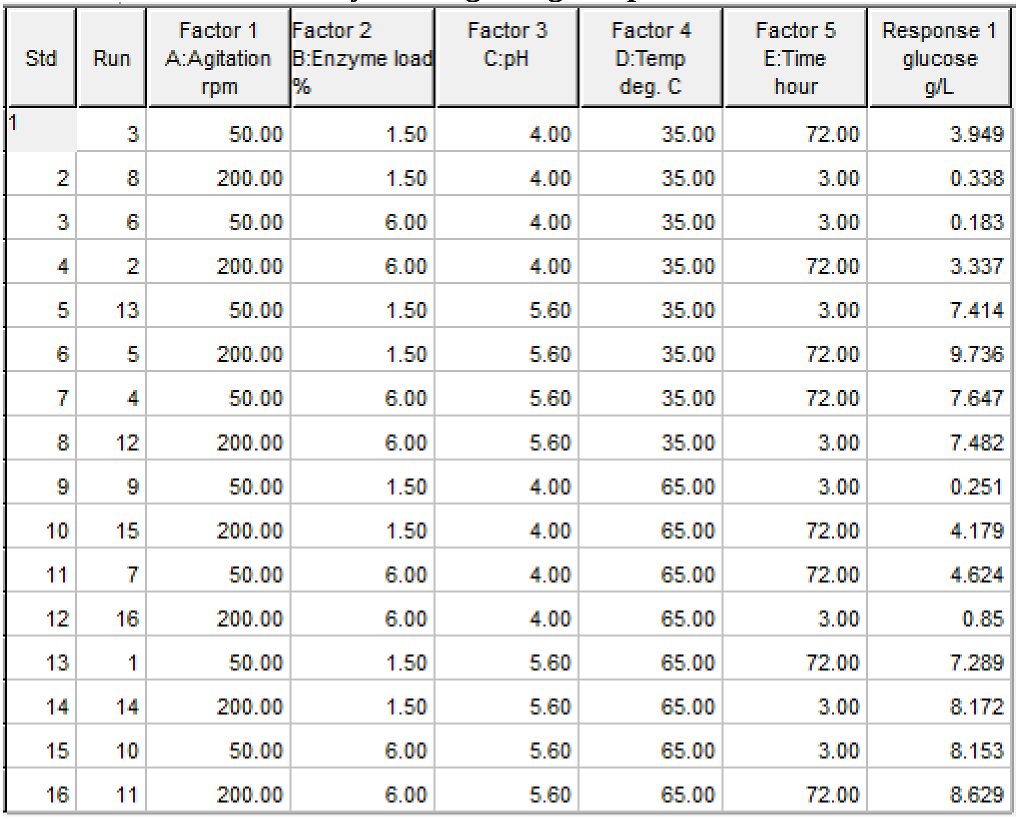


Figure 3.1: Calibration curve of glucose

3.6 *Statistical analysis*

The experimental data was analyzed by using Design Expert Software. Based on two-level factorial, it contains 16 experiments which involving five factors. Each run have different factors combination. After completed all 16 experimental runs, the data were then analyzed using Design Expert Software (Table 3.1).

Table 3.1: Statistical analysis using Design Expert Software



Std	Run	Factor 1 A:Agitation rpm	Factor 2 B:Enzyme load %	Factor 3 C:pH	Factor 4 D:Temp deg. C	Factor 5 E:Time hour	Response 1 glucose g/L
1	3	50.00	1.50	4.00	35.00	72.00	3.949
2	8	200.00	1.50	4.00	35.00	3.00	0.338
3	6	50.00	6.00	4.00	35.00	3.00	0.183
4	2	200.00	6.00	4.00	35.00	72.00	3.337
5	13	50.00	1.50	5.60	35.00	3.00	7.414
6	5	200.00	1.50	5.60	35.00	72.00	9.736
7	4	50.00	6.00	5.60	35.00	72.00	7.647
8	12	200.00	6.00	5.60	35.00	3.00	7.482
9	9	50.00	1.50	4.00	65.00	3.00	0.251
10	15	200.00	1.50	4.00	65.00	72.00	4.179
11	7	50.00	6.00	4.00	65.00	72.00	4.624
12	16	200.00	6.00	4.00	65.00	3.00	0.85
13	1	50.00	1.50	5.60	65.00	72.00	7.289
14	14	200.00	1.50	5.60	65.00	3.00	8.172
15	10	50.00	6.00	5.60	65.00	3.00	8.153
16	11	200.00	6.00	5.60	65.00	72.00	8.629

Chapter 4 Result & Discussion

The model for two level factorial is as follows:

5.14 + 0.20A – 0.026B +2.93C + 0.13D + 1.03E – 0.24AB + 0.24AC + 0.32BD – 0.77CE

A: Agitation speed
B: Enzyme loading
C: pH
D: Temperature
E: Reaction time

The R square obtained was 0.9949.

Based on the model equation, coefficient of the α_1 to α_9 are small compared to constant α_0. This shows that the model equation is good with small errors and can be used for further analysis.

Factors affecting enzymatic hydrolysis

In this research, the most affecting factor in the enzymatic hydrolysis was pH as shown in Table 4.1 with the contribution of 81.07%. The second highest factor affecting the enzyme hydrolysis is reaction time by having value of 10.13%. The other three factors are agitation (0.38%), temperature (0.16%) and finally is enzyme loading (0.00662%). The actual pH values of the substrate suspensions consist of the buffer solution and enzymes (CTec2). Lignin is known as hydrophobic. Through surface functional groups, pH can affect the charge of substrate surface to alter surface hydrophobicity. This may also affect electrostatic interactions between cellulase and lignin (Lan, Zou & Zhu, 2012). The alteration of lignin will not only increase enzyme accessibility but also reduce enzyme nonspecific absorption on lignin.

Table 4.1: Contribution of factors

Factors	**Contribution (%)**
Agitation speed	0.38
Enzyme loading	0.00662
pH	81.07
Temperature	0.16
Reaction time	10.13

Aside affecting the lignin, pH also affect the enzyme activity. Extremely high or low pH values normally result in negative effect for most enzymatic activity. pH is also a factor in the stability of enzymes. As with activity, for each enzyme there is also a region of pH optimal stability.

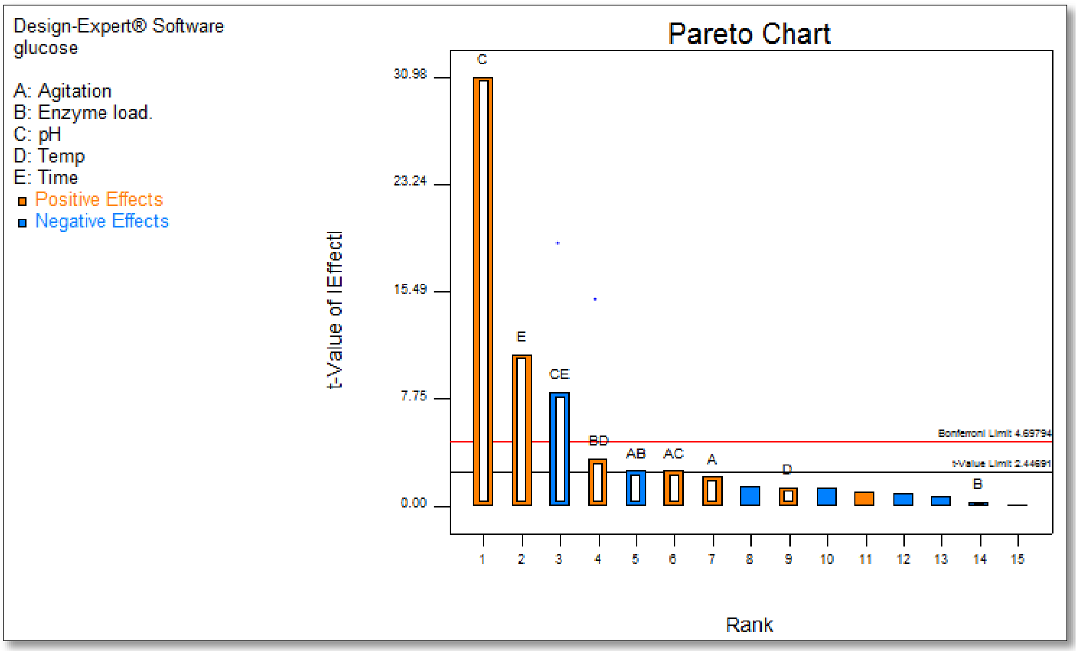
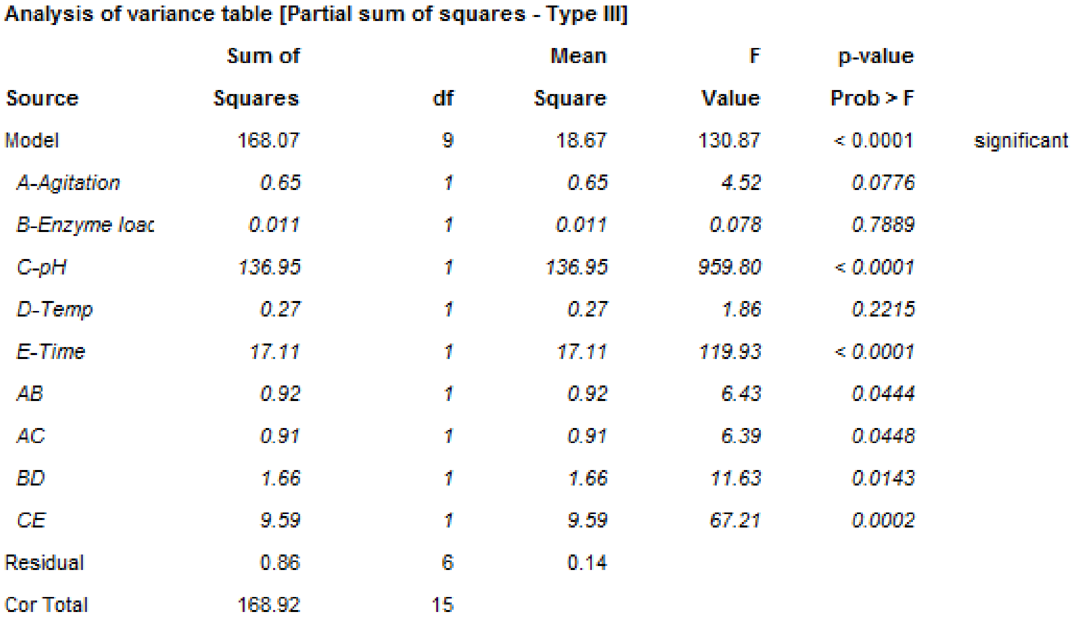


Figure 4.1: Pareto chart

The black vertical line shown in Figure 4.1 indicate that the model terms of BD, AB and AC are significant as they exceed the line. Whereas model terms of C, E and CE are more significant as they exceed the red line.

Table 4.2: Statistical analysis of the effects of enzyme hydrolysis



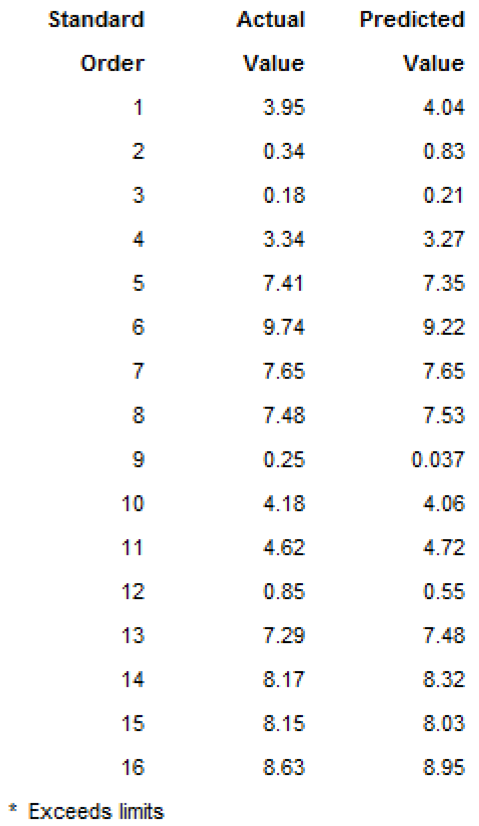
Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	168.07	9	18.67	130.87	< 0.0001	significant
A-Agitation	*0.65*	*1*	*0.65*	*4.52*	*0.0776*	
B-Enzyme loac	*0.011*	*1*	*0.011*	*0.078*	*0.7889*	
C-pH	*136.95*	*1*	*136.95*	*959.80*	*< 0.0001*	
D-Temp	*0.27*	*1*	*0.27*	*1.86*	*0.2215*	
E-Time	*17.11*	*1*	*17.11*	*119.93*	*< 0.0001*	
AB	*0.92*	*1*	*0.92*	*6.43*	*0.0444*	
AC	*0.91*	*1*	*0.91*	*6.39*	*0.0448*	
BD	*1.66*	*1*	*1.66*	*11.63*	*0.0143*	
CE	*9.59*	*1*	*9.59*	*67.21*	*0.0002*	
Residual	0.86	6	0.14			
Cor Total	168.92	15				

Based on Table 4.2, the Model F-value of 130.87 implies the model is significant. Values of “Prob > F” less than 0.0500 indicate that the model terms are significant. In this case C, E, AB, AC, BD and CE are significant model terms. Values greater than 0.1000 indicate the model are not significant.

pH (C) and reaction time (E) had a highly significant effect ($p < 0.0500$), with a positive effect (Figure 4.1), indicating that glucose yield and cellulose conversion were increased when the pH and reaction time increase. Whereas, enzyme loading (B) did not present significant effect for glucose yield. The enzyme loading presented a negative effect ($p > 0.0500$) indicating that lower the enzyme loading used, the higher the cellulose conversion achieved.

Table 4.3: Diagnostics case statistic



Standard Order	Actual Value	Predicted Value
1	3.95	4.04
2	0.34	0.83
3	0.18	0.21
4	3.34	3.27
5	7.41	7.35
6	9.74	9.22
7	7.65	7.65
8	7.48	7.53
9	0.25	0.037
10	4.18	4.06
11	4.62	4.72
12	0.85	0.55
13	7.29	7.48
14	8.17	8.32
15	8.15	8.03
16	8.63	8.95

* Exceeds limits

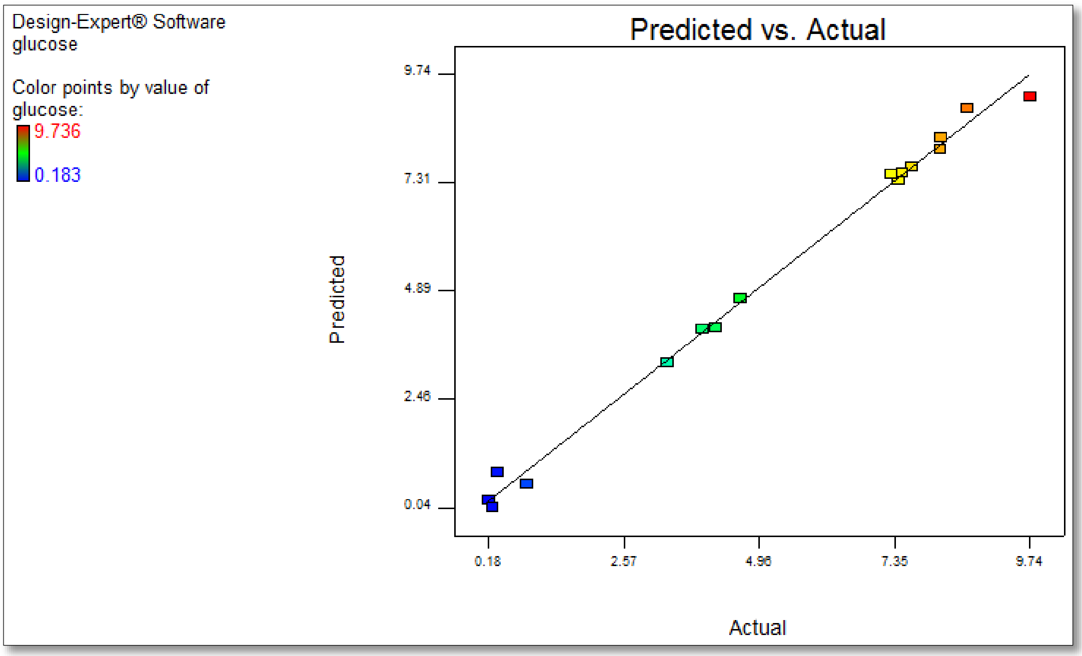
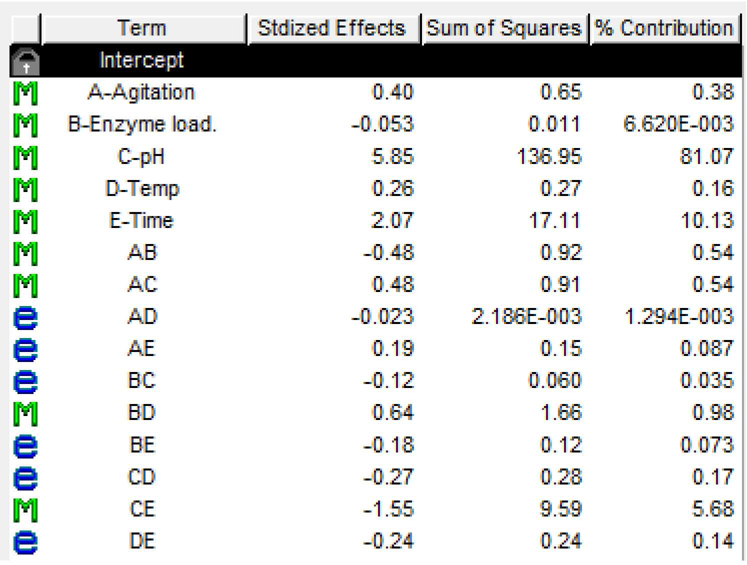


Figure 4.2: Graph of predicted versus actual of glucose conversion

Figure 4.2 illustrated the graph of predicted versus actual of glucose conversion. All of the predicted values of 16 standard runs are close to actual values.

Interaction between factor pH with reaction time

The relations between factors were tested and as results, three interactions provide significant effect towards enzymatic hydrolysis. Based on Figure 4.3, the interaction between pH and reaction time is 5.68% contribution. Whereas, interaction between factor of enzyme loading with temperature and interaction between agitation speed and enzyme loading having a value of 0.98%and 0.54% contribution respectively.



	Term	Stdized Effects	Sum of Squares	% Contribution
🔒	Intercept			
M	A-Agitation	0.40	0.65	0.38
M	B-Enzyme load.	-0.053	0.011	6.620E-003
M	C-pH	5.85	136.95	81.07
M	D-Temp	0.26	0.27	0.16
M	E-Time	2.07	17.11	10.13
M	AB	-0.48	0.92	0.54
M	AC	0.48	0.91	0.54
e	AD	-0.023	2.186E-003	1.294E-003
e	AE	0.19	0.15	0.087
e	BC	-0.12	0.060	0.035
M	BD	0.64	1.66	0.98
e	BE	-0.18	0.12	0.073
e	CD	-0.27	0.28	0.17
M	CE	-1.55	9.59	5.68
e	DE	-0.24	0.24	0.14

Figure 4.3: Percentage contribution of factors

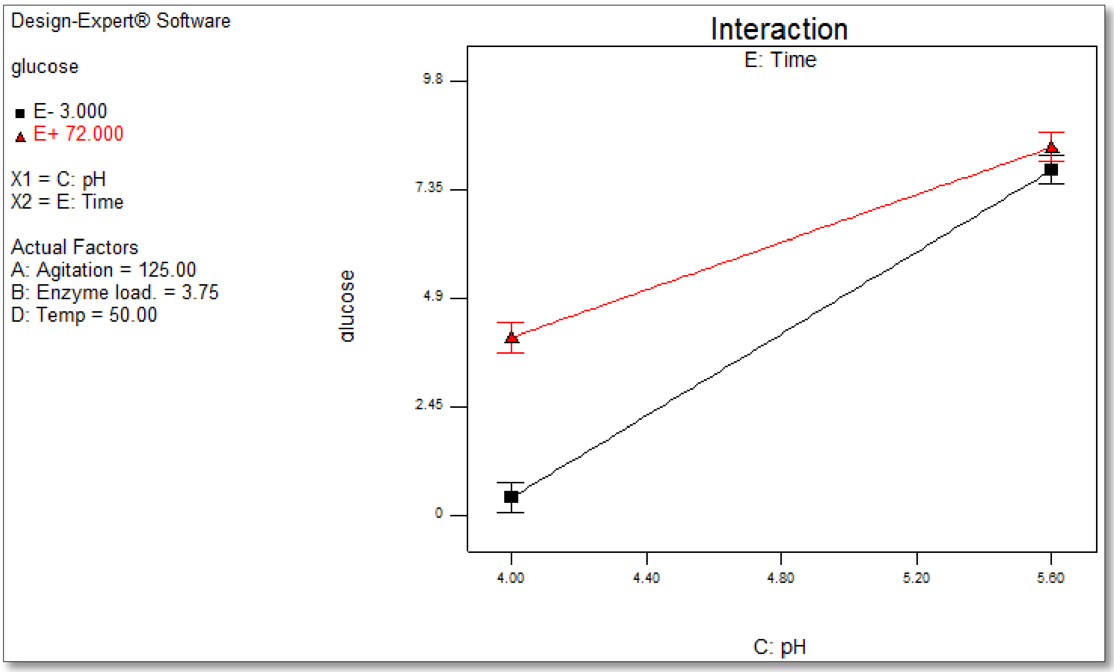


Figure 4.4: Interaction graph between pH and reaction time

The interaction between factor pH and reaction time gives highest contribution of 5.68% to the enzymatic hydrolysis process and the most significant interaction as referred in Figure 4.1. The trend of interaction graph between pH and reaction time was shown in Figure 4.4. As referred to the graph, reaction time gives effect to the enzymatic hydrolysis. Increasing of the pH value resulting increasing of glucose concentration with both reaction time. At pH 4, glucose concentration was increase as reaction time increase. At pH 5.6, glucose concentration was increase too with increasing of reaction time.

Interaction between enzyme loading with temperature

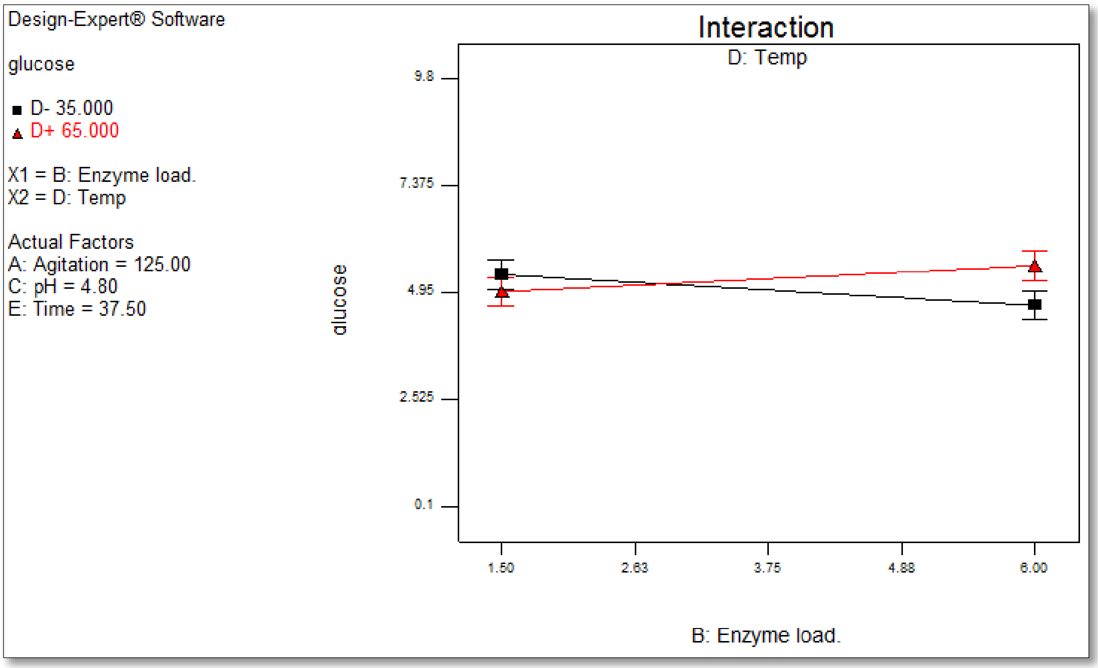


Figure 4.5: Interaction graph between enzyme loading and temperature

This interaction gives second highest contribution of 0.98%. As referred to the Figure 4.5, temperature gives effect to the enzymatic hydrolysis. In Figure 4.1, the interaction between enzyme loading and temperature (BD) exceed half of the black line which is more significant than AB. At enzyme loading 1.5%, glucose concentration was increase as temperature decrease. At enzyme loading 6%, glucose concentration was increase too with increasing of temperature.

Interaction between agitation speed with enzyme loading

Interaction between agitation speed with enzyme loading gives the lowest contribution of 0.54%. As referred to the Figure 4.6, even enzyme loading gives the lowest contribution for single effect but the interaction with agitation speed (AB) is slightly significant (Figure 4.1). At 50 rpm, glucose concentration was increase as enzyme loading increase. At 200 rpm, glucose concentration was decrease with increasing of enzyme loading.

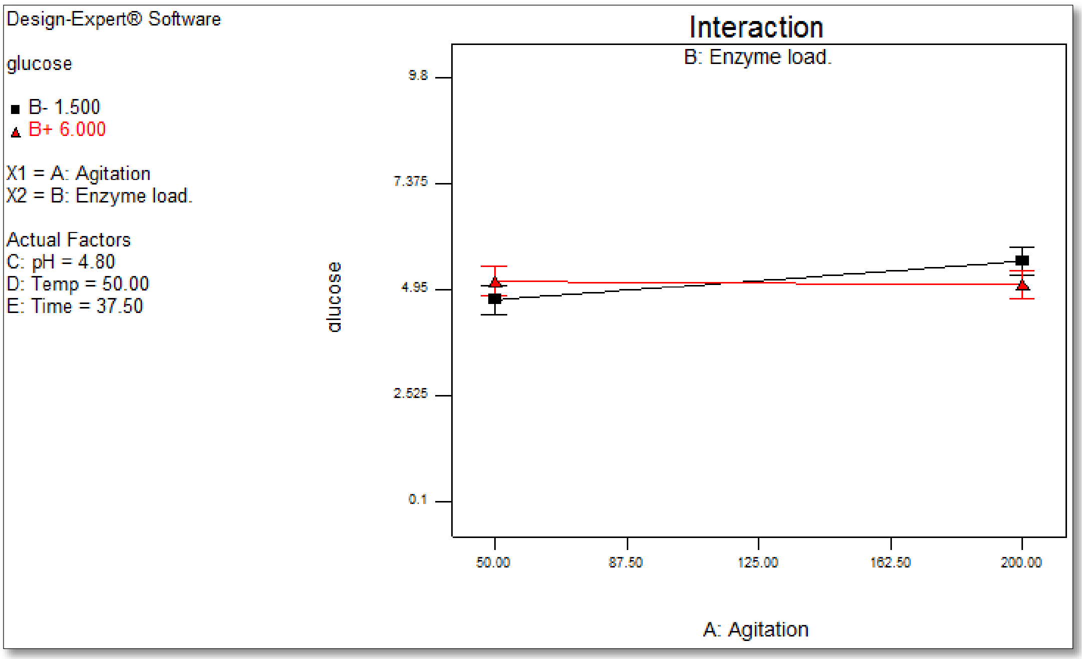


Figure 4.6: Interaction graph between agitation speed and enzyme loading

The cellulose hydrolysis by cellulase enzymes needs a sufficient mixing to make sure adequate contact between the conversion yield, substrate and enzymes, and to provide heat and mass transfer within the reaction vessel. Certain agitation raises the hydrolysis rate and yields, but extreme mixing can deactivate the enzymes and decrease the conversion yield (Ingesson et al., 2001; Musatto et al., 2008).

Chapter 5 Conclusion

Based on the result obtained from the Design Expert Software, pH shows the most affecting factors with 81.07% contribution in enzymatic hydrolysis. pH gives effect on the lignin modification where it increase the accessibility of enzyme to obtain higher concentration of glucose. The factors that shows highest interaction is between pH and reaction time. They give the highest contribution of 5.68%. The model equation is significant and can be used for further analysis.

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